UNIVERSITÉ DE STRASBOURG

UNIVERSITE DE STRASBOURG

THÈSE

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Rôle du niveau d'activité physique dans la régulation de la balance oxydative des lipides exogènes - Inférences dans la physiopathologie de l'obésité.

Jury

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« Aujourd'hui, avant de terminer, nous allons parler d'alimentation. Oubliez cette histoire de régimes. » … « Nous avons survécu depuis des millénaires parce que nous étions capables de manger. Et de nos jours, on dirait que c'est devenu une malédiction. Pourquoi ? Qu'est ce qui nous pousse à vouloir garder, à quarante ans, le corps que nous avions quand nous étions jeunes? Est-il possible d'arrêter cette dimension du temps? Et pourquoi avons-nous besoin d'être maigres ? » ...

« Nous n'en avons pas besoin. Nous achetons des livres, nous fréquentons des salles de gymnastique, nous perdons une part très importante de notre concentration à essayer d'arrêter le temps, alors que nous devrions célébrer le miracle de marcher dans ce monde. Au lieu de réfléchir à la façon de vivre mieux, nous sommes obsédés par le poids. « Oubliez ça ; vous pouvez lire tous les livres que vous voudrez, faire les exercices que vous désirerez, subir toutes les punitions que vous déciderez de vous infliger, vous n'aurez que deux choix - ou vous cessez de vivre ou vous allez grossir»

« Mangez avec modération, mais mangez avec plaisir ; le mal n'est pas ce qui entre dans la bouche de l'homme, mais ce qui en sort. Rappelez-vous que pendant des millénaires nous avons lutté pour ne pas avoir faim. Qui a inventé cette histoire selon laquelle nous devons tous être maigres toute notre vie? » « … Utilisez l'énergie et l'effort que représente un régime pour vous nourrir du pain spirituel. Comprenez que la Grande Mère donne avec abondance et avec sagesse - respectez cela, et vous ne grossirez pas plus que le temps ne l'exige.» Plutôt que de brûler artificiellement ces calories, efforcez-vous d'en faire l'énergie nécessaire pour lutter pour vos rêves ; personne n'a maigri pour très longtemps grâce à un régime. »

Paulo Coelho : *La sorcière de Portobello*

Ce travail de thèse a fait l'objet de 4 articles :

Publication acceptée

- **Edwina Antoun**, Iman Momken, Audrey Bergouignan, Clément Villars, Carine Platat, Dale A. Schoeller, Stéphane Blanc, et Chantal Simon. The [1-13C]acetate recovery factor to correct tracer-derived dietary fat oxidation is lower in overweight insulin-resistant subjects. *e-SPEN, the European e-Journal of Clinical Nutrition and Metabolism 5 (2010) e173–e179*

Publications en révision

- **Edwina Antoun**, Audrey Bergouignan, Iman Momken, Dale A. Schoeller, Guillemette Gauquelin-Koch, Chantal Simon, Stéphane Blanc. Habitual Physical activity level predicts metabolic flexibility. *Diabetologia*

Publications soumises

- **Antoun Edwina**, Momken Iman, Audrey Bergouignan, Zimmer Cedric, Sylvie Normand, Michel Desage, Laure Gabert, Blanc Stephane, Simon Chantal. Effects of two-month training at current recommendation on the trafficking of dietary fat. *American Journal of Clinical Nutrition*

- Momken Iman, **Antoun Edwina**, Platat Carine, Simon Chantal, Blanc Stephane. Effects of moderate physical activity and detraining on exogenous saturated and monounsaturated fatty acids repartition. *Diabetes*

SOMMAIRE

LISTE DES ABREVIATIONS

2-MAG: 2-monoacylglycérol
ACC: acétyl-CoA carboxylase
ACSM: American College of Sports Medicine
AEE: activity energy expenditure – dépense énergétique liée à l'activité physique
AHA: American Heart Association
Akt/PKB: sérine/thréonine protéine kinase
AMP: adénosine monophosphate
ARF: acetate recovery factor – facteur de récupération de l'acétate
ARNm: acide ribonucléique messager
ATP: adénosine triphosphate
AUC: area under the curve – aire sous la courbe
BMI: body mass index – indice de masse corporelle
BR: bed rest – modèle d'alitement
cAMP: cyclic adenosine monophosphate – adénosine monophosphate cyclique
CM: chylomicron
COX4: citrate synthase
CPT1: carnitine palmitoyl-transférase 1
DAG: diacylglycérol
dARF: dietary acetate recovery factor – facteur de récupération de l'acétate alimentaire
DE: dépense énergétique
DER: dépense énergétique de repos
DES1: sphingolipide delta(4)-désaturase
DET: dépense énergétique totale
DNA: deoxyribonucleic acid – acide deoxyribonucléique
FA: fatty acid – acide gras
FABPc: cytoplamic fatty –acid binding proteins – protéines cytoplasmiques de liaison des acides gras
FABP$_{PM}$: plasma membrane fatty-acid binding protein – protéines menbranaires de liaison des acides gras
FAT/CD36: fatty acid transporter CD36 – transporteur CD36 des acides gras
FAT: fatty acid translocase – translocase des acides gras
FATP: fatty-acid transporter protein – transporteur protéique des acides gras
FFM: fat free mass - masse maigre
FM: fat mass - masse grasse
G: glucose
GLUT-4: transporteur de glucose type 4
HDL: high density lipoprotein – lipoprotéine de haute densité
HGPO: hyperglycémie provoquée par voie orale
HSL: hormone sensitive lipase
IGT: sujet intolérant au glucose
IMC: indice de masse corporelle
IMTG: intramuscular triglyceride - triglycéride intramusculaire
Kbr: potassium bromide
LIPOX: lipid oxidation study – étude de l'oxydation des lipides
LPL : lipoprotéine lipase
MET: équivalent métabolique (rapport du coût énergétique d'une activité donnée à la dépense énergétique au repos)
MGL: lipase du monoacylglycérol
MHC: myosin heavy chain – chaîne lourde de myosine

MOSPA-Q: MONICA study of physical activity questionnaire – questionnaire MONICA de l'étude de l'activité physique
MPE: molar percent enrichment – pourcentage d'enrichissement molaire
mtGPAT: mitochondrial Glycerol-3-Phosphate Acyltransferase – acyltransférase mitochondriale du glycerol-3-phosphaste
MTP: intestinal microsomal triacylglyceride transfer protein – protéine intestinale microsomale de transfert du triacylglycéride
MUFA: monounsaturated fatty acid – acide gras monoinsaturé
NAP: niveau d'activité physique
ND: non déterminé
NEAEE: non exercise activity energy expenditure – activité spontanée
NEFA: non-esterified fatty acid – acide gras non esterifié
NEFA: plasmatic free fatty acid – acide gras plasmatique
NPRQ: non protein respiratory quotient - ratio d'échange respiratoire non protéique
OAA: oxaloacetate - oxaloacétate
PAL: physical activity level – niveau d'activité physique
PEP: phosphoenolpyruvate – phosphoénolpyruvate
PGC-1α: peroxisome proliferator-activated receptor coactivator – 1alpha
PPAR-β: peroxisome proliferator-activated receptor beta
PRKAA2: AMP-activated, alpha 2 catalytic subunit protein kinase
REE: resting energy expenditure – dépense énergétique de repos
RMR: resting metabolic rate - dépense énergétique de repos
RNA: ribonucleic acid – acide ribonucléique
RT3: triaxial accelerometer – accéléromètre triaxiale
RT-QPCR : real time polymerase chain reaction – amplification en chaîne par polymérase
SEM: standard error of the mean – erreur standard
SFA: saturated fatty acid – acide gras saturé
SPTLC1, SPTLC2: serine palmitoyltransferase long chain base subunit 1 / 2
SREBP1: Sterol regulatory element-binding protein 1
T2D: diabète du type 2
TAG: intramuscular triacylglycerol – triacylglycérol intramusculaire
TBW: total body water – eau corporelle
TCA: tricarboxylic acid cycle – cycle de l'acide tricarboxylique
TEE: total energy expenditure – dépense énergétique totale
TG: trigylcéride
UCP3: uncoupling protein 3 – protéine 3 de découplage
VLDL: very low density lipoprotein – lipoprotéine de très faible densité
VO2max: consommation maximale d'oxygène
WHO: world Health Organization
WISE2005: Women International Space Simulation for Exploration
α2-AMP: sous-unité α2-protéine kinase activée

PRÉAMBULE

Interprétation solo II d'Alfred Gockel

De récentes données épidémiologiques ont mis en évidence une association entre les comportements sédentaires adoptés par les sociétés occidentales et de nombreuses maladies chroniques tels que les maladies cardiovasculaires, l'obésité et le diabète. En effet, avec l'émergence des innovations technologiques, l'homme n'a plus besoin de dépenser beaucoup d'énergie pour subvenir à ses besoins vitaux. De ce fait, l'adoption des comportements sédentaires s'est répandue dans les populations modernes. En parallèle, de nombreuses maladies chroniques déjà existantes, ont pris une plus grande ampleur ce dernier siècle. Cette simultanéité d'évènements suggère une interaction entre l'adoption d'un mode de vie sédentaire et l'émergence du « Sedentary death syndrome » (Lees and Booth, 2004). Une des théories qui pourrait expliquer cette croissance est que 95% de nos gènes ont été sélectionnés avec l'apparition de l'*Homo sapiens* dans une société de chasseur-cueilleur nécessitant un grand potentiel physique, et que nos gènes, depuis cette ère, ont peu évolué. Ainsi, un décalage entre la réduction du niveau d'activité suite à la révolution industrielle et l'adaptation génétique face à ce phénomène, s'est installé. Il a été estimé que l'homme actuel dépense seulement 65% de la dépense énergétique de ses ancêtres, et notre niveau d'activité est en dessous de celui pour lequel nos gènes ont été génétiquement programmés (Cordain *et al.*, 1998).

En plus, à travers les âges, notre régime alimentaire s'est diversifié; les produits raffinés sont apparus et notre alimentation s'est enrichie progressivement en lipides. Au-delà de la quantité, la nature des acides gras a aussi été modifiée avec des proportions d'acides gras saturés supérieures à ce que consommaient nos ancêtres. Sachant que les acides gras sont différemment utilisés par l'organisme suivant leur nature, cette modification dans la composition des lipides du régime alimentaire constitue également un changement environnemental en soi important à noter. Cependant, cette théorie ne permet pas d'expliquer le fait que certains individus échappent aux maladies du siècle moderne et en outre à l'obésité. Une des hypothèses serait que ces individus s'adaptent à ce phénomène en augmentant leur niveau d'activité physique à un seuil d'équilibre avec la balance énergétique. De ce fait, bien que nos gènes jouent un rôle dans la régulation du poids via la susceptibilité individuelle, la modification des facteurs environnementaux tel le niveau d'activité physique semble être un facteur déterminant face au déclenchement de la prise de poids.

Face à la prévalence alarmante de l'obésité (Annexe 1), il est important d'étudier les relations de cause à effets entre les différents niveaux d'activité et le développement de cette maladie. De telles études permettront de démasquer les mécanismes impliqués dans la régulation du poids et altérés par l'inactivité physique et qui pourront être améliorés avec l'activité physique, mettant ainsi en avant de nouvelles pistes préventives ou thérapeutiques.

En outre, l'obésité est une maladie du stockage des lipides caractérisée par une incapacité à utiliser les lipides alimentaires comme substrat énergétique et par un acheminement préférentiel des lipides dans le tissu adipeux pour y être stockés plutôt que vers le muscle pour y être oxydés. Les anomalies de l'utilisation des acides gras chez l'obèse se sont retrouvées après perte de poids, indiquant qu'elles pourraient être antérieures et participer à la constitution de l'excès de masse. L'activité physique, en tant que composante la plus modulable de la dépense énergétique totale, pourrait jouer un rôle clef dans le devenir des lipides. Toutefois,

hormis les données existantes sur les effets aigus de l'activité physique sur la lipémie, nous disposons de peu de données concernant l'effet de la modulation du niveau habituel d'activité physique sur l'oxydation et la répartition des lipides alimentaires.

Le but de ce travail de thèse a été d'étudier, chez l'homme normopondéré et en surpoids, les mécanismes par lesquels une dépense énergétique liée à l'activité physique, basse ou élevée, module l'oxydation des lipides alimentaires en portant une attention toute particulière à l'évaluation des recommandations actuelles d'activité physique. Pour cela, nous avons soumis deux groupes de sujets sédentaires différant par leur masse corporelle (surpoids et normopondéré) à deux mois d'entraînement basé sur les recommandations actuelles et un troisième groupe d'hommes actifs normopondérés à un mois de désentraînement, avec un maintien d'un poids stable tout au cours de l'étude.

Dans l'**introduction**, le **Chapitre 1** présente les concepts de balances énergétique et oxydative des substrats et les troubles métaboliques de prise de poids en attachant une importance au le métabolisme des lipides alimentaires. Le **Chapitre 2** présente l'impact de la modulation de l'activité physique sur le métabolisme énergétique et oxydatif puis s'attache à mettre en avant les interactions du niveau d'activité physique avec les lipides. À la suite, le **Chapitre 3** expose les objectifs et les hypothèses de ce travail de thèse.

La **partie expérimentale** est composée de 4 parties:

Nous nous sommes tout d'abord intéressés aux effets directs de l'inactivité physique et de l'activité physique sur le métabolisme postprandial des lipides alimentaires en fonction de leur nature. Plus spécifiquement, nous présentons l'impact de l'inactivité physique et de l'activité physique sur l'oxydation des acides gras exogènes saturés et monoinsaturés chez l'homme de poids normal (**Chapitre 4**) et l'effet de l'activité physique chez des hommes en surpoids (**Chapitre 5**). Nous avons aussi cherché à caractériser l'influence de l'inactivité physique et de l'activité physique sur la flexibilité métabolique combinant nos résultats obtenus dans les chapitres 4 et 5 avec ceux d'études spatiales précédemment réalisées sur un modèle d'alitement prolongé (**Chapitre 6**). Les protocoles classiquement utilisés pour l'étude du métabolisme lipidique exogène font appel à l'utilisation d'isotopes stables pour lesquelles des corrections doivent être appliquées. Le **Chapitre 7** vérifie l'emploi de ces corrections, définies pour des sujets normopondérés, dans le cadre de sujets en surpoids.

Enfin, une **discussion** générale rappellera les principales conclusions de ce travail de thèse et permettra d'envisager les perspectives et les implications de nos résultats au niveau de la santé.

Introduction

Parfaitement équilibré, d'Alfred Gockel

Chapitre 1

La régulation pondérale

1. EXISTERAIT –IL UNE ACTIVITE PHYSIQUE SEUIL POUR LA REGULATION PONDERALE ?

La régulation de l'apport alimentaire est flexible. Ainsi, toute augmentation de dépense énergétique due à une activité physique est automatiquement suivie par une équivalente régulation en apport calorique. Cette supposition montre clairement le rôle de l'activité physique comme facteur dans la régulation du poids. Dans les années 1950, Jean Mayer *et al.* (Mayer *et al.*, 1954) ont avancé l'hypothèse qu'il existerait un seuil d'activité physique en dessous duquel les mécanismes de régulation du poids sont inopérants, en se basant sur des études transversales faites chez le rat (Mayer *et al.*, 1954) puis chez l'homme (Mayer *et al.*, 1956).

En effet, Mayer *et al.* (Mayer *et al.*, 1954) ont émis l'hypothèse d'une zone d'activité physique compensatoire dans laquelle les rats actifs réalisant 1 à 6h d'activité physique par jour en courant sur un tapis roulant, augmentent leur prise alimentaire en fonction de la durée de l'exercice physique et par conséquent maintiennent leurs poids stables. Cette zone d'activité a été nommée « zone compensatoire » puisque la quantité d'énergie ingérée augmente en fonction de dépense énergétique engendrée par l'excercise et le poids reste constant. Une cessation de prise alimentaire est observée à des niveaux d'activité physique plus élevés et les rats extrêmement actifs perdent du poids. Néanmoins, la réduction de l'activité physique n'est pas accompagnée d'une diminution de prise alimentaire. En effet, les rats qui passent moins d'1 heure par jour à courir, augmentent leur prise alimentaire ce qui résulte en une prise de poids en comparaison avec les rats de la zone compensatoire. Une déficience dans la régulation de la prise alimentaire a été suggérée pour expliquer le gain d'adiposité observé.

Dans une seconde étude menée sur des Indiens adultes du Bengale classés en fonction de leur niveau d'activité physique en se basant sur leurs catégories professionnelles, Mayer *et al.* (Mayer *et al.*, 1956) ont observé que les hommes employés dans des fonctions qui demandent un travail physique important ont une consommation alimentaire fonction de leur besoins énergétiques mais sont plus minces que les hommes qui ont un travail sédentaire. En revanche, ces derniers ont une prise alimentaire supérieure à celle des hommes ayant un travail physique important et présentent un poids plus élevé. Ce déséquilibre énergétique était comparable à celui observé au préalable chez les rats. Suite à ces observations, Mayer *et al.* (Mayer *et al.*, 1956) ont émis l'hypothèse qu'il existerait un seuil d'activité physique en dessous duquel les mécanismes de régulation du poids sont inopérants, ce qui conduit à une augmentation du poids corporel (**Figure 1**). Cependant, du fait du manque de précisions des mesures de la dépense énergétique totale, aucune conclusion n'a pu être tirée quant au rôle direct de l'inactivité dans la prise du poids. L'existence d'un niveau seuil d'activité physique en dessous duquel les mécanismes de régulation du poids corporel seraient inopérants reste donc à démontrer.

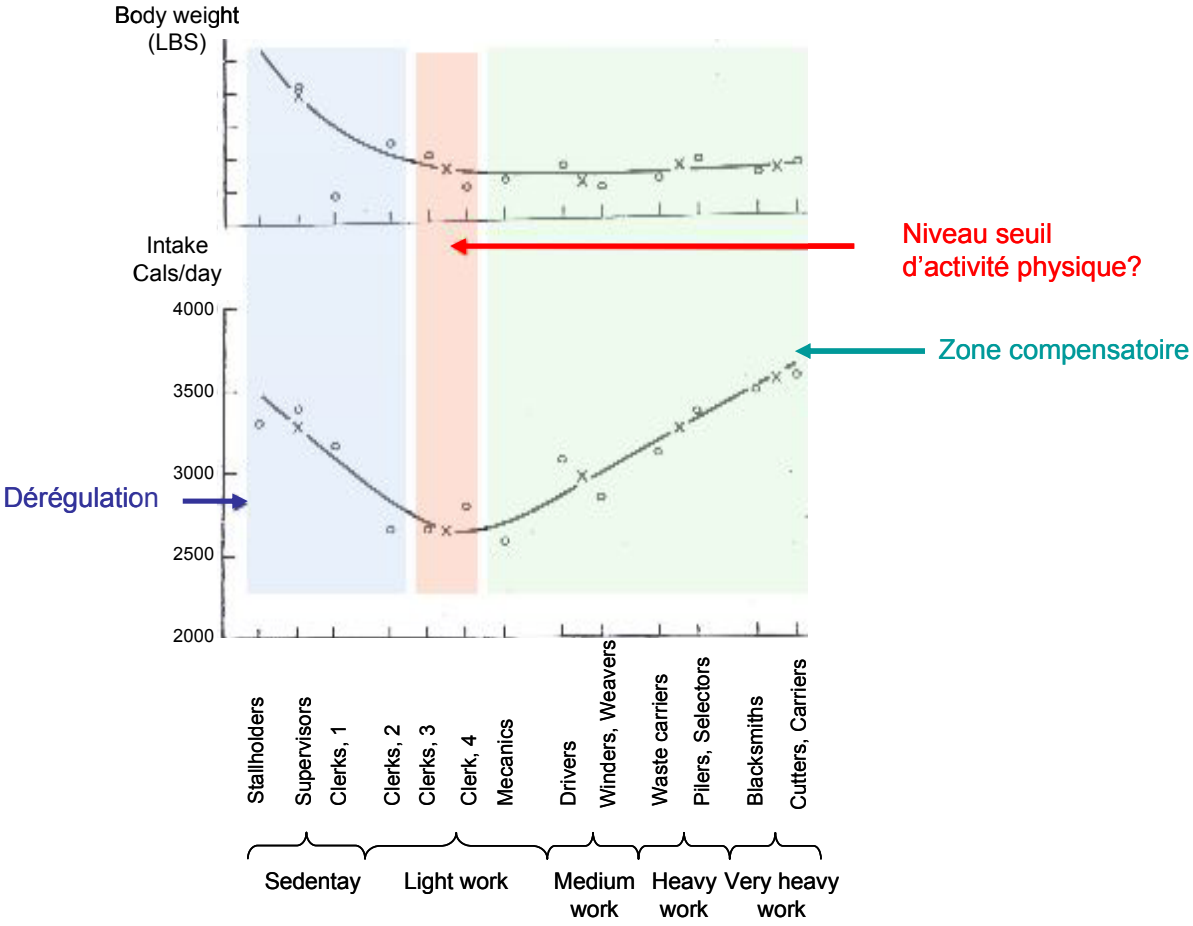


Figure 1 : Relation entre l'apport alimentaire, le poids corporel et le niveau d'activité physique chez les Indiens du Bengale (Mayer *et al.*, 1956).

Récemment, avec les nouvelles techniques de mesure de dépense énergétique comme l'eau doublement marquée, il a été montré que les personnes qui pratiquent une activité physique ayant un coût énergétique inférieur à 4 MJ par jour pendant 14 jours ne modifient pas leurs apports alimentaires (Blundell *et al.*, 2003). En revanche, la pratique d'une activité physique intense s'accompagne d'une réduction de l'appétit à court terme (Blundell and King, 1999). Plus globalement, les résultats analysant la relation entre activité physique et apports alimentaires restent très divergents avec seulement 19 % des études d'intervention rapportant une augmentation des apports nutritionnels après exercice, 65 % ne montrant pas de modification et 16 % faisant état d'une diminution de l'alimentation (Blundell and King, 1999). Des études longitudinales ont démontré que des femmes post-obèses étaient capables de maintenir leurs indices de masse corporelle IMC normaux si et seulement si elles conservaient un niveau d'activité physique PAL d'environ 1,70-1,75 (Schoeller *et al.*, 1997; Weinsier *et al.*, 2002). Aussi, Murgatroyd *et al.* (Murgatroyd *et al.*, 1999) ont observé que pour un régime spécifique (dense *vs.* faible teneur en lipides), les sujets (actifs *vs.* sédentaires) consommaient les mêmes calories quel que soit leur niveau de dépense énergétique. De même, Shepard *et al.* (Shepard *et al.*, 2001) ont observé que la diminution de l'activité physique associée à un environnement sédentaire induit par une journée passée dans une chambre calorimétrique, a généré une balance énergétique positive à la fois chez des sujets de poids normal et obèses.

En accord avec l'hypothèse de Mayer discutée ci-dessus, l'ensemble de ces données révèlent qu'il n'y a pas de diminution de l'apport alimentaire en rapport avec une réduction de la dépense énergétique, pour des périodes courtes et de moyenne durée. A l'opposé des résultats retrouvés dans les études de court et de moyens termes, des études d'alitement ou « bed rest » de longues durées (Ritz *et al.*, 1998; Bergouignan *et al.*, 2009b) montrent que les sujets soumis à une période d'inactivité physique de plus de deux mois, induite par l'alitement, sont capables d'ajuster leur apport alimentaire à leur dépense énergétique et par conséquent, sont capables de maintenir une balance énergétique stable durant toute la période de l'alitement (Blanc *et al.*, 1998). Inversement, les dépenses énergétiques induites par l'exercice chez les sujets alités en « bed rest» en déficience d'activité physique spontanée, n'engendrent pas une sensation de faim et induisent en revanche un bilan énergétique négatif, suggérant un rôle potentiel des activités physiques quotidiennes réalisées dans les activités spontanées de la vie de tous les jours et non induites par un exercice structuré dans la régulation du bilan énergétique (Bergouignan *et al.*, 2010). Ce type d'activité semble adaptatif lors d'expériences de surnutrition, suggérant un rôle dans le maintien de la masse corporelle en réponse à des variations de l'énergie ingérée (Levine *et al.*, 1999) .

Compte tenu des effets contrastés des activités dites spontanées *vs.* structurées, et les périodes de courte/moyennes *vs.* longue durée d'inactivité physique, le rôle direct de la modulation de l'activité physique dans la prise de poids et l'existence d'un niveau seuil d'activité physique en dessous duquel les mécanismes de régulation du poids corporel seraient inopérants restent donc à élucider.

En se basant sur les données de la littérature, l'objectif de ce chapitre est de présenter les effets de la modulation du niveau d'activité physique sur la régulation pondérale. Au préalable, les notions de balances énergétiques et oxydatives des substrats et en particulier les mécanismes de prise de poids sont discutés.

2. LA BALANCE ENERGETIQUE

2.1. Principe

Quantitativement, la source principale de cette énergie alimentaire est la liaison C-H qu'inclut toute molécule de protéines, de lipides, ou d'hydrates de carbone. L'énergie de cette liaison est libérée par les processus d'oxydation internes et aboutit à la formation de CO_2 et d'H_2O. Le principe de conservation d'énergie s'applique sur la balance énergétique : ainsi, tout gain de masse ne peut résulter que d'une augmentation des apports caloriques et/ou d'une réduction de la dépense énergétique totale.

2.2. Energie ingérée

L'énergie ingérée représente l'apport énergétique total de l'ensemble des aliments et boissons qui sont utilisés comme substrats pour le métabolisme corporel. Les aliments sont constitués de trois principaux macronutriments : les glucides, les protides et les lipides, ces derniers présentant la densité énergétique la plus importante. Les lipides fournissent l'énergie la plus élevée par unité de poids (9 kcal/g), les glucides et les protides ont la valeur énergétique la plus faible (4kcal/g).

2.3. La dépense énergétique globale

La dépense énergétique globale est répartie en plusieurs compartiments : la dépense énergétique de repos et les fractions de la dépense énergétique liée à la thermorégulation, à l'acte alimentaire et à l'activité physique. Il convient d'y adjoindre dans les cas correspondants la dépense énergétique liée à la croissance (enfants, ou adultes en cas de prise pondérale après perte de masse maigre ou grasse) et celle liée à la grossesse et à la lactation.

2.3.1. La dépense énergétique de repos

La dépense énergétique de repos DER témoigne des échanges métaboliques de la période dite post absorptive, lorsque les transferts de l'énergie associée aux nutriments sont finalisés dans un environnement thermique neutre, 8 à 12 heures après un repas ou une activité physique. La DER n'est pas équivalente à la dépense énergétique basale (ou métabolisme de base). En effet, le métabolisme de base est mesuré juste avant le réveil et représente environ 90% de la DER. La DER correspond à l'énergie obligatoirement dépensée à travers les transports ioniques actifs, le maintien des fonctions vitales et le tonus musculaire de repos. Elle représente chez l'adulte sédentaire 70 à 75% de la dépense énergétique totale DET. La variation interindividuelle est considérable et est explicable pour 85% par quatre facteurs : âge, sexe, taille et poids corporel. Ces variations sont en fait principalement dues à la variation de la masse maigre. Elle peut expliquer de 70 à 80 % de la variabilité du métabolisme de repos contre à peine 2 % pour la masse grasse (mais cette valeur peut atteindre 10% chez des sujets obèses). Chez l'homme, il a été mis en évidence l'existence d'une relation entre le métabolisme de base et la masse musculaire (Alpert, 2007) de telle sorte que l'on peut prédire le niveau du

métabolisme de base à partir de l'estimation de la masse maigre. Ajustée pour les différences de masse maigre, la variabilité interindividuelle du métabolisme de repos varie entre 3 et 8 %.

2.3.2. La dépense énergétique de thermorégulation

L'homme, homéotherme, doit maintenir sa température corporelle dans d'étroites limites de température (31°C- 41°C) malgré des variations de grande amplitude de la température extérieure. Le maintien d'une température constante est nécessaire pour maintenir les structures anatomiques cohérentes (membranes protéo-lipidiques), et les systèmes enzymatiques fonctionnels. L'énergie nécessaire à l'homéostasie thermique provient de la chaleur émise des transports d'énergie dans les réactions biochimiques. A la température de neutralité (22 -24°) où l'on mesure la DER, il n'y a pas de dépense supplémentaire liée à la thermorégulation.

2.3.3. La thermogenèse postprandiale

Elle représente 10 à 15% de la dépense énergétique globale d'un sujet sédentaire. Elle a trois composantes : la dépense énergétique en rapport avec l'activité musculaire aboutissant à l'ingestion d'aliments (mineure) et les dépenses énergétiques facultative et obligatoire.

- La part obligatoire :

L'essentiel de la dépense énergétique postprandiale est en rapport avec l'effet thermique des aliments. C'est la part obligatoire dans laquelle les processus digestifs déclenchés par l'ingestion (sécrétions exocrines, motricité, absorption) interviennent classiquement pour moins de 10%. Il est admis que la part obligatoire de la dépense énergétique postprandiale est principalement le fait du coût énergétique du stockage des nutriments. Le coût de stockage varie en fonction des réserves énergétiques au moment du repas et de la nature des aliments ingérés.

- La part facultative :

La réponse énergétique globale à un repas varie notablement d'un sujet à l'autre avec des écarts de 30% par rapport à la moyenne. Cette variation est liée à la part de la thermogenèse alimentaire dite facultative ou régulatrice qui est non expliquée par le stockage des nutriments. L'existence d'une dépense énergétique postprandiale facultative pourrait rendre compte partiellement des différences interindividuelles de rendement d'une alimentation hypercalorique (c'est-à-dire excédant les besoins). Une diminution de cette dépense énergétique facultative participe au moins à la genèse de l'obésité. A l'inverse, une importante dépense postprandiale facultative pourrait expliquer pourquoi, soumis à une agression aigue ou chronique, certains sujets maigrissent et d'autres non.

2.3.4. La dépense énergétique liée à l'activité physique

Dans les conditions physiologiques, le principal facteur de modification de la dépense énergétique est l'activité physique en tant qu'élément le plus variable de la dépense énergétique journalière. L'activité physique correspond à l'ensemble des mouvements corporels produits par la contraction des muscles squelettiques qui entraîne une augmentation de la dépense énergétique au-dessus de la dépense énergétique de repos. En fait, les modifications de la dépense énergétique sont surtout le fait de l'intensité et de la durée de l'effort musculaire consenti, plus que celui de la variation de l'énergie dissipée au cours d'un effort d'une puissance donnée. Ceci pourrait être plus explicite dans les conditions pathologiques : un sujet en conditions de dénutrition réduit son activité physique, il diminue aussi le coût énergétique des efforts dépendants de la masse corporelle telle la marche, puisqu'il pèse moins. Un sujet obèse qui a maigri diminue sa dépense énergétique en réponse aux activités physiques dépendantes du poids. Chez un sujet sédentaire, le coût de l'activité physique peut représenter moins de la moitié du métabolisme de repos. Ces proportions peuvent être inversées chez un sujet très actif.

L'intensité de l'activité physique peut être exprimée en valeur absolue (énergie dépensée par unité de temps) ou rapportée à une estimation de la DER. Ainsi, le coût de l'activité physique est souvent exprimé par un index d'activité, le PAL (Physical Activity Level) qui correspond au rapport de la dépense énergétique totale sur le métabolisme de repos. Un PAL de 1,0-1,4 caractérise des individus sédentaires alors qu'un PAL de 1,9-2,5 correspond à des individus très actifs (Black *et al.*, 1996). Par exemple, un individu marchant 3,5 km par jour en 30 minutes, en plus de ces activités quotidiennes, a un PAL d'environ 1,5. Chez l'adulte, le PAL présente une très grande variabilité selon le contexte socio-écologique de l'individu et dépend du type d'emploi, de la localisation géographique, du niveau social, etc. La dépense énergétique liée à l'activité physique pourrait représenter chez un sujet sédentaire 15 à 20 % de la dépense énergétique totale. Un PAL correspondant à une activité physique modérée réduit la prévalence de nombreuses maladies chroniques. Des données obtenues chez des femmes post-obèses suggèrent qu'un PAL de 1,75 est la limite inférieure en dessous de laquelle la régulation de la composition corporelle devient inopérante (Schoeller *et al.*, 1997).

2.4. Processus de prise de poids

Une balance énergétique positive qui est maintenue sur une longue période peut mener à une prise de poids si elle n'est pas contrebalancée par une dépense énergétique équivalente. Un apport alimentaire supérieur aux besoins pourrait être dû soit à un apport énergétique total supérieur, soit à une diminution de la dépense énergétique totale, soit aux deux. On peut distinguer plusieurs phases dans la dynamique de prise de poids (Schutz, 1995). Au cours de la phase de constitution, le bilan d'énergie est positif : les apports dépassent les dépenses mais le poids ne varie pas : c'est la phase pré-obèse statique. L'excès d'énergie est stocké sous forme de masse grasse mais aussi de masse maigre. Dans la phase dynamique le sujet est en balance énergétique positive et commence à prendre du poids sur une longue période. Cette augmentation de la masse et en particulier de la masse maigre entraine une augmentation de la dépense énergétique. Avec le temps, la balance énergétique se rétablie suite à l'augmentation de la dépense énergétique de repos engendrée par l'augmentation de la masse maigre et à la dépense énergétique liée au

déplacement d'un poids plus élevé. C'est la phase de stabilité pondérale. Ainsi, l'individu dépense plus d'énergie qu'avant sa prise de poids mais en ayant alors un bilan d'énergie équilibré où les entrées égalent les sorties.

Au bout d'un certain temps d'évolution, et ce d'autant plus qu'une prédisposition génétique est présente, l'obésité s'organise et devient de moins en moins sensible aux mesures comportementales (régime, activité physique). Cependant, au cours de la phase initiale, la prise de poids est essentiellement liée aux facteurs comportementaux et environnementaux. Les réserves adipeuses augmentent mais cette inflation reste réversible pendant longtemps. Mais au fur et à mesure qu'une obésité progresse, et ce d'autant plus qu'il existe des facteurs de prédisposition génétique, le tissu adipeux recrute non seulement des nouvelles cellules mais il est le site d'une angiogenèse, d'une nouvelle innervation, d'une infiltration macrophagique et, au bout d'un certain temps, d'une fibrose (Clement *et al.*, 2004). Autrement dit une pathologie d'organe s'installe et même une pathologie de système : tout se passe comme si l'inflation du tissu adipeux se déconnectait du système réglant les réserves énergétiques.

2.5. Les déterminants d'une balance énergétique positive

Une balance énergétique positive résulte à la fois d'une prédisposition génétique et également d'un mode de vie favorisant la prise de poids. Ainsi, l'obésité témoigne d'une mise en échec du système de régulation des réserves énergétiques par des facteurs externes (mode de vie, environnement) ou internes (déterminants psychologiques ou biologiques) (**Figure 2**) (Campfield *et al.*, 1998; Hill and Peters, 1998). Les facteurs biologiques, souvent génétiques jouent un rôle le plus souvent permissif; il a été estimé que 40 à 70% de la variabilité interindividuelle dans la prévalence de l'obésité est d'ordre génétique (Bouchard and Perusse, 1993). Les résultats des études concernant de très nombreux gènes candidats sont reportés chaque années (Bouchard *et al.*, 1990; Clement and Ferre, 2003).

Actuellement, on estime plutôt que les gènes impliqués dans la prise de poids augmentent le risque ou la prédisposition d'un sujet à l'obésité lorsqu'il est exposé à un environnement défavorable. Ainsi, selon Roland Wiensier: « *Our genes permit us to become obese, the environment determines if we become obese* » (Weinsier *et al.*, 1998). De ce fait, bien que les gènes jouent un rôle dans la régulation du poids chez l'homme via la susceptibilité individuelle, ils ne peuvent à eux seuls expliquer l'évolution récente de l'obésité. L'un des points sur lequel nous différons notablement de l'*Homo sapiens* est notre mode de vie qui a considérablement changé au cours du dernier siècle. Si la génétique joue manifestement un rôle, elle ne permet cependant pas à elle seule d'expliquer la spectaculaire progression de la prévalence de la maladie de l'obésité sous l'influence de facteurs comportementaux, sociaux et économiques. Ainsi, la génétique détermine une susceptibilité à l'environnement.

Des individus soumis a une même suralimentation pendant 3 mois diffèrent dans leur capacité à prendre du poids : certains gagnent 2kg d'autres plus de 10kg ; mais la prise de poids de jumeaux homozygotes est parfaitement corrélée. La contribution de l'hérédité à l'obésité pourrait donc résulter de l'interaction d'un grand nombre de variants géniques fréquents, associés de manière variable selon les

individus et les populations (hérédité polygénique). Cependant, la contribution de ces gènes de susceptibilité ne devient significative qu'en interaction avec des facteurs environnementaux prédisposant à leur expression phénotypique (suralimentation, baisse d'activité physique). En revanche, les études longitudinales sur l'activité physique montrent que de nombreux facteurs environnementaux et sociaux peuvent aussi induire l'adoption d'un mode de vie actif. Il s'agit notamment de la participation à des sports organisés (Telama and Yang, 2000) , les relations sociales (Yang *et al.*, 1999), l'intérêt et l'encouragement parental à l'activité physique (Kantomaa *et al.*, 2007), l'environnement local (Telama and Yang, 2000), une bonne auto-perception de la santé (Dovey *et al.*, 1998) et une baisse de la consommation d'acides gras saturés (Raitakari *et al.*, 1994).

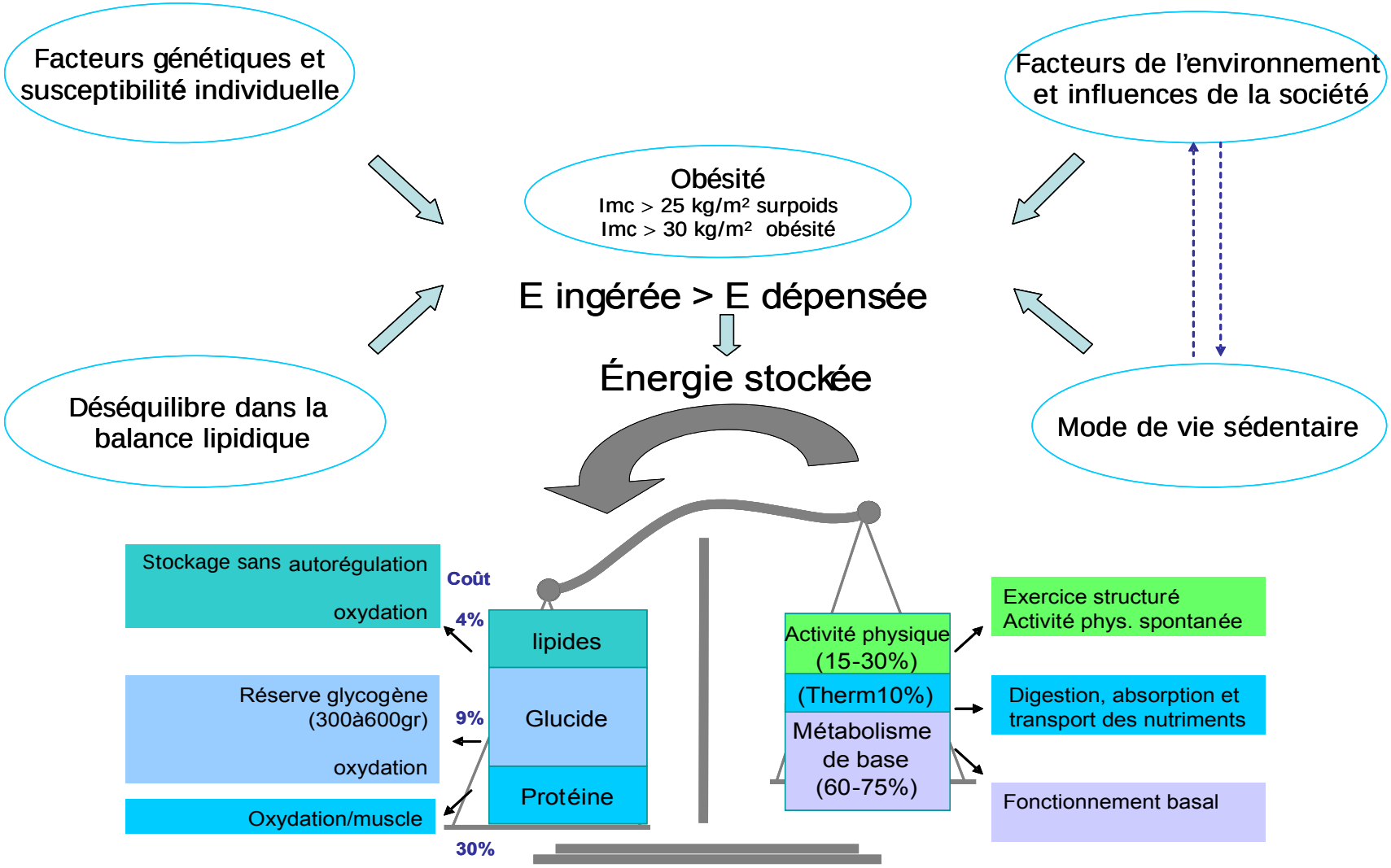


Figure 2 : Influences s'exerçant sur la balance énergétique et la prise de poids.

2.6. Une balance oxydative positive

Hormis le rôle primordial de la régulation de la balance énergétique dans le maintien d'un poids stable, la balance oxydative joue un rôle tout aussi important (Flatt, 1988). La proportion des substrats oxydés doit être représentative de la composition des macronutriments ingérés notamment les lipides, glucides et protéines d'où la notion d'une balance oxydative des substrats.

2.6.1. Les apports énergétiques de l'alimentation moderne

Les apports alimentaires jouent un rôle important dans le bilan énergétique. En effet, des études ont mis en evidence une corrélation positive avec l'apport alimentaire et l'indice de masse corporelle (Miller *et al.*, 1990). L'apport énergétique total est influencé par plusieurs facteurs. L'augmentation de la densité calorique de l'alimentation, l'augmentation de la taille des portions, la diminution de la consommation de glucides complexes (féculents, fibres), la déstructuration des rythmes alimentaires, la diversité et la disponibilité des aliments sont des facteurs susceptibles de prendre en défaut les mécanismes régulant le bilan d'énergie. Il faut aussi considérer les déterminants psychophysiologiques de la prise alimentaire et le plaisir engendré par les déterminants olfactifs, visuels ou cognitifs. La vitesse de consommation au cours et entre les repas est influencée par la sapidité des aliments. Les macronutriments ont des teneurs énergétiques différentes. Le niveau des apports susceptibles d'entrainer un bilan positif est éminemment variable d'un individu à l'autre : la notion d'un dépassement des besoins caloriques est individuelle et non normative. Les modalités d'augmentation des apports alimentaires sont variables. Chez certains sujets, la prise alimentaire est influencée par la disponibilité et la palatabilité des aliments, les habitudes familiales et les sollicitations. Ailleurs, des troubles du comportement alimentaire sont en cause.

Globalement, la densité d'énergie de notre alimentation a augmenté: l'alimentation traditionnelle africaine est à ~ 450 kJ.100g^{-1} par rapport à la densité moyenne de l'alimentation britannique (~ 670 kJ.100g^{-1}) ou la densité moyenne de régime fast-food (~ 1100 kJ.100g^{-1}) (Prentice and Jebb, 2003) et notre alimentation est devenue plus douce et plus raffinée, avec plus de sucres simples et une réduction des fibres alimentaires (Swan, 2004). Bien que l'apport total en matières grasses a diminué, les lipides saturés et artificiels (par exemple, trans) ont augmenté (Swan, 2004). Ces changements ont largement accompagné l'urbanisation, l'accroissement des richesses, l'augmentation des aliments et des boissons transformés et la diminution de la consommation des aliments crus, des grains complets, des fruits et des légumes (Popkin, 2006). Ces changements de la composition de l'alimentation peuvent modifier les processus normaux de métabolisme : par exemple, un homme de 70 kg a ~ 18,3 kg d'énergie stockée, dont 66,5% (12 kg), 32,8% (6 kg) et de 0,7% (0,3 kg) de matières grasses, de protéines et d'hydrates de carbone, respectivement. Cela équivaut à 139, 200 kcals en supposant que l'oxydation des matières grasses, des protéines et des glucides a pour rendement 9.3, 4.4 et 4.0 kcal, respectivement. Pour l'ensemble de la matière grasse, la dose journalière par rapport à cette «réserve» est très faible (35% de ~ 2000 kcal = 700kcal, relativement à la "réserve" de 92.568 kcal ou ~ 0,1%) et donc de très légères variations dans la teneur en matières grasses des produits alimentaires peut être plus difficile à détecter et par conséquent à réguler

efficacement (Gardner and Rhodes, 2009). L'alimentation moderne met d'avantage l'accent sur une adaptation difficile à la consommation alimentaire, en parallèle à la réduction de l'activité physique (Gardner and Rhodes, 2009). Il est clair que, si un tel environnement persiste pour de longues périodes (années - décennies), une augmentation progressive de la masse grasse sera induite.

L'apport énergétique total est aussi influencé par la palatabilité qui joue un rôle important sur le comportement alimentaire (Blundell and King, 1996). Par ailleurs, la sapidité des aliments joue sur la vitesse de consommation des aliments et sur la sensation de faim au cours des repas et entre ceux-ci. Bien que les glucides semblent avoir un impact plus marqué sur le système de satiété de l'organisme (Blundell and King, 1996), le sucré est l'un des goûts les plus puissants et qui procure le plus de plaisir. Les lipides présentant la densité énergétique la plus importante sont responsables de l'effet d'hyperphagie, ou surconsommation passive. Ainsi, un régime dense en calorie et riche en lipides résulte en une hyperphagie par rapport à un régime faible en énergie et en lipides (Kendall *et al.*, 1991; Tremblay *et al.*, 1991; Thomas *et al.*, 1992). Des études nutritionnelles montrent une relation entre la réduction de l'apport lipidique et la réduction de l'apport énergétique total (Kendall *et al.*, 1991; Prewitt *et al.*, 1991; Sheppard *et al.*, 1991). D'autres études suggèrent une corrélation entre les apports en lipides alimentaires et le pourcentage de masse grasse corporelle (Dreon *et al.*, 1988; Miller *et al.*, 1990; Tucker and Kano, 1992).

Mis à part l'effet différentiel que joue le type des macronutriments sur l'apport énergétique total et par conséquent la prise de poids, les substrats présentent aussi une différentiation au niveau métabolique.

2.6.2. Métabolisme différentiel entre les substrats

Le type des macronutriments ingérés agit également sur la quantité d'énergie en excès qui va être stockée dans l'organisme. En effet, l'oxydation et le stockage des substrats ne sont pas égaux entre les macronutriments. Il existe une hiérarchie dans l'oxydation des substrats d'origine exogène qui dépend de la capacité de stockage de l'organisme pour chacun d'entre eux (Flatt, 1995b). Lorsque les apports dépassent les besoins, les substrats qui sont préférentiellement oxydés sont ceux ayant une faible capacité de stockage. La capacité de stockage des protéines coûte environ 25% de la capacité de stockage. Celle des glucides est restreinte et limitée au niveau des stocks de glycogène et coûte 15% de la charge. En revanche, la capacité de stockage des lipides, considérée comme quasi illimitée, ne coûte que 4%. Toute consommation en excès de glucides ou de protides stimule leurs propres oxydations de sorte qu'à court terme, les balances glucidique et protidique sont finement réglées. Cette régulation rapide de l'oxydation des substrats s'explique aussi par l'importance fonctionnelle des protéines et par la dépendance du cerveau envers les glucides.

En revanche, toute surconsommation de lipides n'est pas associée à une augmentation immédiate de l'oxydation lipidique. Quand le rapport lipides/glucides est altéré dans un régime alimentaire, le métabolisme des glucides est autorégulé (Schutz *et al.*, 1989; Shetty *et al.*, 1994; Stubbs *et al.*, 1995a; Jebb *et al.*, 1996), à l'opposé avec la régulation du métabolisme lipidique qui ne l'est pas (Flatt, 1995a; Flatt *et al.*, 1985; Schutz *et al.*, 1989). Dans les conditions d'excès d'apport glucidique, il n'y a pas seulement une augmentation de l'oxydation glucidique

(Horton *et al.*, 1995; Stubbs *et al.*, 1995a; Jebb *et al.*, 1996) mais aussi une diminution dans l'oxydation lipidique (Horton *et al.*, 1995; Jebb *et al.*, 1996). En revanche, lorsqu'il y a un surplus d'apport lipidique, il n'y a quasiment pas d'augmentation d'oxydation lipidique correspondante (Flatt *et al.*, 1985; Schutz *et al.*, 1989; Horton *et al.*, 1995). Donc, en réponse à un surplus d'apport énergétique, les lipides s'accumulent même avec des régimes alimentaires riches en glucides (Flatt, 1995a).

Ainsi, alors que des changements dans les apports en glucides induisent de larges variations au niveau du stock des glycogènes qui sont rapidement ajustés par l'oxydation glucidique, des changements d'apports en lipides ne provoquent que de faibles variations et ne sont donc pas suivis par des modifications équivalentes de l'oxydation lipidique. D'où le rôle important des lipides alimentaires dans la balance énergétique.

3. RÔLE MAJEUR DES LIPIDES DANS LA BALANCE ENERGETIQUE

La lipogenèse étant négligeable chez l'homme, les lipides alimentaires représentent la source majeure de l'apport lipidique. Ainsi, ils jouent un rôle primordial dans la balance lipidique et par conséquent dans la balance énergétique.

3.1. Une balance lipidique positive prédit un déséquilibre de la balance énergétique

La régulation de la balance lipidique en réponse à un repas hyperlipidique ne s'effectue pas par une augmentation de l'oxydation lipidique mais par un stockage initial (Flatt, 1995b). Pour que le stockage soit contrebalancé, une oxydation lipidique correspondante doit être établie pour les lipides ingérés. Ce nouvel équilibre ne sera atteint qu'après une augmentation de la masse grasse. Cette augmentation de l'adiposité engendre, par conséquent d'effet de masse, une augmentation de la masse maigre seule capable métaboliquement d'une oxydation lipidique équivalente à l'excès d'apport de matières grasses. Par conséquent, il existe une relation positive entre la balance lipidique et la balance énergétique (Schrauwen *et al.*, 1998) (**Figure 3**). En addition, puisque les matières grasses sont les macronutriments les plus denses en énergie comme discuté ci-dessus, une augmentation de l'absorption des graisses alimentaires augmente l'apport énergétique et ainsi mène à un bilan énergétique positif (Astrup *et al.*, 2000).

3.2. La régulation de l'oxydation lipidique

Il a été rapporté que les consommateurs habituels de régimes alimentaires à forte teneur en matières grasses ont une plus grande oxydation lipidique au cours du jeûne et ont une oxydation lipidique relativement plus élevée en réponse à une charge riche en matières grasses que des sujets qui consomment moins de matières grasses habituellement (Blundell *et al.*, 2002). Un indice de masse corporelle élevé a été associé à une réduction de la capacité d'augmentation de l'oxydation lipidique, après une charge de matières grasses (95% d'énergie en matières grasses), principalement chez des sujets obèses avec une faible oxydation lipidique à jeun (Blaak *et al.*, 2006). Ainsi, la capacité à augmenter l'oxydation des lipides suite à un régime alimentaire dense en matières grasses peut contribuer à maintenir un poids stable, ou peut prédisposer à l'obésité, si cette capacité est réduite (Schrauwen, 2007; Zurlo *et al.*, 1990). En effet, l'incapacité d'augmenter l'oxydation lipidique suite à un régime alimentaire riche en matières grasses peut être liée à l'incapacité d'augmenter l'oxydation lipidique dans les conditions postabsorptives, et peut être une caractéristique primaire de l'altération de la flexibilité métabolique de l'oxydation du substrat (Ukropcova *et al.*, 2007). Un déséquilibre entre l'apport de matières grasses et l'oxydation lipidique, en raison de l'altération de la capacité d'adapter l'oxydation lipidique, peut promouvoir une balance lipidique positive et par conséquent une balance énergétique positive. Ceci résulte en un stockage des lipides dans d'autres tissus (musculaires) que les tissus adipeux et peut ainsi induire l'obésité et la résistance à l'insuline. C'est ainsi qu'une balance lipidique positive peut déterminer un gain de poids ultérieur.

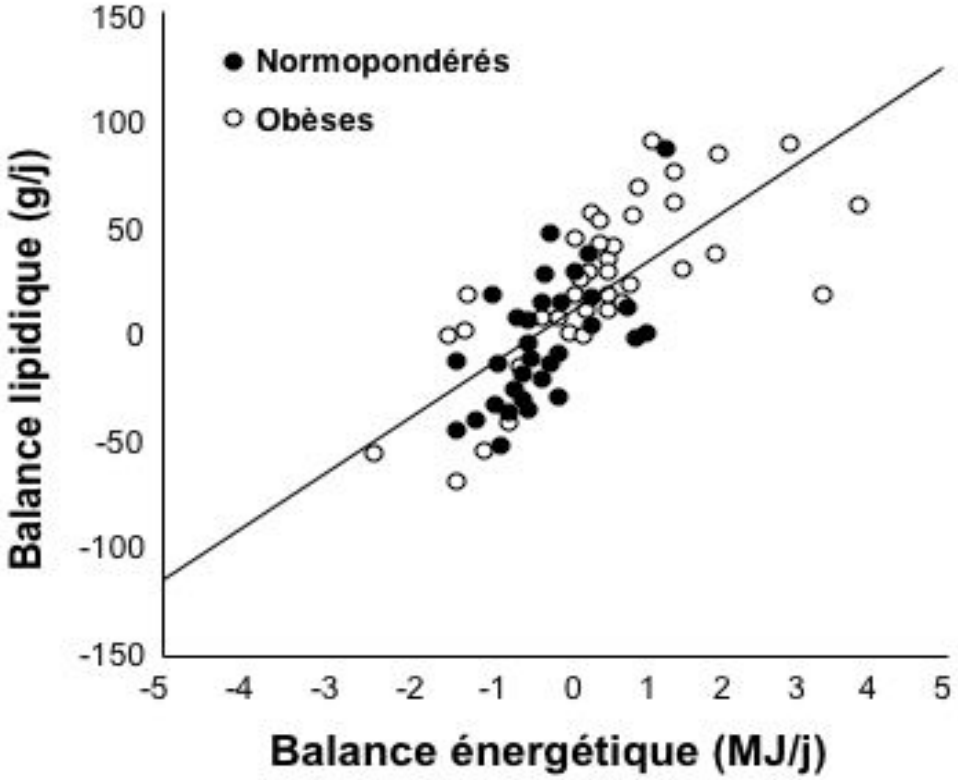


Figure 3 : Relation entre la balance énergétique de 24h et la balance lipidique de 24h chez des sujets obèses et des sujets normopondérés (Schrauwen *et al.*, 1998)

3.3. Impact de la quantité des lipides alimentaires

Des études longitudinales suggèrent qu'une augmentation de la quantité lipidique consommée mène à une élévation du poids corporel (Popkin *et al.*, 1995; Paeratakul *et al.*, 1998). Un effet significatif de la quantité de lipides dans l'alimentation sur l'indice de masse corporelle IMC a été montré : une augmentation de 100kcal est associée à une augmentation d'environ 0.05 et 0.01 de l'IMC chez les adolescents et les adultes, respectivement. Au contraire, une augmentation combinée de 100kcal de glucides et de protides résulte en une augmentation de seulement 0.01 et 0.0007 de l'IMC chez les adolescents et les adultes, respectivement. Ces résultats mettent ainsi en avant le rôle majeur des lipides dans la régulation du poids comparés aux protides et aux glucides. Dans une étude de Bray et collègues (Bray and Popkin, 1998) basées sur des enquêtes alimentaires nationales, la quantité de lipides du régime alimentaire a été associée à la prévalence de l'obésité.

3.4. L'influence de la nature des acides gras

La qualité des graisses alimentaires (saturées ou insaturées) influence l'orientation des acides gras vers le stockage ou l'oxydation (Feskens and van Dam, 1999). Les acides gras poly- et mono-insaturés sont plus oxydés que les acides gras saturés, et plus la longueur de chaîne carbonée est grande, moins importante sera l'oxydation des acides gras (DeLany *et al.*, 2000). Il a été montré que le pourcentage de lipides saturés, mono-insaturés ou poly-insaturés dans le régime alimentaire peut influencer la prise de poids. Certaines études ont montré qu'une augmentation de la part des acides gras polyinsaturés dans l'alimentation s'accompagne d'une réduction de la masse grasse (Couet *et al.*, 1997) et un pourcentage élevé en graisses saturées dans l'alimentation est associé à un tour de taille plus élevé (Doucet *et al.*, 1998). Une réduction de l'oxydation lipidique postprandiale et de la dépense énergétique totale suite à un régime riche en acide palmitique (acide gras saturé) a été observée mais aucun changement après un régime riche en acide oléique (acide gras mono-insaturé) (Kien *et al.*, 2005). En substituant les acides gras saturés (palmitate) par des acides gras mono-insaturés (oléate) avec maintien de l'apport énergétique et lipidique, Piers *et al.* (Piers *et al.*, 2003) ont mis en évidence une perte significative de masse corporelle et de masse grasse. Les différences de la répartition des acides gras entre les différents types d'acides gras ont été démontrées dans des myotubes humains en culture (Gaster *et al.*, 2005). Les captations de l'acide oléique et palmitique sont comparables, mais l'acide oléique est accumulé plutôt comme un acide gras intracellulaire, alors que l'acide palmitique est plus orienté vers le stockage en triglycérides et diglycérides intramusculaires. Dans les myotubes établis de sujets diabétiques de type 2, l'oxydation de l'acide palmitique était intrinsèquement réduite par rapport aux myotubes de sujets sains de poids normal.

3.5. L'obésité : un problème de balance oxydative positive

L'obésité correspond à une perte de contrôle de l'équilibre énergétique de telle sorte que dans le temps, l'excès d'énergie est stocké sous forme de graisse. La

lipogenèse étant négligeable chez l'humain, l'obésité pourrait témoigner d'une dérégulation entre stockage et oxydation des lipides alimentaires.

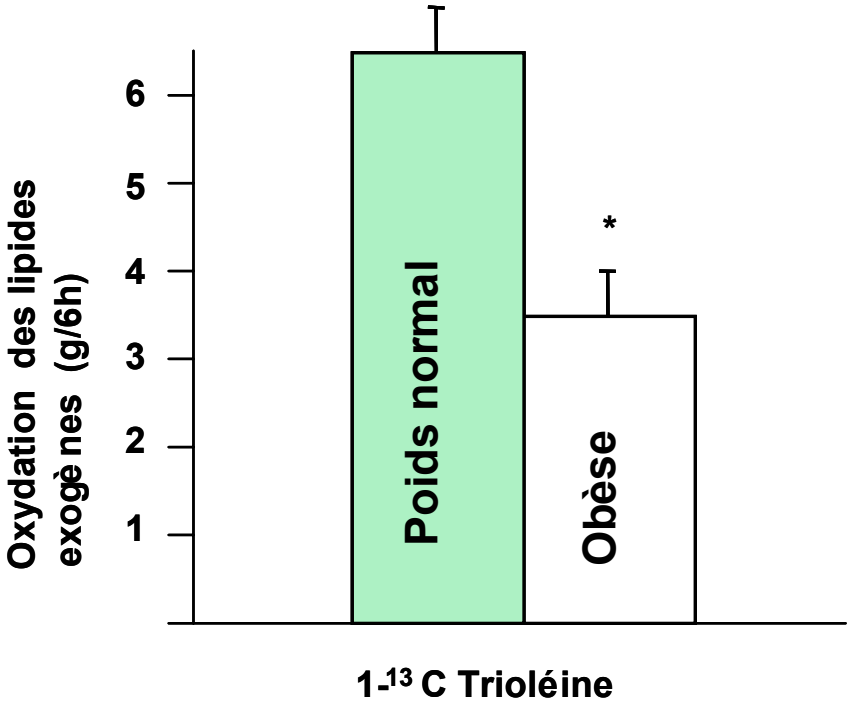
3.4.1. Altérations des capacités d'oxydation et de stockage

Une altération de la capacité de l'oxydation des lipides a été observée chez les obèses, favorisant ainsi le stockage des graisses pendant le jeûne (Colberg *et al.*, 1995) et dans les conditions postprandiales (Binnert *et al.*, 1998), ce qui conduit à la l'excès de poids. Dans l'obésité, une déréglementation entre la capacité de la ß-oxydation, la glycolyse et la capacité oxydative des mitochondries peut privilégier le glucose comme carburant à jeun plutôt que les lipides (Corpeleijn *et al.*, 2009) et postprandialement diminue la capacité à réprimer la lipolyse (Arner, 2005). En effet, l'oxydation des lipides alimentaires après une charge orale de graisses est diminuée de 50% chez le sujet obèse. De façon intéressante, chez les sujets de poids normal, une partie importante des lipides alimentaires est retrouvée au niveau des acides gras libres, alors que cette réponse est diminuée chez les sujets obèses, suggérant une captation plus importante des lipides par le tissu adipeux chez ces derniers (Binnert *et al.*, 1998)(**Figure 4**). De même, chez les rats non-obèses, les lipides exogènes sont orientés préférentiellement vers le muscle pour y être oxydés, alors que chez les rats obèses Zucker, les lipides alimentaires sont essentiellement dirigés vers le tissu adipeux (Bessesen *et al.*, 1995). Ces réductions sont clairement montrées, dans des conditions de stimulation comme la stimulation bêta-adrénergique et l'exercice (Blaak *et al.*, 1994b; Binnert *et al.*, 1998; Blaak *et al.*, 2000a; Blaak *et al.*, 2000b; Bruce *et al.*, 2006). Chez les femmes de poids normal ou obèses, une faible oxydation des lipides observée dans le muscle de la jambe au cours du jeûne, était liée à une diminution du stockage du glucose au cours d'une stimulation par l'insuline (Colberg *et al.*, 1995). Au cours d'une stimulation bêta-adrénergique, la captation et l'oxydation des acides gras ont montré un manque d'augmentation chez des sujets obèses par comparaison aux sujets contrôles de poids normal (Blaak *et al.*, 1994a). Aussi, au cours d'une stimulation par l'insuline, la suppression de l'oxydation des lipides a été réduite chez les hommes et les femmes obèses (Corpeleijn *et al.*, 2008). Ainsi, lorsque l'utilisation d'acides gras est altérée, elle pourrait contribuer au stockage ectopique des matières grasses et à la résistance à l'insuline. Certains auteurs ont suggéré que cette incapacité à oxyder les lipides serait causale dans l'étiologie de l'obésité, plutôt qu'adaptative puisqu'aucune amélioration dans l'oxydation lipidique n'a été observée après retour à un poids normal (Blaak *et al.*, 1994b; Kelley *et al.*, 1999). Par conséquent, il est indispensable de déterminer les causes de cette altération métabolique pour la mise en place de stratégies préventives et thérapeutiques. Bien que la génétique joue un rôle dans la capacité individuelle à oxyder les lipides puisque l'oxydation lipidique a été définie comme un trait familial avec une héritabilité de 30% (Bouchard and Perusse, 1993), les altérations environnementales, qu'ont subies nos sociétés modernes au cours du siècle dernier peuvent être considérées comme un déclencheur de la prévalence de l'obésité.

3.4.2. Quels sont les mécanismes potentiels pour une oxydation lipidique réduite ?

L'incapacité à utiliser les lipides en tant que substrats est due à une dérégulation du métabolisme lipidique qui se caractérise par un acheminement

préférentiel des acides gras alimentaires vers le tissu adipeux plutôt que vers le muscle. Cette altération métabolique pourrait impliquer une régulation différentielle entre le tissu adipeux et le muscle de l'activité de la lipoprotéine lipase (LPL), protéine clef dans la répartition des lipides entre les différents tissus. Aussi, une plus grande proportion des protéines responsables du transport des acides gras a été observée au niveau du tissu adipeux par rapport au muscle chez des individus obèses comparés à des sujets minces (Bonen *et al.*, 2006), indiquant une captation des lipides plus élevée au niveau du tissu adipeux que du muscle. Le profil des types de fibres musculaires des sujets obèses est caractérisé par une quantité plus importante de fibres rapides glycolytiques que de fibres lentes oxydatives par rapport aux sujets sains (Kelley *et al.*, 2001; Oberbach *et al.*, 2006). Aussi, il a été trouvé que le pourcentage de masse grasse est négativement corrélé avec le pourcentage de fibres lentes (Wade *et al.*, 1990). L'entrée dans la mitochondrie est une autre étape qui pourrait être limitante dans l'oxydation des acides gras à chaîne longue pour l'accès à la bêta-oxydation et le cycle tricarboxylique. Ce transport s'effectue grâce à la carnitine palmitoyl-transférase 1 (CPT1). Chez les sujets obèses et insulino-résistants, certaines études ont montré une activité réduite de la CPT1 au niveau des muscles (Kelley *et al.*, 1999).



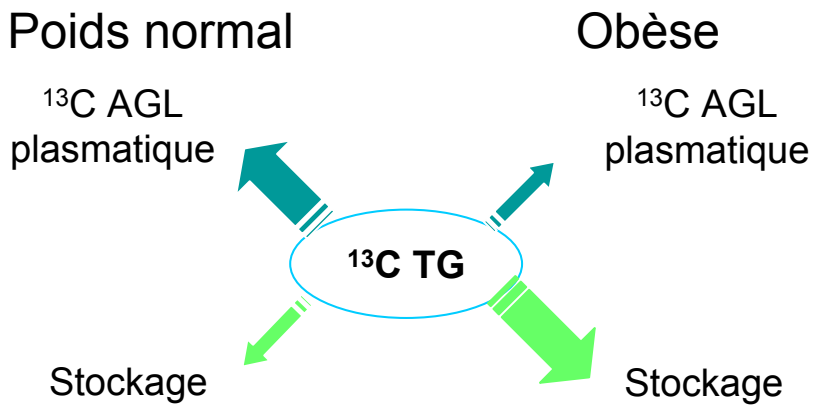


Figure 4 : Oxydation cumulative des lipides exogènes de chaines longues durant 6h chez des sujets obèses (n=8) et des sujets normopondérés (n=8) (d'après Binnert *et al.* Am J Clin Nutr, 1998).

4. CONCLUSION

Bien qu'une vision de l'étiologie de l'obésité repose sur un déséquilibre de la balance énergétique, les données des paragraphes précédents mettent en évidence le rôle primordial de la balance lipidique dans la régulation pondérale. Alors que l'obésité est une maladie de stockage des acides gras à trait héréditaire, elle ne se manifeste pas sans l'interaction des facteurs environnementaux tel un mode de vie sédentaire. Toute intervention visant à améliorer la capacité de l'oxydation des lipides alimentaires est bénéfique. L'activité physique en tant qu'élément modulable de la balance énergétique pourrait jouer un rôle primordial dans une stratégie d'une amélioration métabolique et par conséquent pondérale. Le chapitre suivant met en exergue les effets de l'activité physique en relation avec la balance lipidique.

CHAPITRE 2

L'ACTIVITÉ PHYSIQUE

1. L'ACTIVITE PHYSIQUE

1.1. Relation linéaire inverse entre l'activité physique et les maladies du siècle

L'activité physique est considérée comme un facteur a priori favorable à la santé. Les effets bénéfiques sur la santé des activités physiques et sportives sont connus depuis l'Antiquité.

Au XIXe siècle, les premiers travaux scientifiques, réalisés en 1843 à Londres, montraient que les taux de mortalité de personnes sédentaires étaient plus élevés que ceux de travailleurs physiquement actifs. Au début des années 1950, des auteurs comparant 30000 conducteurs et employés des chemins de fer (supposés peu actifs physiquement) à 20000 contrôleurs (supposés actifs) trouvaient que ces derniers étaient moins exposés à la survenue d'infarctus du myocarde (Taylor *et al.*, 1962). Dès la fin des années 1980, un nombre conséquent de travaux semblent conforter la relation entre activité physique et réduction de la mortalité prématurée et montrent une relation inverse dose-réponse entre l'activité physique et la mortalité. Ces études ont souvent distingué trois groupes de personnes selon l'intensité de leur activité : peu actifs, modérément actifs et très actifs. L'activité physique n'étant pas quantifiée de façon précise au niveau de son intensité, sa fréquence et sa durée, il est difficile de définir un seuil utile à atteindre pour infléchir la mortalité.

Cependant, d'après la revue de Kesaniemi *et al.* (Kesaniemi *et al.*, 2001), la plupart des études décrivent une relation linéaire inverse entre le niveau d'activité physique et le taux de mortalité; la dose minimale effective n'est pas bien définie mais une activité physique qui entraîne une dépense de 1 000 kcal par semaine est associée à une réduction de 30 % de la mortalité toutes causes confondues. La revue d'Oguma *et al.* (Oguma *et al.*, 2002) fait état d'un minimum de 1 680 kcals (4 200 kJ) par semaine pour infléchir la mortalité chez les femmes. Une étude récente a porté sur 252 925 individus retraités de 50 à 71 ans suivis entre 1995 et 2001 (Leitzmann *et al.*, 2007). Elle montre qu'une pratique à un niveau voisin de celui des recommandations pour une activité d'intensité modérée (au moins 3 heures par semaine) ou pour une activité d'intensité élevée (au moins 20 minutes 3 fois par semaine) entraîne une réduction du risque de mortalité de l'ordre de 30 % par rapport au fait d'être inactif.

Les individus sédentaires peuvent réduire leur risque pour de nombreuses maladies chroniques par l'augmentation de l'activité physique. Des études ont démontré que la perte de poids n'est pas nécessaire pour que les individus bénéficient des effets de l'activité physique au niveau de la tolérance au glucose et la sensibilité à l'insuline (Oshida *et al.*, 1989; Kelley and Goodpaster, 1999; Nishida *et al.*, 2001). Un entraînement aérobique d'intensité modérée a eu un effet favorable sur la tolérance au glucose chez les personnes âgées, indépendamment de la variation de l'adiposité abdominale (DiPietro *et al.*, 1998). L'augmentation de l'activité physique d'un niveau inactif à un niveau de 30 min d'activité modérée chaque jour diminue la prévalence de certaines maladies au sein d'un même IMC.

1.2. L'activité physique et le tissu adipeux : aspects physiologiques

Puisque les sujets actifs ont des quantités de tissu adipeux relativement basses, des changements biochimiques favorisant une taille réduite du tissu adipeux seraient favorisés avec l'activité physique. Face aux personnes sans entraînement physique préalable, et en réponse à l'exercice d'une même intensité, les personnes qui ont subi un entraînement d'endurance ont une oxydation lipidique supérieure au cours de l'exercice, sans augmentation de la lipolyse (Horowitz and Klein, 2000). Les niveaux de catécholamines sanguins sont moindres chez les sujets entraînés pour une même charge de travail (Kjaer, 1998). Il semble donc que les cellules adipeuses deviennent plus sensibles aux catécholamines. Les sujets entraînés ont une plus grande efficacité de l'activation de la voie lipolytique-adrénergique dans le tissu adipeux sous-cutané abdominal, même si cette voie ne recrute pas la voie anti-lipolytique 2-adrénergique, en réponse aux catécholamines au cours de l'exercice (De Glisezinski *et al.*, 2001). Une plus petite masse de tissu adipeux consécutive à l'exercice physique suggère une plus faible concentration de la leptine, hormone régulatrice de l'appétit contrôlant la sensation de satiété. Chez des rats qui s'exercent volontairement sur des roues, la leptine a été retrouvée en plus faible quantité par rapport aux rats nourris sans roue (Nara *et al.*, 1999). Des cytokines pourraient également jouer un rôle dans la modulation des effets de l'exercice sur la masse de tissu adipeux. Le TNF-α est augmenté dans les tissus adipeux des rats qui s'exercent volontairement (Baba *et al.*, 2000). Les résultats de plusieurs études cliniques et expérimentales, résumés par Hube et Hauner (Hube and Hauner, 1999), suggèrent que le TNF-α constituera une importante régulation autoparacrine de la fonction des cellules adipeuses, qui sert à limiter l'expansion du tissu adipeux. Le rôle bénéfique de l'activité physique au niveau métabolique et physiologique sur le tissu adipeux détermine par conséquent d'effet de masse, l'effet régulateur de l'activité physique dans la prise de poids.

1.3. L'activité physique et la prise de poids

Des études épidémiologiques prospectives et longitudinales suggèrent un rôle majeur de l'activité dans la prévention de la prise de poids (Williamson *et al.*, 1993; Haapanen *et al.*, 1997a; Schmitz *et al.*, 2000) et montrent que les sujets actifs présentent moins de risque vis-à-vis de la prise de poids au cours du temps que les sujets sédentaires. En effet, le niveau d'activité physique initial est négativement associé à une prise de poids ultérieure (Williamson *et al.*, 1993; Haapanen *et al.*, 1997a; Schmitz *et al.*, 2000). Dans une étude prospective suédoise chez des femmes suivies pendant 6 ans, une interaction était mise en évidence entre l'activité physique habituelle et les apports énergétiques (et en graisses): une prise de poids plus importante était associée avec un apport énergétique (et en graisses) plus élevé seulement dans le groupe des sujets dont l'activité physique initiale était la plus faible (Lissner *et al.*, 1997). Williamson *et al.* (Williamson *et al.*, 1993) ont étudié l'influence de l'activité physique dans la régulation pondérale pour une période de longue durée qui s'étale sur 10 ans d'intervalle chez 3515 hommes et 5810 femmes. Ils ont conclu que le niveau d'activité physique initial et durant le suivi de l'étude est corrélé négativement à la masse corporelle. Un niveau d'activité faible à la fin de

l'étude était très fortement associé au gain de poids acquis durant les 10 ans. Cependant, ils n'ont pas trouvé de relation entre le niveau d'activité initial et le poids ultérieur. Ils ont suggéré qu'une faible activité physique pourrait être à la fois cause et conséquence d'une prise de poids.

Ces résultats montrent le rôle important de l'activité physique sur différents aspects de la santé et sur la régulation pondérale. Ceci suggère que les niveaux d'activité des individus à risque sont inférieurs à un niveau requis pour une régulation pondérale optimale. Les recommandations sur l'activité physique incitent à pratiquer une activité physique durant 30mn au moins 5 fois par semaine, pour maintenir une bonne santé et prévenir un gain de poids ultérieur.

1.4. Recommandations actuelles

Chez l'adulte, plusieurs types de recommandations concernant l'activité physique et destinées à la population générale ont été diffusés au cours des dernières années (Pate *et al.*, 1995) (**tableau 1**). Les recommandations élaborées à la fin des années 80, avaient pour objectif principal d'augmenter la condition physique. Le type d'activité préconisé dans ce cas était d'intensité relativement élevée et basée sur l'évaluation de la fréquence cardiaque maximale. Les recommandations plus récentes, et plus pragmatiques, se sont centrées sur l'activité physique nécessaire pour diminuer le risque de pathologie chronique en général et cardiovasculaire en particulier. La possibilité de réaliser l'activité physique en plusieurs fois au cours de la journée est d'un intérêt pratique évident et l'augmentation de la complaisance dans ce cas a été rapportée (par ex. 3 fois 10 minutes d'activité d'intensité modérée par jour plutôt que 30 minutes en une seule fois). L'effet de ce fractionnement sur le risque cardiovasculaire (et sur le risque d'évènements coronariens en particulier) reste cependant à démontrer.

Une difficulté est de définir ce qu'il faut entendre par « activité d'intensité modérée ». La marche à bonne allure (marche rapide) est prise comme exemple d'activité type dans les recommandations. Une activité d'intensité modérée peut également être définie comme une activité qui s'accompagne d'une accélération de la respiration (à la limite de l'essoufflement) sans que l'individu ne transpire obligatoirement ou de façon subjective (activité moyennement difficile sur l'échelle de Borg) (**tableau 2**). L'activité physique minimum conseillée chez l'adulte correspond donc à la pratique de la marche à un pas soutenu 30 minutes par jour, la plupart, et si possible tous les jours de la semaine. Chez l'enfant, les experts ajoutent qu'un minimum de 60 minutes (et non 30 minutes) par jour d'activités physiques d'intensité modérée ou plus élevée est souhaitable chez les jeunes, sous forme de sports, de jeux ou d'activités de la vie quotidienne (Biddle and Fox, 1998). De nouvelles recommandations issues en 2007 ont souligné l'importance d'augmenter toutes sortes d'activités en plus des 30 minutes d'exercice structuré (ACSM, 2007).

1.5. Les recommmandations actuelles et la régulation pondérale

Après la 1ère Conférence « Stock conference » faite par l'association internationale d'études sur l'obésité IASO à Bangkok en 2002 (Saris *et al.*, 2003) qui s'est tenue principalement pour discuter de la question: «Combien d'activité physique faut-il pour empêcher le gain de poids? », les experts ont conclu que « l'actuelle ligne directrice pour l'activité physique des adultes, suffisante pour limiter les risques pour la santé pour un certain nombre de maladies chroniques y compris les maladies coronariennes et le diabète, est de 30 minutes d'activité d'intensité modérée par jour, de préférence tous les jours de la semaine. Toutefois, pour prévenir le gain ou la reprise de poids, cette directive est susceptible d'être insuffisante pour de nombreuses personnes dans l'environnement actuel ». Il existe des preuves que la prévention de reprise de poids retrouvée chez les personnes post-obèses exige 60-90 minutes d'activité d'intensité modérée ou une moindre durée d'activité d'intensité vigoureuse. Bien que les données définitives fassent encore défaut, il semble probable qu'une activité d'intensité modérée d'environ 45 -60 minutes par jour, soit à un PAL de 1,7 est nécessaire pour empêcher le passage à la surcharge pondérale ou à l'obésité. Ceci a été confirmé en 2007 par l'ACSM et l'association américaine d'étude des maladies du cœur (American Heart Association, AHA) qui ont publié conjointement une mise à jour des recommandations d'activité physique pour le maintien de la santé de 1995 (Haskell *et al.*, 2007).

Il a été prouvé que la marche est un facteur important dans l'amélioration de profil de risques métaboliques. En outre, il semble plus facile pour les sédentaires ou les personnes obèses d'adhérer à des interventions de santé publique promouvant la marche plutôt que l'exercice. Dans cette ligne, Rodearmel *et al.* (Rodearmel *et al.*, 2006) suggère d'ajouter 2000 pas par jour à l'activité quotidienne pour éviter une prise de poids. Toutefois, le déficit de l'activité physique spontanée dans l'obésité est plus proche de 2-3h de marche à pied tout au long de la journée correspondant à 2000-2500 kcal / semaine (Levine and Kotz, 2005). En termes quantitatifs, une heure supplémentaire par jour de marche à un bon pas était associée à une diminution d'environ 25 % du risque de devenir obèse (IMC>30 kg/m2) après 6 ans de suivi chez les femmes de l'étude des infirmières américaines (Nurses' Health Study)(Hu *et al.*, 2003).

	Recommandations « traditionnelles »	Recommandations « actuelles »
Fréquence	3 – 5 jours par semaine	6 – 7 jours par semaine
Intensité	60 – 90 % de la fréquence cardiaque maximale (50-85% de la puissance aérobie maximale, VO_2max)	Modérée (3 – 6 METS* ou 4 – 7 kcal/min)
Durée	20 – 60 minutes en une fois d'activité d'endurance	≥ 30 minutes /jour en une ou plusieurs fois
Type	Toute activité utilisant les grands groupes musculaires (course, vélo, natation…)	Toute activité pouvant être réalisée d'intensité comparable à la marche rapide

Tableau 1 : Evolution des recommandations d'activité physique pour la population générale (Adulte)

*MET : équivalent métabolique (rapport du coût énergétique d'une activité donnée à la dépense énergétique au repos)
Source : Activité physique et santé : Arguments scientifiques, pistes pratiques. Programme National Nutrition Santé .Ministère de la Santé et des Solidarités.2005

Intensité	VO_2max (%) Fréquence cardiaque de réserve (%)	Fréquence cardiaque maximale (%)	Echelle de Borg
Très légère	< 25	< 30	< 9
Légère	25 – 44	30 – 49	9 - 10
Modérée	45 - 59	50 - 69	11 – 12
Intense	60 - 84	70 - 89	13 - 16
Très intense	≥ 85	≥ 90	> 16
Maximale	100	100	20

Tableau 2 : Classification de l'intensité de l'activité physique (activités d'endurance) – intensité relative
Echelle de Borg : échelle d'évaluation de l'effort perçu (de 6 à 20)
Source : Surgeon General Report (consultable sur le site www.healthfinder.gov)

1.6. Mécanismes de l'effet de l'activité physique *per se*

La pratique de l'activité physique peut être accompagnée ou non d'une perte de poids. Toutefois, il n'est pas nécessaire de perdre du poids pour pouvoir bénéficier de certains effets métaboliques de l'activité physique.

L'activité physique *per se* peut améliorer la sensibilité à l'insuline et la flexibilité métabolique postprandiale (Corpeleijn *et al.*, 2009). La flexibilité métabolique est définie comme la capacité de basculer de l'oxydation des lipides à l'oxydation des glucides après stimulation par l'insuline, et d'augmenter l'oxydation des lipides à jeun, qui reflète en quelque sorte la capacité d'augmenter l'oxydation des lipides en général. La capacité à augmenter l'oxydation des lipides au cours de l'exercice peut également refléter la capacité d'augmenter l'oxydation des lipides en général. L'activité physique *per se* peut améliorer la sensibilité à l'insuline et la flexibilité métabolique postprandiale à travers des changements des intermédiaires des lipides comme les diglycérides et les triglycérides (Bruce *et al.*, 2006), par l'intermédiaire des changements de système de détection des substrats du malonyl-CoA (Kuhl *et al.*, 2006), et via les fonctions mitochondriales (Bruce *et al.*, 2006; Kiens, 2006).

L'exercice peut améliorer la sensibilité à l'insuline et la flexibilité métabolique grâce à des mécanismes aigus et chroniques mais les effets de l'exercise sont moins visibles chez des sujets obèses ou diabétiques. En effet, des études chez des sujets diabétiques ou intolérants au glucose montrent que l'utilisation d'acides gras est réduite au cours de l'exercice (Blaak *et al.*, 2000a; Mensink *et al.*, 2001). Au cours de 1 h à vélo, à 50% de la capacité aérobique maximale, la réponse aigue de l'oxydation des acides gras est comparable entre les sujets obèses, les sujets intolérants au glucose IGT et les sujets ayant le diabète du type 2 T2D, mais l'utilisation des acides gras plasmatiques a été réduite pour les sujets IGT et T2D (Blaak *et al.*, 2000a; Mensink *et al.*, 2001). En revanche, chez des hommes normopondérés non diabétiques et moyennement entraînés, un exercice aigu (1 h, à 65% de la capacité aérobique maximale) a stimulé l'activation de la protéine kinase par l'α2-AMP et l'inhibition de l'acétyl-CoA carboxylase ACC. Les concentrations du malonyl-CoA et de l'acétyl-CoA dans le muscle squelettique ont diminué et par conséquent l'oxydation des lipides dans les muscles des jambes a été augmenté (Roepstorff *et al.*, 2005). En effet, une diminution de la production de malonyl-CoA, par la réduction de l'activité ACC- et/ou l'augmentation de l'activité du malonyl-CoA decarboxylase-1, est bénéfique pour la captation des acides gras dans les mitochondries à travers la désinhibition de la carnitine palmitoyltransferase I CPT-1, stimulant ainsi l'oxydation des lipides. En outre, l'exercise aigu a empêché la résistance à l'insuline induite par les acides gras chez des femmes saines accompagné d'une augmentation de l'expression protéique des enzymes de la DAG acyltransférase, l'acyltransférase glycérol-3-phosphate mitochondriale et le Δ9-désaturase. Aussi, l'acheminement des acides gras vers le stockage réduit les niveaux de céramide et des DAG, mais augmente les niveaux des IMTG (Schenk and Horowitz, 2007).

	Effets d'un exercice aigu	Effets de l'entraînement
Tolérance au G (corps entier)		
-post exercice : HGPO	↑	↑
-post exercice : repas	↑	
Utilisation du G (corps entier)	↔	↑
Transport du G musculaire stimulée par insuline	↑	↑
Translocation de GLUT-4	↑	↑
Expression de GLUT-4	↔	↑
Récepteur insuline stimulé par insuline		
-expression de protéine	↔	↔
-phosphorylation de tyrosine	↑	↑
PI-3 kinase stimulée par insuline		
-expression de la protéine	ND	ND
-activité	↔	↑
Akt/PKB stimulée par insuline		
-expression de la protéine	ND	ND
-activité	ND	↔

G : Glucose ; HGPO : Hyperglycémie provoquée par voie orale ; ↑ : Augmenté ; ↔: Inchangé ;
ND : Non déterminé

Tableau 3 : Récapitulatif des effets d'un exercice aigu (mesure en post-exercice immédiat) et de l'entraînement en endurance (mesures 48 h après le dernier exercice) sur la captation du glucose et l'action musculaire de l'insuline chez l'homme et l'animal (d'après Henriksen, 2002; Zierath, 2002)

L'exercice chronique, à faible intensité (40% VO_2max, durant 12 semaines) a tendance à accroître l'oxydation totale des lipides, au cours de l'exercice, mais pas pendant le jeûne, chez des sujets sains entraînés (Schrauwen *et al.*, 2002) et chez les hommes obèses (van Aggel-Leijssen *et al.*, 2002). Cela n'était pas du à l'oxydation des acides gras libres plasmatiques mais plus vraisemblablement à l'augmentation de l'oxydation des dérivés des IMTG ou des dérivés des TG plasmatiques. Chez les hommes sains, une diminution (-36%) des ARNm de l'ACC a été observée après l'entraînement, en faveur de l'oxydation des lipides. Chez les sujets âgés (~ 74 ans), caractérisés par un taux réduit d'oxydation des lipides, un entraînement d'endurance de 16 semaines a augmenté le taux d'oxydation des lipides au cours de l'exercice (Sial *et al.*, 1998). Un fait intéressant a été retrouvé, à savoir que l'exercice chronique a réduit la concentration des IMTG à des niveaux semblables à ceux des sujets de poids normal, et a amélioré l'oxydation totale des lipides au cours de l'exercice, mais a échoué à restaurer complètement la sensibilité à l'insuline chez les diabétiques par rapport aux sujets contrôles avec des concentrations de IMTG correspondants (Bruce *et al.*, 2004). D'autre part, un entraînement d'endurance chez des sujets obèses a permis l'amélioration de l'activité de CPT-1 et a réduit la sensibilité de CPT-1 pour la malonyl-CoA, mais n'a pas changé la concentration des IMTG, bien que les DAG et les DAG saturés ont tendance à être réduits (-15%, P = 0,06 et -27%, P = 0,06 respectivement) (Bruce *et al.*, 2006). Le même manque de variation de la concentration des IMTG après l'exercice a été trouvé précédemment chez des hommes en surpoids et obèses, indiquant néanmoins une amélioration de l'oxydation des lipides à jeun et de la sensibilité à l'insuline (Gan *et al.*, 2003). Une explication probable est que l'amélioration (dans les muscles) de l'oxydation des lipides rétablit l'équilibre entre la captation et l'oxydation des acides gras, réduisant ainsi les taux des lipides intermédiaires (acyl-CoA lipidique, DAG, céramide) et améliorant la sensibilité à l'insuline.

En conclusion, l'exercice chronique améliore la capacité du muscle squelettique à utiliser les acides gras comme carburant au cours de l'exercice, et dans certains cas, permet aussi d'améliorer l'oxydation des lipides au cours du jeûne, ce qui indique une amélioration de la flexibilité métabolique (Corpeleijn *et al.*, 2009). La flexibilité métabolique améliore les régulations de l'équilibre entre la captation des acides gras, l'oxydation et le turnover des IMTG dans le muscle squelettique, la réduction des lipides intermédiaires, ce qui permet d'améliorer ainsi la sensibilité à l'insuline. Cependant, les modifications engendrées par l'effet combiné de la perte de poids et de l'activité physique peuvent différer de l'effet de l'activité physique *per se*.

1.7. Effet combiné de l'activité physique et du régime alimentaire

Dans la plupart des études d'intervention, des conseils diététiques, de l'activité physique et/ou de restriction de l'énergie sont combinés. Goodpaster *et al.* (Goodpaster *et al.*, 2003) ont procédé à une étude de combinaison d'activité physique et d'un régime alimentaire chez des sujets obèses. Ce programme combiné a amélioré l'oxydation de lipides au cours du jeûne ainsi que la bascule médiée par l'insuline de l'oxydation des lipides en oxydation des glucides. Les améliorations apportées à l'oxydation des lipides à jeun ont été fortement liées à l'amélioration de la sensibilité à l'insuline (Goodpaster *et al.*, 2003).

Mensink *et al.* (Mensink *et al.*, 2003) ont montré qu'un régime alimentaire combiné à l'exercice chez des sujets en surpoids intolérants au glucose permet une amélioration de la sensibilité à l'insuline après 1 an d'intervention, accompagnée par une réduction dans le muscle de l'expression de l'ARNm de l'acétyl-CoA carboxylase-2 (ACC2) (Mensink *et al.*, 2003). En outre, les sujets intolérants au glucose qui ont subi l'intervention ont été en mesure de maintenir ou même améliorer légèrement la capacité d'oxyder les acides gras au cours de l'exercice (en raison de l'amélioration de l'oxydation des acides gras plasmatiques), et donc de maintenir la flexibilité métabolique, alors que chez les sujets intolérants au glucose du groupe témoin, l'oxydation des acides gras a été réduite (Mensink *et al.*, 2005).

La perte de poids à elle seule permet l'amélioration de la sensibilité à l'insuline et réduit l'accumulation de triglycérides intramusculaires, mais la perte de poids associée à l'exercice améliore la capacité aérobique, augmente le contenu mitochondrial et améliore la chaîne d'activité de transport d'électrons dans le muscle squelettique des sujets obèses sédentaires (Toledo *et al.*, 2008). Il semble que la perte de poids toute seule est efficace pour améliorer la sensibilité à l'insuline, mais elle est plus susceptible d'améliorer l'oxydation des lipides et les fonctions mitochondriales pendant le jeûne si elle est combinée avec l'activité physique.

2. INTERACTION DE L'ACTIVITE PHYSIQUE ET DES LIPIDES

2.1. Existerait-il un continuum entre l'activité physique et le devenir des lipides alimentaires?

L'exercice physique pourrait favoriser la redistribution des lipides alimentaires au profit des muscles pour y être oxydés, par rapport au tissu adipeux (stockage) (Calles-Escandon *et al.*, 1996; Friedlander *et al.*, 1998). Comme discuté auparavant, l'obésité est caractérisée par une oxydation lipidique réduite suggérant une incapacité à s'adapter rapidement à des régimes riches en lipides (Astrup *et al.*, 1994). Aussi, une association entre le gain de poids et l'apport en lipides a été

observée seulement chez des sujets présentant un niveau d'activité physique faible (Lissner and Heitmann, 1995). Olsen *et al.* (Olsen *et al.*, 2008) ont montré que la réduction de la marche quotidienne pendant 2 à 3 semaines à 1500 pas de la fourchette recommandée pour les adultes d'environ 10 000 ou de 6000 marches développe des changements métaboliques en diminuant la sensibilité à l'insuline, en réduisant le métabolisme lipidique postprandial et en entraînant des changements physiques qui suggèrent que les calories utilisées pour maintenir la masse musculaire durant une marche plus intense ont été reconduites dans la formation de la masse grasse viscérale. Cela suggère que la surcharge pondérale résulte d'une incapacité à ajuster l'oxydation lipidique assez rapidement en réponse à une surconsommation lipidique.

Cette capacité réduite d'adaptation de l'oxydation lipidique face à un régime hyperlipidique pourrait être due à de faibles variations dans les stocks de glycogène. L'exercice pourrait donc moduler l'oxydation lipidique suite à une diminution de la réserve glycogénique. Schrauwen *et al.* (Schrauwen *et al.*, 1998) ont montré que la pratique d'un exercice physique intense destiné à épuiser les stocks de glycogène avant de recevoir un repas riche en lipides permet aux personnes obèses d'ajuster sur une période de temps plus rapide leur balance énergétique et lipidique. Smith *et al.* (Smith *et al.*, 2000b) ont mis en évidence le rôle de l'activité physique dans la vitesse d'ajustement de la balance lipidique. Dans cette étude dans laquelle les sujets sont en inactivité dans une chambre calorimétrique, alors que certains participants sont capables de s'adapter en un jour au régime eucalorique hyperlipidique, d'autres n'ont pas amorcé un semblant d'adaptation. Néanmoins, ils ont mis en évidence que la capacité aérobique (VO_2max) des volontaires est corrélée négativement à la balance lipidique. Smith *et al.* (Smith *et al.*, 2000a) a répété la même expérience mais avec une seule différence qui est le fait que les volontaires devaient pratiquer une activité physique dans la chambre calorimétrique afin d'atteindre un niveau d'activité physique de 1,8. Dans de telles conditions d'activité physique, tous les sujets de l'étude adaptent leur oxydation lipidique en réponse au régime riche en lipides en un seul jour.

En d'autres termes, la variabilité interindividuelle (reflétant la variabilité génétique) observée en réponse à un régime obésigène disparaît lorsqu'un certain niveau d'activité physique est maintenu. Aussi, le temps nécessaire pour ajuster l'oxydation lipidique à la suite d'une augmentation de lipides dans le régime alimentaire est d'autant plus réduit que le niveau d'activité physique est élevé (Hansen *et al.*, 2007) (**figure 5**). Une étude similaire a récemment été menée chez la femme et a testé l'effet de trois niveaux d'activité physique distincts 1,4, 1,6 et 1,8 (Hansen *et al.*, 2007) sur l'adaptation métabolique. Les auteurs ont montré que le temps nécessaire pour ajuster l'oxydation lipidique à la suite d'une augmentation de 30% à 50% de la proportion de lipides dans le régime alimentaire est d'autant plus réduit que le niveau d'activité physique est élevé. Stubbs *et al.* (Stubbs *et al.*, 2004) a effectué une étude de 7 jours au cours de laquelle la dépense énergétique totale était bloquée à environ 1,4 et 1,8 × la dépense énergétique de repos et les sujets ont été nourris *ad libitum*. Le septième jour, la balance énergétique cumulative était de 26,3 et 11,1 MJ, respectivement, et la plupart de l'excès d'énergie était stockée sous la forme de graisse.

Dans cette perspective, Levine *et al.* ont montré que la variabilité interindividuelle dans la prise de poids en réponse à une surnutrition lipidique est liée à la modulation de l'activité spontanée (Levine *et al.*, 1999). Ainsi, les sujets qui prennent le moins de poids lorsqu'ils sont soumis à deux mois de surnutrition de

1000 kcal/j sont ceux qui augmentent le plus leur activité. Ainsi, toute sorte d'activité physique pourrait être bénéfique. Mis à part les activités du type structuré (activité volontaire d'exercices physiques), une corrélation négative a été montrée entre l'adiposité et l'activité du type spontanée (toutes actions de la vie quotidienne) (Kotz and Levine, 2005).

L'ensemble de ces données suggèrent que le niveau d'activité physique module l'oxydation totale des lipides (**figure 6**). Il est moins clair si cette relation s'applique aussi sur l'oxydation des lipides exogènes, qui représentent la seule source lipidique pour le corps en tenant compte que la lipogenèse est négligeable chez l'homme. Une démonstration claire de cette hypothèse reste à mettre en evidence.

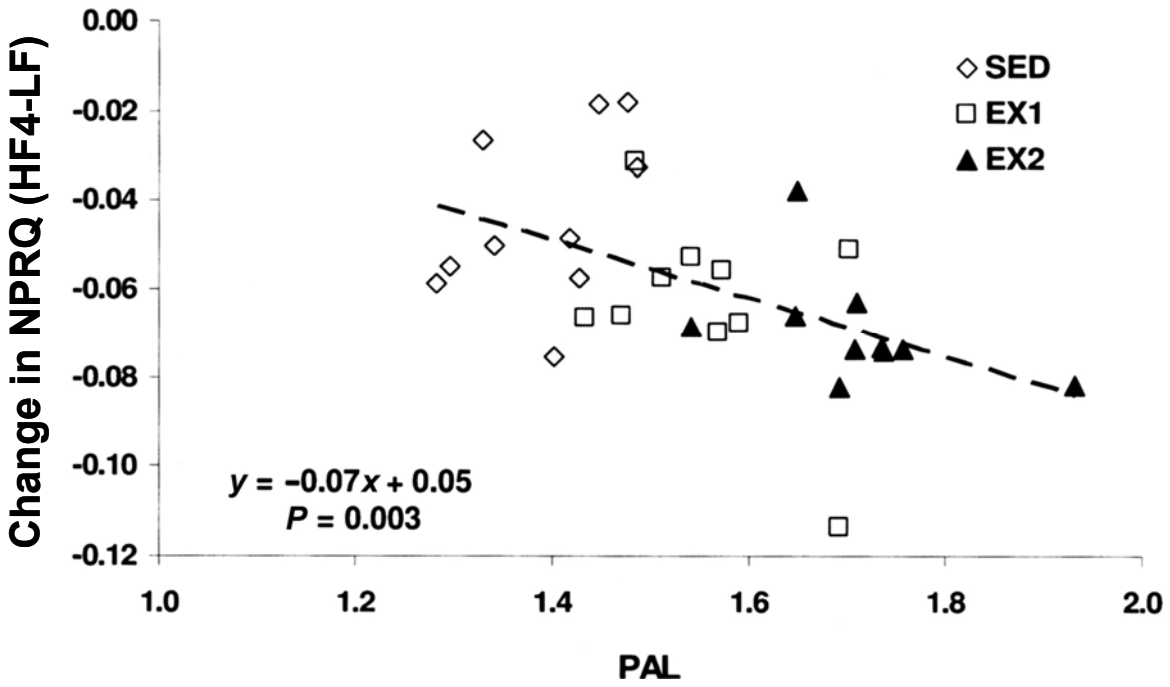


Figure 5 : Les moyennes des niveaux d'activité physique (PAL) en fonction de variations moyennes du ratio d'échange respiratoire non protéique (NPRQ) entre le régime alimentaire de faible teneur en lipides (LF) et le jour 4 de l'alimentation riche en lipides (HF4) pour les traitements individuels: sédentaires (SED), 1 h exercice / j (EX1) et 2 h exercice / j (EX2). Un modèle à effets aléatoires utilisé pour analyser la relation entre la variation de NPRQ et PAL a abouti à une droite de régression avec une pente différente de zéro (d'après Hansen, K. C *et al.* Am J Clin Nutr 2007).

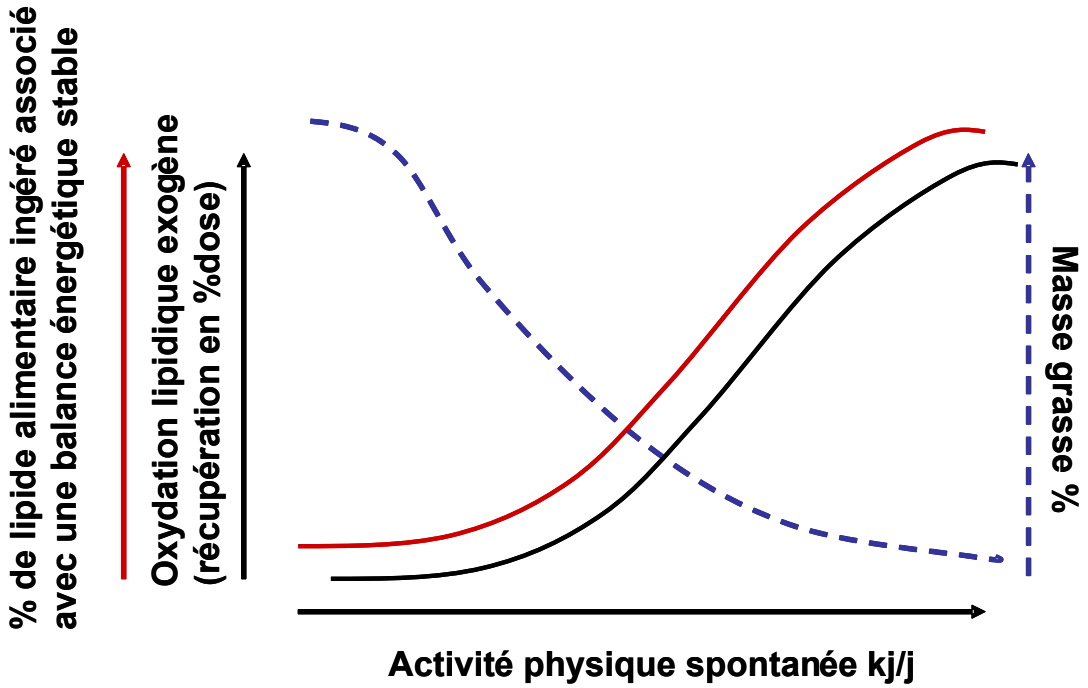


Figure 6: Relations hypothétiques entre la dépense énergétique liée à l'activité physique spontanée, la régulation de la masse grasse, l'oxydation des lipides exogènes et la tolérance à un régime hyperlipidique exprimée en pourcentage de lipide alimentaire ingéré associé avec une balance énergétique stable (adapté de Stubbs *et al.* (Stubbs *et al.*, 1995b) et Levine *et al.* (Levine *et al.*, 1999).

2.2. Durée, intensité et type d'exercice : effet sur l'oxydation lipidique

Les effets d'un exercice physique aigu diffèrent des effets d'un entraînement physique chronique. Il est supposé que l'exercice a un effet de courte durée sur le métabolisme des lipides (Schenk and Horowitz, 2007). Les effets de désentraînement renforcent la constatation que l'exercice n'influence pas significativement le métabolisme des triglycérides, en l'absence de l'effet aigu d'un exercice et que tout effet bénéfique serait de courte durée (Gill and Hardman, 2003). Certaines études n'ont montré aucune modification du taux des acides gras libres ni sur l'expression de gènes impliqués dans la régulation du métabolisme lipidique, après trois mois d'entraînement (Richterova *et al.*, 2004). Néanmoins, l'entraînement pourrait être bénéfique sur le métabolisme des triglycérides, sur de longues durées puisque les sujets actifs dépensent plus d'énergie durant une session d'exercice que les sujets sédentaires (Gill and Hardman, 2003). Votruba *et al.* (Votruba *et al.*, 2003) ont montré qu'un pré-exercice augmente significativement l'oxydation des lipides exogènes. Cependant, les effets d'un entraînement physique, mis à part les effets aigus, sur l'acheminement des acides gras exogènes n'a pas retenu suffisamment d'attention et la plupart des études se sont concentrées sur les effets de l'exercice sur l'oxydation totale ou plasmatique des lipides (Schrauwen *et al.*, 2002).

Compte tenu des différences interindividuelles d'aptitude physique, un exercice de puissance absolue donnée n'entraînera pas les mêmes réponses de l'organisme et n'impliquera pas les mêmes filières énergétiques chez tous les sujets. La puissance maximale aérobie est définie comme la puissance d'exercice qui entraîne la consommation maximale d'oxygène que le sujet est capable d'atteindre (VO_2 max). Le VO_2 max représente les capacités maximales de distribution et de transport de l'oxygène par le sang et d'extraction de l'oxygène par le muscle. Elle est une simple mesure de l'aptitude aérobie. La puissance de travail peut être augmentée au-delà de ce niveau mais en sollicitant le métabolisme anaérobie. Lors d'un exercice en endurance, la proportion de la dépense énergétique dérivée de l'oxydation des lipides diminue au fur et à mesure que l'intensité de l'exercice augmente. L'inverse se produit pour les glucides. En théorie, le niveau le plus élevé d'oxydation des lipides, est observé pour des activités d'intensité moyenne correspondant à 50-60% du VO_2 max (Brooks and Mercier, 1994; Achten *et al.*, 2002). La participation relative de l'un ou l'autre des substrats dépend en grande partie de la puissance développée : la béta-oxydation et l'utilisation des lipides sont prépondérantes pour une puissance faible. La participation glycolytique est de plus en plus importante au fur et à mesure que la puissance augmente, jusqu' à devenir exclusive pour des puissances proches de la puissance aérobie maximale. Il faut noter que pour des puissances comprises entre 50 et 100% de la puissance maximale aérobie, la cellule musculaire est progressivement obligée de faire appel à la glycolyse anaérobie pour couvrir les besoins énergétiques. Aussi, il semble aussi que l'effet bénéfique de l'exercice est maintenu si l'exercice est pratiqué en une seule fois ou si pour une durée équivalente, l'exercice est fractionné. Ainsi, 30 minutes de marche, entrepris dans une session ou accumulé pendant toute une journée, augmente d'un degré similaire l'oxydation postprandiale des lipides (Murphy *et al.*, 2000).

Il est important de noter que les phases post-exercice ont également une influence sur l'oxydation des lipides, probablement en raison de la reconstitution des stocks de glycogène (Borsheim and Bahr, 2003). Des études ont signalé une

augmentation de l'oxydation lipidique et une lipémie postprandiale atténuée après deux types d'exercice : endurance et résistance (Gill and Hardman, 2003; Horton *et al.*, 1998; Petitt *et al.*, 2003). L'exercice de résistance oxyde plus de lipides qu'un exercice aérobique isocalorique. Cependant, l'exercice de résistance augmente moins la dépense énergétique totale que l'exercice en endurance.

En conclusion, la dépense énergétique de l'activité engendrée par l'intensité et la durée de l'exercice semble être le principal facteur influant sur l'oxydation de lipides (Tsetsonis and Hardman, 1996a, 1996b). Néanmoins, en dépit de la période de post-exercice, les effets aigus de l'exercice doivent être clairement distincts des adaptations chroniques à l'entraînement.

2.3. Interaction du niveau d'activité physique avec la nature des acides gras

Une relation positive entre l'oxydation des lipides et le niveau d'activité physique semble être subtile et dépend des propriétés biochimiques des acides gras ingérés. Votruba *et al.* (Votruba *et al.*, 2002) ont reporté que l'exercice augmente significativement l'oxydation de l'acide oléique, qui est l'acide gras mono-insaturé principal dans le régime alimentaire, mais n'affecte pas l'oxydation de l'acide palmitique alimentaire, l'acide gras principal parmi les acides gras saturés. L'exercice est donc associé à une augmentation des fractions d'acide oléique (Andersson *et al.*, 1998). En effet, l'augmentation de l'activité physique est associée avec une augmentation de l'activité de l'Δ5-désaturase dans les phospholipides du muscle squelettique (Andersson *et al.*, 1998; Andersson *et al.*, 2000). L'activité de l'Δ5-désaturase contribue à la production d'acides gras fortement insaturés (Nakamura and Nara, 2004) qui sont des ligands pour les facteurs de transcription comme les PPAR et la protéine régulatrice de la liaison au stérol, impliquée dans la lipogenèse et l'oxydation des acides gras (Jump and Clarke, 1999). Anderson *et al.* (Andersson *et al.*, 1998) ont trouvé qu'un entraînement régulier à une faible intensité affecte la composition des acides gras dans les phospholipides du muscle squelettique chez des hommes sédentaires. Dans les phospholipides du muscle, ils ont noté une réduction du pourcentage de l'acide palmitique (16:0), une augmentation de l'acide oléique [18:1(n-9)] et une diminution dans les n-6 acides gras polyinsaturés après l'entraînement. Contrairement à Anderson *et al.* (Andersson *et al.*, 1998) et à Helge *et al.* (Helge *et al.*, 1999), qui tous les deux ont trouvé une modification du profil des acides gras dans les phospholipides du muscle squelettique après un entraînement, Kriketos *et al.* (Kriketos *et al.*, 1995) n'ont pas observé un changement significatif dans la composition lipidique des membranes chez les rats entraînés.

Dans une autre étude comparant des sujets entraînés à des sujets désentraînés au niveau du profil des acides gras dans le muscle, après 8 semaines d'un régime alimentaire contrôlé, Anderson *et al.* (Andersson *et al.*, 2000) ont trouvé une faible proportion d'acide palmitique (16:0) et d'acide linoléique et une augmentation de l'acide stéarique (18:0) chez le groupe entraîné par rapport au groupe désentraîné. Cette différence n'était pas expliquée par un changement dans la distribution de type des fibres musculaires, mais était plutôt une conséquence directe de changement dans le métabolisme lipidique dû à un niveau d'activité supérieur. Une faible proportion d'acide palmitique (16:0) et une augmentation de l'acide stéarique (18:0) dans le muscle, ont été observées aussi dans une étude comparant

des sujets entraînés à des sujets sédentaires (Thomas *et al.*, 1977). L'exercice régulier induit aussi une réduction de l'acide palmitique 16:0 dans le muscle quadriceps chez les rats (Helge *et al.*, 1999).

La proportion de l'acide palmitique (16:0) dans les phospholipides du muscle squelettique est corrélée négativement à la sensibilité à l'insuline (Vessby *et al.*, 1994). Dans l'étude de Thomas *et al.* (Thomas *et al.*, 1977), la proportion de l'acide stéarique (18:0) dans la membrane du muscle squelettique est corrélée positivement à la distance parcourue par semaine dans le groupe entrainé. Le ratio 18:0/16:0 dans le muscle squelettique, reflétant l'activité de l'enzyme élongase de la biosynthèse des acides gras, est corrélé positivement à la sensibilité à l'insuline (Clore *et al.*, 1998).

Bergouignan *et al.* (Bergouignan *et al.*, 2006; Bergouignan *et al.*, 2009b) ont montré que l'inactivité physique induite par un alitement prolongé n'altère pas l'oxydation de l'oléate mais diminue l'oxydation du palmitate chez des sujets sains. La réduction de l'oxydation du palmitate est corrélée à l'augmentation de la masse grasse dans le muscle. Comme l'absorption et la clairance des acides gras mono-insaturés et saturés n'étaient pas affectées, ces résultats indiquaient que la réduction de l'oxydation du palmitate peut être expliquée en partie par une répartition préférentielle du palmitate pour être stocké dans le muscle (Bergouignan *et al.*, 2009b). Cet acheminement vers le muscle peut être dû à une capacité oxydative réduite des acides gras saturés au niveau des mitochondries et/ou une incorporation élevée des acides gras saturés dans les lipides des myocytes.

Les mécanismes de changements de la composition des acides gras dans le muscle squelettique après une modulation de l'activité physique, qu'ils soient dûs à une oxydation préférentielle de certains acides gras, des changements dans le taux de transport ou les activités enzymatiques, ou d'autres causes, restent cependant à être élucidés. Ceci est d'une grande importance sachant que les acides gras saturés sont associés à une augmentation du risque d'obésité de diabète (Gonzalez *et al.*, 2000; Votruba *et al.*, 2002; Petitt *et al.*, 2003), probablement dû à une oxydation réduite (Williams *et al.*, 2000) et à une accumulation au niveau du tissu adipeux *(* DeLany *et al.*, 2000; *Brunner et al.*, 2001) et dans les lipides intermédiaires du muscle (Garaulet *et al.*, 2001; Sabin *et al.*, 2007), comparés aux acides gras mono-insaturés. Au contraire, les acides gras mono-insaturés sont considérés protecteurs contre le développement de l'obésité et la résistance à l'insuline (Gonzalez *et al.*, 2000; Votruba *et al.*, 2002; Petitt *et al.*, 2003).

Si le niveau d'activité physique est capable de moduler le devenir des lipides alimentaires indépendamment de leur nature reste encore à être élucidé.

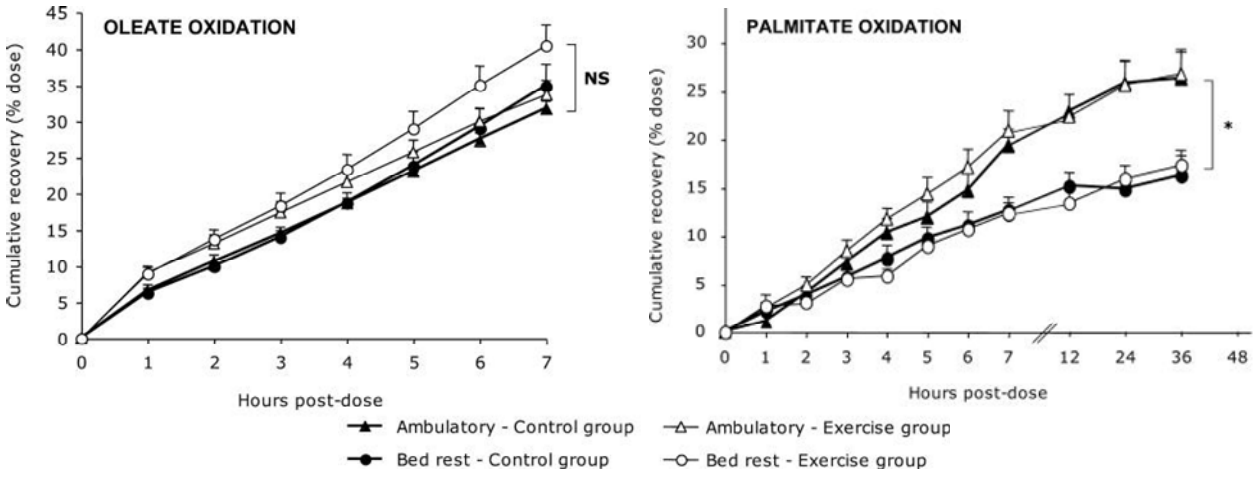


Figure 7 : Effet de l'inactivité extrême (alitement de trois mois) sans (Control group) ou avec exercice (Exercice group) sur l'oxydation du palmitate et de l'oléate. Tandis que l'oxydation de l'oléate est significativement altérée, l'oxydation du palmitate n'est pas affectée (d'après Bergouignan *et al.* Plos Clinical Trials 2006).

3. CONCLUSION

Au cours du siècle dernier, la prévalence de l'obésité a augmenté d'une façon exponentielle. Cette augmentation était parallèle avec la révolution industrielle et les progrès technologiques, induisant une réduction du coût de la vie et incitant les personnes à être moins actives. De ce fait, on peut suggérer que l'augmentation de la proportion des individus qui prennent du poids, au-delà du rôle de la génétique, pourrait être secondaire à une adoption d'un mode de vie sédentaire. Ainsi, le niveau d'activité physique est passé en dessous d'un seuil requis pour assurer une régulation adéquate de la balance énergétique et par conséquent de la régulation pondérale.

Les données de la littérature résumées dans les paragraphes précédents montrent que l'activité physique, en tant que composante la plus modulable de la dépense énergétique totale, pourrait jouer un rôle clef dans la balance énergétique et la balance lipidique. La lipogenèse étant négligeable chez l'homme, les lipides exogènes jouent un rôle clef dans la balance lipidique. Cependant, bien que le rôle de l'activité physique ait été bien démontré en relation avec la balance lipidique, il est moins évident que le niveau d'activité physique module le devenir des lipides alimentaires et nous disposons de peu de données concernant l'effet de la variation du niveau d'activité physique habituelle sur l'oxydation et la répartition des lipides alimentaires. Une telle relation, si elle est démontrée, est primordiale quant au rôle de l'activité physique dans les contextes de prévention et de traitement de l'obésité et des troubles métaboliques associés à la sédentarité.

CHAPITRE 3

OBJECTIFS & HYPOTHÈSES

Alors que la plupart des études se sont limitées à l'étude du métabolisme lipidique total, nous nous sommes intéressés dans cette thèse à l'impact de la modulation du niveau d'activité physique chez l'homme normopondéré et en surpoids sur le métabolisme postprandial des lipides exogènes. L'objectif global de ce travail de thèse a été d'étudier, chez l'homme normopondéré et en surpoids, les mécanismes par lesquels une dépense énergétique liée à l'activité physique, basse ou élevée, module l'oxydation des lipides alimentaires en portant une attention toute particulière à l'évaluation des recommandations actuelles sur le niveau d'activité physique.

Nous avons émis les hypothèses suivantes :

- Le niveau d'activité physique, indépendamment de ses effets sur la balance énergétique, prédit l'oxydation des lipides exogènes en favorisant l'acheminement des graisses alimentaires vers le stockage dans le tissu adipeux au détriment de l'oxydation dans le muscle, lorsqu'il est maintenu à un niveau bas, et en améliorant l'oxydation des lipides exogène lors d'une augmentation de la dépense énergétique.

- Indépendamment de l'état d'obésité, seul un entraînement physique qui augmente de façon significative la dépense énergétique est capable de contrebalancer les effets de l'inactivité physique sur le devenir des lipides alimentaires et sur les mécanismes cellulaires sous-jacents. En d'autres termes, nous n'attendons pas d'effet majeur du seul entraînement physique basé sur les recommandations actuelles.

- Les acides gras alimentaires saturés sont davantage orientés vers le stockage dans les triglycérides musculaires et adipeux que les mono-insaturés, excepté lors de dépenses énergétiques élevées.

Nous avons testé l'effet direct d'un mois d'inactivité physique et de deux mois d'entraînement basé sur les recommandations actuelles sur l'oxydation des lipides totaux et exogènes.

Plus spécifiquement, nous avons cherché à déterminer l'effet de l'inactivité /activité physique sur le devenir des principaux lipides alimentaires de notre alimentation occidentale, à savoir l'acide oléique (38% de l'énergie ingérée) et palmitique (20% de l'énergie ingérée).

Les objectifs spécifiques sont de déterminer les effets de la modulation de l'activité sur :

- La dépense énergétique totale et la dépense énergétique liée à l'activité (activité liée à l'exercice structuré versus. activité spontanée)
- La composition corporelle
- La lipémie
- L'oxydation des lipides totaux en conditions postabsorptives et postprandiales
- L'oxydation de l'oléate et du palmitate exogènes représentant les principaux acides gras monoinsaturés et saturés de l'alimentation humaine

- La répartition de l'oléate et du palmitate dans les différentes fractions lipidiques du plasma
- L'expression génomique des gènes impliqués dans le métabolisme lipidique

RESULTATS

Vélo sous le clair de lune d'Alfred Gockel

CHAPITRE 4

Effects of moderate physical activity and detraining on exogenous saturated and monounsaturated fatty acids repartition

Momken Iman, **Antoun Edwina**, Platat Carine,
Simon Chantal & Blanc Stephane

Diabetes, en préparation

Résumé

Introduction

La régulation de l'apport alimentaire est flexible. Ainsi, toute augmentation de dépense énergétique due à une activité physique est automatiquement suivie par une équivalente régulation en apport calorique. Cette supposition montre clairement le rôle de l'activité physique comme facteur dans la régulation du poids. Dans les années 1950, Jean Mayer *et al.* ont avancé l'hypothèse qu'il existerait un seuil d'activité physique en dessous duquel les mécanismes de régulation du poids sont inopérants, ce qui conduit à une augmentation du poids corporel. Bien qu'une vision d'un gain de poids repose sur un déséquilibre de la balance énergétique, la balance lipidique joue un rôle primordial dans la régulation pondérale. La lipogenèse étant négligeable chez l'homme, les lipides exogènes représentent la majeure source dans la balance lipidique. Cependant, alors que le rôle de l'activité physique a été bien démontré en relation avec la balance lipidique, il est moins évident que le niveau d'activité physique module le devenir des lipides alimentaires et nous disposons de peu de données concernant l'effet de la variation du niveau d'activité physique habituelle sur l'oxydation et la répartition des lipides alimentaires. Une telle relation, si elle est démontrée, est primordiale quant au rôle de l'activité physique dans le contexte de prévention et de traitement de l'obésité et des troubles métaboliques associés à la sédentarité.

Objectifs

Le but majeur de cet article a été d'étudier, chez l'homme normopondéré, les mécanismes par lesquels une dépense énergétique liée à l'activité physique, basse ou élevée, module l'oxydation des lipides alimentaires en portant une attention toute particulière à l'évaluation des recommandations actuelles sur le niveau d'activité physique. Les hypothèses spécifiques sont 1) le niveau d'activité physique, indépendamment de ses effets sur la balance énergétique, prédit l'oxydation des lipides exogènes en favorisant l'acheminement des graisses alimentaires vers le stockage dans le tissu adipeux au détriment de l'oxydation dans le muscle, lorsqu'il est maintenu à un niveau bas, et en améliorant l'oxydation des lipides exogène lors d'une augmentation de la dépense énergétique, 2) Les acides gras alimentaires saturés sont davantage orientés vers le stockage dans les triglycérides musculaires et adipeux que les mono-insaturés, excepté lors de dépenses énergétiques élevées.

Matériel et Méthodes

Nous avons réparti 19 hommes, âgés de 25 à 55 ans et de poids stable, en deux groupes en fonction de leur niveau d'activité physique (NAP). Les NAP étaient obtenus à partir de questionnaires d'activités physiques MOSPA et d'accéléromètres triaxiaux.

- n=10 hommes très actifs (NAP>1,8), normopondérés (IMC<25), sans antécédents personnels ou familiaux d'obésité ou de diabète.

- n=12 hommes sédentaires (NAP<1,5), normopondérés (IMC<25), sans antécédents personnels ou familiaux d'obésité ou de diabète.

Nous avons soumis les sujets sédentaires à 2 mois d'entraînement selon les recommandations actuelles sous forme d'exercices aérobies sur un cyclo-ergomètre pendant 60 min à une intensité modérée (50% VO_2max), 4 fois par semaine pendant deux mois. En dehors des sessions d'entraînements, les sujets ont maintenu leur niveau d'activité physique habituel. Le deuxième groupe formé d'hommes actifs normopondérés à la base a été soumis à quatre semaines d'inactivité en cessant toute activité physique structurée et en réduisant de manière importante les activités de la vie de tous les jours. Une attention particulière était portée sur le maintien de la balance énergétique tout au long des interventions malgré les modifications induites de l'activité physique. Les sujets étaient soumis à deux séries de tests identiques avant et après l'intervention physique afin d'étudier les variations du devenir métabolique des acides gras exogènes (évalué en combinant des molécules marquées par isotopes stables et des microbiopsies de tissus musculaires et de l'expression des gènes cibles au niveau du muscle (RTPCR quantitative) induites par les modifications du niveau d'activité physique en fonction de la nature chimique des acides gras ingérés (saturés ou monoinsaturés).

Résultats

Nous avons montré que la modulation de l'activité physique (basse ou élevée) entraîne une modification de la dépense énergétique totale chez les sujets normopondérés (entraînement +13%, désentraînement -8%). L'inactivité physique, indépendamment des effets mesurables sur la balance énergétique, diminue l'oxydation totale des lipides (- 37%) et des acides gras exogènes (oleate -13%, palmitate – 31%) et entraîne une diminution de la sensibilité à l'insuline. Cette diminution de l'oxydation lipidique totale est probablement due, en partie, à la diminution de la clairance plasmatique des acides gras exogènes et une diminution de l'expression des enzymes participant à la régulation du métabolisme lipidique i.e. nous avons trouvé une baisse de l'expression de la sous-unité catalytique de l'AMPKinase qui favorise l'oxydation lipidique dans le muscle squelettique et une baisse des gènes responsables de la formation des lipides intramusculaires mGPAT et SREBP1. Ces effets sont inversés avec l'activité physique selon les recommandations actuelles, mais avec une moindre amplitude suggérant qu'il n'y a pas eu un effet majeur du seul entraînement physique basé sur les recommandations actuelles. L'activité physique améliore l'oxydation de l'oléate (+15%) mais les oxydations totale et à jeun n'ont pas été modifiées. L'entraînement a eu un effet positif au niveau de l'expression des gènes responsables du transport des acides gras à l'intérieur de la cellule FAT/CD36 et dans la mitochondrie CPT1, ainsi qu'une augmentation de l'expression de PGC-1, responsable de la biogenèse mitochondriale et de SPTLC1, responsable de la formation des céramides. Le résultat majeur obtenu est que le niveau d'activité physique est en étroite relation avec l'oxydation des acides gras exogènes monoinsaturés et saturés chez les sujets normopondérés.

Discussion

Nos résultats montrent que la modulation de l'activité physique est capable de modifier significativement la dépense énergétique totale chez des sujets normopondérés. Après une période courte de un mois de désentraînement en arrêtant tout exercice structuré et en réduisant tout type d'activité spontanée, les oxydations lipidiques à jeun, totale et exogène ont diminuée. D'autre part, un entraînement selon les recommandations actuelles durant la période de deux mois, a réussi seulement à augmenter l'oxydation des acides gras monoinsaturés. Ces résultats montrent que l'inactivité physique a des effets plus marqués sur le métabolisme lipidique que l'activité physique. Cependant, cette étude a montré que le niveau d'activité physique habituelle, indépendamment d'effets quantifiables sur la balance énergétique, prédit l'oxydation des lipides exogènes et que les différents niveaux d'activité physique affectent différentiellement l'orientation des lipides exogènes au profit de l'oxydation. Alors que les données démontrant un rôle causal du niveau d'activité physique dans la physiopathologie de l'obésité restent équivoques, nos résultats apportent des données clefs dans le débat actuel quant au rôle de l'activité physique dans le traitement de l'obésité et des troubles métaboliques associés à la sédentarité, en démontrant une relation positive entre la quantité d'énergie dépensée lors d'activités et le devenir des lipides alimentaires.

1. ABSTRACT

Objective: Although a vision of weight gain is based on a deregulation in energy balance, lipid balance plays a major role in weight regulation. Since lipogenesis in humans is negligible, exogenous lipids represent the major source in lipid balance. However, while the role of physical activity has been well demonstrated in relation to lipid balance, it is less obvious that the level of physical activity modulates the fate of dietary lipids and we have little data on the effect of modulating the level of habitual physical activity on the oxidation and the trafficking of dietary fat. Such relationship, if proven, is essential for the role of physical activity in the context of prevention and treatment of obesity and metabolic disorders associated with physical inactivity. We hypothesized that the level of physical activity, regardless of its effects on energy balance, predicts the oxidation of exogenous lipid by favoring the transport of dietary fat to storage in fat tissue at the expense of oxidation into the muscle, if it is kept at a low level and enhancing exogenous fat oxidation with adequate increase in energy expenditure.

Research design and methods: We investigated the effect of two months training based on current recommendations on sedentary lean men and one month of physical inactivity on active lean subjects. [d_{31}]palmitate and [1-^{13}C]oleate were given through a liquid meal. Total energy expenditure was measured by doubly labeled water before and at the end of the intervention. Total fat oxidation was measured by indirect calorimetry. Measurements and breath and urine sampling were performed at rest 36h after any bout of exercise over 8h following labeled meal ingestion.

Results: Body weight remained stable in both groups, however FFM decreased and FM increased in the detrained subjects. Monounsaturated fatty acid MUFA oxidation increased in trained subjects by 15% ($p<0.05$). Training promoted significantly ($p<0.05$) higher expression of FAT/CD36, PGC1-α, participating in lipid metabolism and oxidation pathways. Detraining reduced exercise capacity and decreased the oxidation of both MUFA (13%, $p<0.05$)) and SFA (31% $p<0.05$). Postprandial insulin concentration increased in detrained subject. The expression of mtGPAT and PRKAA2 (AMPK isoforme) diminished after detraining. Habitual physical activity level correlated with both dietary fatty acid oxidations.

Conclusion: This study showed that the level of habitual physical activity, regardless of quantifiable effects on energy balance, predicts the oxidation of exogenous lipids and various physical activity levels differentially affect the direction of exogenous lipids in favour of oxidation. While data demonstrating a causal role of physical activity in the pathophysiology of obesity remains equivocal, our results provide data keys in the current debate about the role of physical activity in the treatment of obesity and metabolic disorders associated with physical inactivity.

Abbreviations: AEE, activity energy expenditure; BR, bed rest; CPTI, carnitine palmitoyl transferase I; DES1, Sphingolipid *delta*(4)-desaturase, FAT/CD36, fatty acid transporter CD36; FFM, fat free mass; FM, fat mass; IMTG, intramuscular triglyceride; MOSPA-Q, MONICA optional study of physical activity, MUFA, monounsaturated fatty acids; NEFA, non-esterified fatty acid; NPRQ, non protein respiratory quotient; SFA, saturated fatty acids; RMR, resting metabolic rate; PGC-1α, peroxisome proliferator-activated receptor coactivator; PPAR-β, peroxisome proliferator-activated receptor beta; PRKAA2, AMP-activated, alpha 2 catalytic subunit protein kinase; REE, resting energy expenditure; SPTLC1, and SPTLC2, serine palmitoyltransferase long chain base subunit 1 and 2; TBW, total body water, TEE, total energy expenditure; TG, triglyceride; UCP3, uncoupling protein 3; VLDL, very low density lipoprotein.

2. INTRODUCTION

Increased sedentary lifestyle in modern societies has been associated with an increased risk of numerous burden chronic diseases such as cardiovascular and coronary diseases, stroke, cancer, obesity and type 2 diabetes [1, 2]. Such metabolic disorders are frequently associated with a decrease in insulin sensitivity, high plasmatic triglyceride (TG) or hyperlipidemia [3]. Chen *et al.* showed that 2-month cessation of regular training significantly elevated basal and postprandial insulin levels and triglycerides, and were associated with increased basal free fatty acids FFA suggesting that the early development obesity-associated metabolic deregulations due to reduced physical activity may not necessarily reflect on weight status but primarily on metabolic status [4]. While fat oxidation is known to increase slowly to match increased fat intake, increased daily energy expenditure, through the performance of exercise, has been reported to accelerate the adjustment of fat oxidation to the proportion of fat ingested in both men [5] and women [6]. Mayer *et al.* suggested that there is a level of physical activity (PAL as total energy expenditure/ resting metabolic rate) below which mechanisms of body mass regulation are impaired [7]. While The World Health Organization (WHO) have recommended moderated intensity exercise (30 min/day) for optimal health benefits, recent studies suggest that PAL as equal to 1.75-1.8 would be an ideal level to avoid development of weight gain. Such level is reached when exercising intensely for 30min/day or 2h/day of moderate intensity exercise [8].

It is well known that among the main energetic substrates, lipids as dense macronutrient comparing to carbohydrates and proteins, have more limitations to be used in healthy cells [9, 10] and contrary to two other macronutrients, an increase in lipid consummation does not promote higher lipid oxidation. Balance between energy intake and energy expenditure relies mainly on body's fat content and the deleterious consequences of a constant positive energy balance due to a sedentary life style is mainly seen over the regulation and repartition of fatty acid than other substrates [11, 12]. Studies showed that chronic positive energy balance leading to a deregulation in body weight and consequently to overweighteness and /or obesity, is associated with an impairment in the capacity to switch easily from

one fuel to another in response to meal stimulations, while healthy active lean subjects are capable of adjusting substrate [13, 14]utilization. Other effects are also observed such as reduced insulin sensitivity, high intramuscular TG and lower total and exogenous fat oxidation [15, 16]. One of the beneficial effects of physical activity is to reduce plasma triglycerides and to increase total fat oxidation [17, 18]. However, most of the studies have mainly evaluated total and endogenous fat trafficking with regards to physical activity level [19, 20] and only small number of studies such as Votruba *et al.* [21] investigated the effect of exercise on dietary fat. Since lipogenesis is negligible in humans, the majority of fatty acids (endogenous and dietary) are driven from dietary fatty acids, thus playing a major role in lipid balance [12, 22]. Votruba *et al.* investigated the effect of different exercise intensity (low, moderate, and intensive) on dietary fatty acid utilization and showed that exercise increased monounsaturated fat oxidation regardless of intensity while saturated fatty acid oxidation was not affected [21].

On the other hand, sedentary behavior represents one of the main environmental factors triggering predisposed genes to a development of metabolic pathologies and obesity. This observation is more evident when looking at the results of bed rest studies with extreme physical inactivity [23-25]. Indeed, several bed rest studies found similar physiological alterations to those detected in obesity and diabetes type II e.g. a decrease of fat oxidation during fasting and postprandial states, insulin resistance, high plasmatic cholesterol, triglyceride (TG) concentration and accumulation of intramuscular triglyceride (IMTG) and muscle fiber type change from oxidative to more glycolytic [26, 27]. Bergouignan *et al.* found that oxidation of palmitate decreased with BR and palmitate enrichment was enhanced in the pool of intramuscular fatty acid storage [24, 25]. Such variations might be due to a shift of muscle fiber type to use more carbohydrate or a reduction in peripheral insulin sensitivity effecting lipid metabolite profile such as alteration of factors participating in lipid flux or mitochondrial profile [28].

Our aim in this study was to investigate the effect of modulating physical activity level on dietary fat oxidation and trafficking. Further, we hypothesized that saturated fatty acids are more oriented toward storage in muscle and adipose triglyceride than monounsaturated fatty acids, except when sufficient energy expenditure .
We investigated the effect of two months of moderate physical training based on current recommendations in sedentary lean subjects and one month of physical inactivity in high active lean subjects. We measured the fasting and postprandial lipaemia and the cumulative oxidation of dietary SFA and MUFA, and we further quantified the expression of the major factors participating in fat metabolism (oxidation, flow) in the skeletal muscle.

3. METHODS

3.1. Participants and experimental protocol

Data are reported from 19 lean men (20≤ BMI ≤25 kg/m^2) of the lipid oxidation study LIPOX. Volunteers were free of any chronic known diseases and were weight stable (±3kg body weight) for at least 3months before enrolment.

Subjects had no first-degree family history of obesity or type 2 diabetes. Additional criteria were the absence of participation to any structured exercise programs over the 12 months prior to the study for sedentary subjects and the involvement in at least one high level regular sportive activity for active subjects. Sedentary and physically active statuses at inclusion were determined by the MOSPA-Q questionnaire and a RT3 triaxial accelerometer (Stayhealthy, Monrovia, CA, USA) wore for 7 days in free-living conditions. RT3 was used to obtain an estimation of subjects' PALs defined as the ratio between total energy expenditure (TEE) and resting metabolic rate (RMR). Since accelerometers tend to underestimate the PAL, a RT3-estimated-PAL $\leq$1.5 was set to screen sedentary individuals, $\geq$1.7 to screen active ones. Training was performed in sedentary lean and overweight subjects, at the level of current recommendations for two months i.e. four 60-min sessions per week at 50%VO_{2peak} on a cycle ergometer. VO_{2peak} was determined by means of an incremental exercise test to exhaustion performed in an upright position on an electronically braked cycle ergometer (Medifit 1000S, Belgium). Detraining in the active group consisted of stopping all structured physical activity and reducing spontaneous activities of daily living. Throughout the study, research dieticians followed the participants and the diet was regularly adjusted in an effort to maintain subjects in stable energy balance. Informed, written consent was obtained from each subject. The study was approved by the Institutional Review Board of Alsace I.

3.2. Resting energy expenditure, total energy expenditure measurement, body composition

Two sets of identical tests were performed before and after trials (**figure 1**). Subjects were provided with standard meals (50% carbohydrate, 15% protein, 35% lipid) 36 hours prior to each test. On the test day, after an overnight fast, breakfast was served at 9am representing 50% of the RMR (expressed as MJ/24 h) (55% carbohydrates, 15% protein, and 30% fat) and lunch (73% carbohydrate, 10% fat and 18%) representing 27% of RMR was served after 4hours from the first meal ingestion. Resting metabolic rate RMR was measured by indirect calorimetry (Deltatrac II; General Electric, Indianapolis, IN) for 1 h. The non-protein respiratory quotient NPRQ was calculated by using the expired CO_2 and the consumed O_2 measured hourly by indirect calorimetry and the nitrogen excretion. Total energy expenditure (TEE) was measured by doubly labelled water as the daily CO2 production measured from the differential elimination of water labelled with stable isotopes of hydrogen and oxygen [29]. 2 g/kg TBW of doubly labelled water ($^2H_2{}^{18}O$) composed respectively of 0.2 g/kg of 10% ^{18}O and 0.15g/kg of 99.85% ^2H (Cambridge Isotope Laboratories, Andover, MA) was orally administered. Equilibration and end point urines were cleaned as previously described [30]. Deuterium and 18-oxygen isotopic abundances were analyzed by pyrolysis on an elemental analyzer (Flash HT; ThermoFisher) connected to a continuous flow isotope ratio mass spectrometer (Delta V; ThermoFisher, Scwerle, Germany). The results were scaled using laboratory standards. Analyses were performed in quadruplicate and repeated if the SD exceeded 2% for deuterium and 0.5% for 18-oxygen. The total body water and TEE were calculated as previously described [31] using a food quotient of 0.86.
Body composition was measured using the isotope dilution method, as previously described [32]. Total body water (TBW) was calculated for both the deuterium and ^{18}O dilution and the two values were averaged. FFM was calculated from TBW using a constant hydration factor (0.73) [33] and fat mass (FM) was calculated as the difference between body weight and FFM.

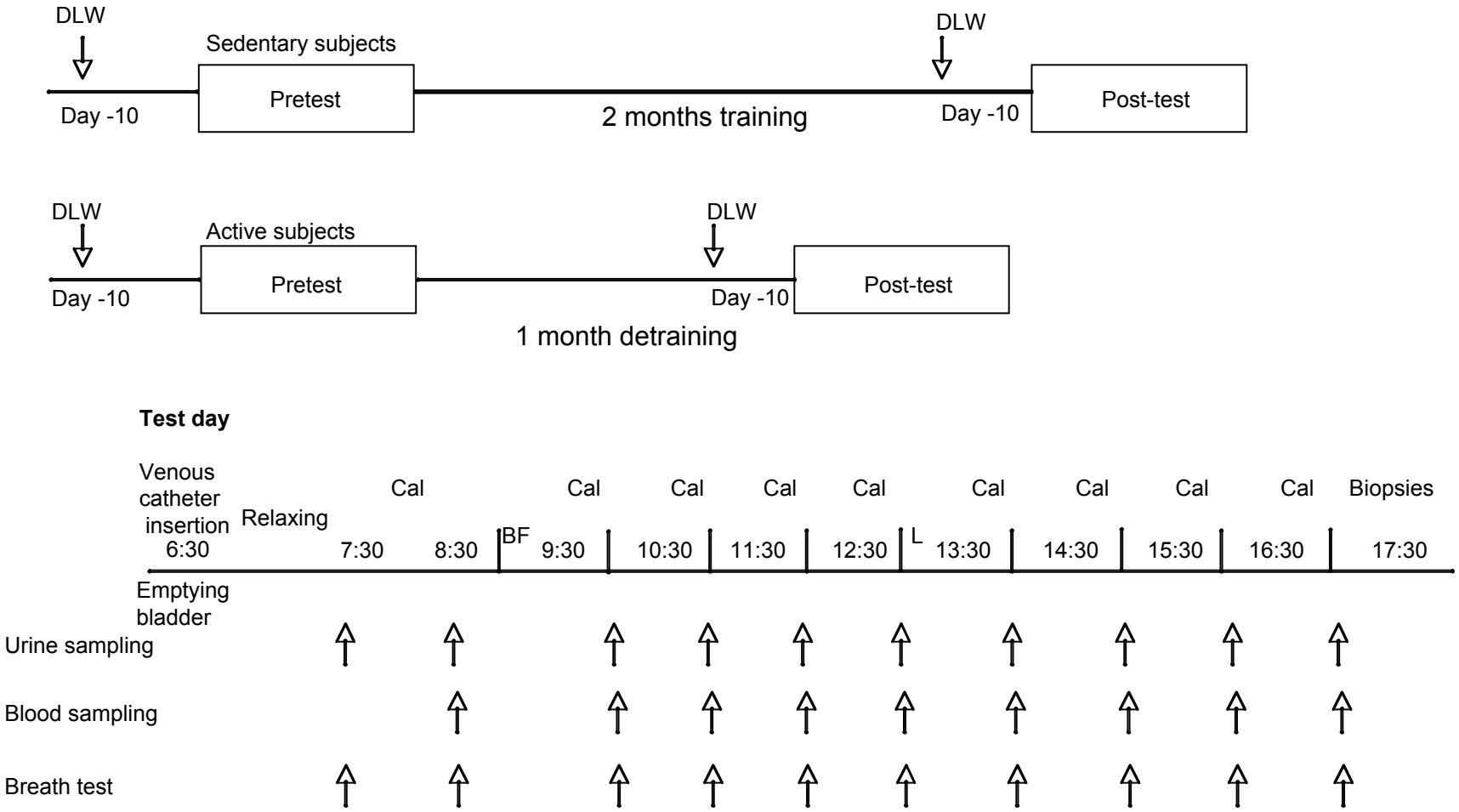


Figure 1: General procedure of the tests is presented. Two groups were selected based on their physical activity level, age and absence of family history for pathologies such as diabetes and obesity. Sedentary group went under 2 months of moderate (30min/d) training and active group under 1 month detraining. Lipid tests were performed before and after trials. Urine, blood and breath samples were collected every hour over 9hours. The breakfast (BF) consisted of [1- ^{13}C]-oleate and d_{31}-palmitate and was served at 9am and lunch (L) served at 1pm. Resting energy expenditure (REE) was measured by indirect calorimeter (Cal) every 30min during the day test. Total energy expenditure (TEE) was measured by doubly labeled water (DLW) technique during 10 days prior to day tests.

3.3. Dietary fat oxidation by isotope ratio mass spectrometry analyses

Dietary fat oxidation and trafficking were measured once before and once after trials. After an overnight fast, an intravenous catheter was inserted retrogradely into the forearm vein, covered with a heated pad for arterialized blood samples. Baseline breath, urine and blood samples were collected (**figure 1**). A liquid replacement meal was provided with breakfast and was enriched with 15 mg/kg of d_{31}-palmitic acid (>98% enriched; CIL, Andover, Massachusetts, United States) and 10 mg/kg of [1-^{13}C]-oleic acid (>99% enriched, CIL) homogenized at 65 °C (melting point of palmitate). These fatty acids represent the main monounsaturated and saturated fatty acids of the human diet (38% and 20% of total intake, respectively).
To determine [1-^{13}C]-oleate oxidation after breakfast ingestion, hourly breath samples were collected for 8 h. Breath samples $^{13}CO_2$-to-$^{12}CO_2$ ratios were measured in triplet on a continuous-flow inlet system connected to an isoprime isotope ratio mass spectrometer (GV Instruments, Isotope Ration MS, Germany). All enrichments were expressed against International Atomic Energy Agency standards. [1-^{13}C]-oleic oxidation was calculated as the instantaneous recovery of ^{13}C in expired CO_2 per hour over 8 hours. Instantaneous recovery of ^{13}C in expired CO_2 expressed as a percentage of the dose was corrected for isotope sequestration by assuming an acetate correction factor of 51% [34]. To measure d_{31}-palmitate oxidation, 2H/1H from urine samples was analyzed, as previously described [31]. The oxidation rate of palmitate/water was inferred from the cumulative recovery by ^{2}H in TBW. The detailed calculations of the percentage recoveries are described in detail elsewhere [35].

3.4. Plasmatic analysis

Plasmatic free fatty acid (NEFA) was measured by NEFA C WAKO kit (SOBIODA S.A.S), plasmatic glucose measured by chemical reaction using GLUCm glucose kit and triglyceride (TG) were assayed by enzymatic reaction using triglyceride GPO kit both from Beckman Coulter System SYNCHORN. Insulin was determined by immunoassay (ADVIA Centaur Insulin IRI Siemens).
To determine dietary d_{31}-palmitate and [1-^{13}C]-oleate trafficking, total lipids were extracted from plasma [36]. NEFA was separated from plasma by solid-phase extraction and derivatized to methyl esters [37]. By slight modification of Chung B [38] protocol for plasmatic lipid separation, VLDL and total chylomicrons fractions were prepared by ultracentrifugation at 80 000 rpm at 12°C (KENDRO S150, rotor S140AT) during 30min using gradient of 0.997 density. A pasture pipette was used to remove gently the supernatant containing the chylomicrons followed by measuring the volume using an automated pipette. To obtain the VLDL the precipitant was suspended in ultra pure water containing Kbr (Potassium bromide) to increase the density to 1.006. By another ultracentrifugation step (1h10 at 10°C in 140 000 rpm) the VLDL were floating and were collected. TG concentrations in both fractions were quantified by Mira automated analyzer (Roch Cobas Chemistry Analyser) using enzymatic reagents.

Gas chromatography was used to analyze the fatty acid composition of plasma NEFA, chylomicron remnant TG, and VLDL TG [37-39]. The absolute concentrations of the individual fatty acids were calculated by reference to internal standards added to the plasma during lipid extraction—heptadecanoic acid for NEFA and triheptadecanoyl glycerol for lipoprotein TG. Isotope enrichment was analyzed

Variables	Sedentary		Active	
	Baseline	Training	Baseline	Detraining
Body weight (kg)	76.7 ± 3.8	76.1 ± 2.9	71.7± 2.9	71.4 ± 3.2
BMI (kg/m^2)	22.9 ± 0.7	22.7 ± 0.6	22.2 ± 0.6	22.0 ± 0.7
FFM (kg)	58.8 ± 1.9	59.2 ± 1.9	61.2 ± 2.5†	60 ± 2.7 *
FM (kg)	17.9 ± 1.9	17 ± 1.7	10.5 ± 1.2††	11.4 ± 1.5*
FM%	22.8 ± 1.8	21.9 ± 1.7	14.5 ± 1.4††	15.8 ± 1.8**
VO_2max (ml/min/kg)	39.7 ± 1.8	44.9 ± 1.9**	50.2 ± 2††	46.4 ± 1.3**
Fasting Variables				
Glucose (g/l)	0.88 ± 0.03	0.87 ± 0.03	0.84 ± 0.01	0.86 ± 0.02
Insulin (mU/l)	5.15 ± 0.65	4.41 ± 0.42	3.63 ± 0.3	5.24 ± 0.73*
TG (g/l)	0.92 ± 0.11	0.84 ± 0.13	0.49 ± 0.04††	0.59 ± 0.13
NEFA (μmol/l)	0.39 ± 0.05	0.38 ± 0.04	0.41 ± 0.03	0.41 ± 0.05

Table 1. Body weight, body composition, maximal respiratory capacity (VO_2max) and fasting plasmatic parameters before and after physical activity interventions for Active (n=9) and Sedentary (n=10) participants. Values are expressed as mean ±SEM. Significant differences are shown between sedentary *vs.* active baselines (†) by using an unpaired t-test, and before *vs.* after trials within each group (*) by paired t-test. *$p<0.05$, †$p<0.05$. BMI, body mass index; FFM, fat free mass; FM, fat mass; TG, triglycerides; NEFA, non-esterified fatty acids.

by gas chromatography-isotope ratio mass spectrometry as described previously by Evans *et al.* [40]. To assess both the isotopic enrichment and the individual fatty acid concentrations (both unlabeled and labeled) in the same gas chromatography/mass spectrometry (Agilent 5975 Inert XL), we designed a dual acquisition program in single-ion monitoring mode. The following m/z ratios were acquired: 296 and 297 for oleate and 270 and 301 for palmitate. The concentration of each labeled fatty acid was calculated by multiplying its molar percent enrichment (MPE) by the concentration of its corresponding unlabeled compound.

3.5. Real Time PCR (RT-QPCR)

8 hours after the labelled meal ingestion, muscle biopsies from the *vastus lateralis* were taken using a Bergström needle. Muscle biopsies were grounded in liquid nitrogen and total RNA was extracted using mirVana™ miRNA Isolation Kit (Applied Biosystems, Courtaboeuf, France). RNA concentration was measured with Nanodrop ND1000 (Labtech, Palaiseau, France) and integrity assessed using an Agilent 2100 Bioanalyzer (Agilent Technologies, Massy, France).

First-strand cDNAs were synthesized from 500 ng of total RNA in the presence of 100 units of Superscript II (Invitrogen, Cergy Pontoise, France) using a mixture of random hexamers and oligo (dT) primers (Promega, Charbonnières, France). Real-time PCR assays were performed using a Rotor-GeneTM 6000 (Corbett Research, Mortlake, Australia) in a final volume of 20µl containing 5µl of a 60-fold dilution of the RT reaction medium, 10µl of reaction buffer from the Absolute QPCR SYBR Green ROX Mix (Abgene, Courtaboeuf, France) and 0.375µM of the specific forward and reverse primers. For quantification, a standard curve was systematically generated with six different amounts of cDNA. Each assay was performed in duplicate, and validation of the real-time PCR runs was assessed by evaluation of the melting temperature of the products and by the slope and error obtained with the standard curve. Hypoxanthine phosphoribosyltransferase mRNA level was determined in each sample and was used as internal standard for normalization of target mRNA expression. The list of the PCR primers and the quantitative PCR assay conditions are available in the table below. The RT-QPCR was applied to detect the expression of, Fatty acid translocase (FAT/CD36), carnitine palmitoyle transferase 1 (CPT-1), peroxisome proliferator-activated receptor coactivator (PGC-1α), peroxisome proliferator-activated receptor beta (PPAR-β), serine palmitoyltransferase, long chain base subunit 1 (SPTLC1), uncoupling protein 3 (UCP3), Sphingolipid *delta*(4)-desaturase DES1, and α2 isoform of the catalytic subunit of AMPK PRKAA2

Variables	Sedentary		Active	
	Baseline	Training	Baseline	Detraining
TEE (MJ/d)	10.7 ± 0.5	12.3 ± 0.7**	14 ± 0.6†††	12.9 ± 0.5**
REE (MJ/d)	6.6 ± 0.2	6.7 ± 0.2	6.3 ± 0.2	6.2 ± 0.2
$REE_{(FFMadj)}$	7.0 ± 0.1	7.1 ± 0.1	6.2 ± 0.2†††	6.2 ± 0.2
AEE (MJ/d)	3.0 ± 0.3	4.4 ± 0.5**	6.3 ± 0.4†††	5.4 ± 0.3**
AEE (kJ/d/kg BW)	38.9 ± 4.6	57.7± 6.3*	88.9 ± 5.7†††	76.8 ± 5.2**
PAL	1.60 ± 0.05	1.83 ± 0.08**	2.23 ± 0.06†††	2.09 ± 0.06*

Table 2. Total energy expenditure (TEE) level, physical activity level (PAL), resting energy expenditure (REE) and activity energy expenditure (AEE) are reported for Active (n=9) and Sedentary (n=10) participants. REE (FFM) adjusted for fat free mass. Values are expressed as mean ±SEM. Significant differences are shown between sedentary *vs.* active baselines (†) by unpaired t-test, and before *vs.* after trials within each group (*) by paired t-test. *p<0.05, †p<0.05 ††p<0.01, ††† p<0.001

CD36 AS	AGACTGTGTTGTCCTCAGCG
CD36 S	TGATGATGAACAGCAGCAACAT
CPT1 tot S	TACAACAGGTGGTTTGACA
CPT1ASMU LC	CAGAGGTGCCCAATGATG
Des1 S	TGATTTCCCCAACATTCCTG
Des1 AS	GCACCATCTCTCCTTTTTGG
PGC1a AS	CTTGGTTGGCTTTATGAGGAGG
PGC1a S	TCCTCTGACCCCAGAGTCAC
UCP3 AS	CTGGGCCACCATCTTTATCA
UCP3 S	ATGGACGCCTACAGAACCAT
SPTLC1 S	GATTTGCCACCATAGCCAG
SPTLC1 AS	GAGCCTTGCGAGGATTCTTTTG
SPTLC2 S	GAGACGCCTGAAAGAGATGG
SPTLC2 AS	TGGTATGAGCTGCTGACAGG
ACC2 AS2	TGATGTCATTGCCGATGACG
ACC2 S	GGTGGAGATGAACCGACTTC
GPAT AS	CTGTGTCTGTAGAGGAGCAG
GPAT S	CTACGAAGGAGGTTGATTGC
SRE1C S	GCAGATCGCGGAGCCATGGATTGC
SRE-AS2	GAGGTGGAGACAAGCTGCCTGG
PRKAA2 S	GAGCTTACAACTTTACCTGG
PRKAA2 AS	GAACCAGATCTCTGCTCCAC
PPAR B AS2	GCCACCAGCTTCCTCTTCTC
PPAR B S2	ACTGCCGCTTCCAGAAGTGC

3.6. Statistical analysis

The normality of the distribution of all parameters was tested using Shapiro-Wilk test. Areas under the curve (AUC) of the concentrations-time curve were calculated using the trapezoid rule. An unpaired Student t-test was used to compare groups' baselines and paired Student t-test to compare before and after intervention within the same group. Further analyses were made with Pearson correlations between the difference of before and after physical activity interventions of parameters. Data are presented as means ± standard error mean (SEM). Significant level was $p<0.05$.

Variables	Sedentary		Active	
	Baseline	Training	Baseline	Detraining
NPRQ	0.856± 0.010	0.852± 0.014	0.872± 0.014	0.902± 0.012*
Fasting Substrate Oxidation (mg/kgFFM)				
Glucose	101± 5	101± 8	108± 10	119± 11
Lipids	38± 4	40± 5	32± 4	22± 3*
Protein	33± 4	33± 4	45± 6	57± 11
Post prandial Substrate oxidation (mg/kgFFM)				
Glucose	1380± 56	1349± 63	1146± 37††	1317± 52*
Lipids	172± 16	174± 21	198± 21	125± 16*
Protein	350± 30	403± 30	503± 40††	487± 40

Table 3: Non-protein respiratory quotient (NPRQ), fasting and postprandial substrate oxidation are represented for Active (n=9) and Sedentary (n=10) participants. Values are expressed as mean ±SEM. Significant differences are shown between sedentary *vs.* active baselines (†) by unpaired t-test and before *vs.* after trials within each group (*) by paired t-test. *$p<0.05$, †$p<0.05$ ††$p<0.01$.

4. RESULTS

4.1. Baseline differences before trials

The anthropometric characteristics of subjects are shown in **table 1**. Body weight and BMI were comparable among the two groups at baseline. Sedentary group showed lower FFM (26%, $p<0.01$) and VO_2max (4%, $p<0.05$) while FM% (8%, $p<0.001$) was higher. As it was expected, the baseline PAL and AEE (kJ/d/kgBW) in sedentary group were significantly ($p<0.0001$) lower compared to active group (**table 2**). Fasting (NPRQ) was not significantly different between groups (**table 3**). Fasting plasmatic glucose and insulin concentrations showed no significant differences between the two groups (**table 1**). Basal plasma concentration for TG (**table 1**) was significantly higher in sedentary subjects ($p<0.0001$) while no significant difference found for NEFA. In response to breakfast, basal carbohydrate oxidation was 17% ($p=0.01$) higher in sedentary group while their lipid oxidation was non-significantly 15% lower than active group. Contrary to carbohydrate, the basal protein oxidation was significantly higher ($p<0.01$) in active that might indicate higher protein turnover.

4.2. Effects of two month training in sedentary participants

Body weight, FFM and FM were not affected by training while VO_2max improved by 13% ($p<0.01$). As **table 2** shows, TEE (MJ/d) level in trained sedentary increased by 15% and approached the TEE (MJ/d) level of basal active group. This improvement was mainly due to an increase in activity energy expenditure (AEE) by 48.5% ($p<0.05$) and PAL by 14% ($p<0.01$) with training while the resting energy expenditures (REE) did not vary (**table 2**).

No significant changes occurred for fasting and postprandial carbohydrate and lipid oxidation after training (**table 3**). Fasting insulin concentration was reduced non-significantly ($p=0.4$) by 14% after training (**table 1**). Postprandial glucose concentration was not significantly modified (**figure 2a**), however postprandial insulin concentration (**figure 2b**) tended to decrease (27% $p=0.1$). Total postprandial TG and NEFA (**figure 2c, d**) concentrations were not modified by training.

Physical activity increased [1-^{13}C]-oleate oxidation by 15% ($p<0.05$) but not d_{31}-palmitate oxidation (**figure 3**). Training significantly ($p\leq0.05$) increased NEFA clearance for both dietary fatty acids (**figure 4c, f**). Conversely, however it did not effect the plasmatic concentrations of d_{31}-palmitate-VLDL and [1-^{13}C]-oleate-VLDL. Training promoted significantly ($p<0.05$) higher expression of FAT/CD36, PGC1-α, DES1 and SPTLC2 ($p<0.05$) all participating in lipid metabolism and oxidation pathways (**figure 5**).

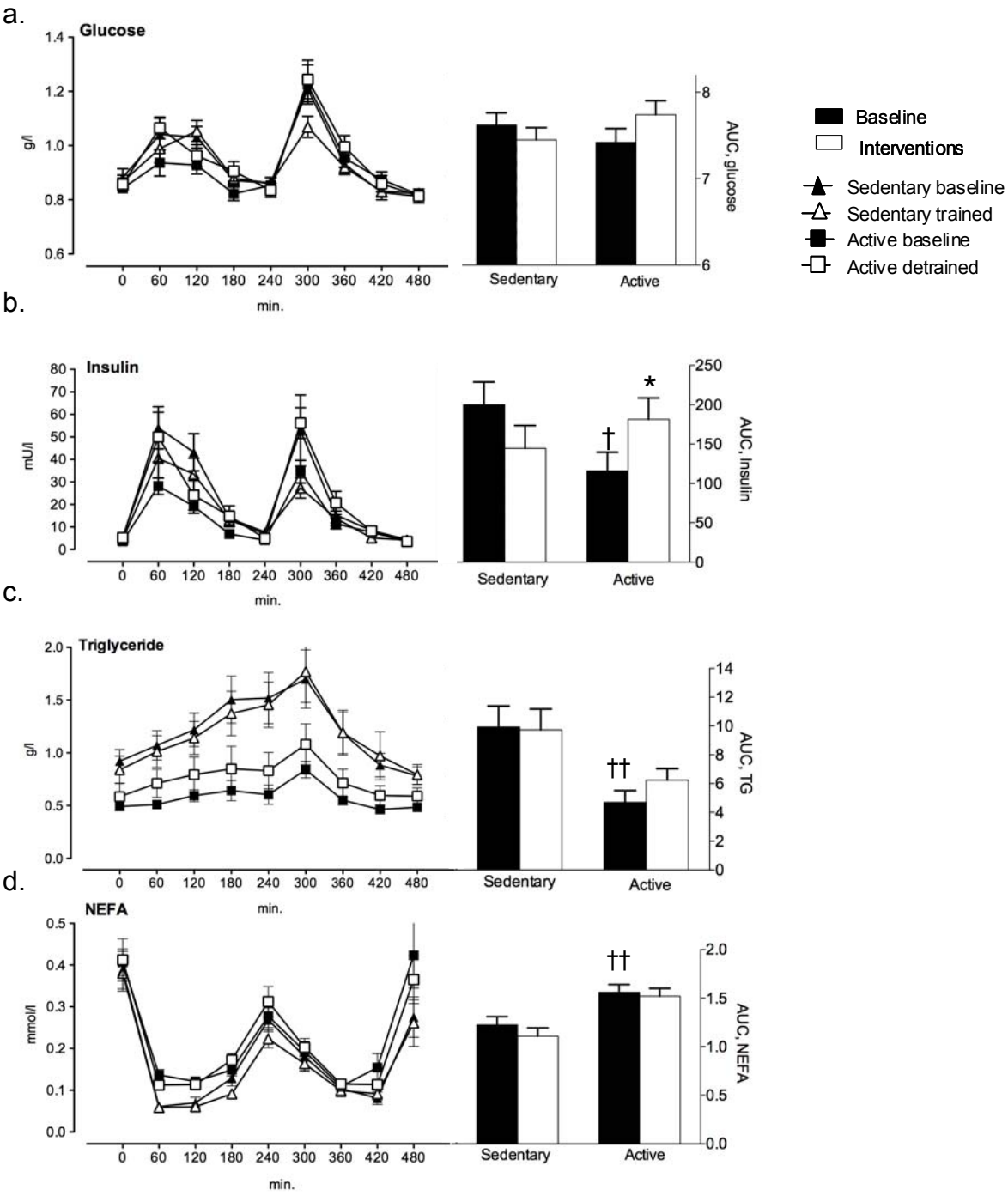


Figure 2: Time course of glucose (a), insulin (b), triglycerides (c) and non-esterified fatty acids NEFA (d) concentrations of active (n=9) and sedentary (n=10) participants. Time 0 corresponds to the breakfast ingestion. The cumulative responses of these parameters were calculated by the area under the curve (AUC). Significant differences are shown between sedentary *vs.* active baselines (†) by unpaired t-test and before *vs.* after trials within each group (*) by paired t-test. *p<0.05, †p<0.05 ††p<0.01

4.3. Effects of one month detraining in active participants

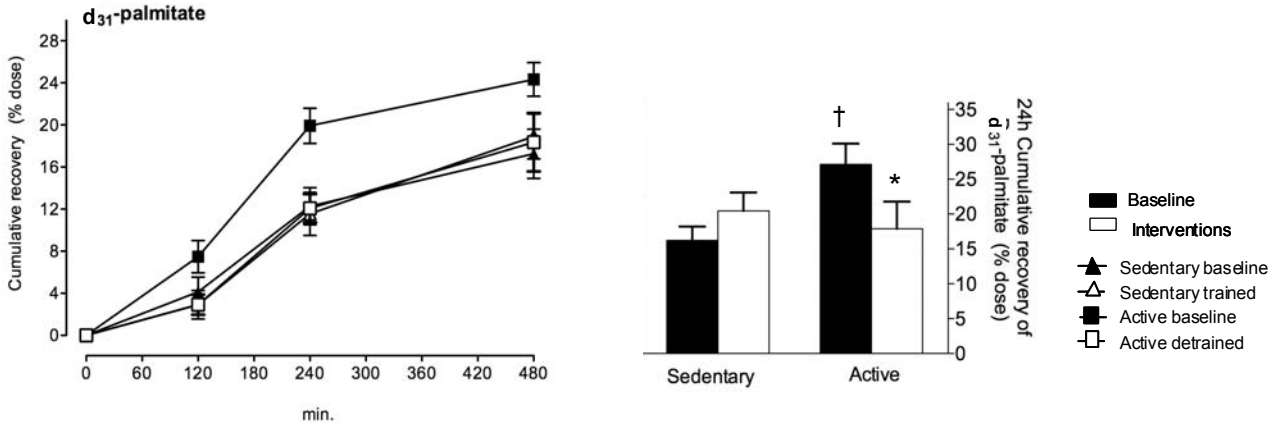
While body weight remained stable, detraining decreased significantly FFM (kg) by 2% and increased FM (%) by 1.3%. VO_2max was reduced by 8% ($p<0.01$). After detraining, TEE decreased by 8% ($p<0.01$), which was mainly due to the reduction of AEE by 14% and PAL by 6.3% ($p<0.05$) yet active detrained subjects maintained higher AEE and PAL comparing to trained group. In fasting conditions, active subjects shifted to use 10% more of carbohydrate; however this value was not significantly different. Fasting lipid oxidation diminished significantly by 31% after detraining (**table 3**).

Postprandial carbohydrate oxidation was increased by 15% ($p<0.05$) and postprandial lipid oxidation was reduced by 37% ($p<0.05$) (**table 3**). The detrained group increased fasting (44%) and postprandial (58%) insulin concentrations indicating lower insulin sensitivity (**figure 2b**). Postprandial glucose concentration was not significantly influenced by detraining. Detraining did not influence postprandial NEFA and TG concentrations (**figure 2c, d**). Detraining reduced the oxidation of both [1-^{13}C]-oleate and d_{31}-palmitate by 13% ($p<0.05$) and 31% ($p<0.05$) respectively and values tended to reach the same level as sedentary baseline (**figure 3a, b).**

The patterns of plasmatic trafficking of [1-^{13}C]-oleate and d_{31}-palmitate were mainly affected between time 0 (breakfast) and time 240 (before lunch) (**figure 4**). The dietary fatty acids packaging in chylomicrons increased significantly by detraining as d_{31}-palmitate rate of appearance in chylomicron increased by 94% (AUC 0-240) and [1-^{13}C]-oleate rate of appearance in chylomicron increased by 36% (**figure 4a, d**). Palmitate rate of appearance increased but not oleate rate of appearance in VLDL (**figure 4b, e**). Both dietary fatty acids rate of appearance in NEFA increased significantly after detraining (**figure 4c, f**). In general, the plasmatic parameters measured in detrained group showed a drift toward the baseline values of the sedentary group.

Detraining lowered significantly the expression of SERBPc, mGPAT and PRKAA2 (catalytic isoforme of AMPK) (**figure 5**). No significant modifications were found for FAT/CD36 or CPT-1. However, the higher or lower expression of FAT/CD36 might not be an indicative of fatty acid transport capacity within the cell without considering other factors inducing the translocation of FAT/CD36 to the cell membrane.

a.



b.

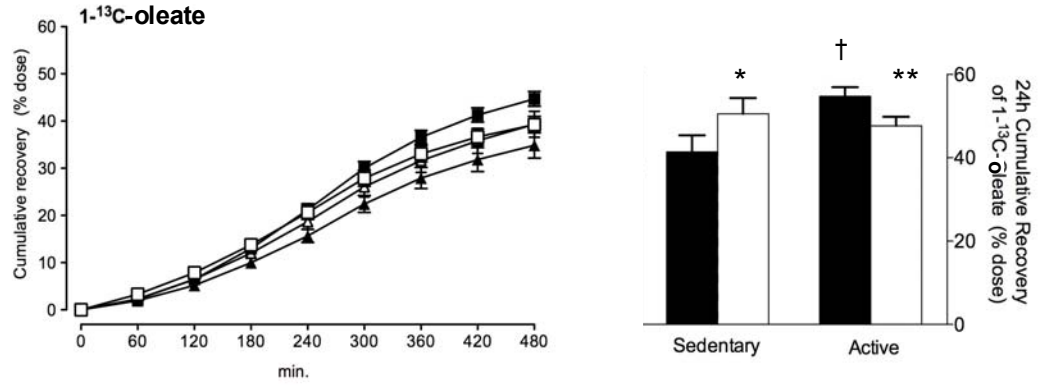


Figure 3: Cumulative recovery for palmitate measured by measuring ^{2}H in total body water hourly sampled through urine, and for oleate were calculated by recovery of [1- ^{13}C]-oleate in expired CO_2. Both recoveries are expressed as a percentage of the dose. At right side, the area under the curve (AUC) representing the 24h fatty acid oxidation. Significant differences are shown between sedentary *vs.* active baselines (†) by unpaired t-test and before *vs.* after trials within each group (*) by paired t-test. *$p<0.05$, †$p<0.05$

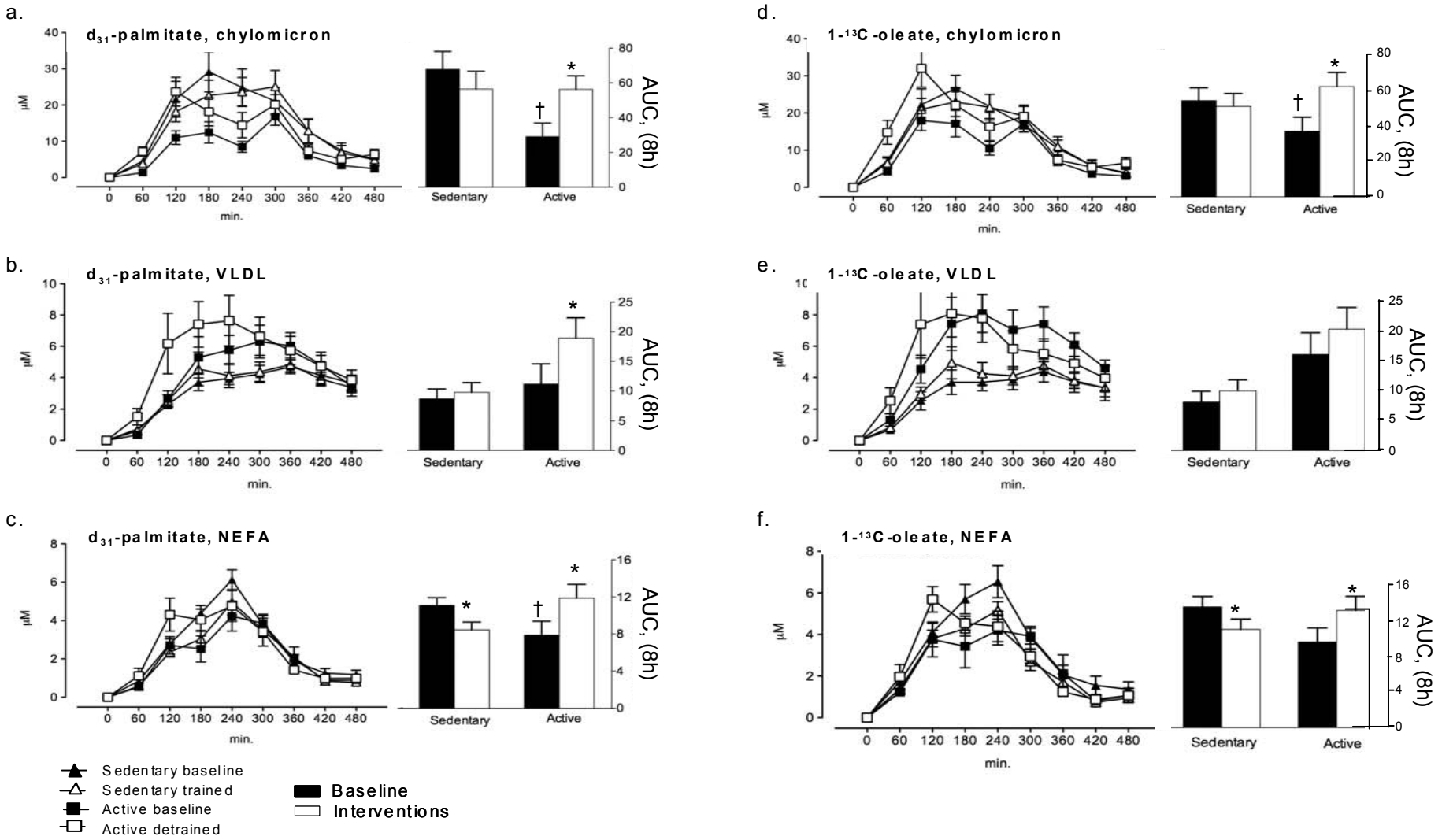


Figure 4: Time course of d_{31}-palmitate and [1- ^{13}C]-oleate incorporated in chylomicron (a, b) very low density lipoprotein VLDL (c, d), non-esterified fatty acids NEFA (e, f). Time 0 corresponds to the breakfast ingestion. The cumulative responses of these parameters were calculated by the area under the curve (AUC), here AUC 0-240 min are shown. Significant differences are shown between sedentary *vs.* active baselines (†) by unpaired t-test and before *vs.* after trials within each group (*) by paired t-test. *p<0.05, †p<0.05 ††p<0.01.

4.4. Correlations between dietary fatty acid oxidation with physical activity level and mRNA expression

Significant positive correlations between the variations of physical activity level and dietary fatty acid oxidation were found (**figure 6a, c**), suggesting a direct relation between physical activity level and the degree of dietary fatty acid oxidation (palmitate, R^2=0.33, p= 0.01, oleate, R^2=0.35, p=0.01). In addition, no significant correlation was found between the variations of dietary fatty acid oxidation with FM (**figure 6b, d**), which suggests that the changes we observed were not due to changes in energy balance.

The variation of PGC-1α expression before and after training correlated positively with palmitate oxidation differences (p=0.025) but not with oleate (**figure 7a, b**) while the mtGPAT variation correlated significantly (p=0.03) with the variation of oleate oxidation but not palmitate (**figure 7c, d**). Further correlation analysis showed that TG variation significantly correlated with the variation in FAT/CD36 (**figure 8a**), and NEFA changes significantly correlated with PRKAA2 and mtGPAT variations (**figure 8b, c**).

5. DISCUSSION

The major finding in this study is the positive linear relationship between the level of physical activity and the degree of the dietary fatty acid oxidation. Even though detraining duration (one month) was shorter than training (2 months), it seems that inactivity had an enhanced effect on dietary lipid metabolism rather than moderate training.

The modulation of physical activity level succeeded in changing total energy expenditure in both lean groups. One month of detraining by stopping any structured exercise and reducing any bodily movement reduces significantly total and activity energy expenditure, while two months training based on currents recommendations succeeded in increasing total and activity energy expenditure in lean subjects. Modulating physical activity, however, is not always followed by effects on total energy expenditure. Indeed, compensatory behaviors may occur, which can reduce the effect of modifying AEE. Introducing exercise training into a sedentary life style might result in a compensatory behavior by reducing the non exercise activity in individuals with predisposition to be sedentary. However, in lean subjects, after training of 18 wk, Van Etten *et al.* [41] found no changes in non training activity. Training improved VO_2max, PAL, AEE, while detraining decreased these parameters. However, the detrained group maintained higher values than trained subjects.

Our results show a positive relationship between AEE and exogenous lipid metabolism in lean subjects. Our finding is in line with Mayer's theory which suggested the presence of a PAL below which mechanism of body mass regulation are impaired. Accordingly, Stubbs *et al.* [42] showed that active individuals tolerated more the high fat diet compared with inactive and this may be related to differences in daily NEAEE. Moreover, Smith *et al.* [5] and Hansen *et al.* [6] reported that the increase in the daily energy expenditure accelerated the adjustment of fat oxidation to the proportion of fat ingested in men and women respectively. The positive relationship we found of AEE and exogenous fatty acid oxidation suggests that improvement can be achieved when sufficient increase in AEE. These increases might be produced in enhancing NEAEE. Thus NEAEE might be a key modulator in body weight regulation through lipid balance.

The variation in dietary fatty acid oxidation was not related to changes in body weight nor in body mass composition. In addition no significant difference appeared with physical activity interventions on total plasmatic insulin, TG and NEFA. It was suggested that exercise has only short term beneficial effect on lipid metabolism [43]. De-training studies provide compelling evidence that exercise training may not markedly influence TG metabolism in the absence of recent exercise and that training induced changes in TG metabolism may be short-lived [44]. After detraining, a higher fasting and postprandial NPRQ was found, suggesting a shift toward more carbohydrate oxidation while reducing lipid oxidation. This was in line with results obtained from obese [45] and subjects in extreme physical inactivity studies (Bed Rest) where a shift of fuel utilization from lipids to carbohydrates during fasting and postprandial were noted [25].

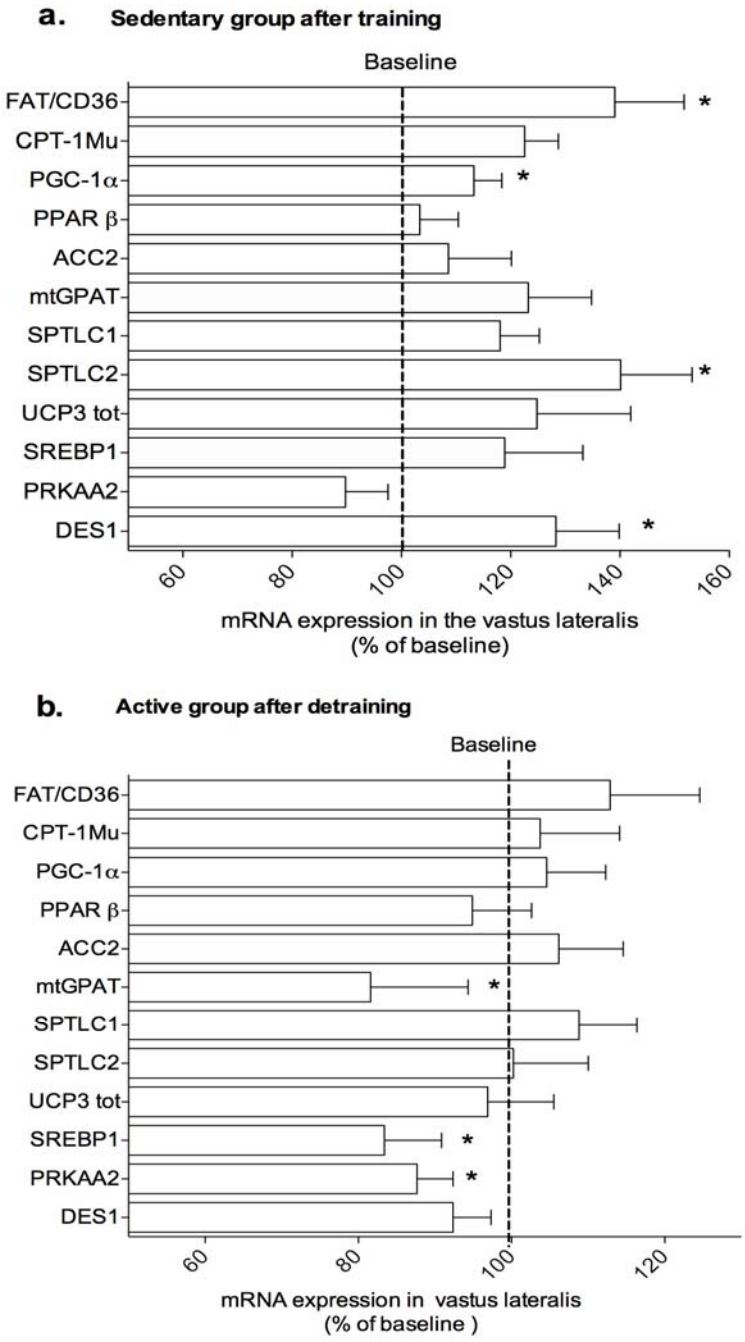


Figure 5: Training increased PGC-1α, FAT/CD36, SPTLC2, DES1 mRNA expression (a) and detraining reduced the expression of mtGPAT, SREBP-1c and PRKAA2 (b). Results expressed in % of the fold change *vs.* the baseline values at 100%. Data are mean ±SEM. * $p<0.05$ *vs.* pre-trial.

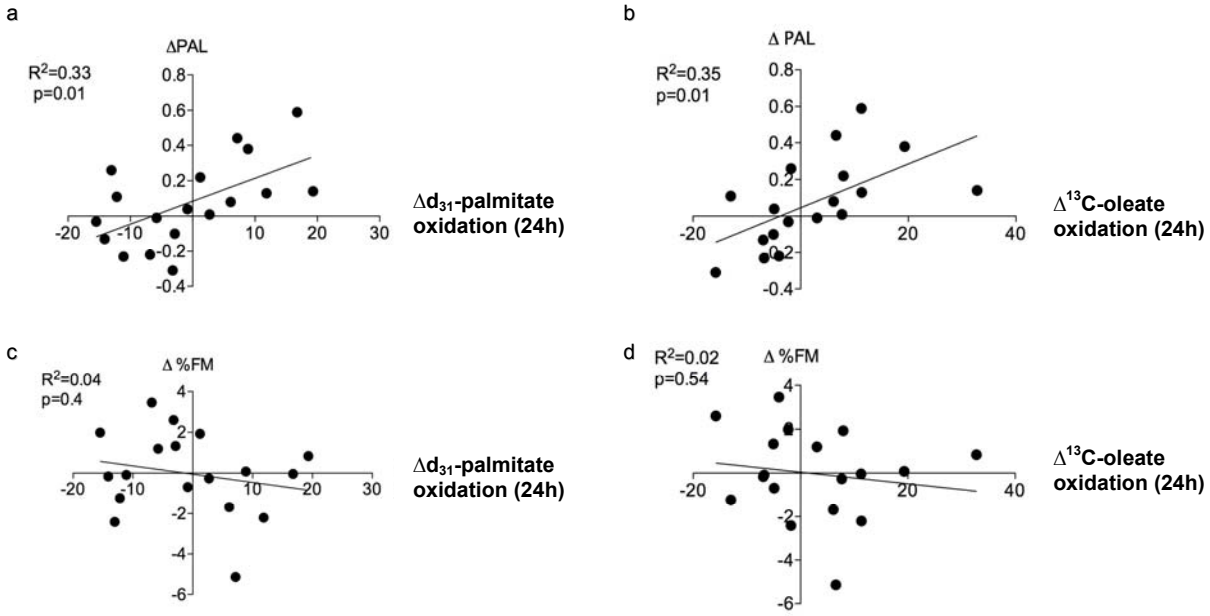


Figure 6: Correlations between *delta* PAL with *delta* d_{31}-palmitate oxidation (a) and *delta* [1-^{13}C]-oleate oxidation (b), correlations between *delta* %FM with *delta* d_{31}-palmitate oxidation (c) and *delta* [1-^{13}C]-oleate oxidation (d).

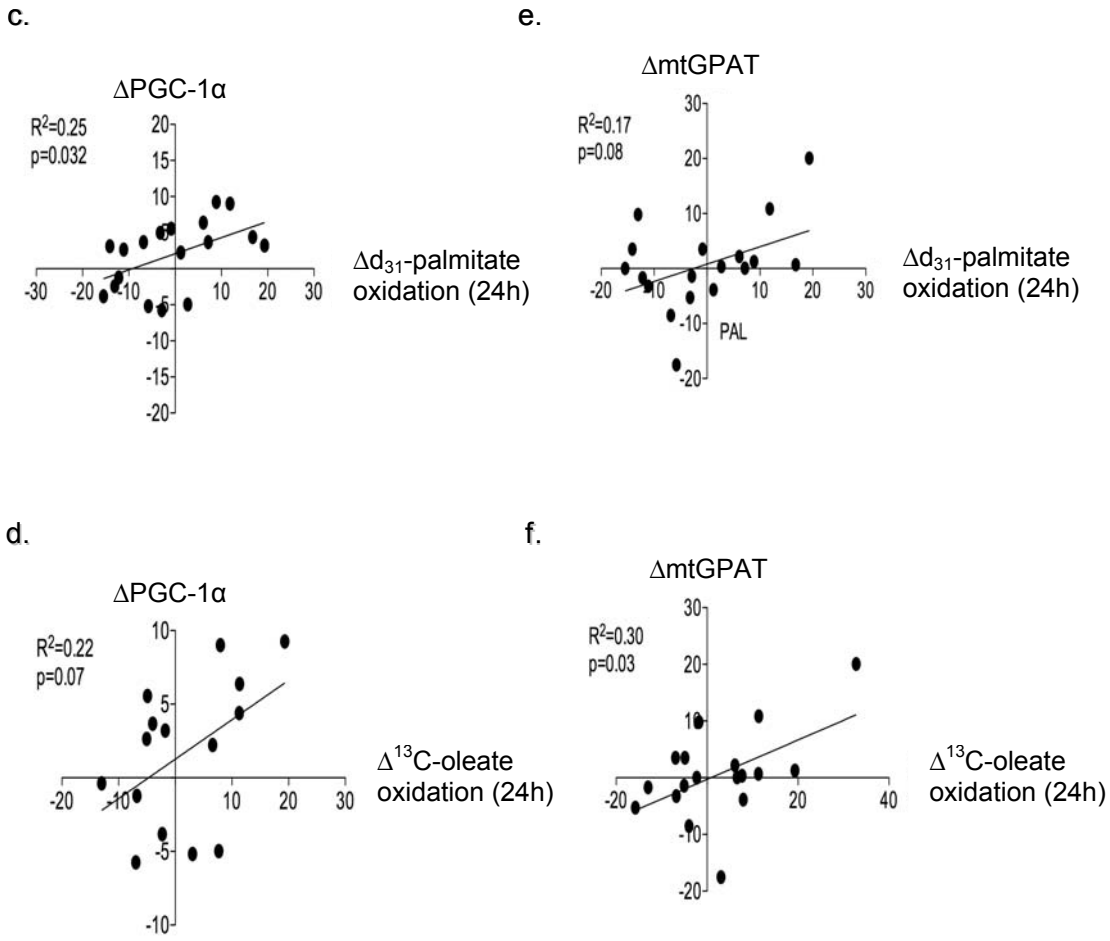


Figure 7: Correlation between changes in cumulative palmitate and oleate recovery (%) dose and changes in mRNA expression of PGC-1α in the skeletal muscle in the left side (a, b) and with changes mRNA expression of mtGPAT in the right side (c, d).

Hyperinsulenimia could be one of the characteristics of sedentary life style [46] and studies showed that it could appear even during short term of bed rest such as 3 to 7 days [47, 48]. The higher insulin concentration in detrained subjects comparing to their baseline would indicate a decrease of insulin sensitivity. The low expression of SREBP-1c found in the skeletal muscle in detrained subjects might also reflects lower insulin sensitivity as a study by Guillet-Deniau I *et al.* proposed that SREBP-1c expression in rat skeletal muscle might transduce better muscle insulin sensitivity and SREBP-1c can mimic the effect of insulin on lipogenic enzyme expression [49]. The higher concentration of insulin in detrained subject might be responsible for lower peripheral lipases activity to break TGs into NEFAS and facilitated fatty acid uptake by tissues, thus reducing lipolysis and consequently promoting lipid biogenesis in adipose tissue [50, 51]. It is known that the high rates of FFA uptake and release are accompanied by higher clearance rate and exercise would increase the FFA release by reducing insulin activity and increasing catecholamine availability [52]. Plasma NEFA concentration are inversely related to the insulin concentration in healthy humans [53]. Recently a study by Jelic and al. [53] proposed that insulin has an important control on increased uptake and reesterfication of NEFA into adipose tissue and eventual stimulatory effect on adipose tissue lipoprotein lipases (LPL) notably in postprandial conditions, however, the effect on LPL is activated with several hours delay. Jelic *et al.* proposed that there is a direct effect between NEFA flow and the rate of NEFA oxidation flux and non oxidative NEFA flux to the liver, while Campbell *et al.* [54] detected no differences between plasma NEFA and total fat oxidation. Campbell *et al.* concluded that insulin regulates FFA rate of appearance by inhibition of lipolysis while maintaining a constant rate of primary FFA reesterification [54].

However, insulin is also known to participate in FAT/CD36 translocation from cytoplasm to the membrane [55-57]. Thus lower insulin sensitivity might lead to a lower flux of fatty acid to the myocytes even if the FAT/CD36 expression remained at its basal values in the detrained group. In addition another regulator of FAT/CD36 translocation during muscle contraction is the AMPK [58]. The lower expression of PRKAA2 (catalytic subunit of AMPK) we found after detraining might affect the translocation of FAT/CD36 since the variation in FAT/CD36 correlated with TG and NEFA concentrations. Our results are in line with other studies showing that cessation of regular training would lead to an increase basal and postprandial insulin , triglycerides, and NEFA concentrations [4, 59].

The type of fatty acids does not influence the intestinal absorption of dietary fat [60], however some studies differentiated between SFA and MUFA packaging in chylomicron [61]. In our study, inactivity increased the plasmatic concentration of both dietary fatty acids in chylomicrons, which suggests that physical activity level effects on the packaging of fatty acids is independent of their type. In the line of the increased appearance rate of dietary fatty acids in chylomicrons after detraining, Mankowitz *et al.*, without distinguishing type of fatty acid, showed that normolipidemic, male runners who ran 30-40 miles/week after 14-22 days of detraining, increased their chylomircon by 41% [62]. Moreover, training did not affect exogenous fatty acid appearance rate in chylomicrons. In this line, no effect was found of prior exercise on postprandial chylomicron concentration [63].

After ingestion of exogenous MUFA and SFA, most differences appeared within the first four hours after ingestion. Although total TG concentrations were not affected by physical activity modulation, detraining increased d_{31}-palmitate-chylomicron and ^{13}C-oleate-chylomicron while only VLDL-palmitate increased after detraining; this might be due to an increased NEFA-palmitate availability to the liver. In addition both palmitate-NEFA and oleate-NEFA were cleared much faster after training while the reverse pattern occurred in the detrained subjects. Higher expression of FAT/CD36, SPTLC2, PGC-1α, DES1 expression in the skeletal muscle of trained sedentary might explain the improvement of clearance. Up regulation of FAT/CD36 expression would specify a better fatty acid transport and higher expression of DES1 and SPTLC2 might indicate a better utilization and synthesis of IMTG. In addition, higher expression of PGC1-α which is known to promote mitochondrial biogenesis and β-oxidation implies elevated skeletal muscle fat oxidation capacity. It seems that daily moderate training for 2 months mainly enhance MUFA oxidation and slightly SFA and this was mainly due to a better uptake of dietary NEFA and improvement of fatty acids oxidation machinery at skeletal muscle level. Endurance exercise training is known to increase the protein expression and activity of factors associated with mitochondria and β oxidation [64] such as PGC-1α, PPAR-β, FAT/CD36 in muscle [65-67]. This suggestion could also be valid by lower insulin sensitivity since insulin has been suggested to regulate FAT/CD36 translocation in myocytes [68]. Moreover, we can suggest that since the fatty acid flux is reduced to enter the detrained skeletal muscle, this might induce a less expression of mtGPAT enzyme located in mitochondria outer membrane which participates in IMTG synthesis [43]. Other studies showed that endurance training increases the expression of SREBP-1 and mtGPAT [69] but we did not detect any change for those factors in trained subject. On the other, both of SREBP-1 and mtGPAT expression were down regulated in the skeletal muscle of detrained subjects suggesting a lower fatty acid turnover [70, 71]. Also, the lower expression of catalytic subunit (PRKAA2) of AMPK we found in the detrained group, consistent with other studies in detrained skeletal muscle, would influence the reduction of dietary fatty acid oxidation and regulation of FAT/CD36 translocation [72, 73]. Fu *et al.* showed that women have higher lipid oxidation capacity during endurance exercise and the authors proposed that this high capacity was due to a higher expression of mtGPAT, CPT-1, SREBP-1 [69]. This role might be true for DES1 and SPTLC2 all participating in lipid synthesis in association of IMTG formation. The increased rate of lipid oxidation within the muscle with training indicates the improvement of lipid turnover in trained sedentary subjects. Altogether, fatty acid transport system plays a crucial role to regulate fatty acid clearance by peripheral tissue. Moderate endurance training increased gene involved in fat metabolism, specifically, mitochondrial biogenesis, transcriptional regulation, cytosolic and mitochondrial fatty acid transport and β-oxidation. Detraining decreased gene involved in the synthesis of intramuscular lipid and oxidative and transport capacity as regulated by AMPK.

In our study, under detraining conditions, both exogenous palmitate and oleate oxidation were deteriorated. Extreme physical inactivity in previous bed rest studies reduced the oxidation of palmitate but not oleate, and this was in accordance with lower fat oxidation [24, 25]. The authors of these studies concluded that while saturated and monounsaturated fats have similar plasma trafficking and clearance, physical inactivity affects the partitioning of saturated fats towards storage, likely leading to an accumulation of palmitate in muscle fat. This reduction in total and exogenous fat oxidation was attributed partly to a lower expression of genes involved in mitochondrial biogenesis and lipid transport FAT/CD36, CPT-1 and

PGC-1α in the skeletal muscle of BR subjects and higher interaction of palmitate with these factors [24, 25]. the difference between our results and Bergouignan *et al.* [24, 25] might be due to the metabolic alterations induced by extreme physical activity such as muscle atrophy and changes in muscle fibers type, which did not occur within one month of detraining. Indeed, with extreme physical activity, there is a shift in the muscle fiber types characterized by a decrease in the oxidative twitch fibers (myosin heavy chain [MHC]-I and MHCIIa) and an increase in the glycolytic twitch fibers (MHCIIx) [74, 75]. Such a fiber type pattern with a higher proportion of glycolytic fibers in skeletal muscle was observed in obese and diabetic subjects [76]. It might be that monounsaturated fatty acid are more oxidized in glycolytic fibers than saturated fatty acid, which compensate the decrease of its oxidation in oxidative fibers, when shift into more glycolytic fibers occur with detraining. Since one month of detraining does not induce such alterations in muscle fiber type, the decrease in oleate oxidation would be concomitant to the reduction in palmitate oxidation.

Altogether, it seems that monounsaturated fatty acid oxidation would be more affected by training while saturated fatty acid oxidation is affected by detraining. Oleate oxidation in active baseline was significantly higher than sedentary baseline. This pattern was reversed by training for oleate oxidation in the trained sedentary group approached the oxidation rate of active subjects' baselines. A study by Votruba *et al.* showed that endurance exercise (120 min, 25% VO_2 max) in sedentary individual prior to food ingestion, was able to increase exogenous oleate oxidation during exercise however, no change observed for [d_{31}-palmitate] [21]. In addition, they showed that the exercise-induced increase in monounsaturated fat oxidation rather than saturated fat oxidation occurs regardless of exercise intensity [77].

mtGPAT and PGC-1α were the only mRNA expression showing significant correlation with oxidation of dietary fatty acids. mtGPAT correlated significantly with oleate oxidation while PGC-1α with palmitate oxidation. A study by Coll *et al.*, after treating the myotubes by palmitate and oleate proposed that palmitate reduced PGC1-α expression but not oleate [78]. mtGPAT has a 3 fold to 10 fold higher activity with palmitoyl –CoA than oleyl [79]. Further studies investigating the differential effect of fatty acid type with mtGPAT are warranted. The negative correlation of mtGPAT with the variation of total NEFA might indicate that the lower flux of NEFA to the cell (thus higher concentration in the plasma) decreased the expression of mtGPAT.

In conclusion, we showed that beyond any differential effect between the effects of modulation in physical activity according to the type of dietary fatty acid, both monounsaturated and saturated fatty acids would be modified with modulation of physical activity as shown by the positive relationship of both of these fats with physical activity level. Nevertheless, it seems that saturated fatty acids are more affected by detraining. While data demonstrating a causative role of physical activity in the pathophysiology of obesity remains equivocal, our results provide key data on the current debate about the role of physical activity in the treatment of obesity and metabolic disorders associated with physical inactivity, demonstrating a positive relationship between the amount of energy expended during activities and fate of dietary lipids.

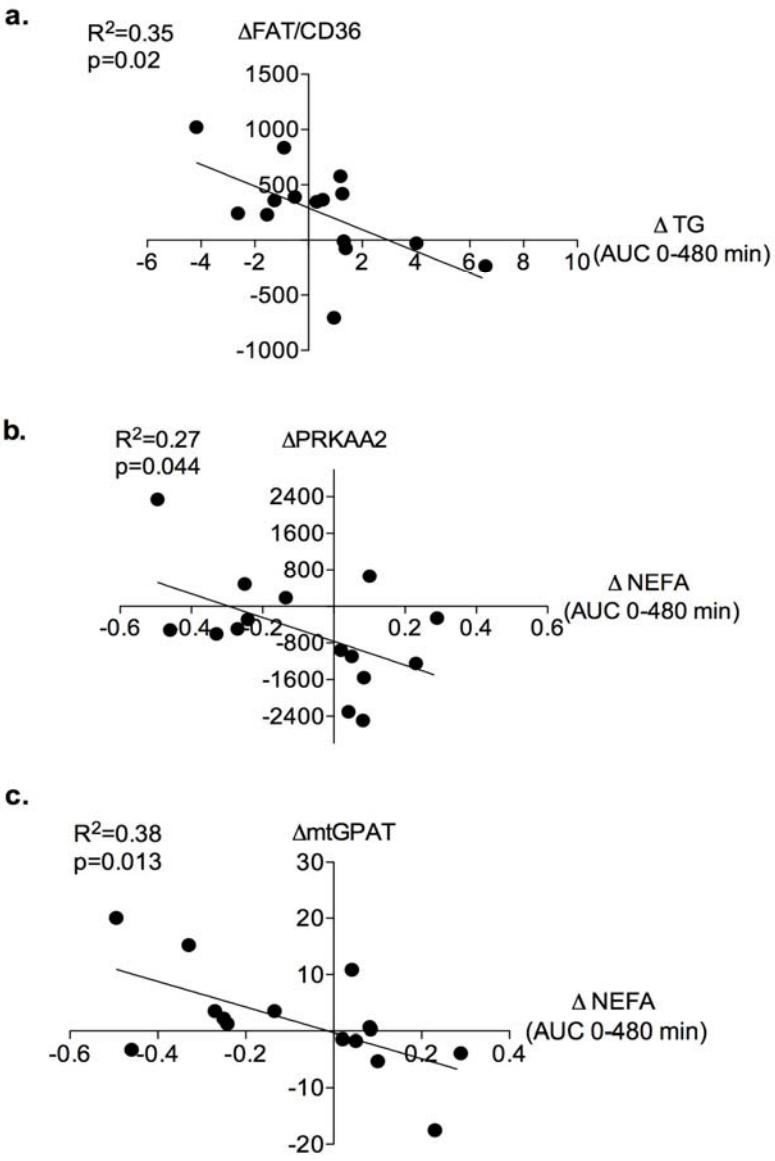


Figure 8: Correlations between *delta* FAT/CD36 *with delta* total triglycerides TG (a), *delta* PRKAA2 with *delta* total non-esterified fatty acids NEFA (b), *delta* mtGPAT with *delta* total NEFA, (c).

6. REFERENCES

[1] Healy GN, Wijndaele K, Dunstan DW, *et al.* (2008) Objectively measured sedentary time, physical activity, and metabolic risk: the Australian Diabetes, Obesity and Lifestyle Study (AusDiab). Diabetes care 31: 369-371

[2] Hamilton MT, Hamilton DG, Zderic TW (2007) Role of low energy expenditure and sitting in obesity, metabolic syndrome, type 2 diabetes, and cardiovascular disease. Diabetes 56: 2655-2667

[3] Bittner V, Johnson BD, Zineh I, *et al.* (2009) The triglyceride/high-density lipoprotein cholesterol ratio predicts all-cause mortality in women with suspected myocardial ischemia: a report from the Women's Ischemia Syndrome Evaluation (WISE). American heart journal 157: 548-555

[4] Chen SY, Chen SM, Chang WH, *et al.* (2006) Effect of 2-month detraining on body composition and insulin sensitivity in young female dancers. International journal of obesity (2005) 30: 40-44

[5] Smith SR, de Jonge L, Zachwieja JJ, *et al.* (2000) Concurrent physical activity increases fat oxidation during the shift to a high-fat diet. The American journal of clinical nutrition 72: 131-138

[6] Hansen KC, Zhang Z, Gomez T, Adams AK, Schoeller DA (2007) Exercise increases the proportion of fat utilization during short-term consumption of a high-fat diet. The American journal of clinical nutrition 85: 109-116

[7] Mayer J, Marshall NB, Vitale JJ, Christensen JH, Mashayekhi MB, Stare FJ (1954) Exercise, food intake and body weight in normal rats and genetically obese adult mice. The American journal of physiology 177: 544-548

[8] Weinsier RL, Hunter GR, Desmond RA, Byrne NM, Zuckerman PA, Darnell BE (2002) Free-living activity energy expenditure in women successful and unsuccessful at maintaining a normal body weight. The American journal of clinical nutrition 75: 499-504

[9] Jequier E, Tappy L (1999) Regulation of body weight in humans. Physiological reviews 79: 451-480

[10] Flatt JP (1987) The difference in the storage capacities for carbohydrate and for fat, and its implications in the regulation of body weight. Annals of the New York Academy of Sciences 499: 104-123

[11] Schrauwen P, Lichtenbelt WD, Saris WH, Westerterp KR (1998) Fat balance in obese subjects: role of glycogen stores. The American journal of physiology 274: E1027-1033

[12] Flatt JP (1995) Body composition, respiratory quotient, and weight maintenance. The American journal of clinical nutrition 62: 1107S-1117S

[13] Schrauwen P, van Marken Lichtenbelt WD, Westerterp KR (1997) Energy balance in a respiration chamber: individual adjustment of energy intake to energy expenditure. Int J Obes Relat Metab Disord 21: 769-774

[14] Colberg SR, Simoneau JA, Thaete FL, Kelley DE (1995) Skeletal muscle utilization of free fatty acids in women with visceral obesity. The Journal of clinical investigation 95: 1846-1853

[15] Galgani JE, Heilbronn LK, Azuma K, *et al.* (2008) Metabolic flexibility in response to glucose is not impaired in people with type 2 diabetes after controlling for glucose disposal rate. Diabetes 57: 841-845

[16] Kelley DE (2002) Skeletal muscle triglycerides: an aspect of regional adiposity and insulin resistance. Annals of the New York Academy of Sciences 967: 135-145

[17] Achten J, Jeukendrup AE (2004) Optimizing fat oxidation through exercise and diet. Nutrition (Burbank, Los Angeles County, Calif 20: 716-727

[18] Kanaley JA, Mottram CD, Scanlon PD, Jensen MD (1995) Fatty acid kinetic responses to running above or below lactate threshold. J Appl Physiol 79: 439-447

[19] Horowitz JF, Klein S (2000) Lipid metabolism during endurance exercise. The American journal of clinical nutrition 72: 558S-563S

[20] Jeukendrup AE (2002) Regulation of fat metabolism in skeletal muscle. Annals of the New York Academy of Sciences 967: 217-235

[21] Votruba SB, Atkinson RL, Hirvonen MD, Schoeller DA (2002) Prior exercise increases subsequent utilization of dietary fat. Medicine and science in sports and exercise 34: 1757-1765

[22] Astrup A (2001) The role of dietary fat in the prevention and treatment of obesity. Efficacy and safety of low-fat diets. Int J Obes Relat Metab Disord 25 Suppl 1: S46-50

[23] Smorawinski J, Kaciuba-Uscilko H, Nazar K, *et al.* (2000) Effects of three-day bed rest on metabolic, hormonal and circulatory responses to an oral glucose load in endurance or strength trained athletes and untrained subjects. J Physiol Pharmacol 51: 279-289

[24] Bergouignan A, Schoeller DA, Normand S, *et al.* (2006) Effect of physical inactivity on the oxidation of saturated and monounsaturated dietary Fatty acids: results of a randomized trial. PLoS clinical trials 1: e27

[25] Bergouignan A, Trudel G, Simon C, et al. (2009) Physical inactivity differentially alters dietary oleate and palmitate trafficking. Diabetes 58: 367-376

[26] Shangraw RE, Stuart CA, Prince MJ, Peters EJ, Wolfe RR (1988) Insulin responsiveness of protein metabolism in vivo following bedrest in humans. The American journal of physiology 255: E548-558
[27] Stuart CA, Shangraw RE, Prince MJ, Peters EJ, Wolfe RR (1988) Bed-rest-induced insulin resistance occurs primarily in muscle. Metabolism: clinical and experimental 37: 802-806
[28] Rimbert V, Boirie Y, Bedu M, Hocquette JF, Ritz P, Morio B (2004) Muscle fat oxidative capacity is not impaired by age but by physical inactivity: association with insulin sensitivity. Faseb J 18: 737-739
[29] Schoeller DA, Ravussin E, Schutz Y, Acheson KJ, Baertschi P, Jequier E (1986) Energy expenditure by doubly labeled water: validation in humans and proposed calculation. The American journal of physiology 250: R823-830
[30] Blanc S, Colligan AS, Trabulsi J, *et al.* (2002) Influence of delayed isotopic equilibration in urine on the accuracy of the (2)H(2)(18)O method in the elderly. J Appl Physiol 92: 1036-1044
[31] Schoeller DA, Colligan AS, Shriver T, Avak H, Bartok-Olson C (2000) Use of an automated chromium reduction system for hydrogen isotope ratio analysis of physiological fluids applied to doubly labeled water analysis. J Mass Spectrom 35: 1128-1132
[32] Schoeller DA (1984) Use of two-point sampling for the doubly-labelled water method. Human nutrition 38: 477-480
[33] Wang Z, Deurenberg P, Wang W, Pietrobelli A, Baumgartner RN, Heymsfield SB (1999) Hydration of fat-free body mass: review and critique of a classic body-composition constant. The American journal of clinical nutrition 69: 833-841
[34] Bergouignan A, Schoeller DA, Votruba S, Simon C, Blanc S (2008) The acetate recovery factor to correct tracer-derived dietary fat oxidation in humans. Am J Physiol Endocrinol Metab 294: E645-653
[35] Votruba SB, Zeddun SM, Schoeller DA (2001) Validation of deuterium labeled fatty acids for the measurement of dietary fat oxidation: a method for measuring fat-oxidation in free-living subjects. Int J Obes Relat Metab Disord 25: 1240-1245
[36] Sperry WM, Brand FC (1955) The determination of total lipides in blood serum. The Journal of biological chemistry 213: 69-76
[37] Burdge GC, Wright P, Jones AE, Wootton SA (2000) A method for separation of phosphatidylcholine, triacylglycerol, non-esterified fatty acids and cholesterol esters from plasma by solid-phase extraction. The British journal of nutrition 84: 781-787
[38] Chung BH, Segrest JP, Ray MJ, *et al.* (1986) Single vertical spin density gradient ultracentrifugation. Methods in enzymology 128: 181-209
[39] Fielding BA, Callow J, Owen RM, Samra JS, Matthews DR, Frayn KN (1996) Postprandial lipemia: the origin of an early peak studied by specific dietary fatty acid intake during sequential meals. The American journal of clinical nutrition 63: 36-41
[40] Evans K, Burdge GC, Wootton SA, Clark ML, Frayn KN (2002) Regulation of dietary fatty acid entrapment in subcutaneous adipose tissue and skeletal muscle. Diabetes 51: 2684-2690
[41] Van Etten LM, Westerterp KR, Verstappen FT, Boon BJ, Saris WH (1997) Effect of an 18-wk weight-training program on energy expenditure and physical activity. J Appl Physiol 82: 298-304
[42] Stubbs RJ, Ritz P, Coward WA, Prentice AM (1995) Covert manipulation of the ratio of dietary fat to carbohydrate and energy density: effect on food intake and energy balance in free-living men eating ad libitum. The American journal of clinical nutrition 62: 330-337
[43] Schenk S, Horowitz JF (2007) Acute exercise increases triglyceride synthesis in skeletal muscle and prevents fatty acid-induced insulin resistance. The Journal of clinical investigation 117: 1690-1698
[44] Gill JM, Hardman AE (2003) Exercise and postprandial lipid metabolism: an update on potential mechanisms and interactions with high-carbohydrate diets (review). The Journal of nutritional biochemistry 14: 122-132
[45] Kelley DE, Goodpaster B, Wing RR, Simoneau JA (1999) Skeletal muscle fatty acid metabolism in association with insulin resistance, obesity, and weight loss. The American journal of physiology 277: E1130-1141
[46] Dunstan DW, Salmon J, Healy GN, *et al.* (2007) Association of television viewing with fasting and 2-h postchallenge plasma glucose levels in adults without diagnosed diabetes. Diabetes care 30: 516-522
[47] Svanfeldt M, Thorell A, Brismar K, Nygren J, Ljungqvist O (2003) Effects of 3 days of "postoperative" low caloric feeding with or without bed rest on insulin sensitivity in healthy subjects. Clinical nutrition (Edinburgh, Scotland) 22: 31-38
[48] Blanc S, Normand S, Pachiaudi C, Fortrat JO, Laville M, Gharib C (2000) Fuel homeostasis during physical inactivity induced by bed rest. The Journal of clinical endocrinology and metabolism 85: 2223-2233
[49] Guillet-Deniau I, Mieulet V, Le Lay S, *et al.* (2002) Sterol regulatory element binding protein-1c expression and action in rat muscles: insulin-like effects on the control of glycolytic and lipogenic enzymes and UCP3 gene expression. Diabetes 51: 1722-1728
[50] Herd SL, Kiens B, Boobis LH, Hardman AE (2001) Moderate exercise, postprandial lipemia, and skeletal muscle lipoprotein lipase activity. Metabolism: clinical and experimental 50: 756-762

[51] Zderic TW, Hamilton MT (2006) Physical inactivity amplifies the sensitivity of skeletal muscle to the lipid-induced downregulation of lipoprotein lipase activity. J Appl Physiol 100: 249-257
[52] Jensen MD (2003) Fate of fatty acids at rest and during exercise: regulatory mechanisms. Acta physiologica Scandinavica 178: 385-390
[53] Jelic K, Hallgreen CE, Colding-Jorgensen M (2009) A model of NEFA dynamics with focus on the postprandial state. Annals of biomedical engineering 37: 1897-1909
[54] Campbell PJ, Carlson MG, Hill JO, Nurjhan N (1992) Regulation of free fatty acid metabolism by insulin in humans: role of lipolysis and reesterification. Am J Physiol 263: E1063-1069
[55] van Oort MM, van Doorn JM, Hasnaoui ME, *et al.* (2009) Effects of AMPK activators on the sub-cellular distribution of fatty acid transporters CD36 and FABPpm. Archives of physiology and biochemistry 115: 137-146
[56] Chabowski A, Baranczuk E, Gorski J (2005) Effect of adrenalin, insulin and contractions on the content of the free fatty acid fraction in skeletal muscle. J Physiol Pharmacol 56: 381-390
[57] Chabowski A, Coort SL, Calles-Escandon J, *et al.* (2005) The subcellular compartmentation of fatty acid transporters is regulated differently by insulin and by AICAR. FEBS letters 579: 2428-2432
[58] Bonen A, Luiken JJ, Arumugam Y, Glatz JF, Tandon NN (2000) Acute regulation of fatty acid uptake involves the cellular redistribution of fatty acid translocase. The Journal of biological chemistry 275: 14501-14508
[59] Stubbs RJ, Hughes DA, Johnstone AM, Horgan GW, King N, Blundell JE (2004) A decrease in physical activity affects appetite, energy, and nutrient balance in lean men feeding ad libitum. The American journal of clinical nutrition 79: 62-69
[60] Bergouignan A, Momken I, Schoeller DA, Simon C, Blanc S (2009) Metabolic fate of saturated and monounsaturated dietary fats: The Mediterranean diet revisited from epidemiological evidence to cellular mechanisms. Progress in lipid research
[61] Ockner RK, Manning JA (1974) Fatty acid-binding protein in small intestine. Identification, isolation, and evidence for its role in cellular fatty acid transport. The Journal of clinical investigation 54: 326-338
[62] Mankowitz K, Seip R, Semenkovich CF, Daugherty A, Schonfeld G (1992) Short-term interruption of training affects both fasting and post-prandial lipoproteins. Atherosclerosis 95: 181-189
[63] James AP, Slivkoff-Clark K, Mamo JC (2007) Prior exercise does not affect chylomicron particle number following a mixed meal of moderate fat content. Lipids in health and disease 6: 8
[64] Coyle EF, Martin WH, 3rd, Bloomfield SA, Lowry OH, Holloszy JO (1985) Effects of detraining on responses to submaximal exercise. J Appl Physiol 59: 853-859
[65] Talanian JL, Holloway GP, Snook LA, Heigenhauser GJ, Bonen A, Spriet LL Exercise training increases sarcolemmal and mitochondrial fatty acid transport proteins in human skeletal muscle. American journal of physiology 299: E180-188
[66] Baar K, Wende AR, Jones TE, *et al.* (2002) Adaptations of skeletal muscle to exercise: rapid increase in the transcriptional coactivator PGC-1. Faseb J 16: 1879-1886
[67] Wang L, Psilander N, Tonkonogi M, Ding S, Sahlin K (2009) Similar expression of oxidative genes after interval and continuous exercise. Medicine and science in sports and exercise 41: 2136-2144
[68] Chabowski A, Coort SL, Calles-Escandon J, *et al.* (2004) Insulin stimulates fatty acid transport by regulating expression of FAT/CD36 but not FABPpm. American journal of physiology 287: E781-789
[69] Fu MH, Maher AC, Hamadeh MJ, Ye C, Tarnopolsky MA (2009) Exercise, sex, menstrual cycle phase, and 17beta-estradiol influence metabolism-related genes in human skeletal muscle. Physiological genomics 40: 34-47
[70] Wendel AA, Lewin TM, Coleman RA (2009) Glycerol-3-phosphate acyltransferases: rate limiting enzymes of triacylglycerol biosynthesis. Biochimica et biophysica acta 1791: 501-506
[71] Clark SA, Chen ZP, Murphy KT, *et al.* (2004) Intensified exercise training does not alter AMPK signaling in human skeletal muscle. American journal of physiology 286: E737-743
[72] McGee SL, Howlett KF, Starkie RL, Cameron-Smith D, Kemp BE, Hargreaves M (2003) Exercise increases nuclear AMPK alpha2 in human skeletal muscle. Diabetes 52: 926-928
[73] Dzamko N, Schertzer JD, Ryall JG, et al. (2008) AMPK-independent pathways regulate skeletal muscle fatty acid oxidation. The Journal of physiology 586: 5819-5831
[74] Trappe S, Creer A, Minchev K, *et al.* (2008) Human soleus single muscle fiber function with exercise or nutrition countermeasures during 60 days of bed rest. Am J Physiol Regul Integr Comp Physiol 294: R939-947
[75] Salanova M, Schiffl G, Puttmann B, Schoser BG, Blottner D (2008) Molecular biomarkers monitoring human skeletal muscle fibres and microvasculature following long-term bed rest with and without countermeasures. Journal of anatomy 212: 306-318
[76] Tanner CJ, Barakat HA, Dohm GL, *et al.* (2002) Muscle fiber type is associated with obesity and weight loss. American journal of physiology 282: E1191-1196
[77] Votruba SB, Atkinson RL, Schoeller DA (2003) Prior exercise increases dietary oleate, but not palmitate oxidation. Obesity research 11: 1509-1518
[78] Coll T, Jove M, Rodriguez-Calvo R, *et al.* (2006) Palmitate-mediated downregulation of peroxisome proliferator-activated receptor-gamma coactivator 1alpha in skeletal muscle cells involves MEK1/2 and nuclear factor-kappaB activation. Diabetes 55: 2779-2787

[79] Coleman RA, Lee DP (2004) Enzymes of triacylglycerol synthesis and their regulation. Progress in lipid research 43: 134-176

CHAPITRE 5

Effects of two-month training at current recommendation on the trafficking of dietary fat

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Résumé

Introduction

L'obésité est une maladie du stockage des lipides caractérisée par une incapacité à utiliser les lipides alimentaires comme substrat énergétique et un acheminement préférentiel des lipides dans le tissu adipeux pour y être stockées plutôt que vers le muscle et oxydées. Les anomalies de l'utilisation des acides gras de l'obèse sont retrouvées après perte de poids, indiquant qu'elles pourraient être primitives et participer à la constitution de l'excès de poids. Bien que l'oxydation lipidique soit une caractéristique héréditaire, ceci n'implique pas que les gènes jouent un rôle déterminant indépendamment des facteurs environnementaux, telle que l'adoption d'un mode de vie sédentaire et/ou une alimentation dense. La compréhension des facteurs qui sous-tendent ces anomalies liées à l'oxydation des lipides constitue un pré-requis indispensable à la mise au point de stratégies de prévention ou thérapeutiques. L'activité physique, en tant qu'élément le plus modulable de la dépense énergétique totale, pourrait jouer un rôle clef dans le devenir des lipides. Toutefois, hormis les données existantes sur les effets aigus de l'activité physique sur la lipémie, nous disposons de peu de données concernant l'effet chronique de l'activité physique sur l'acheminement et l'oxydation des lipides alimentaires est encore à élucider.

Objectifs

Notre objectif était de déterminer l'effet d'un entraînement physique sur l'acheminement et l'oxydation des graisses alimentaires chez des sujets sédentaires normopondérés et en surpoids. Nous avons émis l'hypothèse que l'effet chronique d'une activité physique modérée, indépendamment de son effet sur la balance énergétique et au-delà des effets aigus de l'exercice, sera favorable à un acheminement préférentiel des acides gras vers l'oxydation au lieu d'etre stockés, grâce à une augmentation de l'expression de gènes impliqués dans le métabolisme des lipides, et en partie permettra de corriger les déficiences dans l'oxydation des lipides observés chez des sujets en surpoids. Notre objectif secondaire était de déterminer si une augmentation de l'activité physique affecte différentiellement la dépense énergétique liée à l'activité physique entre des sujets différant en leur poids.

Matériels et Méthodes

10 hommes sédentaires normopondérés et 10 hommes sédentaires en surpoids ont effectué un entraînement aérobic sur un cyclo-ergomètre de 60 minutes quatre fois par semaine pendant deux mois à 50% VO_2max. [d_{31}] palmitate et [1-^{13}C]oléate ont été donnés par un repas sous forme liquide. La dépense énergétique totale a été mesurée par l'eau doublement marquée avant et à la fin de l'intervention. L'oxydation totale des lipides a été mesurée par la calorimétrie indirecte.

Résultats

L'entraînement physique a augmenté la dépense énergétique totale chez les sujets normopondérés de 16% ($p = 0,005$) mais pas chez les sujets en surpoids ($p = 0,20$) probablement en raison de comportements compensatoires réduisant les activités quotidiennes spontanées. Tandis que l'oxydation lipidique à jeun a augmenté uniquement chez les sujets en surpoids (36%, $p = 0,04$), l'oxydation postprandiale totale des lipides et l'oxydation exogène des acides gras saturés n'ont pas été affectées par l'intervention sur l'activité physique dans les deux groupes. L'oxydation des acides gras monoinsaturés a augmenté de 15% chez les sujets normopondérés ($p = 0,049$). Toutefois, le niveau d'activité physique a expliqué l'oxydation des deux acides gras alimentaires chez les sujets normopondérés, mais pas chez les sujets en surpoids L'entraînement a augmenté l'expression des gènes responsables du transport des acides gras CD36 et CPT-1, et le gènes impliqués dans l'oxydation des lipides PGC-α chez les deux groupes.

Discussion

Nos résultats montrent que la modulation de l'activité physique prédit l'oxydation des lipides exogènes chez les sujets normopondérés. Cette constatation est d'une importance particulière car elle suggère qu'une amélioration de l'oxydation des acides gras exogènes peut être obtenue avec une augmentation additionnelle du niveau physique telle qu'une plus longue durée ou une fréquence supplémentaire de l'exercice. Indépendamment de l'état d'obésité, seul un entraînement physique qui augmente de façon significative la dépense énergétique, est capable de contrebalancer les effets de la sédentarité sur le devenir des lipides alimentaires et sur les mécanismes cellulaires sous-jacents. En prenant en compte les mécanismes compensatoires des sujets en surpoids face à l'entraînement, toute augmentation de la dépense énergétique liée à l'activité AEE, que ce soit en dépense énergétique liée à des exercices structurés ou/et en activités spontanées, est bénéfique au niveau du métabolisme lipidique. En d'autres termes, les personnes sédentaires peuvent accroître leur oxydation lipidique en augmentant toute sorte d'activité, sans contrainte des programmes d'activité physique imposée. Cependant, nous ne pouvons pas exclure l'importance du rôle de l'exercice structuré en soi et des investigations complémentaires sont nécessaires pour déterminer l'effet de l'exercice structuré et/ ou activité spontanée. Les recommandations actuelles n'ayant pas permis d'atteindre des changements significatifs dans l'oxydation des lipides totaux, la question cruciale de déterminer quel volume d'activité physique serait suffisant pour susciter de véritables améliorations dans le métabolisme des lipides exogènes, reste ouverte. Des études supplémentaires pour déterminer ce volume sont primordiales dans le contexte de nouvelles implications thérapeutiques dans la prévention de la prise de poids. Compte tenu des effets contrastés des activités dites spontanées versus structurées, il semble essentiel que ces études prennent en compte le milieu socio-écologique dans lequel les populations à risques évoluent.

1. ABSTRACT

Objective: Obesity is the consequence of chronic fat imbalance with fat partitioning in favor of storage rather than oxidation. Exercise is known to be beneficial in preventing weight gain. However, the effect of chronic training on dietary lipid trafficking is yet to be cleared. We tested whether two months training at current guidelines would increase dietary fat oxidation in sedentary lean and overweight subjects.

Research design and methods: Sedentary 10 lean and 10 overweight men performed an aerobic training on a cycle-ergometer for one hour four times per week during two months at 50%$VO_{2\ peak.}$ [d_{31}]palmitate and [1-^{13}C]oleate were given through a liquid meal. Total energy expenditure was measured by doubly labeled water before and at the end of the intervention. Total fat oxidation was measured by indirect calorimetry. Measurements and breath and urine sampling were performed at rest 36h after any bout of exercise over 8h following meal ingestion.

Results: Training increased total energy expenditure TEE in lean by 16% (p=0.005) but not in overweight subjects (p=0.20) probably due to compensatory behaviors. Fat free mass (2%, p=0.03), resting metabolic rate (4%, p=0.003) and fasting fat oxidation (36%, p=0.04) increased in overweight subjects. Postprandial total fat and exogenous [d_{31}]palmitate oxidation were not affected by training in both groups. [1-^{13}C]oleate oxidation increased by 15% in lean subjects (p=0.049). Physical activity level explained both dietary fatty acids oxidation in lean but not in overweight group (lean: [d_{31}]palmitate: p=0.02; [1-^{13}C]oleate: p=0.03; overweight: [d_{31}]palmitate: p=0.84; [1-^{13}C]oleate: p=0.96). Training increased CD36, PGC-α, CPT-1 gene expressions in both groups.

Conclusion: Moderate training independently of energy balance, increased fasting but not postprandial and exogenous fatty acid oxidation in overweight subjects. Monounsaturated fatty acid oxidation was improved in lean subjects while saturated fatty acid oxidation failed to reach statistical significance. Positive relationships of the physical activity level with both exogenous fat oxidations suggest that further improvements in fat oxidation can be achieved when adequately increasing total energy expenditure.

2. INTRODUCTION

Obesity is the consequence of a chronic disequilibrium in energy balance primarily due to a chronic fat imbalance (56). Although genetic factors determine predisposition to obesity (67), environmental factors, such as a high-fat diet and a sedentary lifestyle favor weight gain and likely explain obesity's increased prevalence in westernized societies (74). Since lipogenesis is negligible in humans, weight gain represents the consequence of altered fat partitioning between oxidation and storage, likely due to a preferential directing of dietary fat towards adipose tissue and away from muscle both in fasting (16) and postprandial (8) conditions. These abnormalities in the use of fatty acids observed in obese patients are supposed to be primary rather than an adaptive response to excess weight and may participate in the formation of the latter, because weight reduction was not associated with improvement in fat utilization (22). Consequently, any intervention that would affect dietary fat towards oxidation in muscle and decreases fat deposition in adipose tissue might be of interest in preventing and/or reducing weight gain.

Studies in animals and humans suggest that exercise plays an important role in preventing weight gain (72). While fat oxidation is known to increase slowly to match increased fat intake, increased daily energy expenditure, through the performance of exercise, has been reported to accelerate the adjustment of total and plasma fat oxidation to the proportion of fat ingested in both men (63) and women (27). Thirty minutes of walking, undertaken in one session or accumulated throughout a day, both increase in a similar extent postprandial fat oxidation (47). Chronic moderate intensity training protocols in sedentary lean (1) or obese individuals (25) resulted in increased total fat oxidation during exercise and in some cases also improved fasting fat oxidation (23).

So far, most of the studies focused on the effect of exercise training on total and plasma fatty acid oxidation (60) and only few studies such as Votruba *et al.* (71) investigated the effect of exercise on dietary fat oxidation. Indeed, Votruba *et al.* (71) reported that prior acute exercise at different level of intensity significantly increases the oxidation of dietary monounsaturated fatty acid (oleate). However, the effects of chronic exercise training, beyond the effect of acute exercise, on dietary fat trafficking and oxidation have not received much attention neither in lean nor in overweight subjects.

The current physical activity guideline for adults of 30 minutes of moderate intensity activity daily (28) is efficient for limiting health risks for several chronic diseases including coronary heart diseases and diabetes. However for preventing weight gain or regain, this guideline is likely to be insufficient (53). This might be related to an impaired effect of the current guidelines in improving dietary fat oxidation. Although definitive data are still lacking, it seems likely that moderate intensity activity of approximately 45 to 60 minutes per day, or 1.7 PAL is required to prevent the transition to overweight or obesity (53). Schoeller *et al.* (59) and Weinsier *et al.* (75) showed that prevention of weight regain in former obese individuals requires 60-90 minutes per day of moderate intensity activity or lesser amounts of vigorous intensity activity. If higher recommendations of physical activity are also required in overweight subjects remains to be answered.

We hypothesize that chronic moderate physical activity, independently of its effect on energy balance and beyond the acute effect of exercise, will favor a preferential trafficking of fatty acids towards oxidation and away from storage, through an increased expression of genes implicated in fat oxidation and storage, and will partly correct the impairments in fat oxidation observed in overweight subjects. Sedentary lean (n=10) and overweight (n=10) subjects underwent tests before and after two months of training at current recommendations to investigate dietary fat partitioning of the two main saturated (palmitate) and monounsaturated (oleate) dietary fatty acid in the human diet.

3. RESEARCH DESIGN AND METHODS

3.1. Subjects and inclusion criteria

Data are reported from the Strasbourg lipid oxidation study LIPOX which took place at the University Hospital of Strasbourg (France). The inclusion criteria were a body mass index (BMI) of 25≤ BMI ≤ 35 kg/m^2 and a BMI of 20 ≤ BMI ≤ 25 kg/m^2 for overweight (n=10) and lean (n=10) subjects respectively. Healthy volunteers, free of any chronic known diseases, were weight stable (± 3kg body wt) for at least 3 months before enrolment. Lean subjects had no first-degree family history of obesity nor type 2 diabetes while overweight subjects had at least one overweight or diabetic parent. Sedentary status at inclusion was determined by the MOSPA-Q questionnaire (48), a RT3 triaxial accelerometer (Stayhealthy, Monrovia, CA, USA) and a combined heart rate and motion sensor Actiheart (11, 19) (Cambridge Neurotechnology Ltd) both worn for 7 days in free-living conditions (51). RT3 and Actiheart was used to obtain an estimation of subjects' PALs defined as the ratio between total energy expenditure (TEE) and resting metabolic rate (RMR). Since accelerometers tend to underestimate the PAL, an estimated-PAL ≤1.5 was set to screen sedentary individuals. Additional criteria were the absence of participation to any structured exercise programs over the 12 months prior to the study. Informed, written consent was obtained from each subject. The study was approved by the Institutional Review Board of Alsace I (France).

3.2. Physical activity intervention and weight maintenance diet

Aerobic training at the level of current recommendations (28) consisted of four 60-min sessions per week at 50% $VO_{2\ peak}$ on a cycle ergometer during two months. The peak oxygen uptake V_{O2peak} was determined by means of incremental exercise test (20) to exhaustion performed in an upright position on an electronically braked cycle ergometer (Medifit 1000S, Belgium). Throughout the study, research dieticians followed the participants and the diet was regularly adjusted to maintain subjects in energy balance.

3.3. Dietary fatty acid oxidation protocols

Before and after interventions the participants underwent a series of clinical tests, during which dietary fat oxidation trafficking was assessed using a combination of stable isotope labeled fatty acids mixed in a standard breakfast, indirect calorimetry and hourly blood samples. Thirty-six hours before the test, all subjects were asked not to participate in any structured physical activities and were provided with standardized microwaveable meals calculated to match requirements. After an overnight fast, the subjects were weighed, and an intravenous catheter was inserted into the forearm vein for arterialized blood sampling. Baseline breath, urine and fasting blood samples were collected. RMR was measured for 1h by indirect calorimetry using a Deltatrac metabolic cart (Deltatrac II; GE). Then, standard breakfast covering 35% of the daily energy requirements (55% carbohydrate, 13% protein, 32% lipid) was provided to the participants, which included a homogenized liquid meal labeled with: 15 mg/kg [1-^{13}C]oleate (>99% enriched; Cambridge Isotope Laboratories (CIL), Andover, MA] and 20 mg/kg [d_{31}]palmitate (>98% enriched; CIL). Total substrate use and non protein respiratory quotient (NPRQ) were calculated hourly by indirect calorimetry CO_2 and O_2 measurements and nitrogen excretion. Insulin, glucose, NEFA, triglyceride, cholesterol were collected and measured as previously described (5). After 4h from the first meal ingestion, subjects received a lunch covering 18% of daily requirements (72% carbohydrate, 18% protein, 11% lipid). Following the first meal ingestion, hourly measurements and sample collections were conducted over 8h. *Vastus lateralis* biopsies as previously described (6) and adipose tissue biopsies extracted from needle liposuction, were obtained 8h after the labeled breakfast. Separate breath and urine samples were taken 24h after meal ingestion.

3.4. Body composition and total energy expenditure (TEE)

TEE was measured before and at the end training for a period of 10-d each time, using the doubly labeled water method based on routinely procedures used in our laboratories and described in details elsewhere (10). Subjects ingested a single oral bolus of 2g/kg TBW of doubly labeled water composed of 0.2g/kg of $H_2{}^{18}O$ (99.85% enriched; CIL, ANDOVER, MA) and 0.15g/kg of ^2H_2O (10%enriched; CIL, ANDOVER, MA). After baseline urine sample, equilibration time was taken at 3 and 4h postdose and then 3 subsequent urine samples were collected on days 3, 7 and 10 of the protocol. The mean daily CO_2 production was calculated according to Schoeller *et al.* (58) equation :

rCO2= 0·455N x (1·01 x ko - 1·04 x kh), where N is the TBW calculated from the isotope dilution spaces ((Do / 1·01 + Dh / 1·04) / 2) at the start of the observation period, corrected for the change over the observation period, where ko, Do, kh and Dh are elimination rates and dilution spaces for ^{18}O and ^2H respectively. TEE was subsequently calculated from the carbon dioxide production rate by the use of the standard formula of indirect calorimetry with the Weir equation (76) using an estimated food quotient of 0.86. PAL was calculated as the ratio of TEE over RMR measured for one hour using indirect calorimetry (Deltatrac II; GE) at rest after an overnight fast. Activity energy expenditure (AEE) was calculated as 90% TEE minus RMR; thus assuming a diet induced-thermogenesis of 10%. Body composition was assessed by hydrometry from the DLW-derived total body water (80). FFM was calculated from TBW assuming a hydration factor of 0.732. FM was calculated as the FFM difference form body weight.

3.5. Stable isotopes

The details on the isotope ratio mass spectrometry analyses can be found in previous studies from our laboratories (5, 73). Briefly, the ratio of $^{13}CO_2$ to $^{12}CO_2$ in breath was measured and analyzed in triplicates on a continuous-flow inlet system connected to an isoprime isotope ratio mass spectrometer (GV Instruments, Isotope Ration MS, Germany). [1-^{13}C]oleic oxidation was calculated as the instantaneous recovery of ^{13}C in expired CO_2 per hour and corrected for isotope sequestration by assuming an acetate correction factor of 51% for lean subjects (4), and 45% for overweight subjects, and were expressed as a percentage of the dose. $^{2}H/^{1}H$ from urine samples was analyzed, as previously described (57). Recoveries of [d_{31}]palmitic acid were calculated as the instantaneous recovery of ^{2}H in total body water per hour, expressed as a percentage of the dose, as previously described (3, 5).
^{18}O enrichements in TBW urine samples were decolorized by black carbon and reduced to CO by carbon reduction at 1,400°C in an elemental analyzer (Flash HT; ThermoFisher Germany) coupled to a Delta-V isotope ratio mass spectrometer in Strasbourg and isotopic abundances were measured in quintiplicate. All enrichments were expressed against International Atomic Energy Agency standards.

To determine dietary [d_{31}]palmitate and [1-13C]oleate trafficking, total lipids were extracted from plasma. NEFA fractions were separated by solid-phase extraction and derivatized to methyl esters (Burdge GC 2000). Total VLDL and total chylomicrons were extracted as prevouisly described (12) by ultracentrifugation at 80 000 rpm at 12°C (KENDRO S150, rotor S140AT) during 30min using gradient of 0.997 density. The supernatants of chylomicrons were removed and the volume was measured. The remaining precipitant density was increased by adding Kbr (Potassium brum) and then ultracentrifuged for 1h10 at 10°C at 140 000 rpm, to collect the VLDL. The absolute concentrations of the individual fatty acids were calculated by reference to internal standards. To assess both the isotopic enrichment and the individual fatty acid concentrations (both unlabeled and labeled) in the same gas chromatography/mass spectrometry (Agilent 5975 Inert XL), we designed a dual acquisition program in single-ion monitoring mode. The following m/z ratios were acquired: 296 and 297 for oleate and 270 and 301 for palmitate. The concentration of each labeled fatty acid was calculated by multiplying its molar percent enrichment (MPE) by the concentration of its corresponding unlabeled compound.

3.6. Real Time PCR (RT-QPCR)

Muscle biopsies were grounded in liquid nitrogen and total RNA was extracted using mirVana™ miRNA Isolation Kit (Applied Biosystems, Courtaboeuf, France). RNA concentration was measured with Nanodrop ND1000 (Labtech, Palaiseau, France) and integrity assessed using an Agilent 2100 Bioanalyzer (Agilent Technologies, Massy, France).First-strand cDNAs were synthesized from 500 ng of total RNA in the presence of 100 units of Superscript II (Invitrogen, Cergy Pontoise, France) using a mixture of random hexamers and oligo (dT) primers (Promega, Charbonnières, France). Real-time PCR assays were performed using a Rotor-GeneTM 6000 (Corbett Research, Mortlake, Australia) in a final volume of 20µl containing 5µl of a 60-fold dilution of the RT reaction medium, 10µl of reaction buffer from the Absolute QPCR SYBR Green ROX Mix (Abgene, Courtaboeuf, France) and 0.375µM of the specific forward and reverse primers. For quantification,

a standard curve was systematically generated with six different amounts of cDNA. Each assay was performed in duplicate, and validation of the real-time PCR runs was assessed by evaluation of the melting temperature of the products and by the slope and error obtained with the standard curve. Hypoxanthine phosphoribosyltransferase mRNA level was determined in each sample and was used as internal standard for normalization of target mRNA expression. The list of the PCR primers and the quantitative PCR assay conditions are available in the table below. The RT-QPCR was applied to detect the expression of Fatty acid translocase (FAT/CD36), carnitine palmitoyle transferase 1 (CPT-1), peroxisome proliferator-activated receptor coactivator (PGC-1α), peroxisome proliferator-activated receptor beta (PPAR-β), serine palmitoyltransferase, long chain base subunit 1 (SPTLC1), uncoupling protein 3 (UCP3), Sphingolipid *delta*(4)-desaturase DES1, and α2 isoform of the catalytic subunit of AMPK: PRKAA2.

3.7. Data organization and statistical analysis

The normality of the distribution of all parameters was tested using Shapiro-Wilk test. Paired t-tests were used to test the effect of training within group. Mixed models were used to test putative anthropological and physiological determinants of oleate and palmitate oxidations within group, with the given parameter as a covariable and time as a repeated effect and subject as random effect. Postprandial concentrations of parameters were calculated as the area under the curve over 8hrs after the first meal ingestion. All data are represented as mean ± SE, and the level of significance was set as $P < 0.05$. All statistics were performed using SAS Enterprise Guide 3.0 (SAS Institute Inc., Cary, NC, USA).

4. RESULTS

4.1. The impact of training on body composition and energy expenditure

Subjects' characteristics are shown in **table 1**. Both groups succeeded in maintaining their body weight stable as required in the protocol, however training modestly increased FFM in overweight group (1.2%, p=0.03). In lean subjects, training increased VO_2max (ml/kg/min) by 13.1%, (p=0.005), TEE by 15.8% (p=0.005), AEE normalized for body weight by 48.4% (p=0.004) and PAL by 14.3% (p=0.004). RMR increased in the overweight group by 4% (p= 0.003).

4.2. Fasting and postprandial plasmatic parameters

No changes in fasting and postprandial concentrations of glucose, NEFA, TG and fasting insulin were found in both groups (**Figure 1**). Postprandial insulin decreased in overweight subjects by 21% (p=0.002) but not in lean group (p=0.1).

4.3. Muscle gene expression of key lipid metabolism proteins (Figure 2)

Training increased CD36 (lean 35.9% p=0.009, overweight 57.95% p=0.02), CPT-1 (lean 19.78% p=0.003, overweight 54.28% p=0.008) and PGC-1α (lean 13.06% p=0.03, overweight 25.36% p=0.03) gene expressions. In addition, DES-1 and SPTLC2 gene expression were increased in the lean group (DES-1: 21.95% p=0.04; SPTLC2: 35.81% p=0.02) but not in the overweight group (DES-1: 25.18% p=0.09; SPTLC2: 26.16% p=0.66). SPTLC1 gene expression was increased in the overweight group by 24.93% (p=0.03) and tended to increase in the lean group by 17.66% (p=0.06). Training had no significant effect on PPAR-β, UCP-3, SREBP-1c, ACC2, mtGPAT and PRKAA2 gene expressions.

Table 1. Characteristics of the participants at baseline and after interventions on physical activity.

	sedentary lean men		sedentary overweight men	
	Baseline	After 2 months of training	Baseline	After 2 months of training
n	10	10	10	10
Age (years)	27.2±2.9		27.6±1.1	
Body mass (kg)	76.7±3.0 #	76.1±2.9	99.3±2.9	99.4±3.1
BMI (kg/m^2)	22.9±0.7 #	22.7±0.6	30.4±0.5	30.4±0.6
Fat mass				
kg	17.9±1.9 #	17.0±1.7	32.3±1.5	31.7±1.5
%	22.9±1.8 #	21.9±1.7	32.4±1.1	31.8±1.0
Fat free mass, kg	58.8±1.9 #	59.2±1.9	67.0±2.0	67.8±2.2 *
VO_{2peak}				
l/min	3.0±0.2 #	3.4±0.2 *	3.1±0.2	3.3±0.2
$ml.kg^{-1}.min^{-1}$	39.7±1.8 #	44.9±1.9 *	31.6±1.5	33.2±1.6
NPRQ	0.856±0.010 #	0.852±0.014	0.894±0.007	0.867±0.011
TEE MJ/d	10.7±0.5 #	12.3±0.7 *	14.1±0.7	14.5±0.5
RMR MJ/d	6.6±0.2 #	6.7±0.2	7.5±0.2	7.8±0.2 *
RMR_{adjFFM} MJ/d	7.0±0.1 #	7.1±0.1	7.2±0.2	7.4±0.2 *
AEE MJ/d	3.0±0.4 #	4.4±0.5 *	5.2±0.5	5.2±0.4
AEE (kJ/d/kg)	38.9±4.6 #	57.7±6.3 *	51.6±3.7	51.9±2.9
PAL	1.60±0.07 #	1.83±0.08 *	1.87±0.06	1.85±0.06
Exercise power W	129.2±5.6	159.1±8.9 *	124.5±7.24	169.0±11.1 *
Fasting Glucose (g/l)	0.88±0.03	0.87±0.03	0.89±0.02	0.91±0.02
Fasting Insulin (mU/l)	5.15±0.65 #	4.41±0.42	11.00±2.05	12.07±2.05
Fasting TG (g/l)	0.92±0.11	0.84±0.13	1.03±0.10	1.11±0.10
Fasting NEFA (mmol/l)	0.39±0.05	0.38±0.04	0.36±0.04	0.35±0.04

BMI, body mass index; VO_{2peak}, peak oxygen consumption; NPRQ, non protein respiratory quotient; TEE, total energy expenditure; RMR, resting metabolic rate; RMR_{adjFFM}, RMR adjusted by fat free mass; AEE, activity energy expenditure; PAL, Physical activity level; NEFA, non-esterified fatty acids; TG, triglycerides. * $p < 0.05$ exercise training *vs.* baseline within the same group; # lean *vs.* overweight at baseline.

4.4. Total substrate use

No changes in fasting and postprandial carbohydrate and protein oxidation, and postprandial fat oxidation were noted in both groups (postprandial fat oxidation lean: 2%, p=0.9; overweight: 25%, p = 0.5). Fasting fat oxidation increased in the overweight group by 35.8% (p=0.04) but not in the lean group (p=0.5). CPT-1 explained fasting fat oxidation in overweight group (p=0.03) but not in the lean group (p=0.86). Training increased oleate oxidation by 15% (p=0.049) but not palmitate oxidation (p=0.2) in the lean group. No effect of training on both exogenous fatty acids was noted in the overweight group ([1-^{13}C]oleate p=0.75; [d_{31}]palmitate p=0.51) (**Figure 3**). Activity energy expenditure and PAL explained both dietary fatty acids oxidation and TEE explained only [1-^{13}C]oleate oxidation in the lean group: [d_{31}]palmitate: PAL p=0.02, AEE kJ/d/kg p=0.01, TEE p=0.18; [1-^{13}C]oleate: PAL p = 0.03, AEE kJ/d/kg p=0.03, TEE p=0.03, while these relationships were not significant in the overweight group: [d_{31}]palmitate: PAL p=0.84, AEE kJ/d/kg p =0.87, TEE p=0.42; [1-^{13}C]oleate: PAL p=0.96, AEE kJ/d/kg p=0.93, TEE p=0.30 (**Figure 4**).

4.5. Exogenous [d_{31}]palmitate and [1-^{13}C]oleate trafficking (Figure 5)

After chronic training, no changes in the appearance rate of [d_{31}]palmitate and [1-^{13}C]oleate in chylomicrons and VLDL were found in both groups. Training decreased [1-^{13}C]oleate appearance rate in postprandial NEFA fractions (p=0.008) in the lean group. [1-^{13}C]oleate appearance rate in NEFA was explained by PRKAA2 gene expression (0.047). [d_{31}]palmitate appearance rate in NEFA also tended to decrease (p=0.08) in the lean group.

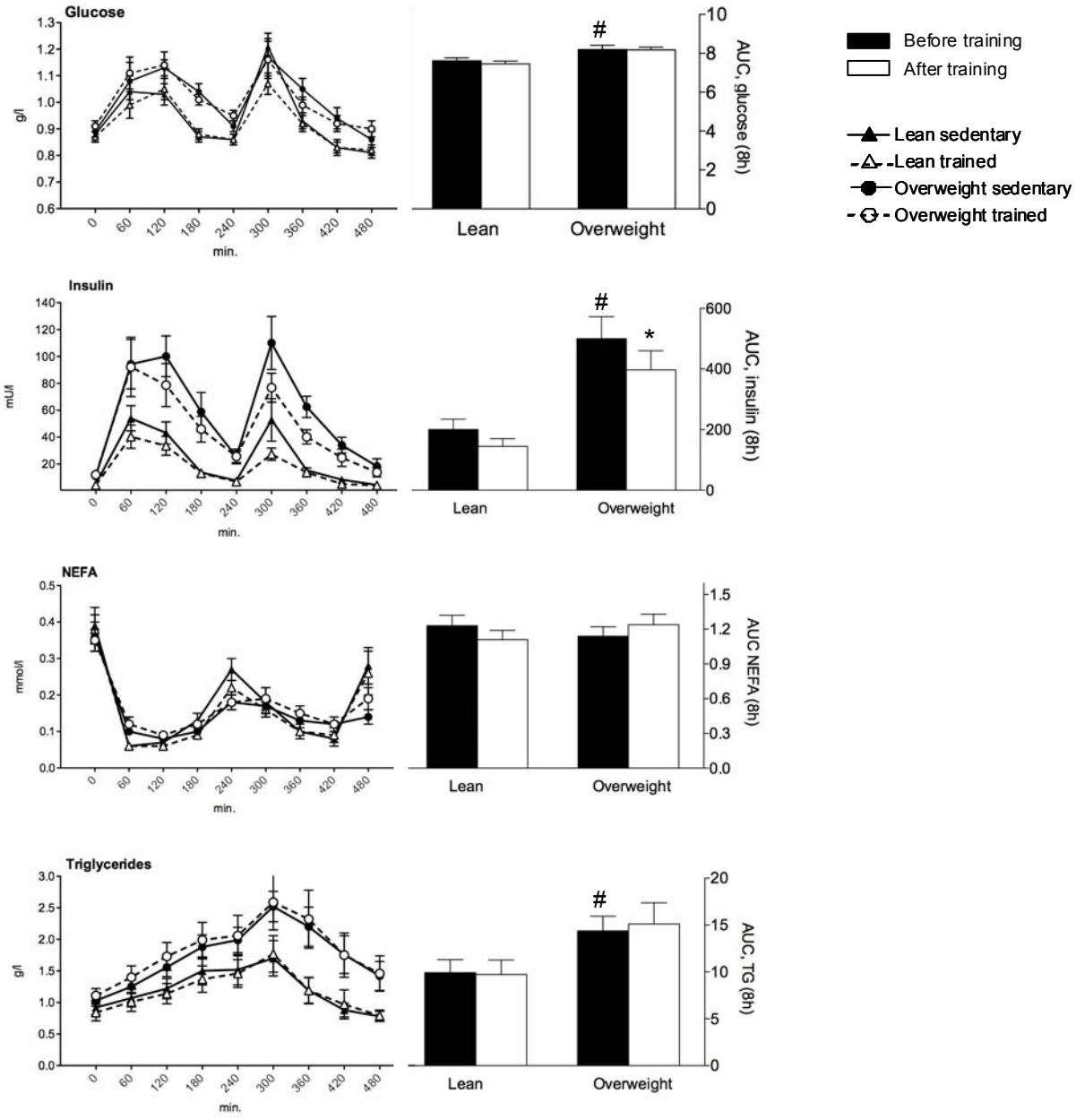

Figure 1: Time course of glucose, insulin, non-esterified fatty acids NEFA and triglycerides concentration for lean (n=10) and overweight subjects (n=10). Time 0 corresponds to the labelled breakfast ingestion, time 240mn represent the ingestion of the second meal. The cumulative responses of these parameters were calculated by the area under the curve (AUC) over 8hrs after first meal ingestion. * exercise training *vs.* baseline within the same group; # lean *vs.* overweight at baseline.

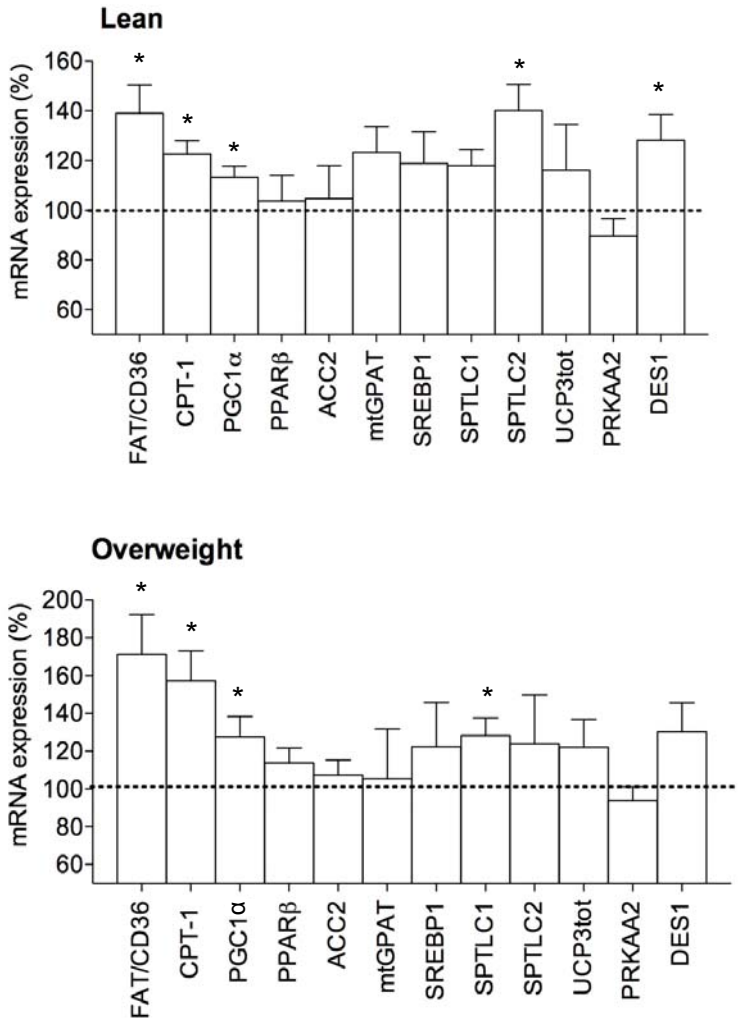

Figure 2: Training effects on mRNA gene expression in the *vastus lateralis* muscle. Results expressed in % of the fold change *vs.* the baseline values at 100%. Data are mean ±SE. * $p<0.05$ *vs.* baseline.

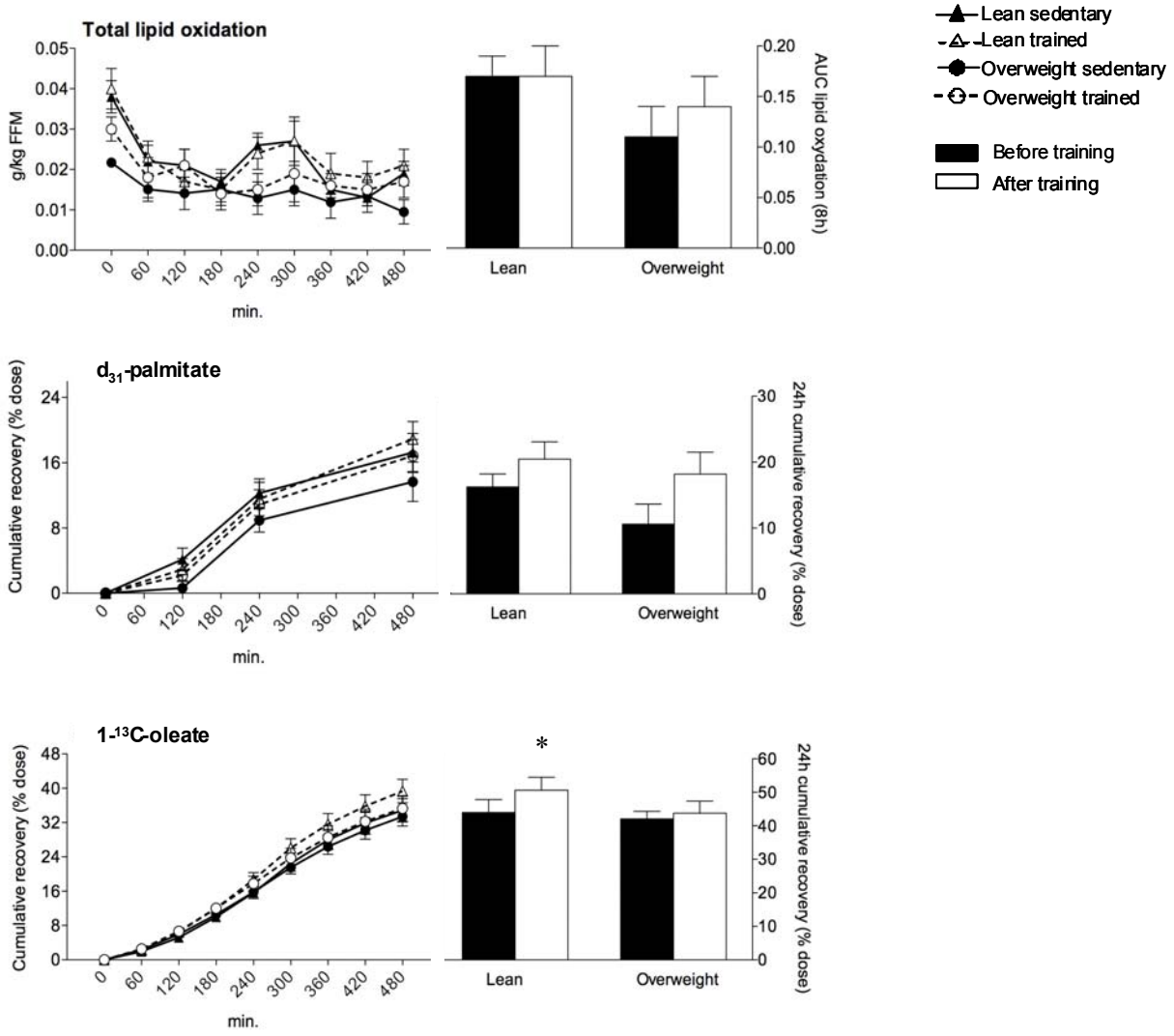

Figure 3: Total lipid oxidation and cumulative recovery for d_{31}-palmitate measured by measuring ^{2}H in total body water hourly sampled through urine, and for ^{13}C-oleate calculated by recovery of ^{13}C in expired CO_2. Both recoveries are expressed as a percentage of the dose. Time 0 corresponds to the labelled breakfast ingestion, time 240mn represent the ingestion of the second meal. At right, the area under the curve (AUC) representing the 8h total lipid oxidation and the 24h palmitate and oleate oxidation. The cumulative responses of these later parameters were calculated over 8hrs after first meal ingestion. * exercise training *vs.* baseline within the same group; # lean *vs.* overweight at baseline.

5. DISCUSSION

Two months of moderate exercise training based on current recommendations, independently of energy balance, failed to increase total energy expenditure, total postprandial and exogenous fat oxidations in overweight subjects while only fasting fat oxidation was increased. In lean subjects, training increased exogenous monounsaturated fatty acid (oleate) oxidation and saturated fatty acid (palmitate) oxidation failed to reach statistical significance, while fasting and postprandial total fat oxidation were unchanged. The major finding of the current study was that PAL explained positively both dietary fatty acid oxidations in lean subjects but not in overweight subjects.

5.1. Fasting and postprandial total fat oxidation

Training increased fasting fat oxidation in overweight subjects at rest, similar to other studies (14, 49). There are several reasons to explain why physical activity might increase the capacity for fat oxidation. While exercise training at moderate intensity induces higher capacity for, and reliance upon, fat oxidation during the exercise session (2), chronic exercise enhances the activity of oxidative enzymes and increases capillary density, thus facilitating fatty acid utilization also at rest (25).

Indeed, CPT-1 explained fasting fat oxidation improvement in overweight subjects. CPT-1 catalyses the transfer of fatty acyl-CoA into mitochondria, where they undergo oxidation (37). Thus we can suggest that increases in fatty acid transport into the mitochondria induced a consequent increase in fasting fat oxidation in the overweight group. A limited fatty acid uptake in myocytes or mitochondria can lead to an impaired ability to stimulate fatty acid oxidation (18). CPT I activity is reduced in skeletal muscle from obese individuals and is likely to contribute to the suppressed rates of FA oxidation in obesity (38). This improvement might also be attributed to an improvement in LPL (17) or alterations in muscles fiber (37). It might not be due to an increase in oxidative capacity because overweight subjects' VO_2 max was unchanged after training. However, they increased their fat free mass and it was shown that changes in fat-free mass correlates with increases in total fat oxidation (49). Increases in RMR in overweight subjects, seem also a potential candidate explaining the improvement in fasting fat oxidation.

Postprandial insulin concentration was reduced in overweight subjects suggesting improvement in insulin sensitivity. This might also have a role in improvement in fat oxidation. Insulin sensitivity is related to fat oxidation (18) and particularly the augmentation of resting rates of fat oxidation is a specific metabolic correlate of the amplitude of improvement in insulin sensitivity (25). Insulin has a antilipolytic effect, and acts on LPL in adipose tissue to store TG and inhibits HSLP and NEFA liberation (30). The improvement in fasting fat oxidation in the overweight group combined with the decrease in insulin concentrations represents the positive effect of exercise in improving metabolic flexibility. Metabolic flexibility is defined as the capacity to switch from fat to carbohydrate oxidation after insulin stimulation, and to increase fat oxidation during fasting, which is a way

to reflect the capacity to increase fat oxidation in general (18). Chronic exercise training was shown to improve the capacity of skeletal muscle to utilize fatty acids for fuel during exercise, and in some cases also improves fasting fat oxidation, which indicates that metabolic flexibility is improved (18).
On the other hand, in lean subjects, training, independently of body composition changes, failed to increase fasting fat oxidation. Similarly, other studies found no improvement in fat oxidation in lean subjects at rest (60) but rather during exercise as response to chronic training (60, 62). Thus, it seems that fasting fat oxidation in lean subjects is less prone to changes than in overweight subjects when no modifications in body weight occur. However, when body weight is reduced by restricted diet, and in particular body weight composition i.e fat mass reduction, it seems that exercise effect is enhanced in improving fasting fat oxidation in lean subjects (18).

Conversely, postprandial fat oxidation does not show significant improvement neither in lean nor overweight groups. It was shown that exercise, in the absence of weight loss or body mass composition changes, does not reduce fatty acid mobilization and uptake (34). In contrast to chronic training, acute prior exercise increases postprandial fat oxidation (47). Thus, chronic training effect on fat oxidation differs from the acute effect of exercise. It was suggested that exercise has only short term beneficial effect on lipid metabolism (54). De-training studies provide compelling evidence that exercise training may not markedly influence TG metabolism in the absence of recent exercise and that training induced changes in TG metabolism may be short-lived (24). In our study, we did not find significant changes in fasting and postprandial NEFA and TG concentrations. Other studies have found no changes in NEFA after three months training nor in the expression of key genes of lipolytic pathway (50). Thus, exercise training in the absence of acute exercise does not appreciably influence postprandial lipemia and TG concentration, and the influence of moderate training on blood lipids is not supported with strong evidence (66).

Exercise parameters i.e the intensity and the duration of the exercise seem to be primary factor influencing fat oxidation (68). We can speculate that modulation of exercise parameters such as longer period (>2months) or frequency (>4times/week) might affect more markedly fat metabolism and its related enzymes. However studies of longer duration (3-6months) and higher frequency (4-5 times/week), in energy balance, found no changes in lipid profile i.e TG concentration both in lean (65) and overweight (39) subjects.

5.2. Gene expressions

Interestingly, looking at values of several gene expressions, while CD36 ranged in the same order between lean and overweight subjects, other gene expressions such as CPT-1 and ACC enzymes responsible for fat oxidation, were divided by two fold or more in overweight subjects. ACC catalyses the carboxylation of acetyl-CoA to form malonyl-CoA, an intermediate that ihibits CPT-1. This suggests that fat uptake facilitated by CD36 is similar (35) between lean and overweight subjects, and reduction in fat oxidation is more related to the reduction in fat oxidation enzymes or uptake into mitochondria (52). In other words, fatty acid oxidation between overweight and lean subjects is limited by factors inside the muscle cell, rather than by limitations in transmembrane transport (37). However, a comparison of lean and obese rat muscles revealed that fatty acid

transport, esterification, and oxidation are upregulated in muscles of obese Zucker rats (32). In addition, it was shown recently, that mitochondrial content, rather than mitochondrial capacity is responsible for the reductions in fat oxidation in obesity (33). Nevertheless, the increase in fatty acid availability, through an increased uptake via the increase in CPT1 gene expression, led to an increase in fasting fat oxidation in the overweight group.

Although no marked changes in total fat oxidation was found in both groups, training increased gene expressions of several enzymes implicated in fat metabolism and transport. Other studies have also shown that exercise enhances the gene expression of proteins involved in fat oxidative pathways (69). This increase in gene expression of fat metabolism enzymes suggests that on a longer term, potential improvement in lipid oxidation might occur with training. Indeed, training increased PGC1α, CD36 and CPT-1 gene expressions. PGC1α is a co-activator involved in adaptive thermogenesis, mitochondrial biogenesis and fatty-acid oxidation (46). Thus, we might suggest that the increase in PGC1α might have led, partly to the increase in exogenous lipid oxidation in lean subjects through a direct role in lipid oxidation in the mitochondria. This was facilitated by the increased availibity of fatty acid through transport into the cell and subsequently into the mitochondria through enhacement of gene expression of fatty acid transporters CD36 and CPT1. CD36 has a major role in fatty acid transport across plasma membranes in muscle (37). Other studies indicate that exercise training alters the localization of FAT/CD36 and increases its association with CPT I, which may help augment fat oxidation (55). Moreover, similar to our results, endurance training in obese individuals improved CPT-1 activity and reduced sensitivity of CPT-1 for malonyl-CoA (13). Consequently, the reduction in oleate and palmitate concentrations in NEFA fractions we found might be attributed to the improvement in transport enzymes inducing an enhanced clearance of exogenous fatty acid into the cell and further into mitochondria to be oxidised. This might explain the positive effect of chronic exercise on exogenous fatty acid oxidation. Altogether, we might suggest that improvement in exogenous fatty acid in the lean group and fasting fat oxidation in the overweight group were enhanced through fatty acid availability facilitated by an increased transport into the myocyte and mitochondria, and through the increase in the oxidative capacity. We can also suppose that gene expression improvement shown during two months is prior to future potential improvement in total fatty acid which may occur later.

Additionally, DES-1 and SPTLC2 were increased in lean group while SPTLC1 gene expression increased in the overweight group. DES1, SPTLC1 and SPTLC2 are responsible of the synthesis of ceramide, an intracellular signaling molecule. In the line of our results, Helge *et al.* (29) reported no difference in the ceramide content of muscle from trained and untrained individuals and found that prolonged exercise elevated the content of ceramide fatty acids both in trained and untrained men. However, some studies have related ceramide concentration to insulin resistance and found that total ceramide content of skeletal muscle decreased in conjunction with enhanced insulin sensitivity in obese individuals after a short-term training program (13). Ceramide, as a second messenger in regulation of different cell functions, could influence muscle cell adaptation to exercise (29). Moreover, we can speculate that since transport of fatty acid into the cell and mitochondria is enhanced through CD36 and CPT1 gene expression found in both groups, fatty acid availability is increased and exceeds further rate of oxidation improvement requiring longer period, thus fatty acids intermediates such as ceramide can accumulate within the cytosol. Further evidence suggest a strong

relationship between fatty acid induced insulin resistance in human skeletal muscle and alterations in the DAG signalling pathways but not with ceramide (52). The finding that ceramide might not be linked to insulin resistance might explain the increase in DES1 and SPTLC1 mRNA gene expression we found after training, when considering that training ameliorated insulin sensitivity in our overweight subjects.

5.3. Oleate and palmitate trafficking and oxidation

While no noticeable improvements were found in overweight groups in exogenous lipid oxidation, training increased exogenous oleate oxidation and palmitate oxidation failed to reach statistically significant values in lean group. Lean subjects decreased oleate concentrations in NEFA fractions, while palmitate tended to decrease in NEFA after training. This suggests that training reduces the appearance rate of labeled fatty acid in lipid fractions and consequently enhances clearance of both exogenous fatty acids and more markedly oleate from plasma NEFA into tissues. In our case, and with respect to the higher exogenous oxidations, palmitate and oleate are probably more vehiculed into myocytes and further into mitochondria to be oxidised. We suggest that the increased availability of fatty acid inside the myocyte and the mitochondria induced a higher oxidation rate. Similar to our results, Votruba *et al.* (71) reported that prior acute exercise significantly increases the oxidation of dietary oleate but does not impact the oxidation of dietary palmitate.

Recent data suggests that oleate, compared with other fatty acids, is predominantly used for VLDL-TG synthesis (31). Thus, VLDL might act as a reservoir for exogenous oleate waiting to be oxidised at a later stage. This is interesting to note, taking into account that whole TG and chylomicrons concentrations in oleate and palmitate were not modified by training suggesting that the two fatty acids are similarly absorbed and uptaked as others have found (21), and that compensatory effects of oleate and palmitate might be regulated between NEFA and VLDL fractions. Other studies, however, found differential packaging between SFA and MUFA (31). Further studies are warranted to elucidate the mechanisms related to the difference in SFA and MUFA metabolism.

We also found decreases in NEFA oleate and palmitate concentrations while total NEFA remained unchanged. Beyond the enhanced clearance of exogenous fatty acid from plasma into the myocyte, this might suggest that lipolysis of endogenous NEFA i.e NEFA hydrolyzed from adipose tissue is favored and endogenous NEFA are available to be oxidized in parallel of the exogenous fatty acids at rest. Maffeis *et al.* (45) found that exogenous fat oxidation was directly related to fat mass, while endogenous fat oxidation was inversely related to fat mass (45). They explained these relationships as an attempt on behalf of the organism to counteract the increase in body fat. Schrauwen *et al.* (60) found that training tends to specifically increase the capacity to oxidize endogenous fatty acid both at rest and during exercise. In addition, exogenous fat in the postprandial phase is mostly stored (in adipose tissue) (61). During moderate exercise, obese sedentary men were shown to increase their rates of fatty-acid oxidation from non plasma sources presumably, from intramuscular sources compared with lean sedentary men during exercise (26). However, our results show that exogenous fatty acid oxidation (oleate) is increased with training in the lean group, and thus it is more likely that both endogenous and exogenous fatty acid oxidation are favored by training.

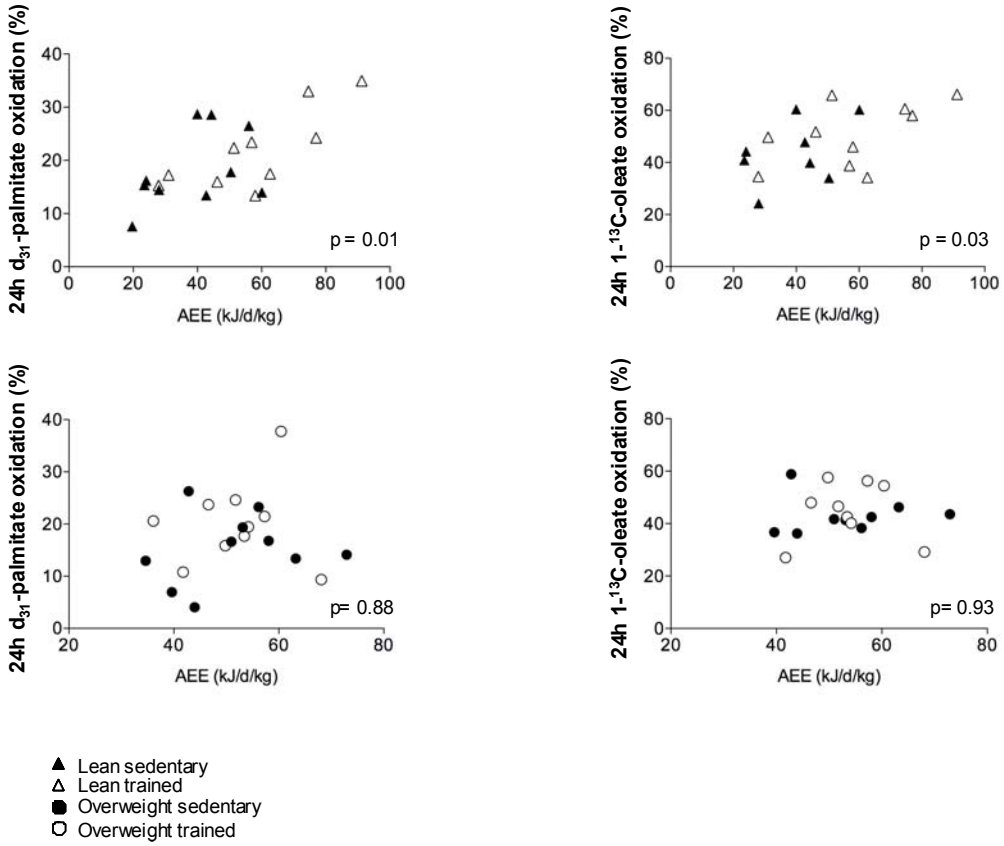

Figure 4: Mixed models tests showed that oleate (p=0.006) and palmitate oxidations (p=0.006) were both explained by activity energy expenditure AEE (KJ/d/kg).

5.4. Total energy expenditure as the driver for exogenous fat oxidation

Training increased total energy expenditure TEE, activity energy expenditure AEE and physical activity level PAL in lean subjects. In opposite and although overweight subjects, were trained similarly to lean counterparts, their TEE, AEE and PAL did not increase. An explanation for this, is the compensatory behavior noted in overweight subjects by reduction of the non exercise related daily activity (78) in opposite to the imposed structured exercise. Classically, TEE can be divided into resting metabolic rate, diet induced thermogenesis and AEE. AEE is the main compartment that can be modulated voluntary by individuals and can also be differentiated between spontaneous NEAEE defined as any bodily movement and exercise defined as a subset of physical activity that is characterized by planned and purposeful training (15). The importance of NEAEE to health outcomes i.e obesity has been recently highlighted (40) and NEAEE has been reported to be a predictor factor in weight gain (43). It was shown that increasing total energy expenditure by exercise is moderate and there is a limited number of studies that found an increase in energy expenditure, as measured by doubly labelled water, through exercise training (7, 9, 70, 77).

Modulating physical activity, however, is not always followed by effects on total energy expenditure. Indeed, compensatory behaviours might occur, which can reduce the effect of modifying AEE. Introducing exercise training into a sedentary life style might result in a compensatory behaviour by reducing the non exercise activity in individuals with predisposition to be sedentary. However, in lean subjects, after training of 18 wk, Van Etten *et al.* (70) found no changes in non training activity. In this line, our trained group was not given any instructions about modifying NEAEE. The positive increase in their TEE suggest that they did not compensate the imposed structured by decreasing their NEAEE.

Nevertheless, the effect of modulating physical activity is less obvious in overweight subjects. Our results show that although our overweight subjects were trained similarly as lean subjects with 3xtimes controlled exercise sessions plus one uncontrolled session per week, they failed to increase their TEE. This suggests that overweight individuals adopted a compensatory behaviour of reducing their NEAEE as opposite to the increase in energy expenditure imposed structured exercise, and thus equilibrating any increase in total and AEE. The fact that this compensatory behaviour was not observed in lean subjects might suggest that a predisposition of overweight subjects to sedentary behaviours may be primary to weight gain. In this line, Levine et al reported a very significant relationship between NEAEE and adiposity in lean and obese subjects (40, 44). While both of these groups spend the same time in lying down and sleeping, obese subjects spend 2.5 times more in sitting position. On the other hand, studies on the effectiveness of diet+exercise interventions versus diet-only interventions for weight loss show it is difficult to overcome diet-induced reduction of physical activity with exercise training. A meta analyze of a total of 18 randomized controlled trials on weight loss conducted for a minimum of 6 months showed an overall difference of only 0.25kg between the two groups (79). At a negative energy balance, the additional exercise was compensated by a reduction of non training activity, resulting in comparable decrease of total energy expenditure in the diet plus exercise and the diet only groups (36).

Recently, Bergouignan *et al.* (5) during bed rest studies with subjects of NEAEE deficiency, found that whereas the combined resistive and aerobic exercise partially maintained the total fat oxidation likely due to the exercise induced energy expenditure, it did not mitigate the decrease in exogenous palmitate oxidation. Taking these results together, they supposed that this lack of impact on exogenous fat oxidation is rather caused by the NEAEE deficiency rather by insufficient exercise induced energy expenditure. They further extrapolated their results in assuming that whereas total lipid oxidation may be a function of exercise induced energy expenditure, dietary fat oxidation would be function of NEAEE. Our results show a positive relationship between AEE and exogenous lipid metabolism in lean subjects. Although we measured overall AEE and we did not differentiate between AEE spent with NEAEE and AEE spent with the structured exercise, however, the lack of improvement in overweight subjects of exogenous fatty acid oxidation in parallel with reduction in NEAEE suggests that predominantly the relation we found between AEE and exogenous fatty acid oxidation can be extrapolated indeed to a relationship between NEAEE and exogenous fatty acid oxidation. In this line, Stubbs *et al.* (64) showed that active individuals tolerated more the high fat diet compared with inactive and this may be related to difference in daily NEAEE. Moreover, Smith *et al.* (63) and Hansen *et al.* (27) reported that the increase in the daily energy expenditure accelerated the adjustment of fat oxidation to the proportion of fat ingested in men and women respectively.

In other words, the lack of improvement in exogenous fatty acid we observed in overweight subjects is not surprising, if we can suggest that this mainly due to the lack of NEAEE, independently of an increase in structured exercise. The positive relationship we found of AEE and exogenous fatty acid oxidation suggests that improvement can be achieved when sufficient increase in AEE. These increases might be produced in enhancing NEAEE. Thus NEAEE might be a key modulator in body weight regulation through lipid balance.
Moreover, modulation in NEAEE can exceed TEE obtained by current recommendation (42). However, NEAEE deficit in obesity is closer to 2-3h of walking throughout the day corresponding to 2000-2500Kcal/week (44). Moreover, other studies showed that NEAEE failed to improve lipid profile (41).

Altogether, our results show that exogenous oxidation is predicted by modulation in physical activity. This finding is of a particular importance for it suggests that improvement in exogenous fatty acid oxidations can be achieved with further modulations of physical level such as longer duration or frequency of the exercise applied. However, the relationship of exogenous fatty acid oxidation with the PAL suggests that increases in total energy expenditure by any intervention i.e increases in NEAEE is of benefit for fat oxidation improvement, regardless of structured exercise. In other words, sedentary individuals may beneficially increase their fat oxidation by increasing any sort of activity without constraint of physical activity imposed programs. However, we can not rule out the importance of the role of structured exercise *per se* and further investigations of a combined or separate structured and/or NEAEE are required. These findings reveal a potential new extent for physical activity guidelines and health outcomes in general population. While current recommendations did not achieve significant changes in total fat oxidation, the question remains how much physical activity would be sufficient to induce improvements in fat metabolism. Further studies to determine such level are warranted in opening new therapeutic implications in preventing weight gain.

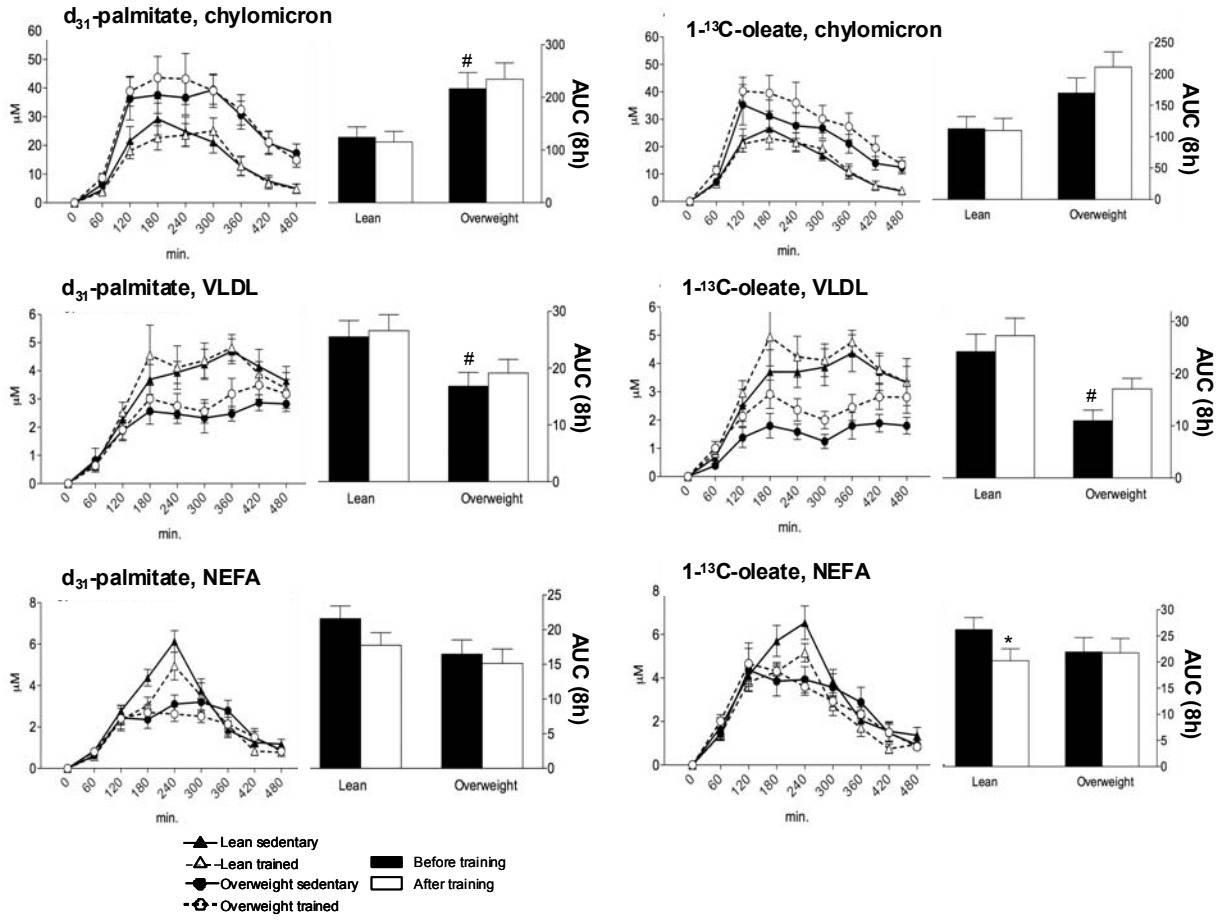

Figure 5: Time course of d_{31}-palmitate and ^{13}C-oleate incorporated in chylomicron, VLDL and NEFA. Time 0 corresponds to the labelled breakfast ingestion, time 240mn represent the ingestion of the second meal. The cumulative responses of these parameters were calculated by the area under the curve (AUC) over 8hrs after first meal ingestion. * exercise training *vs.* baseline within the same group; # lean *vs.* overweight at baseline.

In conclusion, training increased TEE in lean but not in overweight subjects due to compensatory behaviors. Moderate training without weight loss, increased fasting but postprandial and exogenous fat oxidation were not affected in overweight subjects. Mono-saturated fatty acid was increased in lean subjects. Positive relationships of the physical activity level with both exogenous fat oxidations suggest that further improvements in fat oxidation can be achieved when sufficiently increasing total energy expenditure regardless of structured exercise. Further studies are warranted to determine how much physical activity (structured and/or NEAEE) is needed to induce significant fat oxidation improvement. While data demonstrating a causal role of physical activity in the pathophysiology of obesity are unambiguous, our results provide key data on the current debate about the role of physical activity in the treatment of obesity and metabolic disorders associated with physical inactivity, demonstrating a positive relationship between the amount of energy expended during activities and the fate of dietary lipids. This suggests that research efforts on the types of physical activities to promote in different populations are imperative in the context of treatment and prevention. Given the contrasting effects of so-called spontaneous activities versus structured, it seems essential that these studies take into account the socio-ecological environment to which populations at risks evolve.

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6. REFERENCES

1. **Achten J and Jeukendrup AE.** Optimizing fat oxidation through exercise and diet. *Nutrition (Burbank, Los Angeles County, Calif* 20: 716-727, 2004.
2. **Bergman BC, Butterfield GE, Wolfel EE, Casazza GA, Lopaschuk GD, and Brooks GA.** Evaluation of exercise and training on muscle lipid metabolism. *The American journal of physiology* 276: E106-117, 1999.
3. **Bergouignan A, Schoeller DA, Normand S, Gauquelin-Koch G, Laville M, Shriver T, Desage M, Le Maho Y, Ohshima H, Gharib C, and Blanc S.** Effect of physical inactivity on the oxidation of saturated and monounsaturated dietary Fatty acids: results of a randomized trial. *PLoS clinical trials* 1: e27, 2006.
4. **Bergouignan A, Schoeller DA, Votruba S, Simon C, and Blanc S.** The acetate recovery factor to correct tracer-derived dietary fat oxidation in humans. *American journal of physiology* 294: E645-653, 2008.
5. **Bergouignan A, Trudel G, Simon C, Chopard A, Schoeller DA, Momken I, Votruba SB, Desage M, Burdge GC, Gauquelin-Koch G, Normand S, and Blanc S.** Physical inactivity differentially alters dietary oleate and palmitate trafficking. *Diabetes* 58: 367-376, 2009.
6. **Bergstrom J.** Muscle electrolytes in man. *Scand J Lab Invest* 68:7-110, 1962.
7. **Bingham SA, Goldberg GR, Coward WA, Prentice AM, and Cummings JH.** The effect of exercise and improved physical fitness on basal metabolic rate. *The British journal of nutrition* 61: 155-173, 1989.
8. **Binnert C, Pachiaudi C, Beylot M, Hans D, Vandermander J, Chantre P, Riou JP, and Laville M.** Influence of human obesity on the metabolic fate of dietary long- and medium-chain triacylglycerols. *The American journal of clinical nutrition* 67: 595-601, 1998.
9. **Blaak EE, Westerterp KR, Bar-Or O, Wouters LJ, and Saris WH.** Total energy expenditure and spontaneous activity in relation to training in obese boys. *The American journal of clinical nutrition* 55: 777-782, 1992.
10. **Blanc S, Colligan AS, Trabulsi J, Harris T, Everhart JE, Bauer D, and Schoeller DA.** Influence of delayed isotopic equilibration in urine on the accuracy of the (2)H(2)(18)O method in the elderly. *J Appl Physiol* 92: 1036-1044, 2002.
11. **Brage S, Brage N, Franks PW, Ekelund U, and Wareham NJ.** Reliability and validity of the combined heart rate and movement sensor Actiheart. *Eur J Clin Nutr* 59: 561-570, 2005.
12. **Brossard N, Croset M, Normand S, Pousin J, Lecerf J, Laville M, Tayot JL, and Lagarde M.** Human plasma albumin transports [13C]docosahexaenoic acid in two lipid forms to blood cells. *Journal of lipid research* 38: 1571-1582, 1997.
13. **Bruce CR, Thrush AB, Mertz VA, Bezaire V, Chabowski A, Heigenhauser GJ, and Dyck DJ.** Endurance training in obese humans improves glucose tolerance and mitochondrial fatty acid oxidation and alters muscle lipid content. *American journal of physiology* 291: E99-E107, 2006.
14. **Calles-Escandon J, Goran MI, O'Connell M, Nair KS, and Danforth E, Jr.** Exercise increases fat oxidation at rest unrelated to changes in energy balance or lipolysis. *The American journal of physiology* 270: E1009-1014, 1996.
15. **Caspersen CJ, Powell KE, and Christenson GM.** Physical activity, exercise, and physical fitness: definitions and distinctions for health-related research. *Public Health Rep* 100: 126-131, 1985.
16. **Colberg SR, Simoneau JA, Thaete FL, and Kelley DE.** Skeletal muscle utilization of free fatty acids in women with visceral obesity. *The Journal of clinical investigation* 95: 1846-1853, 1995.
17. **Coppack SW, Yost TJ, Fisher RM, Eckel RH, and Miles JM.** Periprandial systemic and regional lipase activity in normal humans. *The American journal of physiology* 270: E718-722, 1996.
18. **Corpeleijn E, Saris WH, and Blaak EE.** Metabolic flexibility in the development of insulin resistance and type 2 diabetes: effects of lifestyle. *Obes Rev* 10: 178-193, 2009.
19. **Crouter SE, Churilla JR, and Bassett DR, Jr.** Accuracy of the Actiheart for the assessment of energy expenditure in adults. *Eur J Clin Nutr* 62: 704-711, 2008.
20. **Daussin FN, Zoll J, Ponsot E, Dufour SP, Doutreleau S, Lonsdorfer E, Ventura-Clapier R, Mettauer B, Piquard F, Geny B, and Richard R.** Training at high exercise intensity promotes qualitative adaptations of mitochondrial function in human skeletal muscle. *J Appl Physiol* 104: 1436-1441, 2008.
21. **Evans K, Burdge GC, Wootton SA, Clark ML, and Frayn KN.** Regulation of dietary fatty acid entrapment in subcutaneous adipose tissue and skeletal muscle. *Diabetes* 51: 2684-2690, 2002.
22. **Faraj M, Jones P, Sniderman AD, and Cianflone K.** Enhanced dietary fat clearance in postobese women. *Journal of lipid research* 42: 571-580, 2001.
23. **Gan SK, Kriketos AD, Ellis BA, Thompson CH, Kraegen EW, and Chisholm DJ.** Changes in aerobic capacity and visceral fat but not myocyte lipid levels predict increased insulin action after exercise in overweight and obese men. *Diabetes care* 26: 1706-1713, 2003.
24. **Gill JM and Hardman AE.** Exercise and postprandial lipid metabolism: an update on potential mechanisms and interactions with high-carbohydrate diets (review). *The Journal of nutritional biochemistry* 14: 122-132, 2003.

25. **Goodpaster BH, Katsiaras A, and Kelley DE.** Enhanced fat oxidation through physical activity is associated with improvements in insulin sensitivity in obesity. *Diabetes* 52: 2191-2197, 2003.
26. **Goodpaster BH, Wolfe RR, and Kelley DE.** Effects of obesity on substrate utilization during exercise. *Obesity research* 10: 575-584, 2002.
27. **Hansen KC, Zhang Z, Gomez T, Adams AK, and Schoeller DA.** Exercise increases the proportion of fat utilization during short-term consumption of a high-fat diet. *The American journal of clinical nutrition* 85: 109-116, 2007.
28. **Haskell WL, Lee IM, Pate RR, Powell KE, Blair SN, Franklin BA, Macera CA, Heath GW, Thompson PD, and Bauman A.** Physical activity and public health: updated recommendation for adults from the American College of Sports Medicine and the American Heart Association. *Medicine and science in sports and exercise* 39: 1423-1434, 2007.
29. **Helge JW, Dobrzyn A, Saltin B, and Gorski J.** Exercise and training effects on ceramide metabolism in human skeletal muscle. *Experimental physiology* 89: 119-127, 2004.
30. **Herd SL, Kiens B, Boobis LH, and Hardman AE.** Moderate exercise, postprandial lipemia, and skeletal muscle lipoprotein lipase activity. *Metabolism: clinical and experimental* 50: 756-762, 2001.
31. **Hodson L, McQuaid SE, Karpe F, Frayn KN, and Fielding BA.** Differences in partitioning of meal fatty acids into blood lipid fractions: a comparison of linoleate, oleate, and palmitate. *American journal of physiology* 296: E64-71, 2009.
32. **Holloway GP, Benton CR, Mullen KL, Yoshida Y, Snook LA, Han XX, Glatz JF, Luiken JJ, Lally J, Dyck DJ, and Bonen A.** In obese rat muscle transport of palmitate is increased and is channeled to triacylglycerol storage despite an increase in mitochondrial palmitate oxidation. *American journal of physiology* 296: E738-747, 2009.
33. **Holloway GP, Bonen A, and Spriet LL.** Regulation of skeletal muscle mitochondrial fatty acid metabolism in lean and obese individuals. *The American journal of clinical nutrition* 89: 455S-462S, 2009.
34. **Horowitz JF, Leone TC, Feng W, Kelly DP, and Klein S.** Effect of endurance training on lipid metabolism in women: a potential role for PPARalpha in the metabolic response to training. *American journal of physiology* 279: E348-355, 2000.
35. **Kelley DE, Goodpaster B, Wing RR, and Simoneau JA.** Skeletal muscle fatty acid metabolism in association with insulin resistance, obesity, and weight loss. *The American journal of physiology* 277: E1130-1141, 1999.
36. **Kempen KP, Saris WH, and Westerterp KR.** Energy balance during an 8-wk energy-restricted diet with and without exercise in obese women. *The American journal of clinical nutrition* 62: 722-729, 1995.
37. **Kiens B.** Skeletal muscle lipid metabolism in exercise and insulin resistance. *Physiological reviews* 86: 205-243, 2006.
38. **Kim JY, Hickner RC, Cortright RL, Dohm GL, and Houmard JA.** Lipid oxidation is reduced in obese human skeletal muscle. *American journal of physiology* 279: E1039-1044, 2000.
39. **King AC, Haskell WL, Taylor CB, Kraemer HC, and DeBusk RF.** Group- vs home-based exercise training in healthy older men and women. A community-based clinical trial. *Jama* 266: 1535-1542, 1991.
40. **Kotz CM and Levine JA.** Role of nonexercise activity thermogenesis (NEAT) in obesity. *Minnesota medicine* 88: 54-57, 2005.
41. **Leon AS and Sanchez OA.** Response of blood lipids to exercise training alone or combined with dietary intervention. *Medicine and science in sports and exercise* 33: S502-515; discussion S528-509, 2001.
42. **Levine JA.** Nonexercise activity thermogenesis--liberating the life-force. *Journal of internal medicine* 262: 273-287, 2007.
43. **Levine JA, Eberhardt NL, and Jensen MD.** Role of nonexercise activity thermogenesis in resistance to fat gain in humans. *Science (New York, NY* 283: 212-214, 1999.
44. **Levine JA and Kotz CM.** NEAT--non-exercise activity thermogenesis--egocentric & geocentric environmental factors *vs.* biological regulation. *Acta physiologica Scandinavica* 184: 309-318, 2005.
45. **Maffeis C, Armellini F, Tato L, and Schutz Y.** Fat oxidation and adiposity in prepubertal children: exogenous versus endogenous fat utilization. *The Journal of clinical endocrinology and metabolism* 84: 654-658, 1999.
46. **Meirhaeghe A, Crowley V, Lenaghan C, Lelliott C, Green K, Stewart A, Hart K, Schinner S, Sethi JK, Yeo G, Brand MD, Cortright RN, O'Rahilly S, Montague C, and Vidal-Puig AJ.** Characterization of the human, mouse and rat PGC1 beta (peroxisome-proliferator-activated receptor-gamma co-activator 1 beta) gene in vitro and in vivo. *The Biochemical journal* 373: 155-165, 2003.
47. **Murphy MH, Nevill AM, and Hardman AE.** Different patterns of brisk walking are equally effective in decreasing postprandial lipaemia. *Int J Obes Relat Metab Disord* 24: 1303-1309, 2000.
48. **Pereira MA, FitzerGerald SJ, Gregg EW, Joswiak ML, Ryan WJ, Suminski RR, Utter AC, and Zmuda JM.** A collection of Physical Activity Questionnaires for health-related research. *Medicine and science in sports and exercise* 29: S1-205, 1997.
49. **Poehlman ET, Gardner AW, Arciero PJ, Goran MI, and Calles-Escandon J.** Effects of endurance training on total fat oxidation in elderly persons. *J Appl Physiol* 76: 2281-2287, 1994.

50. **Richterova B, Stich V, Moro C, Polak J, Klimcakova E, Majercik M, Harant I, Viguerie N, Crampes F, Langin D, Lafontan M, and Berlan M.** Effect of endurance training on adrenergic control of lipolysis in adipose tissue of obese women. *The Journal of clinical endocrinology and metabolism* 89: 1325-1331, 2004.
51. **Rowlands AV, Thomas PW, Eston RG, and Topping R.** Validation of the RT3 triaxial accelerometer for the assessment of physical activity. *Medicine and science in sports and exercise* 36: 518-524, 2004.
52. **Russell AP.** Lipotoxicity: the obese and endurance-trained paradox. *Int J Obes Relat Metab Disord* 28 Suppl 4: S66-71, 2004.
53. **Saris WH, Blair SN, van Baak MA, Eaton SB, Davies PS, Di Pietro L, Fogelholm M, Rissanen A, Schoeller D, Swinburn B, Tremblay A, Westerterp KR, and Wyatt H.** How much physical activity is enough to prevent unhealthy weight gain? Outcome of the IASO 1st Stock Conference and consensus statement. *Obes Rev* 4: 101-114, 2003.
54. **Schenk S and Horowitz JF.** Acute exercise increases triglyceride synthesis in skeletal muscle and prevents fatty acid-induced insulin resistance. *The Journal of clinical investigation* 117: 1690-1698, 2007.
55. **Schenk S and Horowitz JF.** Coimmunoprecipitation of FAT/CD36 and CPT I in skeletal muscle increases proportionally with fat oxidation after endurance exercise training. *American journal of physiology* 291: E254-260, 2006.
56. **Schoeller DA.** The energy balance equation: looking back and looking forward are two very different views. *Nutrition reviews* 67: 249-254, 2009.
57. **Schoeller DA, Colligan AS, Shriver T, Avak H, and Bartok-Olson C.** Use of an automated chromium reduction system for hydrogen isotope ratio analysis of physiological fluids applied to doubly labeled water analysis. *J Mass Spectrom* 35: 1128-1132, 2000.
58. **Schoeller DA, Ravussin E, Schutz Y, Acheson KJ, Baertschi P, and Jequier E.** Energy expenditure by doubly labeled water: validation in humans and proposed calculation. *The American journal of physiology* 250: R823-830, 1986.
59. **Schoeller DA, Shay K, and Kushner RF.** How much physical activity is needed to minimize weight gain in previously obese women? *The American journal of clinical nutrition* 66: 551-556, 1997.
60. **Schrauwen P, van Aggel-Leijssen DP, Hul G, Wagenmakers AJ, Vidal H, Saris WH, and van Baak MA.** The effect of a 3-month low-intensity endurance training program on fat oxidation and acetyl-CoA carboxylase-2 expression. *Diabetes* 51: 2220-2226, 2002.
61. **Schutz Y.** Dietary fat, lipogenesis and energy balance. *Physiology & behavior* 83: 557-564, 2004.
62. **Sial S, Coggan AR, Hickner RC, and Klein S.** Training-induced alterations in fat and carbohydrate metabolism during exercise in elderly subjects. *The American journal of physiology* 274: E785-790, 1998.
63. **Smith SR, de Jonge L, Zachwieja JJ, Roy H, Nguyen T, Rood J, Windhauser M, Volaufova J, and Bray GA.** Concurrent physical activity increases fat oxidation during the shift to a high-fat diet. *The American journal of clinical nutrition* 72: 131-138, 2000.
64. **Stubbs RJ, Ritz P, Coward WA, and Prentice AM.** Covert manipulation of the ratio of dietary fat to carbohydrate and energy density: effect on food intake and energy balance in free-living men eating ad libitum. *The American journal of clinical nutrition* 62: 330-337, 1995.
65. **Sunami Y, Motoyama M, Kinoshita F, Mizooka Y, Sueta K, Matsunaga A, Sasaki J, Tanaka H, and Shindo M.** Effects of low-intensity aerobic training on the high-density lipoprotein cholesterol concentration in healthy elderly subjects. *Metabolism: clinical and experimental* 48: 984-988, 1999.
66. **Tambalis K, Panagiotakos DB, Kavouras SA, and Sidossis LS.** Responses of blood lipids to aerobic, resistance, and combined aerobic with resistance exercise training: a systematic review of current evidence. *Angiology* 60: 614-632, 2009.
67. **Toubro S, Sorensen TI, Hindsberger C, Christensen NJ, and Astrup A.** Twenty-four-hour respiratory quotient: the role of diet and familial resemblance. *J Clin Endocrinol Metab* 83: 2758-2764, 1998.
68. **Tsetsonis NV and Hardman AE.** Reduction in postprandial lipemia after walking: influence of exercise intensity. *Medicine and science in sports and exercise* 28: 1235-1242, 1996.
69. **Tunstall RJ, Mehan KA, Wadley GD, Collier GR, Bonen A, Hargreaves M, and Cameron-Smith D.** Exercise training increases lipid metabolism gene expression in human skeletal muscle. *American journal of physiology* 283: E66-72, 2002.
70. **Van Etten LM, Westerterp KR, Verstappen FT, Boon BJ, and Saris WH.** Effect of an 18-wk weight-training program on energy expenditure and physical activity. *J Appl Physiol* 82: 298-304, 1997.
71. **Votruba SB, Atkinson RL, and Schoeller DA.** Prior exercise increases dietary oleate, but not palmitate oxidation. *Obesity research* 11: 1509-1518, 2003.
72. **Votruba SB, Horvitz MA, and Schoeller DA.** The role of exercise in the treatment of obesity. *Nutrition (Burbank, Los Angeles County, Calif* 16: 179-188, 2000.
73. **Votruba SB, Zeddun SM, and Schoeller DA.** Validation of deuterium labeled fatty acids for the measurement of dietary fat oxidation: a method for measuring fat-oxidation in free-living subjects. *Int J Obes Relat Metab Disord* 25: 1240-1245, 2001.

74. **Weinsier RL, Hunter GR, Heini AF, Goran MI, and Sell SM.** The etiology of obesity: relative contribution of metabolic factors, diet, and physical activity. *The American journal of medicine* 105: 145-150, 1998.
75. **Weinsier RL, Hunter GR, Schutz Y, Zuckerman PA, and Darnell BE.** Physical activity in free-living, overweight white and black women: divergent responses by race to diet-induced weight loss. *The American journal of clinical nutrition* 76: 736-742, 2002.
76. **Weir JB.** New methods for calculating metabolic rate with special reference to protein metabolism. 1949. *Nutrition (Burbank, Los Angeles County, Calif* 6: 213-221, 1990.
77. **Westerterp KR, Meijer GA, Janssen EM, Saris WH, and Ten Hoor F.** Long-term effect of physical activity on energy balance and body composition. *The British journal of nutrition* 68: 21-30, 1992.
78. **Whybrow S, Hughes DA, Ritz P, Johnstone AM, Horgan GW, King N, Blundell JE, and Stubbs RJ.** The effect of an incremental increase in exercise on appetite, eating behaviour and energy balance in lean men and women feeding ad libitum. *The British journal of nutrition* 100: 1109-1115, 2008.
79. **Wu T, Gao X, Chen M, and van Dam RM.** Long-term effectiveness of diet-plus-exercise interventions *vs.* diet-only interventions for weight loss: a meta-analysis. *Obes Rev* 10: 313-323, 2009.
80. **Zahariev A, Bergouignan A, Caloin M, Normand S, Gauquelin-Koch G, Gharib C, and Blanc S.** Skinfold thickness versus isotope dilution for body fat assessment during simulated microgravity: results from three bed-rest campaigns in men and women with and without countermeasures. *Eur J Appl Physiol* 95: 344-350, 2005.

CHAPITRE 6

Habitual Physical activity level predicts metabolic flexibility

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Diabetologia, en révision

Résumé

Introduction

La flexibilité métabolique est définie comme la capacité d'augmenter l'oxydation des lipides en réponse à une augmentation de la disponibilité accrue en acides gras et de relayer entre les lipides et les glucides comme carburant primaire suite à une stimulation par l'insuline. En revanche, comme l'ont suggéré les études sur l'activité physique et/ou les interventions de régime alimentaire spécifique, l'inflexibilité métabolique peut être favorisée par des facteurs de style de vie tels que les comportements sédentaires et le régime alimentaire à haute densité énergétique ou riche en matières grasses. L'observation selon laquelle une activité physique chronique améliore la capacité du muscle squelettique à utiliser les acides gras comme carburant durant l'exercice et, dans certains cas améliore également l'oxydation des lipides à jeun, suggère indirectement que la flexibilité métabolique pourrait être améliorée avec l'exercice régulier. Alors que les données démontrant un effet significatif de l'activité physique combiné avec une perte de masse sur la flexibilité métabolique restent univoques, l'effet du niveau d'activité physique *per se* indépendamment de la balance énergétique sur la flexibilité métabolique n'est pas encore clarifié. L'évaluation de la flexibilité métabolique n'est cependant pas clairement établie. Parce que de nombreuses définitions de la flexibilité métabolique peuvent être trouvées dans la littérature, il n'existe aucune méthode standard à appliquer. La plupart des études ont utilisé la méthode du clamp hyperinsulinémique euglycémique, car elle permet d'effectuer des mesures dans un environnement contrôlé. Toutefois, une telle condition pharmacologique envahissante ne reflète pas la dynamique des réponses physiologiques postprandiales. D'autres approches ont été utilisées pour évaluer la flexibilité métabolique telles que les changements entre le quotient respiratoire (QR) à jeun et postprandial et l'aire sous la courbe du QR à la suite de l'ingestion de repas. Cependant, le quotient respiratoire QR à jeun est fortement influencé par le bilan énergétique et la composition en macronutriments des repas consommés les jours précédents les tests.

Objectifs

L'objectif de cette étude était de déterminer 1) si la flexibilité métabolique varie dans les groupes de sujets avec différents niveaux d'activité physique habituel et 2) si une intervention sur l'activité physique (entraînement / désentraînement) peut moduler la flexibilité métabolique. Un objectif secondaire était de tester de nouvelles méthodes d'évaluation de la flexibilité métabolique fondées sur la nature dynamique des réponses métaboliques à des repas standardisés.

Matériels et méthodes

On a évalué la flexibilité métabolique chez 47 sujets: hommes sédentaires normopondérés et en surpoids soumis à 2-mois d'exercice selon les recommandations actuelles, hommes actifs normopondérés soumis à 1 mois de réduction du niveau d'activité physique et des femmes normopondérées soumis à deux mois d'alitement d'extrême inactivité avec ou sans exercice. Les mesures de la

flexibilité métabolique ont été effectuées avant et après les interventions, indépendamment de l'effet d'un exercice aigu. La flexibilité métabolique a été évaluée après deux repas standards 1) individuellement en tant que la relation entre les variances des taux d'insuline et du quotient respiratoire non-protéique NPRQ et 2) au niveau du groupe en tant que les pentes linéarisées des percentiles de l'insuline et NPRQ.

Résultats

Les variances de l'insuline et du NPRQ étaient négativement corrélée avec le continuum du niveau d'activité physique chez les sujets normopondérés ($p = 0,046$) mais pas chez les sujets insulino-résistants en surpoids. L'inactivité physique réduit la flexibilité métabolique ($p < 0,01$). L'exercice combiné avec une balance énergétique négative a contrebalancé l'effet de l'alitement sur la flexibilité métabolique. L'entraînement, sans perte de poids, était associé à une amélioration significative de la flexibilité métabolique chez les sujets normopondérés ($p = 0,06$), mais n'a pas amélioré la flexibilité métabolique chez les sujets en surpoids.

Discussion

En utilisant deux nouveaux indices qui tiennent compte de la dynamique quotidienne des réponses du quotient respiratoire et de l'insuline à l'ingestion de repas séquentiels, notre résultat majeur est que le niveau de l'activité physique habituelle prédit la flexibilité métabolique chez les sujets normopondérés indépendamment de tout effet d'un exercice récent et d'effets quantifiables de la balance énergétique. Ainsi, les personnes normopondérés actives ont une plus grande capacité à basculer de l'utilisation d'acides gras libres vers le glucose comme carburant primaire entre le jeûne et postprandialement, que leurs homologues inactifs. Une telle relation suggère qu'une augmentation de l'activité physique doit être associée à une amélioration de la flexibilité métabolique alors qu'une réduction du niveau d'activité physique devrait la diminuer et que cela est plus efficace chez les personnes normopondérés. Si l'inactivité physique affecte d'une manière efficace la flexibilité métabolique chez les sujets normopondérés actifs, les effets de l'entraînement physique, sans perte de poids, sur la flexibilité métabolique, sont moins marqués chez les personnes sédentaires. On peut suggérer que la durée de l'entraînement et de l'intensité utilisée dans la présente étude, c'est à dire sur la base des recommandations actuelles développées à l'origine pour diminuer les risques cardio-vasculaires, n'ont pas suffi à avoir une incidence significative sur la flexibilité métabolique. Enfin, l'absence d'effet de l'exercice chez les sujets insulino-résistants en surpoids pourrait simplement refléter le fait que l'entraînement physique n'a pas augmenté la dépense énergétique totale dans ce groupe. En conclusion, le niveau d'activité physique habituel prédit la flexibilité métabolique chez les sujets normopondérés mais pas chez les sujets insulino-résistants en surpoids. La forte détérioration de la flexibilité métabolique observée après l'inactivité physique, par rapport à une absence d'amélioration significative de la flexibilité métabolique après un entraînement selon les recommandations actuelles, à la fois chez les sujets normopondérés et les sujets en surpoids souligne l'importance des activités de la vie quotidienne.

1. ABSTRACT

Objective: Although insulin resistance and type 2 diabetes are associated with metabolic inflexibility and sedentarism, the effect of habitual physical activity level (PAL) on metabolic flexibility (MF) regulation is poorly characterized. We investigated how PAL affects MF in cross-sectional and interventional studies.

Research design and methods: MF was assessed in 47subjects: lean and overweight sedentary men submitted to 2-months of exercise at current recommendations, lean active men submitted to 1-month of reduced PAL and lean women submitted to 1-month of bed rest with or without exercise. MF measurements were performed before and after interventions independently of acute exercise. MF was evaluated following two standard meals 1) individually as the relationship between variances in insulin and non-protein respiratory quotient (NPRQ) and 2) at the group-level as the slopes of linearized insulin and NPRQ percentile/percentile plots.

Results: Insulin and NPRQ variances were negatively related along the continuum of PAL in lean subjects ($p=0.046$) but not in overweight insulin resistant subjects. Physical inactivity reduced MF ($p<0.01$). Exercise combined with negative energy balance prevented the bed rest effect on MF. Training without weight loss was associated with a nearly significant MF improvement in lean subjects ($p=0.06$), but failed to improve MF in overweight subjects.

Conclusion: Habitual PAL predicts MF in lean but not overweight subjects. The stronger deterioration of MF observed after physical inactivity, when compared to the lack of significant improvement in MF after training at current recommendations in both lean and overweight subjects, highlights the importance of daily living activities.

2. INTRODUCTION

Metabolic flexibility is defined as the capacity to increase fat oxidation upon increased fatty acid availability and to switch between fat and glucose as the primary fuel [1]. In healthy individuals, during fasting, the body relies mainly on fatty acid oxidation while glucose is saved for glucose-dependent organs. Postprandially, lipolysis is rapidly suppressed by insulin and glucose is used as fuel.

Several metabolic disorders such as obesity, insulin-resistance and type 2 diabetes are characterized by a deregulation between the capacity for ß-oxidation, glycolysis and mitochondrial oxidative capacity that switches fuel preference towards glucose during fasting [1] and impairs the capacity to switch between fat and glucose as the primary fuel source after a meal [1] and during stimulated conditions such as beta-adrenergic stimulation or exercise [2, 3]. Such impaired capacity to switch between fuel utilization is defined as metabolic inflexibility.

As suggested by physical training and/or specific diet interventions, metabolic inflexibility may be favored by lifestyle factors such as sedentary behaviors and high fat, energy-dense diet [1]. During the shift to isoenergetic high-fat diet, Smith *et al.* [4] showed that the ability of lean men to adapt fat oxidation was related to physical fitness and fasting insulin concentration. Combined with weight loss, exercise training improves insulin sensitivity [5], fasting fat oxidation and increases mitochondrial content [6]. Training without weight loss [7] increases the activity of muscle oxidative enzymes and fat oxidation. Although the evidence that exercise *per se* affects insulin sensitivity independently of body mass loss is not straightforward [8], the observation that chronic exercise improves the capacity of skeletal muscle to utilize fatty acids as fuel during exercise and in some cases also improves fasting fat oxidation, indirectly suggests that metabolic flexibility might be improved with regular exercise [1]. Nevertheless, as highlighted recently [9], the effect of physical activity level on metabolic flexibility has yet to be clarified. The objective of this study was to investigate 1) whether metabolic flexibility varies in groups of subjects with different habitual physical activity patterns and 2) whether physical activity training/detraining interventions can modulate metabolic flexibility.

The assessment of metabolic flexibility is however not clearly established. Because numerous definitions of metabolic flexibility can be found in the literature, there is no standard method to apply. A common agreement is that it can be determined during challenging situations. Most of the previous studies used the method of the euglycemic-hyperinsulinemic clamp, because it allows to perform measurements in a controlled environment [10]. However, such pharmacological invasive condition do not reflect the dynamic of physiological responses to meal and metabolic flexibility during the clamp is mostly the consequence of variability in glucose disposal rate [10]. Other approaches have been used to assess metabolic flexibility such as changes between fasting and postprandial respiratory quotient (RQ) [11] and the area under the curve of RQ following meal ingestion [12]. However, fasting RQ has been shown to be a weak indicator of lipid oxidation capacity, since it is greatly influenced by the energy balance and dietary macronutrient composition of the meals consumed on the previous days [10].

To investigate how physical activity modulates metabolic flexibility, we considered an approach based on the dynamic nature of daily postprandial metabolic responses to standardized meals rather than on RQ changes in response to supraphysiological doses of insulin during a clamp, or in response to high fat/carbohydrate meal or diet. Based on the above definition of metabolic flexibility, we used two indexes that account for the daily post-prandial relationship between RQ and insulin. The first index was established from the overall intra-individual variances in the insulin and non-protein respiratory quotient (NPRQ) responses to standard meals. A sedentary metabolically inflexible subject was hypothesized to be characterized by a large daily variance in insulin for a small variance in NPRQ i.e. a small shift in the fuel mix being oxidized at a high insulin signal. The direct corollary is that improvement in metabolic flexibility by exercise training will increase the daily variance in NPRQ and decrease the daily variance in insulin. The second index we used was based on the slope of the linearized percentile/percentile relationship between the postprandial NPRQ and insulin distributions at the group-level. This slope was hypothesized to reflect the sensibility of the metabolic flexibility.

We took advantages of two of our studies investigating the effects of very contrasted interventions on physical activities (training, detraining, bed resting) on dietary fat metabolism, in subjects already segregated at baseline based on their habitual physical activity levels (trained, untrained) and body mass (normally weight and overweight). Between-study comparison was made possible because the clinical investigations of dietary fat metabolism were strictly identical in two protocols. In this report, we therefore investigated metabolic flexibility along a wide range of habitual physical activity levels, and determined whether metabolic flexibility can be modulated by contrasted changes in the exercise level.

3. METHODS

We combined data from the LIPOX (Strasbourg Lipid Oxidation study) and WISE2005 (Women International Space Simulation for Exploration) studies that aimed to determine the effect of chronic physical activity interventions (training and detraining) on dietary lipid oxidation and trafficking in lean and overweight, sedentary or physically active individuals. Here, we only focused on the methods and data necessary to assess the relationship between physical activity and metabolic flexibility. These include the measure of physical activity levels (PAL), activity energy expenditure (AEE), and descriptions of the training/detraining interventions.

3.1. The LIPOX study

The LIPOX study was conducted on 10 sedentary and 9 physically active lean male subjects (20≤ BMI ≤25 kg/m^2) and on 12 sedentary overweight subjects (27≤ BMI ≤35 kg/m^2; Table 1). Volunteers were free of any chronic known diseases and were weight stable (±3kg body weight) for at least 3months before enrolment. Lean subjects had no first-degree family history of obesity or type 2 diabetes while overweight subjects had at least one overweight or diabetic parent. Sedentary and

physically active statuses at inclusion were determined by the MOSPA-Q questionnaire and a RT3 triaxial accelerometer (Stayhealthy, Monrovia, CA, USA) wore for 7 days in free-living conditions. RT3 was used to obtain an estimation of subjects' PALs defined as the ratio between total energy expenditure (TEE) and resting metabolic rate (RMR). Since accelerometers tend to underestimate the PAL, a RT3-estimated-PAL ≤1.5 was set to screen sedentary individuals, ≥1.7 to screen active ones. Additional criteria were the absence of participation to any structured exercise programs over the 12months prior to the study for sedentary subjects and the involvement in at least one regular sportive activity for active subjects.

Physical inactivity was induced in physically active individuals during one month by stopping all structured physical activities and reducing spontaneous activities of daily living. Training was performed in sedentary lean and overweight subjects, at the level of current recommendations [13] for two months i.e. four 60-min sessions per week at 50%VO_{2peak} on a cycle ergometer. VO_{2peak} was determined by means of an incremental exercise test to exhaustion performed in an upright position on an electronically braked cycle ergometer (Medifit 1000S, Belgium). Throughout the study, research dieticians followed the participants and the diet was regularly adjusted to maintain subjects in stable energy balance. Informed, written consent was obtained from each subject. The study was approved by the Institutional Review Board of Alsace I (France). The main outcomes of the LIPOX study have not been published yet.

3.2. The WISE2005 study

Sixteen healthy women (18≤ BMI ≤25 kg/m^2) participated in a 2-month bed rest (Table 1). Subjects engaged in at least 30minutes of moderate activity per day (as structured exercise or as daily-life activities), but did not engage in regular high volume physical activities. The aim was to recruit normally active subjects with a PAL around 1.75.

During the bed rest period, the volunteers were randomly divided into two groups (n=8, each), one severely detrained group that strictly remained in bed and one exercise group subjected to a high volume combined resistive and aerobic exercise training protocol (35 min sessions every third day) on an inertial flywheel ergometer allowing subjects to perform maximal concentric and eccentric actions in the supine squat and calf press and 40 min aerobic supine sessions three to four times per week, using a specially design vertical treadmill sequentially graded between 40 and 80%VO_2 peak. WISE2005 design, methods and main outcomes have been recently published [14].

Table 1. Characteristics of the participants at baseline and after interventions on physical activity

	WISE normally active lean women			LIPOX high active lean Men		LIPOX sedentary lean men		LIPOX sedentary overweight men	
	Baseline	After 1month of bed rest	After 1month of Bed rest+exercise	Baseline	After 1month of detraining	Baseline	After 2months of exercise training	Baseline	After 2months of exercise training
n	16	8	8	9	9	10	10	12	12
Age (years)	33.5±0.9			23.6±1.1		27.2±2.9		29.4±1.5	
Body mass (kg)	57.0±1.3	52.6±1.4*	55.5±2.1*	71.7±2.9[a]	71.4±3.2	76.7±3.1[a]	76.1±2.9	97.8±2.8[b]	97.7±2.9
BMI (kg/m^2)	21.5±0.3	19.8±0.4*	20.3±0.6*	22.2±0.6[a]	22.1±0.7	22.9±0.7[a]	22.7±0.6	30.1±0.5[b]	30.0±0.5
Fat mass									
kg	14.7±0.8	14.3±1.3	13.0±1.3*	10.5±1.2[a]	11.4±1.5	17.9±1.9[b]	17.0±1.7	31.2±1.5[c]	30.5±1.5
%	25.7±1.3	27.0±1.9	23.2±2.1*	14.5±1.4[a]	15.8±1.8*	22.9±1.8[b]	21.9±1.7	31.8±1.0[c]	31.1±1.0
Fat free mass, kg	42.3±1.2	36.1±1.0*	40.3±1.9*	61.2±2.5 [ab]	60.0±2.7*	58.8±1.9[a]	59.2±1.9	66.6±1.8[b]	67.2±2.0*
PAL	1.82±0.07	1.37±0.06*	1.64±0.07	2.23±0.06[a]	2.09±0.06*	1.6±0.06[b]	1.83±0.08*	1.87±0.07 [c]	1.85±0.05
VO_{2peak}									
l/min	2.0±0.1	1.5±0.1*	1.8±0.1*	3.6±0.2	3.3±0.2*	3.0±0.2	3.4±0.2*	3.0±0.2	3.2±0.2
ml.kg^{-1}.min^{-1}	34.8±1.5	28.3±1.9*	32.8±1.2	50.2±2.0[a]	46.4±1.3*	39.7±1.8[b]	44.9±1.8*	31.0±1.3[c]	32.8±1.4*

LIPOX, Lipid Oxidation study; BMI, body mass index; PAL, Physical activity level; VO_{2peak}, peak oxygen consumption. * $p < 0.05$ *vs.* baseline (paired t-test). [a],[b],[c] $p < 0.05$ PLSD Fisher's post hoc comparison following one way ANOVA within LIPOX groups prior to physical activity interventions.

3.3. Energetic and metabolic assessments

Before and after the interventions (one month of bed rest for the WISE study, one month of detraining and two months of training for the LIPOX study), the participants underwent a series of identical clinical tests.

TEE was measured using the doubly labeled water (DLW) method as routinely used in our laboratories and described elsewhere [15]. PAL was calculated as the ratio of TEE over RMR measured for one hour using indirect calorimetry (Deltatrac II; GE). AEE was calculated as 90% of TEE minus RMR. Body composition was assessed by hydrometry from the DLW-derived total body water.

NPRQ was calculated by using the expired CO_2 and the consumed O_2 measured hourly by indirect calorimetry and the nitrogen excretion. Thirty-six hours before the tests, all subjects were asked to stop any structured physical activities and were provided with microwaveable equilibrated meals calculated to match requirements. On the test day, subjects were provided with a standard breakfast covering 35% of the daily energy requirements (55% carbohydrate, 13% protein, 32% lipid). After 4 hours, subjects received a lunch covering 18% of daily requirements (72% carbohydrate, 18% protein, 11% lipid). Blood samples were collected hourly to measure plasma insulin, glucose and NEFA as previously described [14]. Here are reported the results of NPRQ, insulin, glucose and NEFA over the postprandial period during which data were collected (8hr and 10hr for LIPOX and WISE2005 studies, respectively).

3.4. Indexes of metabolic flexibility

We calculated indexes of daily metabolic flexibility derived from the intra-individual variances in insulin and NPRQ in response to two consecutive meals, as hourly measured over the LIPOX and WISE2005 studies. With such calculations any potentially confounding fasting contributions [10] is minimized for their weight becomes similar to that of any other data point collected over the day. The variance-derived indexes assumed a metabolically flexible state when the variance in insulin is low and the variance in NPRQ is high; and a metabolically inflexible state when the opposite is observed.

We also calculated a complementary index of metabolic flexibility derived for each group being studied rather than individually estimated. In this approach, more specifically used to study the effect of training/detraining, we used the overall data from the 8-10hr tests from each group (on average 3660 data point per group) and calculated the 1^{st}, 5^{th}, 10^{th}, inferior quartile, median, superior quartile, 90^{th}, 95^{th}, and 99^{th} percentiles for both insulin and NPRQ. The NPRQ and insulin percentiles were linearized by logarithm transformation and percentile/percentile plots were constructed for each group. We assumed that the slope of the relationship between NPRQ and insulin percentiles gives us a quantitative insight of the effect of the physical activity intervention on metabolic flexibility. Thus, an increase in the slope reflects a higher shift in NPRQ associated with a lower insulin concentration, which is representative of an improvement in metabolic flexibility.

3.5. Statistical analyses

All data are represented as mean ± SEM, and the level of significance was set as $p < 0.05$. All statistics were performed using SAS Enterprise Guide3.0. (SAS Institute Inc., Cary, NC, USA). Baseline data (including classical indexes of metabolic flexibility and indexes we used in the present study) among physical activity groups were compared by using a one-way ANCOVA. For this ANCOVA, the two groups of women (bed rest and bed rest plus exercise) were pooled since, by design, they were homogenous at baseline. Gender was added as a covariable.

The changes in the variance indexes due to training/detraining interventions were first tested in each group by a paired t-test. To more precisely investigate whether the changes in metabolic flexibility, as assessed by our indexes, is related to the changes in physical activity, we then pooled data from all groups and tested for a relationship between the changes in insulin and NPRQ variances and the changes in AEE normalized per kg of body mass by simple regressions. We also investigated the relationship between the variances in insulin and NPRQ with the habitual physical activity pattern as assessed by the measured PAL. To test this relationship, we used a mixed ANCOVA model with the individuals as aleatory variables and group as main effect that account for the repeated measures (pre vs post training/detraining). PAL was used as the covariable. The degree of association between variances indexes and PAL was assessed by the p value associated to the covariable in the model. Lastly, as metabolic flexibility is often represented in the literature through an inverse relationship between the changes in NPRQ and the changes in insulin during a hyperinsulinemic clamp, we represented the average variance of insulin for each group versus their respective average variances in NPRQ and tested for potential relationships by a similar mixed repeated-measures ANCOVA model with NPRQ as the covariable.
The overall changes of the insulin percentile/NPRQ percentile plots were tested by comparing slopes by using an analysis of covariance with group as main effect and insulin percentiles as covariable. The slope homogeneity was assessed by the group-by-insulin percentiles interaction.

4. RESULTS

4.1. Anthropometric and biologic data

Overweight sedentary subjects presented the highest fat mass, followed by the sedentary lean subjects and active lean men and women (Table 1). No difference was found in fat free mass between sedentary lean and active lean subjects. LIPOX lean and overweight subjects maintained a stable body weight throughout the intervention, while in WISE2005, subjects significantly lost body mass due to a decrease in fat mass in the exercise group but to a decrease in fat free mass in the control group, as expected in this model of physical inactivity (**Table 1**). **Figure 1** depicts the kinetics of NPRQ, NEFA, insulin and glucose concentrations during the test days, before and after intervention.

4.2. Effect of habitual baseline physical activity on indexes of metabolic flexibility

The kinetics of plasma NEFA, insulin, glucose and NPRQ in response to the experimental conditions are indicated in **Figure 1**. Of note is the flat response of NPRQ during the postprandial period in the overweight group suggesting metabolic inflexibility.
The postprandial NPRQ and insulin variance indexes significantly differed according to the habitual baseline physical activity groups (effects of physical activity level: p=0.047 and p=0.004, respectively; **Figure 2)**.

4.3. Effect of physical activity/inactivity interventions on metabolic flexibility

Detraining had opposed effects in postprandial NPRQ and insulin variances, with a 2-fold decrease in NPRQ variance ($p<0.001$) and a concomitantly 2-fold increase in insulin variance ($p<0.01$, **Figure 2**). Although an improvement was found, the effect of training performed at current recommendation levels on both postprandial NPRQ and insulin variances failed to reach statistical significance in lean and overweight sedentary subjects (p=NS for both groups). In normally active women, the combined resistive and aerobic exercise training maintained postprandial variance of insulin at its basal value but did not counteract the bed rest-induced decrease in NPRQ variance, which was reduced by 1.5 fold (p=0.052, **Figure 2**). PAL appeared a significant determinant of NPRQ variance (covariation p=0.007, **Figure 3**). No such relationship was observed between insulin variance and PAL. All groups combined, we found that the changes in AEE normalized by body mass explained 10% of the changes in postprandial NPRQ variance ($r^2=0.10$; p=0.04) (**supplemental data Figure 1**).

To further address how metabolic flexibility is affected by interventions on physical activity, we determined the relationship between the postprandial variances in insulin and NPRQ along the PAL continuum accessible in our groups of subjects. The concept was to represent our results similarly to the classical representation used in studies investigating metabolic flexibility by using a hyperinsulinemic euglycemic clamp. As illustrated in **Figure 4**, training/detraining interventions in lean subjects are linearly associated with changes in metabolic flexibility (covariation p=0.046). Overweight sedentary subjects showed a parallel slope of response but with an offset in the relationship at higher insulin concentrations.

Looking at the effects of physical activity interventions on metabolic flexibility through percentiles/percentiles relationship gave similar results. Detraining decreased the slope of the daily insulin/NPRQ percentile relationships (trained men (p=0.002); normally active women ($p<0.0001$)), indicating an overall negative effect of physical inactivity on metabolic flexibility (**Figure 5**). Resistive and aerobic exercise performed concomitantly to bed rest efficiently prevented physical inactivity-induced metabolic inflexibility (p=0.16). Although exercise training performed at current recommendation levels failed to improve metabolic flexibility in overweight subjects, it significantly increased the percentile/percentile slope in sedentary men (p=0.04). However, the overall effect of this physical activity intervention did not reach significance (p=0.06, **Figure 5**).

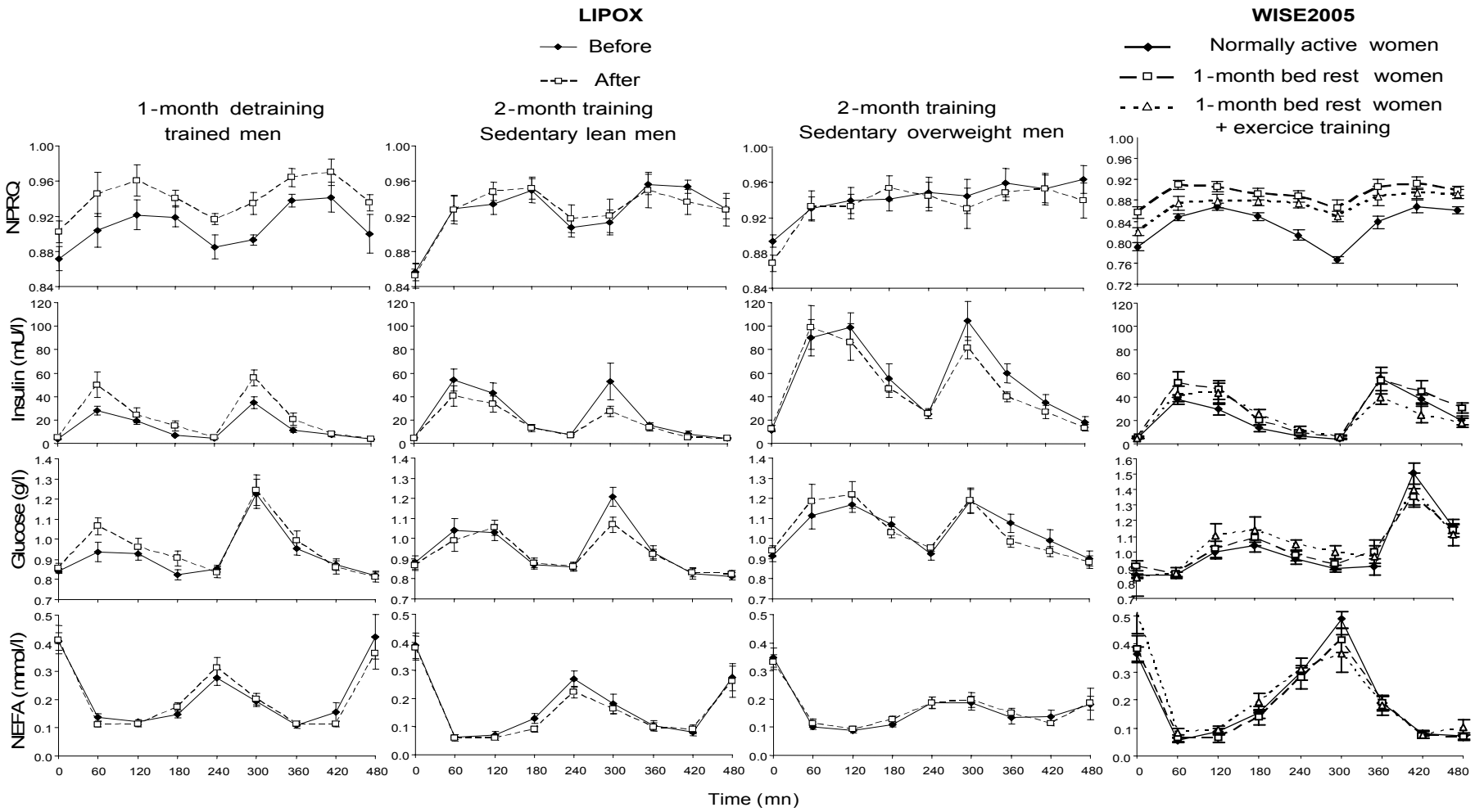


Figure 1: Time course of NPRQ, insulin, glucose and NEFAs concentrations in LIPOX trained lean men submitted to one month of detraining (n=9), LIPOX sedentary lean men submitted to two months of exercise training (n=10) and LIPOX sedentary overweight men submitted to two months of exercise training (n=12) and WISE2005 normally active women (n=16) submitted to bed rest with (n=8) or without (n=8) exercise training. Time 0 corresponds to breakfast ingestion. Time 240 min and 300 min correspond to lunch ingestion in LIPOX and Wise2005 studies respectively.

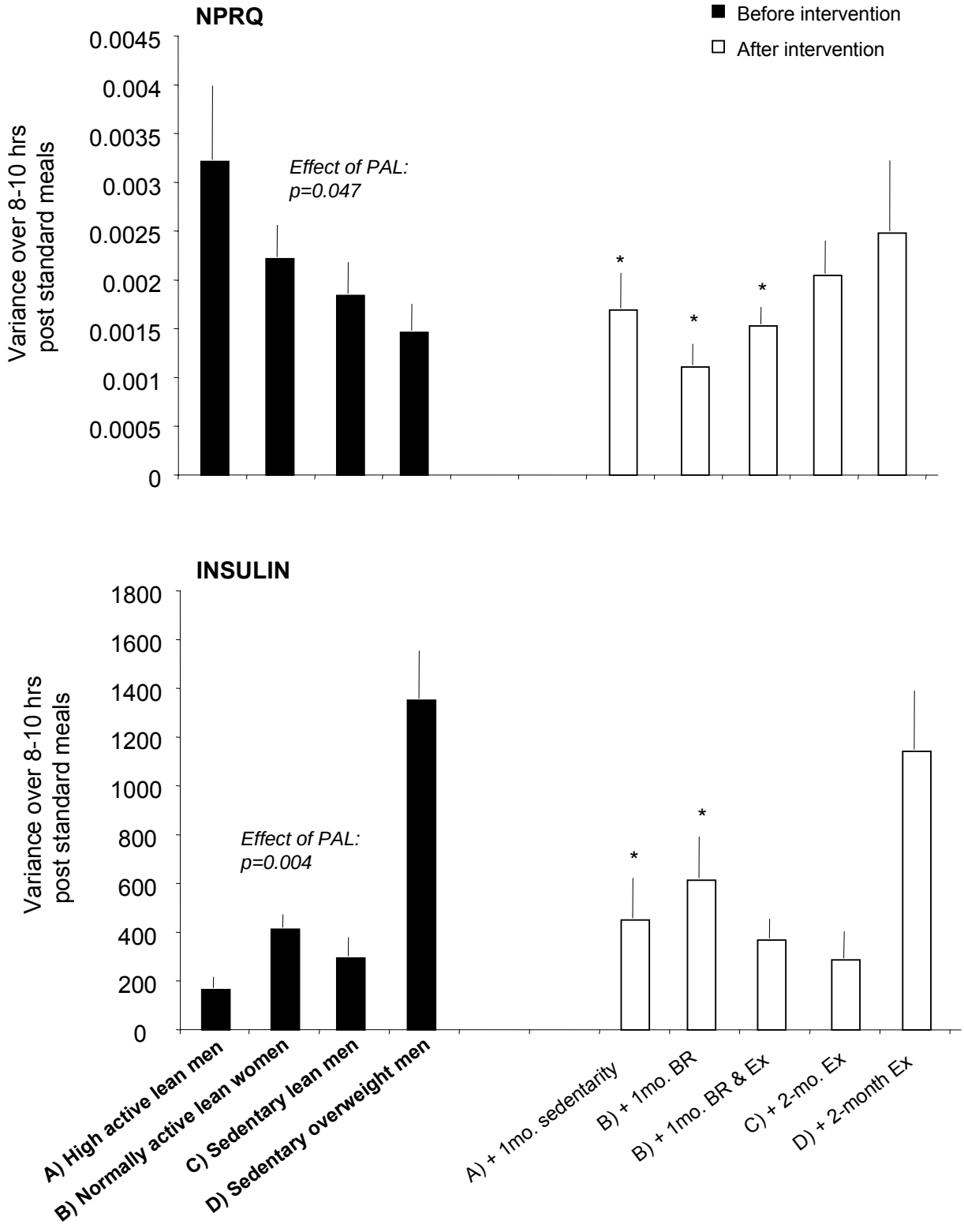


Figure 2: Postprandial variance in NPRQ and insulin in LIPOX and WISE subjects. ANCOVA main effects (habitual physical activity level prior interventions) are indicated in the figure. * $p<0.05$ *vs.* baseline (paired t-tests). NPRQ, non protein respiratory quotient; PAL, physical activity level; BR, bed rest; Ex, exercise.

5. DISCUSSION

In this study, we combined cross-sectional and interventional approaches to investigate how habitual physical activity levels, as well as changes in this level, modulate metabolic flexibility. As recently highlighted by Galgani *et al.* [10] such data are still surprisingly missing. The originality of this study was to use indexes of metabolic flexibility reflecting the overall daily response to standards meals rather than classical approaches which are based on NPRQ *delta* values between a fasting and a stimulated state, e.g. responses to surpraphysiological doses of insulin during a clamp or a high fat load [10, 12]. Such measures are likely to better reflect the metabolic events of daily living and thus should theoretically better predict chronic disease outcomes.

We showed that both a reduction in spontaneous and structured physical activity and an extreme physical inactivity decreased the variance in postprandial NPRQ and increased that of insulin. Both of these changes suggest a decreased metabolic flexibility. This statement is based on our observations being consistent with those from previous detraining and bed rest studies, which reported that a reduction in physical activity induces an increased reliance on carbohydrate as an energy substrate associated with a decrease in fat oxidation, an increase in plasma insulin concentration or a decrease in insulin action regardless of the energy balance conditions [16]. Even changes in spontaneous physical activity, as low as a decrease from 6000 to 1500 in daily steps, significantly impairs insulin sensitivity in healthy lean men [17].

On the other hand, exercise training performed at current recommendations [13] provided mitigated results, as only trends were noted with both indexes. Although an obvious positive effect was observed in lean men, it clearly failed to improve metabolic flexibility in overweight subjects and as a consequence, the overall effect of this exercise on metabolic flexibility did not reach statistical significance. In contrast with our results, Smith *et al.* in men [18] and Hansen at al. in women [19] reported that an increase in PAL from 1.4 to 1.8 improved the capacity to adjust fat oxidation after a shift to an eucaloric high-fat diet. The discrepancy with our results may be explained by the fact that the volunteers in Smith's study were active on the day test and that the measurements of NPRQ in Hansen's study were calculated over 24hrs including the last session of exercise whereas our volunteers had stopped structured exercise 36 hours before the test. In support of that, it has been suggested that exercise has only a short-term beneficial effect on lipid metabolism [20], as well as on insulin sensitivity [21]. Moreover, the high fat eucaloric 4-days diet imposed in both of these studies induced a shift in fat oxidation to eventually balance intake with oxidation which contrasts with our 3-day controlled standard diet period [4].

We can also speculate that the intensity (50%VO_2peak), the duration (1h), the rate (4 times/week) of the sessions and/or the duration of our protocol were insufficient to positively impact metabolic flexibility. The improvement in insulin sensitivity after 7 days of exercise at 70% VO_{2peak}, but not at 50% VO_{2peak}, in obese subjects support such an hypothesis [22]. The fact that training following current recommendation affects metabolic flexibility along with the linear relationship we observed for habitual baseline physical activity levels supports the idea that a greater modulation in the exercise parameters may promote a higher improvement in metabolic flexibility.

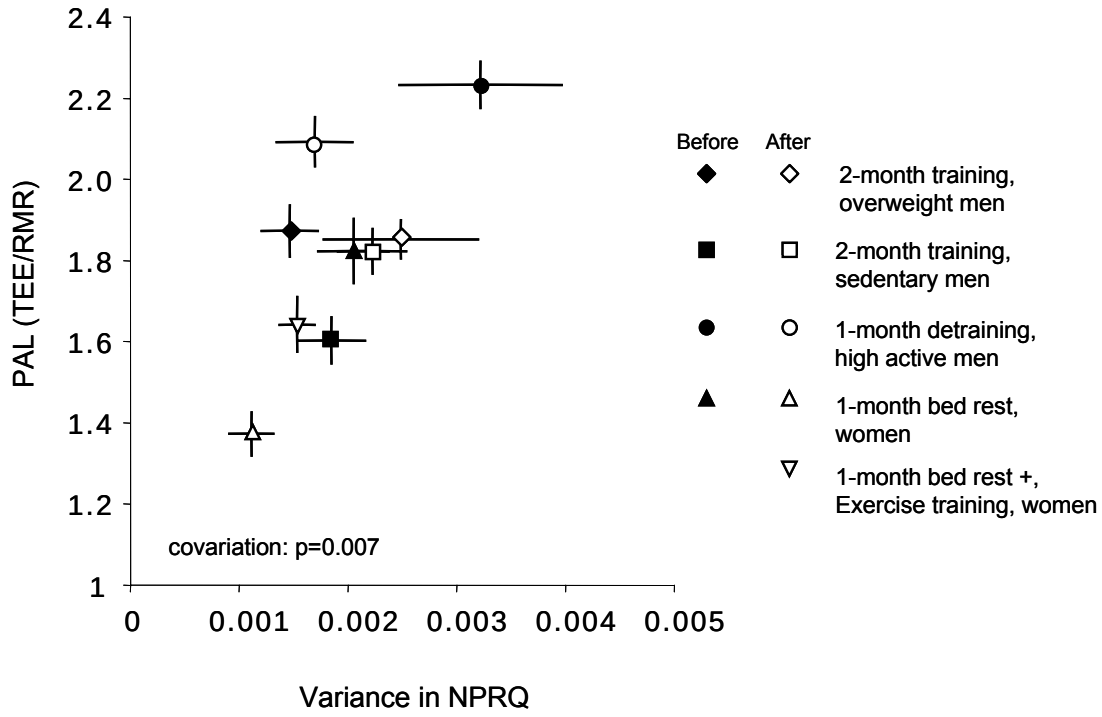


Figure 3: NPRQ variance is a direct function of PAL (interaction group x PAL variance p=0.0066) in the 47 subjects from WISE and LIPOX studies.

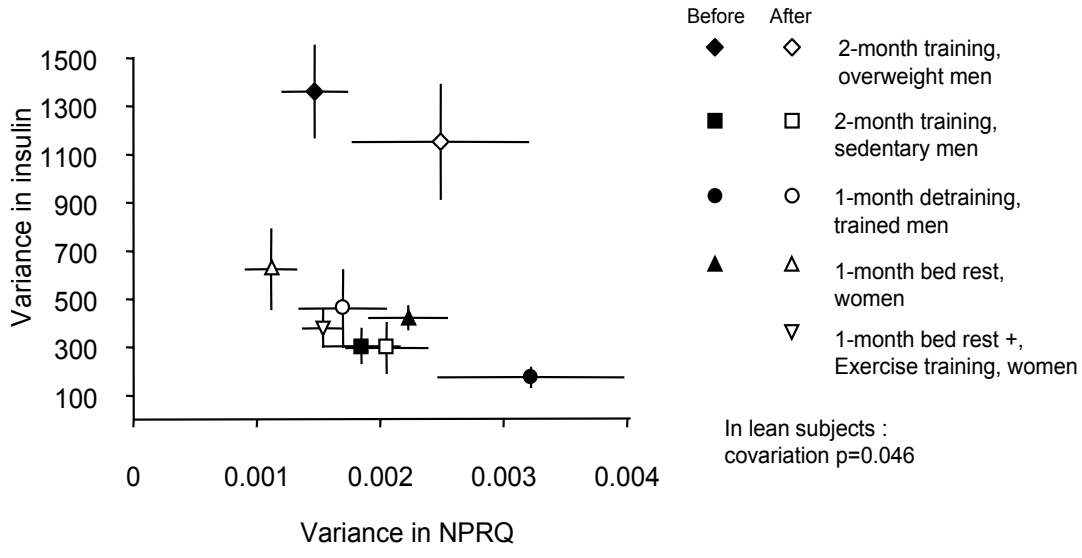


Figure 4: Metabolic flexibility is a function of the physical activity level. NPRQ and insulin variances were related along the continuum of physical activity (group x NPRQ variances p=0.046, n=35). Overweight sedentary subjects (n=12) showed the same slope of response but with an offset in the relationship at higher insulin concentrations.

The fact that the training performed at current recommendations only tend to, but not significantly, improve metabolic flexibility in both lean and overweight men may be simply due to the absence of associated body composition changes – as required by the protocol. Indeed, several studies reported that exercise in absence of body mass loss has a relatively modest effect [8] or no effect [23] on insulin sensitivity. Only few studies [7, 24] showed that exercise, without weight loss, increases insulin sensitivity in previously sedentary adults. In fact, we can argue that only one study [24] reported so far a beneficial effect of exercise *per se* on the response to insulin, since in the other study [7], body mass was effectively stable but body composition was modified by exercise with a reduction in fat mass and an increase in fat-free mass. Based on these evidence, we can assume that the combined resistive and aerobic exercise training performed during the bed rest study had a greater effect on metabolic flexibility than the exercise training at current recommendation not because of differences in the experimental conditions, they were similar (i.e. 36hours after the last bout of exercise), but because of a significant decrease in fat mass, indicating a negative energy balance. In support of that, the existence of a significant correlation between insulin sensitivity and adiposity is well established and several reports showed that combined exercise training and fat mass loss improves insulin sensitivity [25] and fasting fat oxidation [6]. Altogether, these results suggest that the prevention of metabolic inflexibility is related to the combined effect of exercise and the loss of fat mass rather than to the effect of exercise *per se*.

The effect of habitual physical activity on metabolic flexibility appears, however, convincing. By looking at the metabolic flexibility in our different groups as it is classically done, i.e. using the relationship between NPRQ and insulin changes, our major finding is the observation that habitual physical activity predicts metabolic flexibility in lean subjects independent of any overlapping effect of recent exercise and energy balance. Thus, active, lean individuals have a greater ability to switch from free fatty acid to glucose as the primary fuel between the fasting and the fed states than their inactive counterparts. Such relationship suggests that an increase in physical activity on chronic basis should be associated with an improvement of metabolic flexibility whereas a reduction in physical activity level should decrease it and that this is more effective in lean individuals. If enforced physical inactivity effectively impairs metabolic flexibility in lean active subjects, the effects of exercise training without weight loss on metabolic flexibility was not straightforward in sedentary individuals. The observation, however, that training affected metabolic flexibility along the overall same linear relationship than detraining suggests that the training duration and intensity used in the present study, i.e. on the basis of the current recommendations developed originally for cardiovascular risks, were not enough to significantly affect metabolic flexibility. Finally, the lack of effect of this exercise training on overweight insulin resistant subjects might simply reflect the fact that exercise training failed in this group to increase TEE.

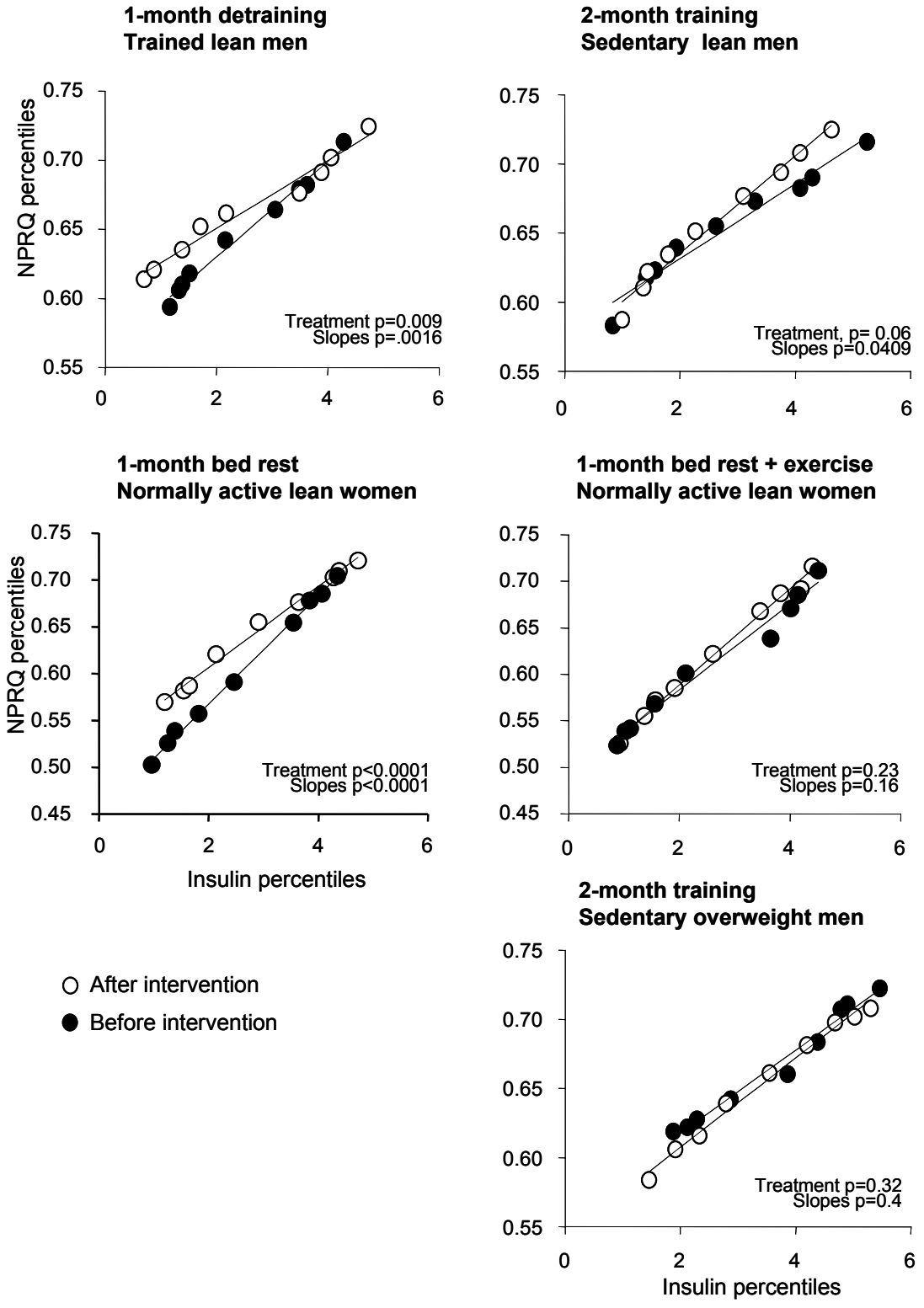


Figure 5: NPRQ and insulin percentiles/percentiles plots prior and after physical activity interventions for each subgroup of intervention. For each group, the 1st, 5th, 10th, the lower quartile, the median, the upper quartile, 90th, 95th and 99th percentiles for NPRQ and insulin are plotted. ANCOVA tests were used to compare slopes and are indicated in the figure.

One limit of the present study is the lack of standard measurement of insulin sensitivity through an euglycemic hyperinsulinemic clamp or an oral glucose tolerance test. We think however, that our conditions using standards meals mimic free-living conditions. However, to confirm our results in a controlled insulin stimulated condition, we reanalyzed data from a 7-day bed-rest study [26] conducted in 8 healthy men and 8 healthy women using an oral glucose tolerance test. We found a significant reduction in the slope of percentiles of NPRQ and insulin in both groups and lower variances of NPRQ were noted concomitantly to higher variances insulin, supporting our above observations (Figure 6).

In conclusion, by using two indexes that account for by the daily kinetics NPRQ and insulin responses to meal ingestion we showed that the habitual physical activity level predicts metabolic flexibility.

ACKNOWLEDGMENTS

We are grateful for the outstanding contributions of all the volunteers who committed themselves to the success of these studies. The LIPOX study was sponsored by a National Program in Human Nutrition (INSERM and INRA), the Fondation Coeur et Artères, the Fondation Louis D. and the Hôpitaux Universitaire de Strabsourg (API 2005 – HUS n°3493) (France). The WISE2005 study was funded by the CNES and the ESA. E. ANTOUN was funded by a presidential grant from the university Louis Pasteur Strasbourg (France) and by the Fondation pour la Recherche Médicale. The authors have no relevant conflict of interest to disclose.

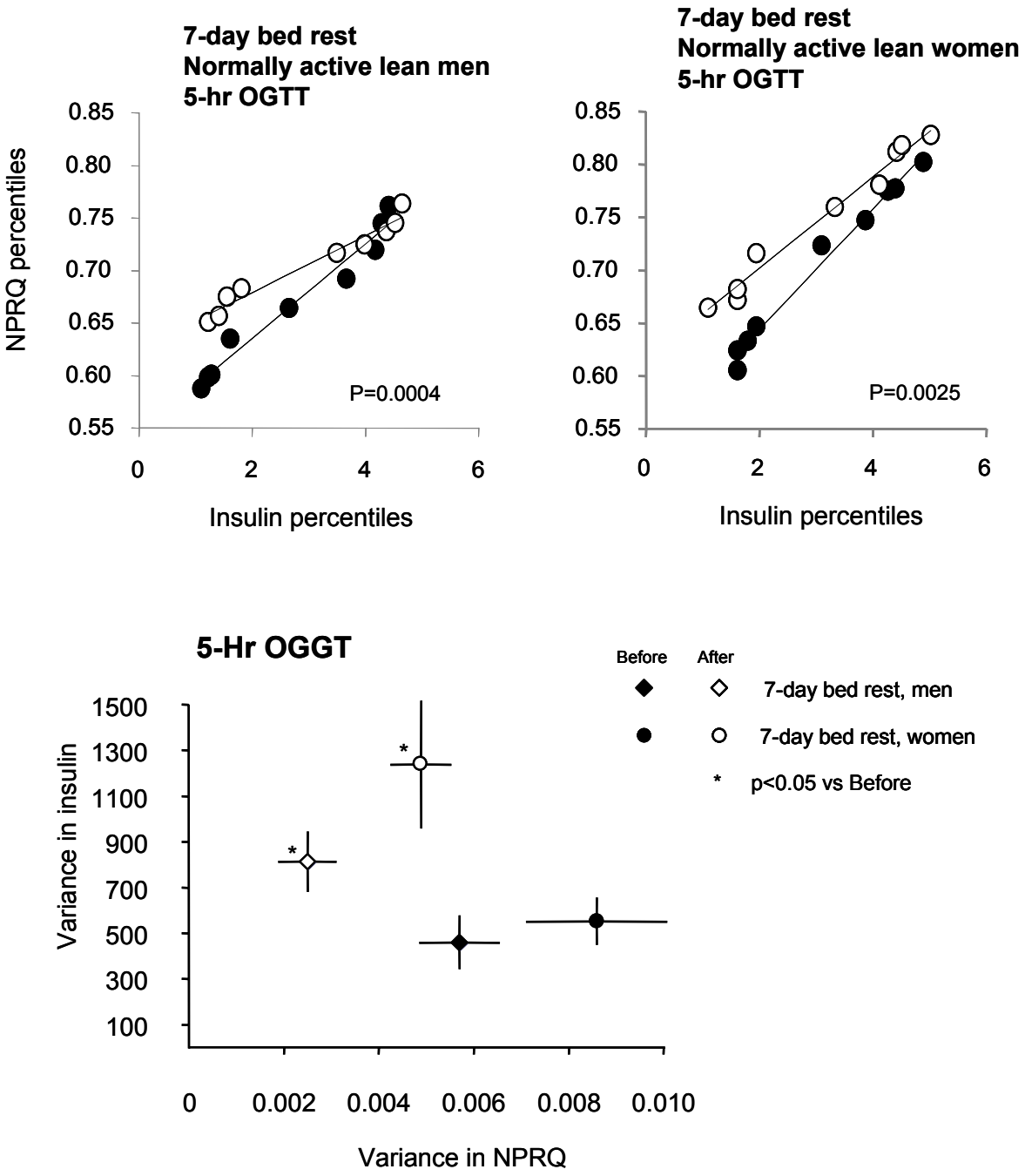
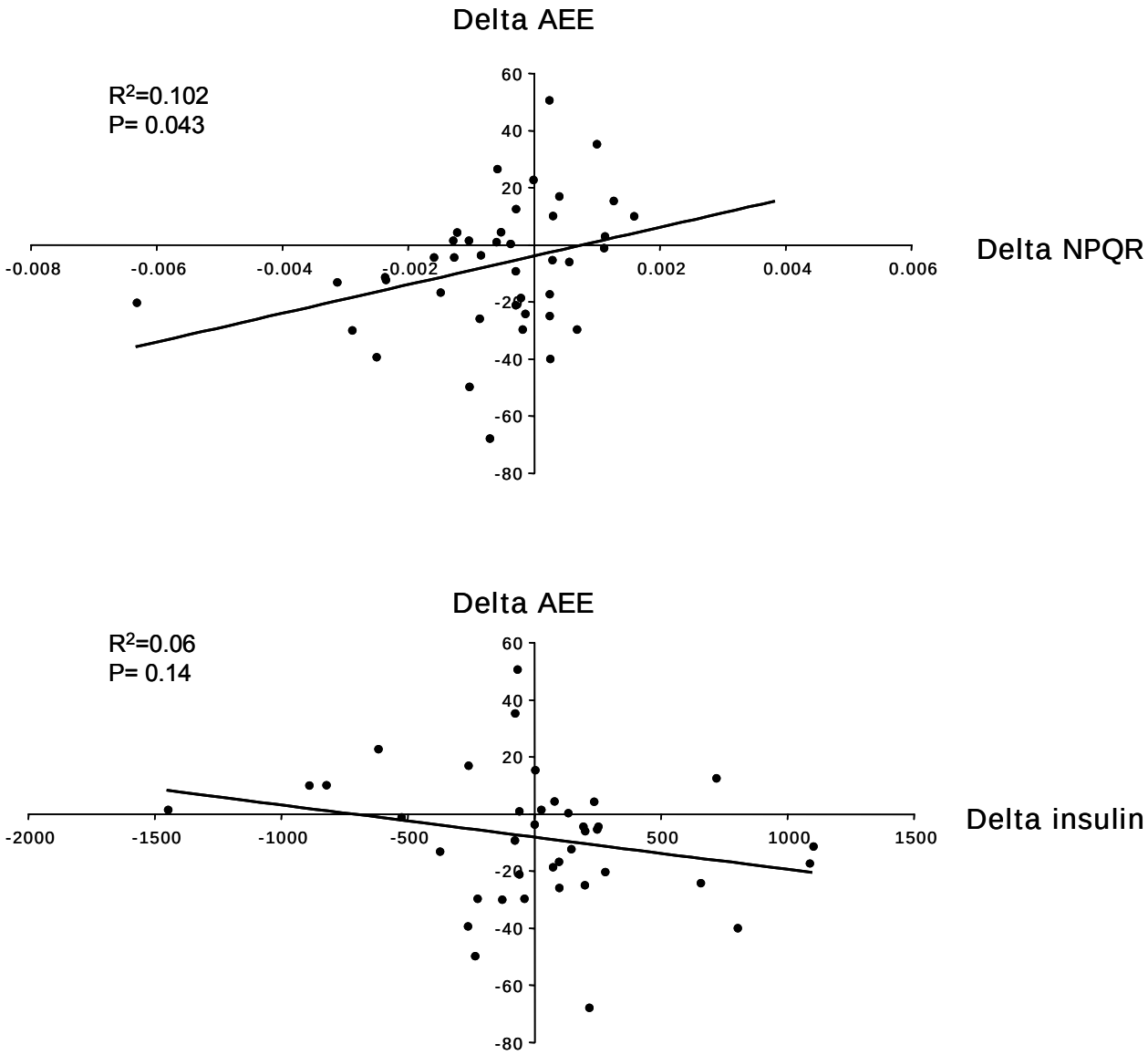


Figure 6: Metabolic flexibility represented by variances and percentile/percentile plots of NPRQ and insulin in 8 women and 8 men submitted to 7 days of bed rest from BLANC at al. [26] study over 5 hrs after an oral glucose tolerance test. Values represent the 1st, 5th, 10th, the lower quartile, the median, the upper quartile, 90th, 95th and 99th percentiles of NPRQ and insulin. Metabolic flexibility decreased in both men and women after 7 days of bed rest. * $p<0.05$ baseline (paired t-tests) in NPRQ variance. # $p<0.05$ of paired *t*-tests in insulin variance between before and after physical activity interventions.



Supplemental data: Figure 1: The changes in physical activity level correlates with the changes in NPRQ and insulin variance induced by the interventions. Physical activity explained 10% of the difference in NPRQ and insulin variance.

6. REFERENCES

[1] Corpeleijn E, Saris WH, Blaak EE (2009) Metabolic flexibility in the development of insulin resistance and type 2 diabetes: effects of lifestyle. Obes Rev 10: 178-193

[2] Blaak EE, van Aggel-Leijssen DP, Wagenmakers AJ, Saris WH, van Baak MA (2000) Impaired oxidation of plasma-derived fatty acids in type 2 diabetic subjects during moderate-intensity exercise. Diabetes 49: 2102-2107

[3] Blaak EE, Van Baak MA, Kemerink GJ, Pakbiers MT, Heidendal GA, Saris WH (1994) Beta-adrenergic stimulation of energy expenditure and forearm skeletal muscle metabolism in lean and obese men. The American journal of physiology 267: E306-315

[4] Smith SR, de Jonge L, Zachwieja JJ, *et al.* (2000) Fat and carbohydrate balances during adaptation to a high-fat. The American journal of clinical nutrition 71: 450-457

[5] Mensink M, Blaak EE, Vidal H, De Bruin TW, Glatz JF, Saris WH (2003) Lifestyle changes and lipid metabolism gene expression and protein content in skeletal muscle of subjects with impaired glucose tolerance. Diabetologia 46: 1082-1089

[6] Goodpaster BH, Katsiaras A, Kelley DE (2003) Enhanced fat oxidation through physical activity is associated with improvements in insulin sensitivity in obesity. Diabetes 52: 2191-2197

[7] Duncan GE, Perri MG, Theriaque DW, Hutson AD, Eckel RH, Stacpoole PW (2003) Exercise training, without weight loss, increases insulin sensitivity and postheparin plasma lipase activity in previously sedentary adults. Diabetes care 26: 557-562

[8] Venables MC, Jeukendrup AE (2008) Endurance training and obesity: effect on substrate metabolism and insulin sensitivity. Medicine and science in sports and exercise 40: 495-502

[9] Ross R (2003) Does exercise without weight loss improve insulin sensitivity? Diabetes care 26: 944-945

[10] Galgani JE, Moro C, Ravussin E (2008) Metabolic flexibility and insulin resistance. American journal of physiology 295: E1009-1017

[11] Heilbronn LK, Gregersen S, Shirkhedkar D, Hu D, Campbell LV (2007) Impaired fat oxidation after a single high-fat meal in insulin-sensitive nondiabetic individuals with a family history of type 2 diabetes. Diabetes 56: 2046-2053

[12] Corpeleijn E, Mensink M, Kooi ME, Roekaerts PM, Saris WH, Blaak EE (2008) Impaired skeletal muscle substrate oxidation in glucose-intolerant men improves after weight loss. Obesity (Silver Spring, Md 16: 1025-1032

[13] Haskell WL, Lee IM, Pate RR, *et al.* (2007) Physical activity and public health: updated recommendation for adults from the American College of Sports Medicine and the American Heart Association. Medicine and science in sports and exercise 39: 1423-1434

[14] Bergouignan A, Trudel G, Simon C, *et al.* (2009) Physical inactivity differentially alters dietary oleate and palmitate trafficking. Diabetes 58: 367-376

[15] Blanc S, Colligan AS, Trabulsi J, *et al.* (2002) Influence of delayed isotopic equilibration in urine on the accuracy of the (2)H(2)(18)O method in the elderly. J Appl Physiol 92: 1036-1044

[16] Stein TP, Wade CE (2005) Metabolic consequences of muscle disuse atrophy. The Journal of nutrition 135: 1824S-1828S

[17] Olsen RH, Krogh-Madsen R, Thomsen C, Booth FW, Pedersen BK (2008) Metabolic responses to reduced daily steps in healthy nonexercising men. Jama 299: 1261-1263

[18] Smith SR, de Jonge L, Zachwieja JJ, *et al.* (2000) Concurrent physical activity increases fat oxidation during the shift to a high-fat diet. The American journal of clinical nutrition 72: 131-138

[19] Hansen KC, Zhang Z, Gomez T, Adams AK, Schoeller DA (2007) Exercise increases the proportion of fat utilization during short-term consumption of a high-fat diet. The American journal of clinical nutrition 85: 109-116

[20] Schenk S, Horowitz JF (2007) Acute exercise increases triglyceride synthesis in skeletal muscle and prevents fatty acid-induced insulin resistance. The Journal of clinical investigation 117: 1690-1698

[21] Perseghin G, Price TB, Petersen KF, *et al.* (1996) Increased glucose transport-phosphorylation and muscle glycogen synthesis after exercise training in insulin-resistant subjects. The New England journal of medicine 335: 1357-1362

[22] Kang J, Robertson RJ, Hagberg JM, *et al.* (1996) Effect of exercise intensity on glucose and insulin metabolism in obese individuals and obese NIDDM patients. Diabetes care 19: 341-349

[23] Dekker MJ, Lee S, Hudson R, *et al.* (2007) An exercise intervention without weight loss decreases circulating interleukin-6 in lean and obese men with and without type 2 diabetes mellitus. Metabolism: clinical and experimental 56: 332-338

[24] Houmard JA, Shinebarger MH, Dolan PL, *et al.* (1993) Exercise training increases GLUT-4 protein concentration in previously sedentary middle-aged men. The American journal of physiology 264: E896-901

[25] Schenk S, Harber MP, Shrivastava CR, Burant CF, Horowitz JF (2009) Improved insulin sensitivity after weight loss and exercise training is mediated by a reduction in plasma fatty acid mobilization, not enhanced oxidative capacity. The Journal of physiology

[26] Blanc S, Normand S, Pachiaudi C, Fortrat JO, Laville M, Gharib C (2000) Fuel homeostasis during physical inactivity induced by bed rest. The Journal of clinical endocrinology and metabolism 85: 2223-2233

CHAPITRE 7

The [1-^{13}C]acetate recovery factor to correct tracer-derived dietary fat oxidation is lower in overweight insulin-resistant subjects

Edwina Antoun, Iman Momken, Audrey Bergouignan, Clément Villars, Carine Platat, Dale A. Schoeller, Stéphane Blanc, et Chantal Simon.

e-SPEN, the European e-Journal of Clinical Nutrition and Metabolism 5 (2010) e173 –e179

Résumé

Introduction

L'oxydation des lipides est classiquement mesurée par les isotopes stables dans les études nutritionnelles. Le taux d'excrétion du $^{13}CO_2$ à la suite d'une perfusion ou d'une ingestion d'acides gras marqués en ^{13}C permet de déterminer la quantité de traceurs oxydés. Toutefois, une partie de ce ^{13}C est séquestrée dans l'organisme dans le pool des bicarbonates et au niveau du cycle tricarboxylique, ce qui induit une sous-estimation des taux d'oxydation des lipides. Comme l'acétate est converti en acétyl-CoA et entre directement dans le cycle tricarboxylique, un facteur acétate est généralement utilisé pour corriger la séquestration de l'isotope marqué. En conditions de perfusion, il a été montré que ce facteur, bien que reproductible, présentait une importante variation interindividuelle. Il faut donc le déterminer pour chaque sujet par des expériences en double avec des conditions similaires à quelques jours d'intervalle, afin de déterminer l'oxydation lipidique et le facteur de correction acétate pour chaque sujet, vu que l'usage des isotopes radioactifs est limité.

En revanche, en conditions d'ingestion, il a été montré qu'un facteur de correction acétate exogène égal à 50,6 ± 5,4% peut être appliqué et ne modifie pas la moyenne et la variabilité des valeurs d'oxydation exogène aussi bien au repos qu'après modifications de l'activité physique chez les sujets normopondérés. Ceci permet de s'affranchir du protocole pour déterminer le facteur acétate de manière individuelle et représente un compromis scientifique acceptable entre le coût des expériences, la lourdeur des protocoles et l'exactitude de la mesure où les tests durent généralement une dizaine d'heures après l'ingestion des traceurs du fait des processus de digestion et d'acheminement des lipides. Cependant, l'application de ce facteur chez des sujets insulino-resistants en surpoids n'a pas encore été vérifiée.

Objectifs

En combinant les données provenant de 5 études distinctes (n=70 sujets normopondérés et n=11 sujets insulino-résistants en surpoids) sur l'oxydation des acides gras exogènes, nous nous proposons dans cette étude de :

- Déterminer si le facteur de correction acétate exogène diffère entre les sujets normopondérés et les sujets insulino-résistants et, le cas échéant, d'enquêter sur les facteurs qui pourraient expliquer cette différence.

- Déterminer si le facteur de correction acétate exogène appliqué chez les sujets normopondérés peut être appliqué chez des sujets insulino-résistants en surpoids afin de corriger l'oxydation des acides gras alimentaires.

- Déterminer l'effet d'une augmentation de l'activité physique sur le facteur de correction acétate exogène chez les sujets insulino-résistants en surpoids.

Matériel et Méthodes

Le facteur de correction [1-^{13}C] acétate exogène et les récupérations d'acides gras marqués en [1-^{13}C] ont été évaluées avant et après les interventions sur l'activité physique entraînement/désentraînement chez des sujets normopondérés et en surpoids. Les sujets en surpoids ont été entrainés à un rythme de 4 séances d'exercice physique à 50% VO_2max par semaine durant deux mois.

Résultats

Le facteur de correction [1-^{13}C] acétate exogène chez les sujets insulino-résistants en surpoids est inférieur à celui des sujets normopondérés (45.3 ± 1,5% contre 50,6 ± 0,6%; P = 0,002) avec une différence de 5,2% de la dose de récupération qui représente 11,6% du facteur de correction [1-^{13}C] acétate exogène chez les sujets insulino-résistants en surpoids. Les interventions sur le niveau d'activité physique n'affectent pas ce facteur de correction, malgré une augmentation du coefficient de variabilité interindividuelle de 17,0% qui est 1,4 fois plus élevée que ce qu'on a observé avant l'intervention physique. Nous avons pu mettre en évidence que le facteur de correction [1-^{13}C] acétate exogène est corrélé au pourcentage de masse grasse (r^2 = 0.10; P = 0.005) et l'insulinémie à jeun représentée par le ratio insuline sur glucose (r^2 = 0.08; P = 0.02). Le fait d'appliquer le facteur de correction [1-^{13}C] acétate exogène des sujets normopondérés induit un taux d'erreur moyen de 11,5% (P = 0,006) de l'oxydation des acides gras des sujets en surpoids.

Discussion

Cette étude montre que les sujets insulino-résistants en surpoids présentent un facteur de correction acétate moyen inférieur à celui des sujets normopondérés. La réduction de ce facteur chez les sujets en surpoids est probablement due à un accroissement des voies métaboliques où le carbone marqué peut être perdu en raison soit d'une plus grande dispersion dans le pool de bicarbonate ou perdu par un plus grand transfert isotopique à travers les réactions d'échange isotopique dans le cycle tricarboxylique ou cycle de Krebs. Nous proposons aussi, que cette réduction est probablement due en partie à une réduction du flux du cycle tricarboxylique, le point final et commun du catabolisme des glucides, lipides et les acides aminés. Un facteur de correction acétate moyen obtenu pour les sujets normopondérés ne peut pas être appliqué chez des sujets avec un excès de poids pour calculer l'oxydation des graisses alimentaires. Avant que d'autres études soient menées sur une grande échelle de degré d'obésité et d'insulino-résistance, nous recommandons de mesurer un facteur de correction acétate individuel chez les sujets présentant des troubles métaboliques.

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Original Article

The [1-^{13}C]acetate recovery factor to correct tracer-derived dietary fat oxidation is lower in overweight insulin-resistant subjects

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SUMMARY

Background&aims: An acetate recovery factor (ARF) is utilized to correct tracer-derived fat oxidation when ^{13}C is used. We showed that when ^{13}C labelled fatty acid are given orally, dietary fat oxidation can be accurately corrected by using an averaged dietary ARF (dARF) derived from 56 lean healthy subjects, instead of individual dARF. The extent to which this factor is valid in overweight insulin resistant subjects is unknown.

Methods: [1-^{13}C]dARF and [1-^{13}C]fatty acid recoveries were assessed before and after physical activity/inactivity interventions in overweight insulin-resistant ($n = 11$) and lean subjects ($n = 70$) in five studies herein compiled.

Results: Overweight dARF was lower compared to lean subjects (45.3 ± 1.5% vs. 50.6 ± 0.6%; $P = 0.002$). Physical activity intervention did not impact dARF. dARF correlated negatively with %body fat ($r^2 = 0.10$; $P = 0.005$) and fasting insulin to glucose ratio ($r^2 = 0.08$; $P = 0.02$). Applying the lean average [1-^{13}C] dARF induced an 11.5% ($P = 0.006$) average error in fatty acid oxidation rate.

Conclusions: Overweight insulin resistant subjects have lower dARF than lean individuals. An average dARF derived from lean subjects cannot be applied in overweight subjects to calculate dietary fat oxidation. We recommend that individual dARF are measured in subjects with metabolic disorders.

1. Introduction

The acetate recovery factor (ARF) was proposed to correct tracer-derived (^{13}C)-fat oxidation for the loss of label to sequestration within the bicarbonate pool and small metabolites that exchange with the tricarboxylic acid cycle intermediates (TCA cycle).[1–3] This correction can be cumbersome and costly as it requires two separate tests performed under identical conditions: one test with labelled acetate and another test with labelled fatty acid,[4] unless radio-labelled acetate can be used along with the stable isotope labelled fatty acid. Given these concerns, there has been interest in the use of a constant ARF under different study conditions and for subjects with different metabolic conditions.

After compiling data on 69 subjects, Schrauwen et al.[4] found that the infused ARF (iARF) is not constant between subjects. At rest, 37% of the variance of (iARF) was accounted for by resting metabolic rate (RMR) adjusted for fat free mass (FFM), percentage body fat and fasting non-protein respiratory quotient (NPRQ). This increased to 69% during exercise. Although iARF was reproducible within individuals, the authors concluded that it should be measured in every subject because of the between individual variance. Under oral administration conditions, the dietary acetate recovery factor (dARF) measured in lean subjects presented similar inter-individual CV (10.6%)[5] as was reported for iARF (12.0%),[4] however, no major anthropological or physiological determinants for dARF were observed. This difference between infused and oral ARF may be due to a buffering effect of the digestion processes and/or a longer post-dose measurement period, both reducing inter-

Abbreviations: dARF, dietary acetate recovery factor; iARF, infused acetate recovery factor; RMR, resting metabolic rate; NPRQ, fasting non-protein respiratory quotient; FM, fat mass; LIPOX, lipid oxidation study.

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individual differences. These conclusions were not affected by chronic interventions on physical activity. We further showed that using individual dARF, or an average dARF of 51% derived from 56 healthy lean men and women, did not affect the variability of the derived dietary fatty acid oxidation rates. Contrary to the iARF, individually measured dARF values, therefore, did not seem required Bergouignan et al.[5]

Metabolic disorders appeared to further affect ARF. In fasting conditions and after controlled diet 3 days prior the tests, Schrauwen et al.[4] showed that after 1 h of cycling exercise at 40–50% of maximal oxygen uptake, iARF was lower in obese diabetic (59.2 ± 2.1%) and obese (69.9 ± 1.7%) subjects than in lean (78.5 ± 2.1%) subjects. Conversely no difference was noted at rest.[4] These findings were interpreted, in a recent re-evaluating study by Schrauwen et al.[6] as a reduction in TCA cycle flux, reflecting mitochondrial dysfunction in obese type 2 diabetes mellitus patients. This emphasizes that special attention should be placed on iARF when measuring plasma-fatty acid oxidation. So far, whether or not dARF differs between lean and overweight insulin resistant subjects, has not been investigated yet.

In the present study we combined data from five of our studies and included data from 11 overweight insulin resistant and 70 lean subjects to determine 1) whether dARF differed between lean and overweight individuals and, if any, to investigate the factors that might explain such difference 2) whether an average dARF can be applied to correct dietary fatty acid oxidation in overweight subjects, and 3) the effect of two months of exercise training at 50%VO2max on dARF realized by overweight subjects four times per week.

2. Materials and methods

2.1. Study design

We compiled dARF data from five studies performed in our laboratories: Votruba et al.,[7] Votruba et al.,[8] which were performed at the University of Wisconsin-Madison (US) and Bergouignan et al.,[9] Bergouignan et al.,[10] and the Strasbourg Lipid Oxidation study (LIPOX) which was performed in France. Votruba et al.[7] was a study designed to validate deuterium-labelled fatty acids for the measurement of dietary fat oxidation in five lean women and two lean men at rest. The second Votruba et al.[8] study investigated the effect of prior acute exercise (85% of $VO_{2\ peak}$) on dietary fat utilization from seven lean women. Bergouignan et al.[9] and Bergouignan et al.[10] were longitudinal studies aimed at determining the effect of chronic physical inactivity interventions (2 and 3 months of bed rest, respectively), with and without physical exercise countermeasures, on dietary lipid oxidation. Details on the exercise countermeasures can be found elsewhere.[9,10] The LIPOX study was completed in 2009. It investigated the effect of exercise training in sedentary lean and overweight men and the effect of detraining in lean active men on dietary lipid partitioning and took place at the University Hospital of Strasbourg (France). The inclusion criterions in LIPOX study was a body mass index (BMI) of $27 \leq BMI \leq 35$ kg/m^2 and a BMI of $20 \leq BMI \leq 25$ kg/m^2 for overweight and lean subjects respectively and the habitual physical activity assessed from their physical activity level (PAL) defined as the ratio of total energy expenditure to resting metabolic rate (RMR).[11] Exercise training for lean and overweight men was conducted in sedentary subjects with an estimated $PAL \leq 1.5$. Detraining was performed on trained subjects with a estimated $PAL \geq 1.7$.[12] Usual PAL was assessed using MOSPA-Q questionnaire, whose validity and reliability has been reported elsewhere.[13] Physical activity was further confirmed by a triaxial accelerometer device[14] (RT3, Stayhealthy, Monrovia, CA, USA) and a combined heart rate and motion sensor[15] (Actiheart, Cambridge Neurotechnology Ltd). Detraining consisted of 1 mo of voluntary reduction in structured and spontaneous physical activities. Aerobic training consisted of four 60-min sessions per week at 50% $VO_{2\ peak}$. The peak oxygen uptake $VO_{2\ peak}$ was determined by means of incremental exercise test[16] to exhaustion performed in an upright position on an electronically braked cycle ergometer (Medifit 1000S, Belgium). LIPOX was approved by the Institutional Review Board of Alsace I (France). For all but the validation studies, dietary acetate and lipids recoveries were measured before and after the intervention on physical activities.

2.2. Subjects

Data from 70 lean participants (28 women and 42 men) and 11 overweight men were compiled. Of these, 68 subjects were studied before and after physical activity interventions. From these five studies, we collected the following parameters: body weight and composition, height, RMR, $VO_{2\ peak}$, and NPRQ. Fasting insulin and glucose were also measured in all but the Votruba's et al. studies.[7,8] The initial subject characteristics are shown in Table 1. Some data are missing because of differences in the protocols objectives.

2.3. Acetate recovery factor and dietary fatty acid oxidation protocols

dARF and the dietary fatty acid oxidation were measured in similar experimental protocols (Table 2) in all studies included here and have been described in details elsewhere.[7–10] Thirty-six hours before the test, all subjects were asked not to participate in any structured physical activities and were provided with standardized microwaveable meals at breakfast, lunch, and dinner. After an overnight fast, the subjects were weighed, and baseline breath and urine samples were collected. RMR, NPRQ and VCO_2 were measured for 1 h by indirect calorimetry using a Deltatrac metabolic cart (Deltatrac II; GE) (LIPOX and Bergouignan et al.[9,10] studies) or a room calorimeter (respiratory chamber of the University of Wisconsin-General Clinical Research Center) (Votruba et al. studies[7,8]) and then hourly throughout the test. Afterwards, a standard breakfast was provided to the participants, which included a homogenized liquid meal labelled with one of the following: 2 mg/kg (lean subjects) or 3 mg/kg (overweight subjects) [1-^{13}C]acetate [>99% enriched; Cambridge Isotope Laboratories (CIL), Andover, MA], 10 mg/kg or 15 mg/kg (LIPOX study) [1-^{13}C] oleate (>99% enriched; CIL), or 10 mg/kg [1-^{13}C]palmitate (>98% enriched; CIL), depending on the test day and the study. Breath samples were collected before the meal, every 30 min for the first 2 h, and then hourly for 7–10 h after the meal according to the study. Tests were performed in exact similar experimental conditions in the lean and overweight individuals.

2.4. Body composition

Body composition was assessed by the $H_2^{18}O$ isotope dilution method, as routinely used in our laboratories, in the Votruba et al.,[7] Votruba et al.[8] and LIPOX study and by dual energy X-ray absorptiometry (DXA, QDR 4500 W scanner using the version software 11.2, Hologic France) for the Bergouignan et al.[9] and Bergouignan et al.[10] bed rests. During the bed rests, we observed good agreement between isotope dilution and DXA. FFM measured by ^{2}H and ^{18}O dilutions indeed agreed within 2.5 ± 2.6% with FFM measured by DXA ($y = 1.025\times$, $R^2 = 0.95$, $p < 0.0001$ with a non significant intercept of −0.3 kg removed from the equation).

Table 1
Characteristics of the participants of each study.

	Votruba et al.,[8]	Votruba et al.,[7]		Bergouignan et al.[9]	Bergouignan et al.[10]	LIPOX lean subjects	LIPOX overweight subjects	All Studies lean subjects	
	F	F	M	M	F	M	M	F	M
n	7	5	2	15	16	25	11	28	42
Age, yr	23 ± 3	25 ± 3	30 ± 7	32 ± 4	34 ± 4	26 ± 7	30 ± 6	29 ± 6	29 ± 7
BMI	21.4 ± 1.7	22.6 ± 0.9	26.1 ± 0.1	23.4 ± 1.6	21.5 ± 1.3	22.5 ± 1.7	30.1 ± 1.7	21.7 ± 1.4	23.0 ± 1.8
Body mass, kg	61.1 ± 5.5	59.2 ± 4.2	85.1 ± 6.4	71.0 ± 5.9	57.0 ± 5.4	73.1 ± 8.5	99.0 ± 9.2	58.4 ± 5.4	73.0 ± 8.0
Fat mass									
kg	10.9 ± 3.0	12.4 ± 2.9	19.4 ± 2.9	12.4 ± 3.6	14.7 ± 3.3	14.9 ± 5.6	31.3 ± 5.5	13.3 ± 3.5	14.2 ± 5.0
%	17.9 ± 4.9	21.3 ± 6.2	22.8 ± 1.6	17.3 ± 4.1	25.7 ± 5.1	19.8 ± 6.1	31.5 ± 3.6	22.9 ± 6.1	19.0 ± 5.4
Fat free mass, kg	50.2 ± 5.7	46.8 ± 7	65.6 ± 3.6	58.6 ± 4.2	42.3 ± 4.7	59.0 ± 6.4	67.6 ± 5.5	45.1 ± 6.2	59.2 ± 5.7
RMR, MJ/d	6.6 ± 0.5	5.8 ± 0.5	7.9 ± 0.1	6.4 ± 0.4	6.0 ± 0.5	6.5 ± 0.5	7.5 ± 0.7	6.1 ± 0.6	6.5 ± 0.5
$VO_{2\ peak}$									
l/min	2.3 ± 0.2			3.0 ± 0.3	2.0 ± 0.4	3.3 ± 0.6	3.1 ± 0.6	2.1 ± 0.4	3.2 ± 0.5
ml kg^{-1} min^{-1}	37.5 ± 2.3			42.5 ± 4.5	34.8 ± 6.1	44.6 ± 7.1	31.4 ± 4.6	35.6 ± 5.3	43.8 ± 6.2
NPRQ	0.877 ± 0.018			0.879 ± 0.057	0.747 ± 0.037	0.868 ± 0.041	0.892 ± 0.024	0.787 ± 0.069	0.872 ± 0.047
Fasting Insulin mU/l				3.3 ± 1.3	4.5 ± 1.3	4.4 ± 1.8	10.5 ± 3.8	4.5 ± 1.3	3.9 ± 1.6
Fasting Insulin/glucose				3.9 ± 1.4	5.3 ± 1.6	5.2 ± 2.1	11.7 ± 4.7	5.3 ± 1.6	4.6 ± 1.9
^{13}C-dARF	51.8 ± 7.3	51.6 ± 6.6	50.3 ± 6.4	50.4 ± 6.1	49.9 ± 2.5	50.6 ± 5.2	45.3 ± 5	50.7 ± 4.7	50.5 ± 5.4

Values are means ± SD. LIPOX, Lipid Oxidation study; F, females; M, males; BMI, body mass index; RMR, resting metabolic rate; VO_2peak, peak oxygen consumption; NPRQ, non-protein respiratory quotient.

2.5. Sample analysis

The details on the isotope ratio mass spectrometry analyses can be found in previous studies from our laboratories.[7,8] Briefly, the ratio of $^{13}CO_2$ to $^{12}CO_2$ in breath from the Votruba et al.[7] and Votruba et al.[8] studies was measured and analyzed in triplicates on a Delta-S isotope ratio mass spectrometer (Finnigan MAT, San Jose, CA) using a continuous flow inlet system developed at the Department of Nutritional Sciences at the University of Wisconsin-Madison.[17] For LIPOX and the two bed rest studies, a continuous flow system connected to GV Instruments Isoprime was used. The ^{18}O enrichments in urinary water were measured by CO_2 equilibration in quadruplicates in a continuous-flow isotope ratio mass spectrometer consisting of the above-cited Finnigan MAT Delta-S [Votruba et al.[7] and Votruba et al.[8]]. For the Bergouignan et al.[10] and LIPOX studies, $H_2^{18}O$ was reduced to CO by carbon reduction at 1400 °C in an elemental analyzer (Flash HT; ThermoFisher Germany) coupled to a Delta-V isotope ratio mass spectrometer in Strasbourg, and isotopic abundances were measured in quintuplicate. All enrichments were expressed against International Atomic Energy Agency standards.

2.6. Calculations

Recoveries of [1-^{13}C]acetate, [1-^{13}C]oleic acid, or [1-^{13}C]palmitic acid were calculated as the instantaneous recovery of ^{13}C in expired CO_2 per hour, expressed as a percentage of the dose and as previously described.[7–10] Cumulative recovery was calculated using the trapezoid rule. Cumulated acetate recoveries were further extrapolated to infinity.[7–10] Corrected fatty acid oxidation rates were calculated by dividing the cumulated fatty acid recovery by the cumulated acetate recovery extrapolated to infinity times 100.

2.7. Data organization and statistical analysis

We first considered the results from the subjects in resting conditions, i.e., the control (rest) tests from all the studies. A one way ANOVA was used on the studies focusing on lean subjects to test whether the dARF differs according to sex and study. Since no differences in dARF were noted between studies ($F = 0.18$, $P = 0.94$) and between sex ($F = 0.02$, $P = 0.89$), we combined data from all lean subjects. During all the following analyses, two groups were accordingly compared: lean subjects and LIPOX overweight subjects. First, the kinetics of the instantaneous and cumulative acetate percentage recoveries were analyzed by a MANOVA with time as repeated measurement, subject as random effect and group (overweight vs. lean) as main effect. The between subject CV was calculated for each group. A *t*-test was used to test if dARF differed between overweight and lean subjects and simple linear regression analyses performed on all lean and overweight subjects were used to test if physiological determinants explain the inter-individual variability of dARF among subjects. We then investigated the influence of different methods to calculate the dARF on final fatty acid oxidation rates in the overweight group, as previously done in lean subjects.[5] The objective was to study the variability in estimated dietary fat oxidation when individual, overweight subjects' mean or lean subjects' mean dARFs which was similar to that found by Bergouignan et al.[5] were used. The corresponding corrected fatty

Table 2
Procedure description of the studies.

	Fasting duration	Rest during procedure	Nutrition	Total duration	Prior days conditions
Lipox	Overnight fast	Quietly in bed rest position	Labelled acetate in breakfast at time 0, lunch at time 4- hour post-dose.	8 h	3 Days of standarized meals with no structured physical activity for 36 h
Bergouignan[9]	Overnight fast	Quietly in bed rest position	Labelled acetate in breakfast at time 0, lunch at time 5- hours post-dose.	7 h	3 Days of standarized meals with no structured physical activity for 36 h
Bergouignan[10]	Overnight fast	Quietly in bed rest position	Labelled acetate in breakfast at time 0, lunch at time 5- hour post-dose.	10 h	3 Days of standarized meals with no structured physical activity for 36 h
Votruba[7]	Overnight fast	Quietly sitting upright	Labelled acetate in breakfast at time 0, lunch at time 4- hour post-dose.	8 h	2 Days of standarized meals with no structured physical activity
Votruba[8]	Overnight fast	Quietly sitting upright	Labelled acetate in breakfast at time 0, lunch at time 4- hour post-dose.	10 h	2 days of standarized meals with no structured physical activity

acid oxidation rates were identified as ^{13}C-FA$_{OVERWEIGHT}$, ^{13}C-FA$_{OVERWEIGHTmean}$ and ^{13}C-FA$_{LEANmean}$ respectively. The differences in calculated oxidation rates were compared using repeated measures ANOVA followed by the Bonferroni post hoc test.

In a third analysis, we determined the effect of physical activity interventions on the dARF and on the estimated dietary lipid oxidation rates. The analyses included subjects from Bergouignan et al.,[9] Bergouignan et al.,[10] LIPOX and Votruba et al.[8] studies. Dietary lipid percentage recoveries corrected by individual dARF were not available for the Votruba et al.[8] study after physical activity intervention. The effect of the physical activity intervention between groups was determined by a multiple analysis of variance (MANOVA) with time as repeated measure (before vs. after intervention), subject as random effects and group (overweight vs. lean) as main effect and by paired *t*-test within the same group. Because we did not observe an effect of the physical activity modifications on the dARF ($t = 0.40$; $P = 0.70$) in overweight subjects, we calculated the within-subject CV in the overweight group.

All data are represented as mean ± SEM, and the level of significance was set as $P < 0.05$. All statistics were performed using SAS Enterprise Guide 3.0 (SAS Institute Inc., Cary, NC, USA).

3. Results

3.1. Lean vs overweight dARF at rest prior intervention on physical activity

Fig. 1A depicts the instantaneous and cumulative acetate percentage dose recoveries for the overweight and lean subjects. We observed a significant difference in the kinetics of both instantaneous (group: overweight vs. lean: $F = 10.41$, $P = 0.001$; group × time interaction: $F = 1.40$, $P = 0.185$) and cumulative (group: $F = 5.99$, $P = 0.014$; group × time interaction: $F = 3.97$, $P < 0.0001$) acetate percentage recoveries between the overweight and the lean groups.

For lean subjects, the average dARF extrapolated to infinite was 50.6 ± 0.6% ($n = 70$) at rest, ranging between 39.5 and 63.8% with a CV of 10.2%. In overweight subjects, the average dARF was 45.3 ± 1.5% ($n = 11$), ranging between 36.7 and 50.2% with a CV of 11.0%. dARF differed significantly between overweight and lean subjects ($F = 10.0$, $P = 0.002$) with a difference of 5.2% dose recovery representing 11.6% of the overweight mean dARF (Fig. 1B). Correlation analyses showed that the variability of dARF was not explained by age, BMI, NPRQ, FFM, RMR, RMR adjusted for FFM, $VO_{2\ peak}$ nor $VO_{2\ peak}$ normalized for body mass (Table 3). dARF correlated negatively with the percentage of fat mass (Table 3). We also observed a significant and negative correlation between dARF and both fasting insulin and fasting insulin to glucose ratio (Table 3).

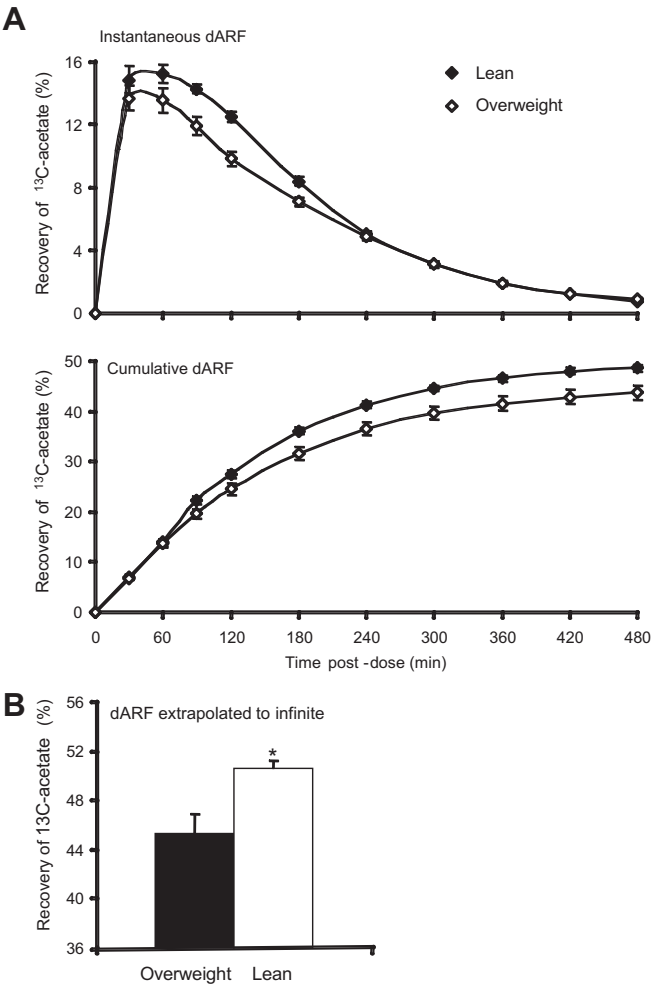

Fig. 1. Kinetics of instantaneous and cumulative (1A) acetate percentage dose recoveries in overweight and lean groups. Multiple ANOVA performed until 420 min post-dose, the last common point between all the studies, showed a significant difference in the kinetics of both instantaneous (group: overweight vs. lean: $F = 10.41$, $P = 0.001$; group × time interaction: $F = 1.40$, $P = 0.185$) and cumulative (group: $F = 5.99$, $P = 0.014$; group × time interaction: $F = 3.97$, $P < 0.0001$) acetate percentage recoveries between the overweight and the lean groups. (1B) represents the dietary acetate percentage between overweight and lean subjects before and after physical activity interventions on the physical activity. Dietary acetate recovery factor was significantly different between overweight and lean subjects (overweight: 45.3 ± 1.5; lean 50.6 ± 0.6; $F = 10.01$, $P = 0.002$).

3.2. Effect of overweight individual, overweight mean or lean mean dARF use on the dietary fat oxidation and its variability in the overweight subjects (Fig. 2)

The mean dARF corrected dietary fatty acid oxidation was 38.2 ± 1.4%, 37.7 ± 1.8% and 33.8 ± 1.6% dose recovery for ^{13}C-FA$_{OVERWEIGHT}$, ^{13}C-FA$_{OVERWEIGHTmean}$ and ^{13}C-FA$_{LEANmean}$, respectively. The CV was 11.7% for the ^{13}C-FA$_{OVERWEIGHT}$ but 15.4% for both ^{13}C-FA$_{OVERWEIGHTmean}$ and ^{13}C-FA$_{LEANmean}$. Applying the lean subjects' dARF to correct ^{13}C-FA oxidation generated a significant 12% underestimation of dietary fatty acid oxidation in overweight participants. Post hoc tests indeed showed a significant difference between ^{13}C-FA$_{OVERWEIGHT}$ vs. ^{13}C-FA$_{LEANmean}$ (−4.4 ± 1.2%; $t = -4.2$; $P = 0.001$) and ^{13}C-FA$_{OVERWEIGHTmean}$ vs. ^{13}C-FA$_{LEANmean}$ (−3.9 ± 0.2%; $t = -3.71$; $P = 0.002$). Although the variability in ^{13}C-FA oxidation increased 1.3 fold when using the mean overweight dARF, no significant difference between ^{13}C-FA$_{OVERWEIGHT}$ and ^{13}C-FA$_{OVERWEIGHTmean}$ calculations was noted (−0.5 ± 1.3%; $t = -0.5$; $P = 0.7$).

3.3. Effect of chronic interventions on physical activity on dARF and the related dietary fat oxidation rates

The effect of physical activity interventions on dARF and the related dietary oxidation rates are summarized in Table 4. In overweight subjects, the post training average dARF was 46.4 ± 2.4%, ranging from 31.5% to 60.1%. Physical activity intervention had no effect in overweight subjects dARF with an average difference between before and after exercise training of 1.1 ± 2.7% ($t = 0.4$, $P = 0.7$). The resulting within-subject CV was 6.5%. The difference in dARF between overweight and lean subjects remained significant ($F = 12.0$, $P = 0.001$, Fig. 3). The between subject CV was 17.0% after exercise training which is 1.4-fold higher than what was observed prior training. The ^{13}C-FA percentage recoveries corrected by either

Table 3
Determinants of dARF.

	r^2	*P* value
Age, yr	0.02	0.25
BMI, kg/m^2	0.03	0.10
Fat mass, kg	0.08	0.01*
Fat mass, %	0.10	0.00*
RMR, MJ/d	0.00	0.89
RMRFFM, MJ/d	0.00	0.46
NPQR	0.02	0.22
VO2max, L/min	0.00	0.68
VO2max, mL/kg/min	0.03	0.14
Fasting insulin, mU/L[a]	0.06	0.04*
Fasting insulin/glucose[a]	0.08	0.02*
Fasting NEFA[b]	0.02	0.40
Fasting TG[c]	0.30	0.00*

BMI: body mass index, RMR: resting metablic rate, RMRFFM : RMR adjusted for fat free–free mass, NPQR: non proteic respiratory quotient, TG: triglyceride, NEFA: non esterified fatty acid. *$P < 0.05$. Regressions were used on 81 subjects.

[a] Regressions used on $n = 67$ from Lipox, Bergouignan et al.[9] and Bergouignan et al.[10] studies.

[b] Regressions used on $n = 52$ from Lipox and Bergouignan et al.[10] studies.

[c] Regression used on $n = 36$ from Lipox study. Few data are missing from studies because of differences in protocols' objectives.

the overweight individual dARF (37.3 ± 2.4%, $t = -0.3$, $P = 0.8$) or by the overweight subjects mean dARF (37.1 ± 3.3%, $t = -0.1$, $P = 0.9$) were not modified by the interventions on physical activity.

4. Discussion

4.1. Overweight vs. lean ARF

Since the introduction of the acetate correction factor by Sidossis et al.,[3] estimations of ^{13}C-derived plasma-fatty acid oxidation are corrected for the sequestration of the tracer in the TCA cycle and in the bicarbonate pool. Although the characteristics of ARF measured under infusion conditions to correct plasma fat oxidation has been widely studied, the variation and the reproducibility of the dietary ARF have not been yet fully investigated. We previously showed that a unique dARF can be applied in lean subjects. However, whether the same dARF, another unique dARF or an individual dARF has be to used to correct fatty acid oxidation for labelled carbon sequestration in the organism of overweight insulin resistant individuals still needs to be determined.

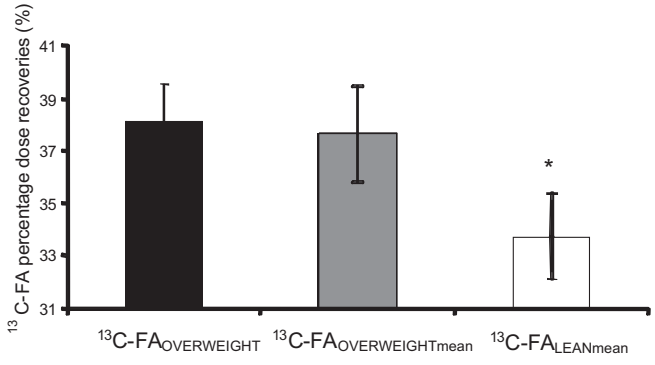

Fig. 2. [^{13}C]fatty acid percentage dose recoveries corrected for label sequestration by overweight individual (^{13}C-FA$_{OVERWEIGHT}$), overweight mean (^{13}C-FA$_{OVERWEIGHTmean}$) and lean mean (^{13}C-FA$_{LEANmean}$) acetate recovery factor before and after physical activity intervention in the overweight group. The mean dietary lipid oxidations are 38.2 ± 1.4, 37.7 ± 1.8 and 33.8 ± 1.6 for ^{13}C-FA$_{OVERWEIGHT}$, ^{13}C-FA$_{OVERWEIGHTmean}$ and ^{13}C-FA$_{LEANmean}$ respectively. A repeated measure ANOVA showed a significant difference between the three methods of corrections ($F = 10.45$; $P = 0.001$) and post hoc analyses showed a significant difference between ^{13}C-FA$_{OVERWEIGHT}$ vs. ^{13}C-FA$_{LEANmean}$ ($P = 0.001$) and ^{13}C-FA$_{OVERWEIGHTmean}$ vs. ^{13}C-FA$_{LEANmean}$ ($P = 0.002$) but not between ^{13}C-FA$_{OVERWEIGHT}$ and ^{13}C-FA$_{OVERWEIGHTmean}$ ($P = 0.648$).

In this present study, we compiled data from five distinct studies, in which we measured the exogenous [1-^{13}C]ARF, and we found an average dARF of 45.3 ± 1.5% in overweight subjects that was significantly lower than the 50.6 ± 0.6% observed in lean subjects.

The reduction in dARF in overweight subjects is likely due to enhanced metabolic pathways where the labelled carbon can be lost due to either a greater dispersion into the bicarbonate pool or lost via isotopic exchange reactions in the TCA cycle. Acetate is immediately converted into acetyl coenzyme A (acetyl-CoA), and it is assumed that almost 100% of the labelled [^{13}C] acetyl-CoA will enter the TCA cycle.[18] Under normal resting conditions, only a fraction of the infused [^{13}C]acetate is recovered as $^{13}CO_2$ in breath, indicating that part of the ^{13}C label is lost in the TCA cycle i.e. formation of glutamate and glutamine and in lower quantity in phosphoenolpyruvate(PEP), and eventually glucose and lactate, in exchange for an unlabeled oxaloacetate (OAA) from gluconeogenic precursors,[2] or buffered within the bicarbonate pool. Thus, the extent of underestimation of the true rate of oxidation depends on the rate of the TCA cycle in relation to the rate of exchange reactions[2] and the dilution in the bicarbonate pool. The kinetics of $^{13}CO_2$ suggest that the differences in dARF between overweight and lean subjects are unlikely to be due to differences in bicarbonate pool sizes (data not shown). A review combining 34 human bicarbonate studies involving 480 subjects and investigating the variability in recovery of labelled CO_2 after administration of [^{13}C]bicarbonate or [^{14}C]bicarbonate, found no significant differences in ^{14}C and ^{13}C recovery between obese and lean subjects.[19] On the other hand, in animals, there is evidence for cytosolic activation of acetate, especially in the liver, leading to the formation of cytosolic (lipogenic) acetyl-CoA pool.[20] Thus, some labelled acetate goes into endogenous cholesterol synthesis[21,22] by the condensation of two acetyl-coA molecules into acetoacetyl-coA and then jointly with a third acetyl-coA molecule to form HMG-coA or into fatty acids formation. Oral ^{13}C-acetate was shown to label fatty acids and cholesterol 3-to 4-fold higher than IV ^{13}C-acetate[21] and is higher during fasting[22] which is not the case of our subjects. Thus, fatty acids and cholesterol synthesis in our experiment' conditions represents a minor way of loss of label.

The flux of the TCA cycle was shown to be influenced by metabolic disorders due to obesity and diabetes.[4,6] Schrauwen et al.[6] hypothesized that a reduced TCA flux occurs in type 2 diabetes mellitus. Further, when skeletal muscle mitochondrial TCA activity was accelerated during exercise, iARF was found to be the highest in lean healthy individuals,[4] followed by obese than type 2 diabetic patients.[23] At rest, Blaak et al.[24] found that iARF was significantly lower in obese type 2 diabetic muscle compared to obese healthy controls muscle. Furthermore, the β-adrenergic infusion stimulation-induced increase in iARF was significantly blunted in obese type 2 diabetes. In addition, a mismatch between the rate of β-oxidation showed by increased levels of acyl-carnitines (β-oxidation intermediates)[25] and the rate of TCA was found to explain the incomplete oxidation in obese and diabetic myotubes.[26] Recently, using ^{13}C magnetic resonance, Befroy et al.[27] assessed rates of substrates oxidation in muscle between lean insulin resistant offspring of type 2 diabetic patients ($n = 7$) and insulin sensitive control subjects ($n = 12$) by monitoring the incorporation of ^{13}C label into C_4 glutamate during (2-^{13}C)acetate infusion. They found that rates of muscle mitochondrial substrate oxidation were decreased in lean, insulin resistant offspring subjects. Taking these results together and the lower dARF we found in our overweight insulin resistant subjects, we can extend the hypothesis of Schrauwen et al.[6] for reduction in TCA flux in type

Table 4
Effect of physical activity interventions on acetate recovery factor and the related dietary fat oxidation rates in overweight subjects.

	Before intervention	After intervention	Difference between after and before intervention	*P* Value of *t*-test
^{13}C-ARF	45.3 ± 1.5 (36.7–50.2)	46.4 ± 2.4 (31.5–60.1)	1.1 ± 2.7 (−18.6 to 13.9)	0.70
^{13}C-FA	17.1 ± 0.8 (14.1–22.9)	17.3 ± 1.4 (11.3–24.7)	0.2 ± 1.8 (−9.9 to 8.0)	0.93
^{13}C-FA$_{OVERWEIGHT}$	38.2 ± 1.4 (31.6–46.3)	37.3 ± 2.4 (27.7–49.7)	−0.9 ± 3.0 (−18.7 to 10.7)	0.77
^{13}C-FA$_{OVERWEIGHTmean}$	37.7 ± 1.8 (31.1–50.6)	37.1 ± 3.3 (24.4–53.1)	−0.5 ± 3.9 (−22.6 to 16.5)	0.89
^{13}C-FA$_{LEANmean}$	33.8 ± 1.6 (27.8–45.4)	33.8 ± 3.0 (22.2–48.4)	−0.0 ± 3.6 (−19.8 to 15.5)	0.99

Values are means ± SEM (minimum/maximum is in the parentheses). ^{13}C-ARF, [1-^{13}C]acetate recovery factor calculated by extrapolation to infinite of the cumulative acetate % recovery; ^{13}C-FA, uncorrected dietary [1-^{13}C]fatty acid %recovery, ^{13} CFA$_{OVERWEIGHT}$, dietary [1-^{13}C]fatty acid %recovery corrected by individual overweight ^{13}C-ARF; ^{13}C-FA$_{OVERWEIGHTmean}$, dietary [1-^{13}C]fatty acid %recovery corrected by overweight subjects' mean ^{13}C-ARF; ^{13}C-FA$_{LEANmean}$, dietary [1-^{13}C]fatty acid %recovery corrected by lean subjects' mean ARF.

2 diabetes mellitus to a reduction in TCA flux in overweight insulin resistant subjects.

In healthy subjects, an increase in insulin concentration might decrease the rate of glycogenic pathway in relation to the TCA cycle, resulting in a higher acetate recovery.[2] However, in the light of significant negative correlations observed between dARF in overweight insulin resistant subjects and indexes of insulin resistance i.e. fasting insulin and fasting insulin to glucose ratio, we found an impaired effect of high insulin concentrations on TCA cycle in overweight insulin resistant subjects. Furthermore, dARF correlated negatively with percentage body fat. A similar negative correlation was observed by Schrauwen et al.[4] infused ARF (iARF). Mitochondrial function could potentially become impaired as a consequence of insulin resistance. Gaster et al.[26] found that a reduced TCA flux does not per se induce insulin resistance. However, it is possible that the decrease in TCA cycle flux observed in overweight insulin resistant subjects may occur as a cause, rather than a consequence, of insulin resistance. Indeed, an inherited defect in mitochondrial biogenesis or an acquired defect in mitochondrial activity due to age or sedentary lifestyle might lead to impaired lipid oxidation, predisposing individuals to accumulation of intramyocellular fatty acids metabolites.[27,28] Increases in concentrations of fatty-acyl CoA and diacylglycerol impairs insulin signaling and causes insulin resistance.[29,30] In that study, the reduction in dARF is associated with adiposity and insulin resistance but it is only correlative data. Further studies are warranted to discern the primary cause of the decrease in TCA cycle flux in overweight insulin resistant subjects.

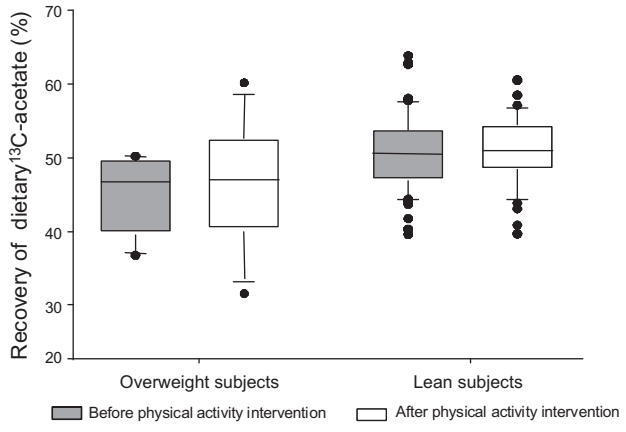

Fig. 3. Box plot representation of the variability of the %recoveries of [1-^{13}C]acetate between overweight and lean subjects before and after the interventions on the physical activity. The box plot represents the median of these data, the 25th and 75th percentiles at each side of the box, and the 10th and 90th percentiles at the extremes, and the black points are the values above and below the 90th and 10th respectively. Dietary acetate recovery factor was significantly different between overweight and lean subjects before (overweight: 45.3 ± 1.5; lean 50.6 ± 0.6; $F = 10.01$, $P = 0.002$) and after the intervention (overweight: 46.4 ± 2.4; lean 51.1 ± 0.6; $F = 7.24$, $P = 0.009$). The physical activity intervention had no effect in among groups (group: overweight vs. lean: $F = 11.97$, $P = 0.001$; Time: before vs. after: $F = 0.65$, $P = 0.422$; Time × group interaction: $F = 0.10$, $P = 0.754$).

4.2. dARF vs. iARF in overweight subjects

The average dARF observed in the overweight group is twofold higher than iARF reported for obese subjects. Indeed, after 120 min of tracer infusion, Schrauwen et al.[4] reported an iARF of 22.9 ± 0.5% in a compilation study involving 31 obese subjects and Van Aggel-Leijssen[31] a iARF of 25.4 ± 2.2% in 21 individuals. Of note, whereas Schrauwen et al.[4] and Van Aggel-Leijssen et al.[31] employed the [1,2-^{13}C]acetate, we used the [1-^{13}C]acetate. The difference in the acetate-labeling pattern partly explains the difference between dARF and iARF values, as previously discussed.[5] The lower values of iARF may also be related to the shorter infusion time (2 h) compared to the 8 h–11.5 h of measure under ingestion conditions.

Effects of a lower dARF in overweight and calculations methods on fatty acid oxidation.

Using the lean subjects 51% dARF suggested by Bergouignan et al.[5] in overweight subjects resulted in an average underestimation of 11.5% of the tracer-derived fatty acid oxidation rates. This is an important effect because it is comparable to the reported differences in fat oxidation. For example, Votruba et al.[8] reported an increase in label recovery of 5–15% between exercise and rest conditions. Bergouignan et al.[9,10] observed decreases in fat oxidation of 11 and 8% when subjects were placed at bed rest.

According to Bergouignan et al. (2), we further tested how using individual or overweight average dARF affected the dietary lipid oxidation rates and their variabilities. As observed for lean subjects, no effects were observed as the average percentage recovery of ^{13}C-FA corrected by either individual or overweight mean acetate recovery factor were not significantly different (within 1%). Using a mean dARF for the overweight group leads to a variability in individual fatty acid oxidation < 12%. Bergouignan et al.[5] reported a difference < 10% in lean subjects between the dietary fatty acid oxidations corrected by the individual dARF and those corrected by an average lean group recovery factor whereas Schrauwen et al.[32] reported a difference of >30% for iARF. Such difference might be attributed to the assumed buffering digestion effect reducing the inter-individual differences resulting in a lower variability for dietary acetate and lipid metabolism compared to plasma acetate and lipids.

4.3. Physical activity intervention effect

The present study also analyzes the effect of physical activity intervention on dARF in overweight subjects. No significant difference was found between before and after the chronic exercise training. However, exercise training increased the between subject coefficient of variability of dARF among overweight subjects. Insulin sensitivity might partly contribute to this observation. The larger variability in fasting insulin due to differences in

the overweight subjects response to training might contribute to the larger variability in dARF recovery after training. Thus, even if our results are in accordance with Bergouignan et al.,[5] special attention should be paid in using a standard dARF during intervention studies in overweight insulin resistant subjects, especially in studies looking at determinants of dietary fat oxidations.

One limitation of our study is the relatively small sample size of the overweight insulin resistant group. If the present study clearly shows that dARF is lower in overweight than in lean subjects (with a posteriori power calculation of 92.4%), further studies are clearly needed in subjects presenting a larger range of obesity and insulin resistance degrees. Nevertheless, based on the above review of the literature convincingly showing that the TCA flux is lower in insulin resistant type 2 diabetes patients, such studies will likely confirm our results. They will be important, however, in determining whether or not an average dARF can be used independently of obesity and insulin resistance degrees. Although we found no difference when using individual or overweight mean dARF of 45.3 to correct for overweight fatty acid oxidation, the small number of subjects and the increased between subjects variability observed after exercise training suggest that special caution should be taken in using the average overweight dARF value from the present study to other experimental settings.

In conclusion, we showed that dARF is lower in overweight insulino-resistant individuals compared to lean individuals. Using a dARF derived from lean subjects for measuring dietary fat oxidation in overweight/obese insulin resistant subjects will results in significant underestimation of the tracer-derived oxidation rates. Before further studies are conducted on a larger scale of obesity and insulin resistance degrees, we recommend to measure individual dARF in subjects presenting metabolic disorders.

Conflict of interest statement

None of the authors had a personal or financial conflict of interest.

Statement of authorship

The authors' responsibilities were as follows: CS and SB: design of LIPOX study, provide funding and analysis of data and writing the manuscript. AE and IM collection and analysis of data and writing this manuscript. AB, collection and analysis of data. CP, collection of data. CV analysis of LIPOX VO2 data. DAS collection of data and drafting the manuscript. All authors read and approved the final manuscript.

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References

1. Pouteau E, Maugere P, Darmaun D, Marchini JS, Piloquet H, Dumon H, et al. Role of glucose and glutamine synthesis in the differential recovery of $13CO_2$ from infused [2-^{13}C] versus [1-^{13}C]acetate. *Metabolism* 1998;**47**:549–54.
2. Sidossis LS, Coggan AR, Gastaldelli A, Wolfe RR. Pathway of free fatty acid oxidation in human subjects. Implications for tracer studies. *J Clin Invest* 1995;**95**:278–84.
3. Sidossis LS, Coggan AR, Gastaldelli A, Wolfe RR. A new correction factor for use in tracer estimations of plasma fatty acid oxidation. *Am J Physiol* 1995;**269**:E649–56.
4. Schrauwen P, Blaak EE, Van Aggel-Leijssen DP, Borghouts LB, Wagenmakers AJ. Determinants of the acetate recovery factor: implications for estimation of [^{13}C]substrate oxidation. *Clin Sci (Lond)* 2000;**98**:587–92.
5. Bergouignan A, Schoeller DA, Votruba S, Simon C, Blanc S. The acetate recovery factor to correct tracer-derived dietary fat oxidation in humans. *Am J Physiol Endocrinol Metab* 2008;**294**:E645–53.
6. Schrauwen P, Hesselink MK. Reduced tricarboxylic acid cycle flux in type 2 diabetes mellitus? *Diabetologia* 2008;**51**:1694–7.
7. Votruba SB, Zeddun SM, Schoeller DA. Validation of deuterium labelled fatty acids for the measurement of dietary fat oxidation: a method for measuring fat-oxidation in free-living subjects. *Int J Obes Relat Metab Disord* 2001;**25**:1240–5.
8. Votruba SB, Atkinson RL, Hirvonen MD, Schoeller DA. Prior exercise increases subsequent utilization of dietary fat. *Med Sci Sports Exerc* 2002;**34**:1757–65.
9. Bergouignan A, Schoeller DA, Normand S, Gauquelin-Koch G, Laville M, Shriver T, et al. Effect of physical inactivity on the oxidation of saturated and monounsaturated dietary Fatty acids: results of a randomized trial. *PLoS Clin Trials* 2006;**1**:e27.
10. Bergouignan A, Trudel G, Simon C, Chopard A, Schoeller DA, Momken I, et al. Physical inactivity differentially alters dietary oleate and palmitate trafficking. *Diabetes*; 2008.
11. den Hoed M, Hesselink MK, van Kranenburg GP, Westerterp KR. Habitual physical activity in daily life correlates positively with markers for mitochondrial capacity. *J Appl Physiol* 2008;**105**:561–8.
12. Westerterp KR. Pattern and intensity of physical activity. *Nature* 2001;**410**:539.
13. Iqbal R, Rafique G, Badruddin S, Qureshi R, Gray-Donald K. Validating MOSPA questionnaire for measuring physical activity in Pakistani women. *Nutr J* 2006;**5**:18.
14. Rothney MP, Schaefer EV, Neumann MM, Choi L, Chen KY. Validity of physical activity intensity predictions by ActiGraph, Actical, and RT3 accelerometers. *Obes (Silver Spring)* 2008;**16**:1946–52.
15. Crouter SE, Churilla JR, Bassett Jr DR. Accuracy of the Actiheart for the assessment of energy expenditure in adults. *Eur J Clin Nutr* 2008;**62**:704–11.
16. Daussin FN, Zoll J, Ponsot E, Dufour SP, Doutreleau S, Lonsdorfer E, et al. Training at high exercise intensity promotes qualitative adaptations of mitochondrial function in human skeletal muscle. *J Appl Physiol* 2008;**104**:1436–41.
17. Schoeller DA, Luke AH. Rapid 18O analysis of CO_2 samples by continuous-flow isotope ratio mass spectrometry. *J Mass Spectrom* 1997;**32**:1332–6.
18. Mion CM, Hegstrom RM, Boen ST, Scribner BH. Substitution of sodium acetate for sodium bicarbonate in the bath fluid for hemodialysis. *Trans Am Soc Artif Intern Organs* 1964;**10**:110–5.
19. Leijssen DP, Elia M. Recovery of $13CO_2$ and $14CO_2$ in human bicarbonate studies: a critical review with original data. *Clin Sci (Lond)* 1996;**91**:665–77.
20. Zambell KL, Fitch MD, Fleming SE. Acetate and butyrate are the major substrates for de novo lipogenesis in rat colonic epithelial cells. *J Nutr* 2003;**133**:3509–15.
21. Faix D, Neese R, Kletke C, Wolden S, Cesar D, Coutlangus M, et al. Quantification of menstrual and diurnal periodicities in rates of cholesterol and fat synthesis in humans. *J Lipid Res* 1993;**34**:2063–75.
22. Neese RA, Faix D, Kletke C, Wu K, Wang AC, Shackleton CH, et al. Measurement of endogenous synthesis of plasma cholesterol in rats and humans using MIDA. *Am J Physiol* 1993;**264**:E136–47.
23. Mensink M, Blaak EE, van Baak MA, Wagenmakers AJ, Saris WH. Plasma free fatty acid uptake and oxidation are already diminished in subjects at high risk for developing type 2 diabetes. *Diabetes* 2001;**50**:2548–54.
24. Blaak EE, Wagenmakers AJ, Glatz JF, Wolffenbuttel BH, Kemerink GJ, Langenberg CJ, et al. Plasma FFA utilization and fatty acid-binding protein content are diminished in type 2 diabetic muscle. *Am J Physiol Endocrinol Metab* 2000;**279**:E146–54.
25. Koves TR, Ussher JR, Noland RC, Slentz D, Mosedale M, Ilkayeva O, et al. Mitochondrial overload and incomplete fatty acid oxidation contribute to skeletal muscle insulin resistance. *Cell Metab* 2008;**7**:45–56.
26. Gaster M. Reduced lipid oxidation in myotubes established from obese and type 2 diabetic subjects. *Biochem Biophys Res Commun* 2009;**382**:766–70.
27. Befroy DE, Petersen KF, Dufour S, Mason GF, de Graaf RA, Rothman DL, et al. Impaired mitochondrial substrate oxidation in muscle of insulin-resistant offspring of type 2 diabetic patients. *Diabetes* 2007;**56**:1376–81.
28. Petersen KF, Befroy D, Dufour S, Dziura J, Ariyan C, Rothman DL, et al. Mitochondrial dysfunction in the elderly: possible role in insulin resistance. *Science* 2003;**300**:1140–2.
29. Morino K, Petersen KF, Shulman GI. Molecular mechanisms of insulin resistance in humans and their potential links with mitochondrial dysfunction. *Diabetes* 2006;**5**(Suppl. 2):S9–15.
30. Itani SI, Ruderman NB, Schmieder F, Boden G. Lipid-induced insulin resistance in human muscle is associated with changes in diacylglycerol, protein kinase C, and IkappaB-alpha. *Diabetes* 2002;**51**:2005–11.
31. van Aggel-Leijssen DP, Saris WH, Wagenmakers AJ, Hul GB, van Baak MA. The effect of low-intensity exercise training on fat metabolism of obese women. *Obes Res* 2001;**9**:86–96.
32. Schrauwen P, van Aggel-Leijssen DP, van Marken Lichtenbelt WD, van Baak MA, Gijsen AP, Wagenmakers AJ. Validation of the [1,2-^{13}C]acetate recovery factor for correction of [U-^{13}C]palmitate oxidation rates in humans. *J Physiol* 1998;**513**(Pt1):215–23.

Discussion

Course de relais d'Alfred Gockel

Since lipogenesis is negligible in humans, weight gain represents a consequence of a positive lipid balance mainly due to exogenous lipid partitioning towards storage rather than oxidation. A positive energy balance might be achieved when there is a lack of compromise in the voluntary modulable compartment of total energy expenditure, the physical activity, in face of an increased energy uptake. Although lipid oxidation is a hereditary trait, this does not imply that genes play a role regardless of environmental factors, such as physical inactivity. This relationship is more obvious when looking at the parallel prevalence of obesity with the increase of sedentary behavioural induced by modern technology, creating a favourable environment to weight gain, and in particular, in individuals with pregenetic disposal for obesity. However, the mechanisms of complex interactions between genetic predisposition and environmental and behavioural factors remain speculative.

The main objective of this work was to longitudinally and cross-sectionally investigate in healthy adults and in overweight subjects a potential relationship of modulating habitual physical activity at different levels with dietary exogenous fatty acid oxidation. We believe by finding such a continuum relationship, a potential area of clinical implications for weight gain prevention and treatment is possible. Indeed, such relationship would confirm that by modulating a specific level of physical activity, improvement can occur in lipid metabolism. However, future work would be warranted to determine such level.

To investigate how physical activity modulation impacts exogenous lipid oxidation, we tested whether:

- Modulating physical activity levels would impact total energy expenditure in adult men dependently on weight (lean *vs.* overweight) or lifestyle (active *vs.* sedentary).
- Because stored lipids essentially originate from the diet and because the partitioning of lipids between oxidation and storage depends on the whole body energy turnover, dietary fat oxidation linearly responds to habitual physical activity independently of energy balance.

To test our hypothesis, we chose the two main dietary fatty acids in human diet: monosaturated (oleate) and saturated (palmitate) fatty acids and we checked if they are similarly affected by physical activity. We also aimed to evaluate our result with the current guidelines for physical activity recommendations.

These objectives, which represent the main frame of the discussion, were:

- To investigate the impact of habitual physical activity in the light of current recommendations on lipid metabolism and discuss potential beneficial effect of NEAEE.

- to determine how metabolic flexibility which represent the overall body metabolism response to environmental challenges is affected by physical activity modulation.

- to determine the impact of physical activity /inactivity on the oxidation of fatty acid of different chemical nature.

1. PHYSICAL ACTIVITY LEVEL AND ENERGY BALANCE REGULATION

In this work, we showed that modulating physical activity *per se* induces differential metabolic modifications between healthy lean and overweight subjects. We summarized our findings and the associated conclusions and perspectives in **Figure 1**.

1.1. Physical activity modulation and energy expenditure

1.1.1. Lean subjects follow modulations in activity energy expenditure

One month of detraining by stopping any structured exercise and reducing any bodily movement reduces significantly total and activity energy expenditure, while two months training based on currents recommendations succeeded in increasing total and activity energy expenditure in lean but not in overweight subjects (**Chapter 1** and **Chapter 2**). Total energy expenditure TEE is classically divided into three components: resting metabolic rate RMR, dietary induced thermogenesis DIT and activity energy expenditure AEE. AEE is the main compartment that can be modulated voluntary by individuals i.e. by increasing any type of physical activity either structured or non exercise physical activity NEAEE or on the contrary by stopping any kind of exercise or body movement. Caspersen *et al.* (Caspersen *et al.*, 1985) gave a very subtile definition to this differentiation by defining the exercise as a subset of physical activity that is characterized by planned and purposeful training, and NEAEE as any bodily movement. Some studies have highlighted the importance of physical activity in body weight regulation and have shown an inverse relationship between physical activity level PAL and body mass index BMI or adiposity and others studies have not (Hunter *et al.*, 1996; Hunter *et al.*, 1997; Ekelund *et al.*, 2002; Salbe *et al.*, 2003).

However, there is a potential benefit to health metabolism by increasing total energy expenditure in means of increasing any type of physical activity beyond the effect of structured exercise. Indeed, Levine *et al.* (Levine *et al.*, 1999) have highlighted the importance of NEAEE, as a predictor of weight gain and it was shown that modulation in NEAEE can exceed TEE obtained by current recommendations (Levine, 2007). On the other hand, increasing total energy expenditure by exercise is moderate and there is a limited number of studies that found an increase in energy expenditure, as measured by doubly labelled water, through exercise training (Bingham *et al.*, 1989; Blaak *et al.*, 1992; Westerterp *et al.*, 1992; Van Etten *et al.*, 1997). Modulating physical activity, however, is not always followed by effects on total energy expenditure. Indeed, compensatory behaviours may occur, which can reduce the effect of modifying AEE.

	Body composition	Energy expenditure	Lipemia	Insulin	exogenous fatty acid concentrations in lipid fractions (palmitate)	exogenous fatty acid concentrations in lipid fractions (oleate)	Entrance into myocyte FAT/CD36	transport and oxidative capacity via AMPK, PRKAA2	Entrance into mitochondria CPT1	oxidative capacity PGC-1α	Exogenous lipid oxidation	Total lipid oxidation
Inactivity effect in lean subjects	BM ns FFM - FM ++	TEE - AEE - RMR ns PAL -	Total NEFA ns Total TG ns	Fasting ns Postprandial +	chylomicron + NEFA + VLDL +	chylomicron + NEFA + VLDL ns	ns	-	ns	ns	Monosaturated (oleate) - Saturated (palmitate) -	Fasting - Postprandial -
Activity effect in lean subjects	BM ns FFM ns FM ns	TEE + AEE + RMR ns PAL +	Total NEFA ns Total TG ns	Fasting ns Postprandial ns	chylomicron ns NEFA ns VLDL ns	chylomicron ns NEFA - VLDL ns	+	ns	+	+	Monosaturated (oleate) + Saturated (palmitate) ns	Fasting ns Postprandial ns
Activity effect in overweight subjects	BM ns FFM + FM ns	TEE ns AEE ns RMR + PAL ns	Total NEFA ns Total TG ns	Fasting ns Postprandial -	chylomicron ns NEFA ns VLDL ns	chylomicron ns NEFA ns VLDL ns	+	ns	+	+	Monosaturated (oleate) ns Saturated (palmitate) ns	Fasting + Postprandial ns

Physical activity level impacts modestly body composition when in energy balance

Physical activity modulation differentialy impacts TEE, AEE and NEAEE between lean and overweight (compensatory behaviors)

Physical activity level predicts metabolic flexibility and impacts insulin sensitivity

Sedentarism induces more pronounced modifications than physical activity , similarly to alterations found in obesity

Physical activity level impacts oxidative capacity and fatty acid transport

Physical activity level predicts exogenous fatty acid oxidation

These continuum- relationships opens a new perspective for futur studies to determine what is the minimum and the optimal level of physical activity to enhance lipid metabolism improvement

- to investigate the role of body composition in relationship with physical activity /inactivity impacts on metabolism

- to investigate the effect of current physical activity guidelines with attention to the of NEAEE and compensatory behaviours in groups with different weight and physical activity pattern: structured exercise per se, NEAEE per se, or both combined on lipid metabolism

- to investigate the relationship between the level of physical activity with metabolic flexibility and insulin sensitivity .

Figure 1: Summary of physical activity modulation-induced metabolic effects observed in our study and of the associated conclusions and perspectives. TEE, total energy expenditure; AEE, activity-related energy expenditure; RMR, resting metabolic rate; PAL, physical activity level; TG, triglycerides; NEFA, non esterified fatty acid; VLDL, very low density lipoprotein; BM, body mass; FFM, fat free mass; FM, fat mass; NEAEE, non exercise activity energy expenditure; CPT1, carnitine palmitoyl transferase1; FAT/CD36, fatty acid translocase CD36; PGC1α, peroxisome proliferator-activated receptor coactivator; AMPK, adenosine monophosphate-activated protein kinase; PRKAA2, α2 isoform of the catalytic subunit of AMPK.

Introducing exercise training into a sedentary life style might result in a compensatory behaviour by reducing the non exercise activity in individuals with predisposition to be sedentary. However, in lean subjects, after training of 18 wk, Van Etten *et al.* (Van Etten *et al.*, 1997) found no changes in non training activity.

In this line, our trained group was not given any instructions about modifying NEAEE. The positive increase in their TEE suggest that they did not compensate the imposed structured by decreasing their NEAEE. On the other hand, our detrained group was asked to strictly abandon any structured exercise and to decrease their NEAEE. Although the PAL of the active detrained group decreased to 2.09, however this value is above general population PAL (Black and Cole, 2000). This observation suggests that active persons preserved their initial level of NEAEE and that this level was initially higher than general population habitual activities. Beyond a supposition that the active group did not comply to our instructions to reduce their NEAEE, it is interesting to speculate that these individuals might have pregenetic disposition to remain active throughout the day in all sort of bodily movement. We can also suppose that these individuals do not have the same perception of physical activity as sedentary subjects and that they recognize differentially the cost of energy required for a particular body movement in a less extent than do sedentary individuals. Accordingly, although they might voluntary reduce high energy required activity such as structured exercise and high NEAEE, they do not reduce other type of moderate or low level of NEAEE because they do not conceive its energy cost.

Thus, also we did not measure separately the NEAEE in our subjects, when looking at the significant changes in TEE both in lean trained and detrained groups; we can assume that their NEAEE did not show significant modifications. Nevertheless, the effect of modulating physical activity is less obvious in overweight subjects.

1.1.2. Overweight subjects adopt compensatory behaviours facing the increase in activity energy expenditure.

We have shown that while modulation of physical activity (high or low) causes a change in the total energy expenditure in normal-weighted patients, physical training under the current recommendations did not increase total energy expenditure in overweight patients. In overweight subjects, training increased fasting fat oxidation but had no effect on postprandial and exogenous lipid oxidation. Current recommendations for physical activity seem to be insufficient to improve exogenous lipid oxidation in overweight subjects. However, despite regular participation in physical training program set by the protocol, the ineffectiveness of physical training to increase total energy expenditure among overweight subjects, suggests that they were offset by a reduction in the spontaneous activity (any type of movement) that compensated the energy expenditure induced by exercise. This compensatory behaviour was not observed in lean subjects suggesting that a predisposition of overweight subjects to sedentary behaviours may be primary to weight gain.

In this line, Levine et al reported a very significant relationship between NEAEE and adiposity in lean and obese subjects (Kotz and Levine, 2005; Levine and

Kotz, 2005). While both of these groups spend the same time in lying down and sleeping, obese subjects spend 2.5 times more in sitting positions. This result is not modified even after weight loss to a normal BMI range, which suggests that obese people might have a pregenetic predisposition to the non adherence to physical activity. Indeed, when lean subjects gained weight through overfeeding, their tendency to be stand/ambulate persisted. When obese subjects became lighter, their tendency to sit did not change. The same authors showed high interindividuel variability in weight gain in response to a high dense lipid overnutrition related to modulation in NEAEE (Levine *et al.*, 1999). Thus, subjects who gained less weight after two months of overnutrition of 100kcal/d were those who had the highest NEAEE.

On the other hand, studies on the effectiveness of diet+exercise interventions versus diet-only interventions for weight loss show that it is difficult to overcome diet-induced reduction of physical activity with exercise training. A meta analysis of a total of 18 randomized controlled trials on weight loss conducted for a minimum of 6 months showed an overall difference of only 0.25kg between the two groups (Wu *et al.*, 2009). At a negative energy balance, the additional exercise was compensated by a reduction of non training activity, resulting in comparable decrease of total energy expenditure in the diet plus exercise and the diet only groups (Kempen *et al.*, 1995). On the contrary, training leads to an appreciable augmentation in the overall energy expenditure of obese children, even with a lack of change in spontaneous physical activity (Blaak *et al.*, 1992). Beyond the weight effect, age seems also to affect NEAEE. Exercise training does not seem to prevent the age associated decline in physical activity because it is compensated by a decrease in non-training physical activity (Westerterp and Plasqui, 2004). Thus, a physically active lifestyle inevitably results in a proportionally larger decrease of daily energy expenditure at later ages than sedentary lifestyle.

Altogether, these studies highlight, jointly with our results, the importance of NEAEE in lipid metabolism. However, a question arises: what can explain this pattern for overweight individuals to be sedentary?

1.2. What triggers reductions in NEAEE (sedentarism) in individuals?

The word "sedentary" is derived from the latin word "sedentarius" which means who sits a lot. Booth *et al.* (Booth *et al.*, 2002) have speculated that there is a biological base for behavioural desire to be sedentary, in other words lack of desire to be active. Approximately, 70% of adults in USA do not adhere to 30 min of moderate physical activity of 5xtimes or more per week, including the 24% who do not engage in any sort of activity (Mu *et al.*, 2001). This behaviour is a part of psychological pattern; however its mechanisms remain unclear. Currently, sedentary behaviours became an ultimate consequence of the development of technology and devices decreasing the cost of living.

1.2.1. Ability to undertake moderate physical activity without fatigue

Physical inactivity reduces the duration of moderate physical activity until exhaustion, preventing the continuation of the exercise at the same intensity of

work (Padilla *et al.*, 2001). It is also possible that a reduced ability to undertake moderate physical activity could lead to reduced voluntary activity for some psychological reasons. Less activity produces more deconditioning, less ability to exercise without fatigue, and even more inactivity, in a negative cycle. While activity induced energy expenditure is similar in subjects with a normal weight and those with a higher weight (Westerterp, 2010), an overweight condition is limiting for high intensity physical activity and physical performance. In a study of Blaak *et al.* (Blaak *et al.*, 1992) investigating the effect of an increase in physical activity on energy balance and body composition, subjects were prepared to run a half marathon competition after 44 weeks of training. Although manifestly obese subjects were excluded by the selection criteria, most of the subjects with a BMI above 24 experienced difficulties with training that led to their withdrawal (Westerterp, 1999). Similarly, in a study of voluntary recruits for military service, an overweight condition was the most important factor limiting high level training (Tanskanen *et al.*, 2008).

1.2.2. Voluntary physical activity

The desire of active individuals to preserving high or moderate level of physical activity by engaging either into structured exercise or maintaining high level of NEAEE, and on the contrary, the predisposition of individuals to become or remain sedentary might have a mechanistic explanation.

Transgenic data suggest that the level of expression of certain genes in skeletal muscle can be associated with the amount of voluntary activity in mice. The expression in mouse muscle of a dominant mutant inhibitor of AMPK, partially blocking the contraction due to glucose, reduced voluntary running by 20-30% (Mu *et al.*, 2001). The mice designed to overexpress the GLUT-4 in the muscle has a fourfold multiplication of the distance travelled voluntarily on wheels (Tsao *et al.*, 2001). These mice ate more than 45% but had a lower weight than sedentary mice without the transgene. The authors of this study (Tsao *et al.*, 2001) assumed that it is possible that the increased availability of glucose and / or glycogen to oxidation allows mouse-MLC-GLUT-4 to practice for a period longer than the mouse controls.

In this line, our active group, when detrained showed a decrease in PRKAA2, the catalytic subunit of AMPK. However, one month of detraining might not be enough to induce voluntary desire to become sedentary and that this pattern might need longer period to manifest and induce voluntary sedentary behaviours. However, the decrease in PRKAA2 suggests that this is probably bound to happen and that sedentary behaviours might engender more sedentarism, thus increasing the risk of weight gain and other metabolic alterations. These findings suggest that the pattern of protein expression of skeletal muscle affects a voluntary control of the central nervous system through either a direct response from skeletal muscle or indirectly due to reduction in the size of adipose tissue. Our late Paleolithic ancestors probably undertook physical activity for survival (food gathering, shelter, etc.), and we thus speculate that metabolic adaptations to physical activity within skeletal muscle may have evolved some type of feed-forward mechanism to provide for the desire for additional voluntary physical activity. Thus, metabolic adaptations to exercise (e.g., increased AMPK activity and GLUT-4 protein) could be

biochemical clues of great importance in a culture in which insufficient activity for the prevention of chronic health conditions occurs.

Therefore, a question arises: how does an environmental factor, such as physical inactivity, trigger a modification of the underlying genetic composition of an individual and encourage the sensitivity to detrimental health conditions, although our genes have been programmed for the maximum preservation natural selection?

1.2.3. An advantageous or detrimental genetic predisposition?

To provide a hypothesis on the genetic evolution to the question above, we will focus on the physical inactivity and the sedentary lifestyle as a potent environmental trigger for the development of several chronic diseases. Environmental factors could exert their influence by altering the expression of a subset of genes that leads to a phenotype that crosses the threshold of biological significance to clinical symptoms (Geisbrecht and Gould, 1999). Physical inactivity is an important component of these environmental factors. The modern *Homo sapiens* is genetically more suited to a preagriculture lifestyle of hunter-gatherer (Fernandez-Real and Ricart, 1999) because all the genetic material of *Homo sapiens* has changed little over the past 10,000 years (Eaton *et al.*, 1988). The societies of hunter-gatherers probably had to undertake physical activity for more than 30 minutes each day in order to provide basic necessities such as food, water, shelter, materials for heat and so on, to survive. One can speculate, but not prove, that any phenotype of hunter-gatherers to prevent from engaging in physical activity increases the probability of the random elimination of this organism or its progeny, at a certain time (Booth *et al.*, 2002). On the other hand, a phenotype that would favour a moderate physical activity, allowing greater capacity for the flow of substrates for ATP production as fuel for physical labour would have been more likely to survive and its gene pool would be transferred to future generations. Thus, it is likely that many metabolic functions of modern humans have evolved as an adaptation to a physically active lifestyle with a diet rich in protein and low in fat, interspersed with frequent periods of famine (Fernandez-Real and Ricart, 1999; Wendorf and Goldfine, 1991).

- **“Thrifty Gene" hypothesis applied to physical inactivity**

The notion of cycles of abundance and famine has generated the hypothesis of "economic gene" or "thrifty gene" by Neel (Neel, 1962, 1999). According to this hypothesis, people with “thrifty” metabolic adaptations probably convert more of their calories as fat during periods of abundance (Cockram, 2000). Consequently, those who have the thrifty phenotype would be less likely to be eliminated at random during periods of food storage (Wendorf and Goldfine, 1991), that is to say, during times of plenty, they will be more efficient and store more food calories fat because of their efficient metabolic process. The ability of an organism to adapt to a decrease in energy intake is beneficial to survival (Shetty, 1999). This concept also involves cycles of metabolic processes with the flow of abundance and famine. A reduction in energy intake below a threshold level results in a series of physiological responses, biochemical and behavioural, which are an adaptation to low energy intake (Shetty, 1999).

In other words, physical inactivity is an unusual event for a genome programmed to expect physical activity, which partly explains the genesis of how physical inactivity leads to metabolic dysfunction and possibly, metabolic disorders such as atherosclerosis, hypertension, obesity, diabetes type 2, and so on. The estimated energy expenditure of daily physical activity is much less today than the society of hunter-gatherers. The total energy expenditure of modern man is ~ 65% of that of the late Paleolithic age, with the assumption that comparisons are possible (Cordain *et al.*, 1998). However, when size differences are taken into account, the energy expenditure per unit body mass of physical activity for contemporary U.S. adult is ~ 38% lower than our ancestors (Cordain *et al.*, 1998). The 30 minutes of moderate exercise per day from the guidelines represent the energy expenditure of only 44% of calories from hunter-gatherers of the 20th century, which, according to Cordain *et al.* (Cordain *et al.*, 1998) is well below estimates for the energy expenditure of our preagricultural ancestors. The thrifty phenotype thus represents a disadvantage for sedentary people who have the right to free access to food (Wendorf and Goldfine, 1991). They store fat in anticipation of a famine which does not produce, because food is available on request. Some of those who develop obesity and type 2 diabetes probably have the "thrifty gene". Also, the metabolic processes have evolved to withstand a significant physical activity. When physical inactivity accompanies periods of continuous nutrition, there is a deregulation of the active phenotype with maintenance of the « thrifty » phenotype resulting in several diseases such as obesity (Hansen, 1999).

This mechanism would have conferred a survival advantages during the regular famines and natural disasters that were interspersed with feast periods. With Westernization, these populations now have a plentiful supply of a diet with an excess of energy, simple carbohydrates and saturated fats. This has been accompanied by a reduction in both occupational and leisure-based physical activity. Both factors may therefore cause the previously favourable metabolic profile seen in survivors to become a handicap, which results in obesity (Dowse and Zimmet, 1993). There exists an excellent model of this phenomen - the Israeli sand rat (Psammomys obesus) (Zimmet, 1999). When this animal is removed from the sparse diet of its natural environment and given an abundant, high calorie diet, it develops all of the components of the metabolic syndrome, including diabetes and obesity. Recently, several new obesity and diabetes related genes have been isolated and sequenced from P.obesus, including the “beacon” gene (Collier *et al.*, 2000). Intracerebroventricular infusion of beacon protein results in a dose dependent increase in food intake and body weight and an increase in hypothalamic expression of neuropeptideY, a brain peptide that stimulates food intake.

- **“Drifty gene”: a nonadaptive scenario explaining the genetic predisposition to obesity**

Speakman (Speakman, 2007) called into question some fundamental assumptions of the hypothesis of thrifty gene - in particular focusing, if the difference in survival between individuals of normal weight and obese individuals during the famine, provides adequate selection for the spread of the so-called "thrifty gene". These arguments were criticized for that famine, affecting not only survival but also fecundity, and obese people should support greater fertility facing food shortage. Speakman (Speakman, 2007), however, showed that the argument of the reduction in fertility is wrong because famines are almost universally followed by periods of improvement in fertility, which offsets the decline observed during the famine itself. The net effect of the famine on fecundity is consequently

insufficient to rescue the idea of the thrifty gene. He suggested an alternative scenario that subsections of the population have a genetic predisposition to obesity due to a lack of selection, coupled with genetic drift. This scenario is based on evidence from the prehistory concerning the release of our ancestors of the predation pressure of about 2 million years. In addition, he added that it could be one of a number of scenarios based on random genetic drift, which could explain the specific etiology of the obesity epidemic. Together, these alternatives on the basis of the concept of genetic drift rather than positive selection as the dominant factor, may be called the hypothesis of " drifty gene " (Speakman, 2008).

In fact, according to Speakman (Speakman, 2007), the great challenge of the assumptions of any explanation of the evolution of the genetic predisposition to obesity is not to explain why we are obese, but rather to explain why only a fraction of the population becomes obese. Even in the United States, 35% of the population still has a BMI in the "normal" range of BMI: 17,5 to 25 (Flegal *et al.*, 2002; Ogden *et al.*, 2006). Any scenario that postulates a selective advantage for obesity due to thrifty genes must explain why 35% of the population does not seem to inherit these genes. The key aspect of this scenario of "drift" is that the genetic predisposition to obesity is not interpreted as an advantageous feature favoured by natural selection (as in the case of the thrifty gene). Instead, it must be regarded as a consequence of the absence of selection. As such, this model is a non-adaptive scenario, which argues that obesity has been consistently hostile to man (Speakman, 2008).

1.2.4. Application of the theory of genetic predisposition to NEAEE

There is a substantial NEAEE defect in human obesity, which may be undcaused by a profound and subtle biology (Levine, 2007). An inference would be that NEAEE is genetically driven (Gustafson and Rhodes, 2006) as twin data suggest (Joosen *et al.*, 2005b; Carlsson *et al.*, 2006) and this conjecture is supported by the overall recognition that obesity is, to a substantial degree, genetically determined (Mutch and Clement, 2006). Obesity is associated with a NEAEE -defect that predisposes obese people to sit (Tryon *et al.*, 1992). Lean people have an innate tendency to stand and walk. Overall, it is likely that there is a numerically substantial NEAEE defect in obesity. This may reflect an ill-defined biology whereby those with obesity have a greater likelihood to respond to sedentary cues to sit. A novel theory has been recently discussed in a review by Levine *et al.* (Levine, 2007) stating that leanness may be a state whereby the signals that stimulate NEAEE are plentiful and potent even in the presence of caloric excess; these animals could be termed, NEAEE activators. Conversely, obesity may be a state of central NEAEE resistance, whereby signals go through the nervous system to stimulate NEAEE, but in obesity the response to these signals is reduced; these animals are NEAEE conservers whereby they dissipate minimal energy through NEAEE and become obese (Levine, 2007).

The migration of humans over the globe enabled them to find nutrition and shelter and the species was perpetuated in the presence of adequate fuel and safety (Morgan, 1993). This time course is consistent with the calculated, spontaneous mutation rate in DNA that explains the selective forces that explain our phenotype (Drake, 1999). Thus, a fundamental characteristic of *Homo sapiens* is the time-driven design to walk. If walking, which is the predominant component of NEAEE,

served as an evolutionary selective force, a bimodal response in early *Homo sapiens* to famine with respect to NEAEE could be imagined. One response to famine would be for humans to search for food beyond their pre-existing physical boundaries whereby NEAEE increases; this person would be a NEAEE activator. An alternate response to famine in early humans might be to conserve fuel and stop moving so that NEAEE decreases and body fat stores are not depleted; this person is a NEAEE conserver (Levine, 2007).

NEAEE activators may therefore be people who are genetically programmed to be more responsive to NEAEE cues, have high NEAEE and are lean-walkers. In contrast NEAEE conservers may be people who are genetically programmed to have blunted central responses to NEAEE signals and may thereby be prone to sit, have low NEAEE and develop obesity. Although, this is only a theoretical model, it illustrates the appropriate time course to consider genetic influences on the human obesity phenotype.

1.3. The role of NEAEE in energy balance regulation

1.3.1. Do reductions in NEAEE induce weight gain?

The current NEAEE has been estimated to be approximately 1500kcal/d lower than our paleolithic ancestors (Hayes *et al.*, 2005). As a result, there was population-wide positive energy balance. People maintained their energy intake at constant levels in the face of declining energy expenditure through NEAEE (Kant and Graubard, 2006). Therefore, our society suffers from physical activity deficiency primary to NEAEE lack. As discussed above, Levine *et al.* (Levine, 2007) argued that there might be a bimodal response to starvation which can be applied to a bimodal response to energy excess. NEAEE activators have genetic programming for high NEAEE. As the environment imposed pressures over 150 years to decrease day-long activity, NEAEE activators found approaches to dissipate NEAEE; for example they walked to work. NEAEE activators may also have mechanisms to be energetically inefficient for example through myocyte uncoupling (Kopecky *et al.*, 1995; Gura, 1998) whereby more energy is dissipated per mile of walking (corrected for weight). Regardless of the precise mechanism, NEAEE Activators retained their high NEAEE -drive despite a flood of opportunities to sit. The NEAEE conservers, on the other hand, are genetically programmed not to waste energy through movement or NEAEE. NEAEE conservers over the last 150 years found their predilection to minimize NEAEE fulfilled; they became able to sit at work, sit whilst in locomotion and sit during leisure. With the emergence of chair-based living, NEAEE conservers 'naturally' (genetically) sat and thereby conserved 1500 kcal day^{-1} (Hayes *et al.*, 2005) whilst their energy intake was unchanged. It is not a surprise that they became obese.

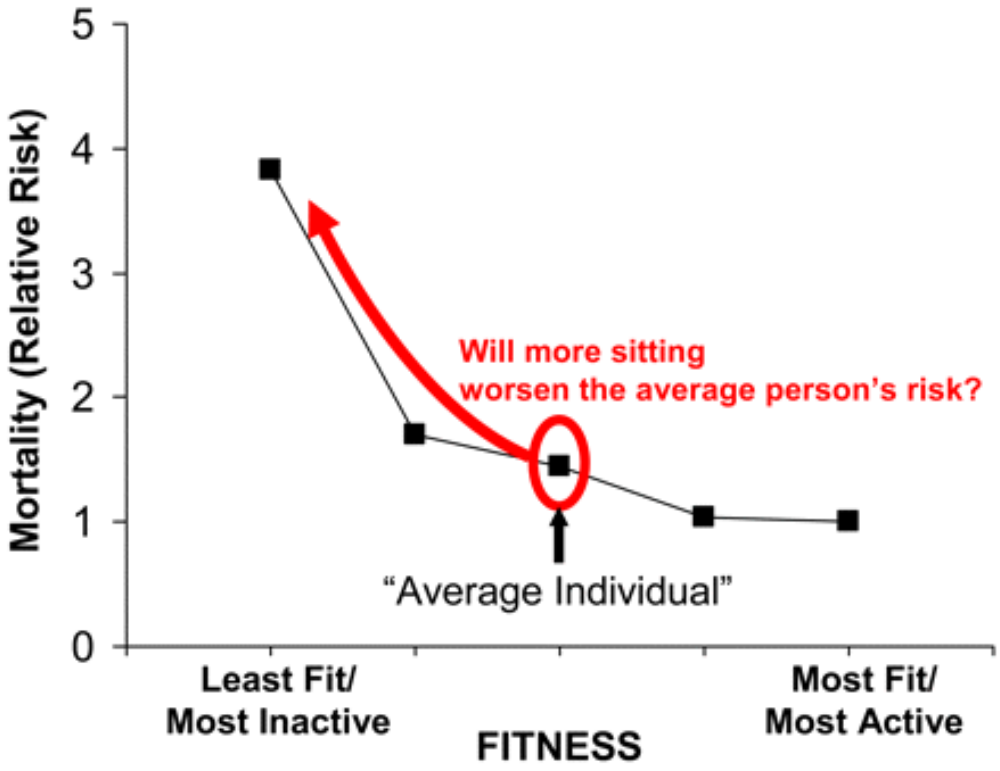


Figure 2: A major question raised by the inactivity physiology paradigm is whether the typical person who already does not perform structured exercise regularly will have increased risks of metabolic diseases in the coming years as a result of too much sitting. The red circle shadows the median of 13,344 middle-aged men and women (adapted from Blair *et al.* (Blair *et al.*, 1989). The majority of people in the general population already do not follow the prescription for enough moderate-vigorous exercise. It logically follows that in people who already do not exercise; it is impossible for higher rates of age-adjusted metabolic syndrome, type 2 diabetes, obesity, and CVD over the coming years to be caused by further exercise deficiency. Inactivity physiology is a discipline concerned with the future of people who may be sitting too much (Hamilton *et al.* 2007).

1.3.2. Non exercise activity deficiency can still increase: implications

Recent National Health and Nutrition Examination Survey data (as reported by Kruger *et al.* (Kruger *et al.*, 2007) reveal that only 28% of Americans are "regularly active" by meeting the current minimal exercise recommendation for 30 min/day for 5 days/week of at least moderate activity. For minority racial groups and less educated people, the numbers are one-half of that (Kruger *et al.*, 2007). It logically follows that in people who already do not exercise, it is absolutely impossible for further rates of age-adjusted overweight/obesity, metabolic syndrome, type 2 diabetes, and cardiovascular disease (CVD) over the coming years to be caused by further structured exercise deficiency. In contrast, non exercise activity deficiency can still increase profoundly because nearly all people stand and move at least 1 h/day and generally for many hours each day. Thus, many people are potentially at greater risk for disease in the future by sitting more.

Taking in account that obesity is continuously increasing with the constant technological progress and the fact that obese persons have been reported to "sit" more than lean persons (Tryon *et al.*, 1992), our results in **Chapter 1** reveal that physical inactivity induces metabolic alterations similar to those found in obesity such as reduction in total, fasting and exogenous lipid oxidation although not to a similar extent. Taken together and when looking at the left side of the positive relationship of AEE with exogenous fat oxidation we found, we can suggest that more sedentary behaviours will worsen the average person risk of developing weight gain and eventually obesity, a question recently highlighted by Hamilton *et al.* (Hamilton *et al.*, 2007). However, our results give further answers by looking in the opposite side of this relationship; we can open a new hope that by increasing physical activity, potential improvements can be achieved in metabolic health, when sufficient increase in energy expenditure is achieved.

In this line, it is not surprising to note that there is no obesity in the high active individuals, while those who abandon their activity, frequently show weight gain and increases in adiposity (Williamson, 1996; Haapanen *et al.*, 1997b). Interestingly although our active group maintained an average PAL after detraining of 2.09 which is above the general population PAL, it was submitted to significant metabolic reductions in total and exogenous lipid oxidation suggesting that these alterations may occur independently of energy balance, through specific mechanisms related to stopping exercise *per se*. We can suppose that our active subjects maintained their usual NEAEE and only stopped their habitual structured exercise. This finding raises the importance of NEAEE combined to structured exercise for metabolic health.

1.3.3. Does NEAEE modulation predict dietary lipid metabolism?

Recently, Bergouignan *et al.* (Bergouignan *et al.*, 2009b) during bed rest studies in subjects with NEAEE deficiency, found that whereas the combined resistive and aerobic exercise partially maintained the total fat oxidation likely due to the exercise induced energy expenditure, it did not mitigate the decrease in exogenous palmitate oxidation. Taking these results together, they supposed that this lack of impact on exogenous fat oxidation is rather caused by the NEAEE deficiency rather by insufficient exercise induced energy expenditure. They further extrapolated their results in assuming that whereas total lipid oxidation may be a function of exercise induced energy expenditure, dietary fat oxidation would be function of NEAEE.

Our results show a positive relationship between AEE and exogenous lipid metabolism in lean subjects. Although we measured overall AEE and we did not differentiate between AEE spent with NEAEE and AEE spent with the structured exercise, the lack of improvement in overweight subjects of exogenous fatty acid oxidation in parallel with reduction in NEAEE suggests that predominantly the relation we found between AEE and exogenous fatty acid oxidation can be extrapolated indeed to a relationship between NEAEE and exogenous fatty acid oxidation. In this line, Stubbs *et al.* (Stubbs *et al.*, 1995b) showed that active individuals tolerated more the high fat diet compared with inactive and this may be related to differences in daily NEAEE. Moreover, Smith *et al.* (Smith *et al.*, 2000a) and Hansen *et al.* (Hansen *et al.*, 2007) reported that the increase in the daily energy expenditure accelerated the adjustment of fat oxidation to the proportion of fat ingested in men and women respectively.

In other words, the lack of improvement in exogenous fatty acid we observed in overweight subjects is not surprising, if we can suggest that this is mainly due to the lack of NEAEE, independently of an increase in structured exercise. The positive relationship we found of AEE and exogenous fatty acid oxidation suggests that improvement can be achieved when sufficient increase in AEE. These increases might be produced in enhancing NEAEE. Thus NEAEE might be a key modulator in body weight regulation through lipid balance. On the other hand, we cannot rule out the beneficial effect *per se* on lipid metabolism. Indeed, even though with deficiency in NEAEE, overweight subjects succeeded when training with structured exercise, in ameliorating their fasting fat oxidation, which represents the whole body metabolic flexibility according to Corpeleijn *et al.* (Corpeleijn *et al.*, 2009). Thus, AEE according to its type (structured *vs.* NEAEE) might have differential effect either on total or on exogenous lipid metabolism. In accordance, previous studies showed that dietary fat oxidation is differentially affected by physical activity than global fat oxidation (Bergouignan *et al.*, 2006), and we confirmed this result in the lean and overweight trained group. A question thus arises, is there differential effect on lipid metabolism between NEAEE and structured exercise? In the next paragraph, we propose protocols for further studies that might answer such a question.

1.3.4. NEAEE *vs.* structured exercise: future researches perspectives

The total amount of time and energy expended during exercise is less than that during non exercise activity. Whereas most of exercisers of our society participate in exercise for less than 2h/week, which corresponds to an average energy expenditure of less than 100 kcal/d, NEAEE has been reported to vary up to 2000 kcal/d (Levine, 2007). In attaining weight loss, exercise seems less efficacious compared with dietary restrictions (Miller *et al.*, 1997). This observation is beyond the negative relationship between physical activity level and body weight or fat mass. This deficiency in results is likely due to a non adherence to exercise prescriptions in overweight subjects (Whybrow *et al.*, 2008) and as shown by our results, might be due to a decrease in the daily AEE similarly to other studies (Stubbs *et al.*, 2004). Therefore, further studies are warranted to investigate the role of the non exercise in the prevention and treatment weight gain, in order to reduce heavy exercise prescriptions which are difficult to follow and are discouraging for sedentary persons. Interestingly, while overweight subjects failed to increase their exogenous fatty acid oxidation, they succeeded in increasing their fasting fatty acid oxidation, enhancing thus the role of structured exercise *per se* on total lipid metabolism. In the light of these results, we think that future studies investigating the role of structured exercise *per se*, NEAEE *per se* and the combined exercise + NEAEE would highlight the importance of each component of AEE on lipid metabolism i.e. total and dietary fat metabolism, in particular:

- A group of sedentary overweight subjects to be trained with controlled non compensatory behaviours in opposite of structured exercise, thus with increased NEAEE, would confirm if physical activity is able to affect exogenous lipid metabolism in overweight subjects such it does in lean ones (**Figure 3A**)

- Two groups of sedentary lean subjects to be trained with a similar amount of total physical activity either spent in structured exercise *per se* or NEAEE *per se* (**Figure 3B**) would permit to differentiate between the effect of NEAEE and structured exercise.

- Two groups of active lean subjects to be detrained, with keeping a low level of NEAEE, and adding either exercise or no exercise (**Figure 3C**). These groups might highlight the effect of detraining combined or not with exercise. The low level of NEAEE would allow these groups to be compared to general sedentary population preventing the interference of metabolic alterations induced by extreme physical inactivity, such in the bed rest studies i.g. muscle atrophy.

Figure 3 is a summary of our results on total energy expenditure induced by our protocols and in accordance, proposals for future researches in order to investigate the potential differential role between NEAEE and structured exercise. While increased energy expenditure through NEAEE and structured exercise has a beneficial role in weight prevention through improvements in lipid metabolism, the direct corollary of this observation is that reduction in energy expenditure leads to the opposite effect and thus may promote weight gain. Modulating activity energy expenditure, beyong its effects on total energy expenditure induces changes in the other compoment of energy balance: the energy intake.

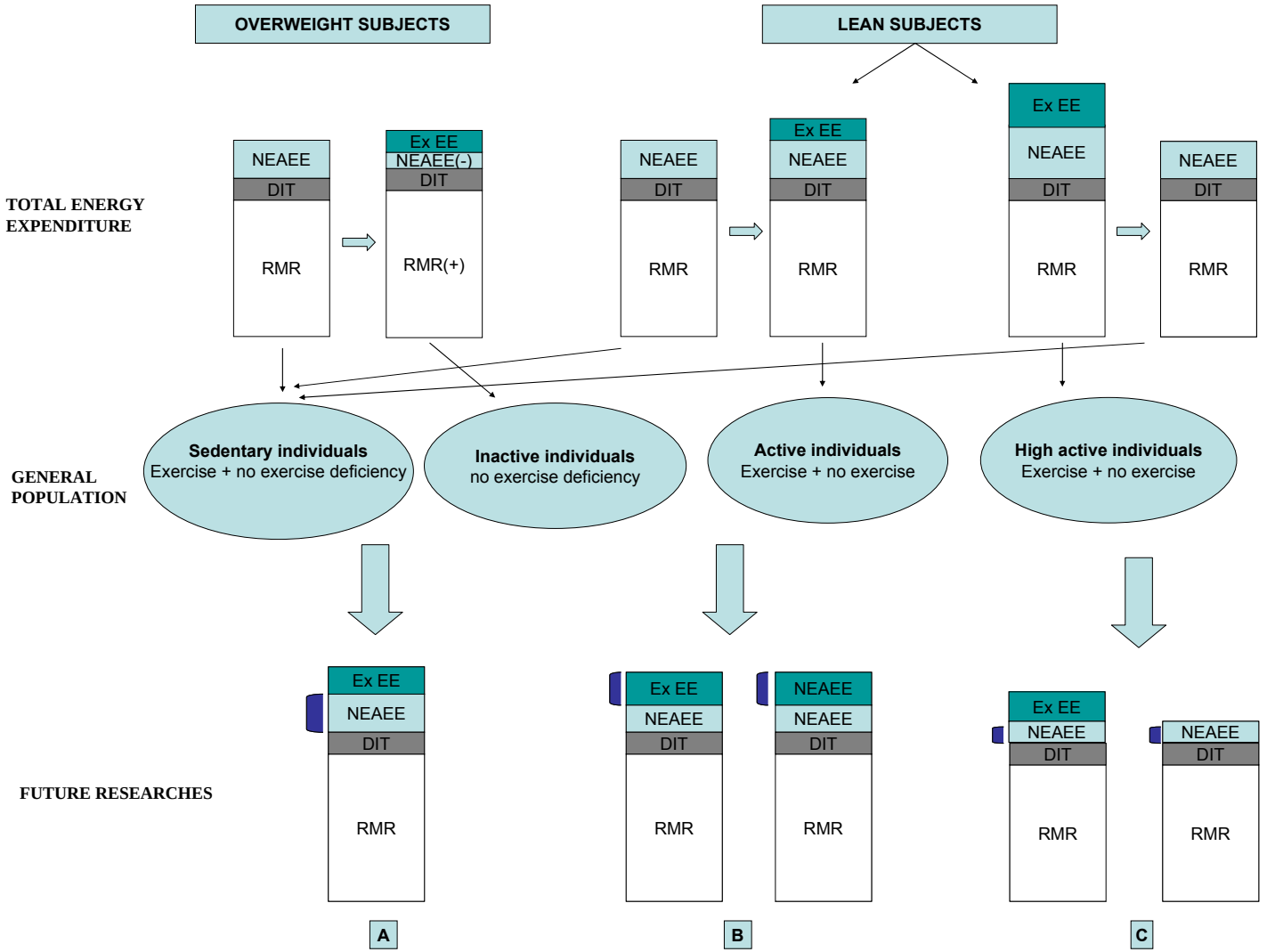


Figure 3: Comparison of the total energy expenditure of overweight and lean subjects before and after physical activity interventions with that of general population, and propositions for future researches perspectives to investigate the role of the two components of physical activity: non exercise physical activity (any bodily movement) and structured exercise activity. Ex EE, exercise energy expenditure; NEAEE, non exercise activity energy expenditure, DIT, diet induced thermogenesis, RMR, resting metabolic rate.

1.4. Effect of modulating physical activity on energy intake

Body weight and energy balance can be maintained by adapting energy intake to changes in energy expenditure, whereas short-term changes in energy expenditure are mainly caused by physical activity. Energy intake can be affected by modulation in physical activity and vice versa.

1.4.1. Does energy intake follow energy expenditure reduction?

Studies have shown differential results when physical activity is combined with weight loss, weight loss *per se* or physical activity per se (Corpeleijn *et al.*, 2009). Therefore, our aim in this work was to investigate the effect of modulating the physical activity *per se* on total energy expenditure and metabolism, independently of energy balance. Particular attention was paid to the maintenance of energy balance throughout the intervention despite the changes induced by physical activity modulation. This was achieved through appropriate dietary advice by dieteticians and the combined measurement of weight and body composition during the period of physical activity intervention. All three groups, lean detrained, lean and overweight trained groups succeeded in maintaining their body weight as required by the protocol. However, we have shown that after one month of detraining, subjects reduced significantly their energy intake; while trained groups did not significantly change their energy intake. These results suggest that on long term, individuals can adjust their energy intake to imposed physical inactivity. This result is of a particular interest when looking at the epidemiological data that suggest that during the last decades, energy intake and fat consumption increased (Popkin, 2006) and physical activity decreased (Egger *et al.*, 2001). These changes were considered to act in the direction of promoting obesity and diabetes.

Indeed, these observations represent a proof of the impaired capacity in the mechanisms of energy balance regulation which leads to weight gain among sedentary lifestyle individuals in population at risk. However, extrapolation of results into general population has to be done with caution for our subjects were followed by professionals and the changes in their energy intake do not represent probably a voluntary choice. However, similar to our results, even when subjects are fed to *ad libitum*, Bergouignan *et al.* (Bergouignan *et al.*, 2010) showed that energy balance is regulated during two months of physical inactivity with subjects reducing voluntarily their energy intake. Also, during a 6-wk bed rest-induced severe inactivity, a decrease in energy intake was noted gradually to be adjusted with energy requirements (Blanc *et al.*, 1998). Indeed, there is growing evidence that energy intake does decrease to re-establish energy balance but that it may take 2-4 wk for energy intake to match the changes in TEE (Blundell *et al.*, 2003).

On the contrary, short term physical inactivity showed no adjustment in energy intake and suggested that energy intake poorly follows modifications in total energy expenditure, thus resulting in positive energy balance during conditions of reduced physical activity (Murgatroyd *et al.*, 1999; Shepard *et al.*, 2001; Stubbs *et al.*, 2004). Although decreases in activity induces decreases in TEE without compensatory changes in energy intake which leads to a positive energy balance, it seems that enhancing activity engender differential energy induced intake.

Modulating physical activity either by increasing or decreasing engenders differential results in individuals.

1.4.2. Physical activity and food intake

Humans who maintain their body weight stable are those who can adjust their energy intake to their energy requirements as determined by body size and function, which leads to a constancy in body weight and body composition. Human can change their energy intake by a factor at least of three when adapting to the energy expenditure (Westerterp, 2010). Westerterp *et al.* (Westerterp, 2010), combined the results of several studies (Bingham *et al.*, 1989; Blaak *et al.*, 1992; Westerterp *et al.*, 1992; Van Etten *et al.*, 1997) which investigated the effect of exercise training in a total of 40 subjects with *ad libitum* access to food. They concluded, that an exercise-induced increase in energy expenditure induces increased energy intake, thus compensating for the additional requirements, especially at higher exercise loads. At physical activity levels higher than approximately 2.5, subjects have problems maintaining energy balance (Westerterp, 1998) (DeLany *et al.*, 1989; Forbes-Ewan *et al.*, 1989; Hoyt *et al.*, 1991; Jones *et al.*, 1993; Burstein *et al.*, 1996).

On the other hand, Stubbs *et al.* (Stubbs *et al.*, 2004) showed that a reduction in the PAL from 1.8 to 1.4 over 7 days markedly affected energy balance. A change to a sedentary routine did not induce compensatory reduction of energy intake and most of the excess energy was stored as fat. Similarly, weight gain was observed in runners because of reduction in weekly exercise, and it was not reversed by resuming prior activity; this shows that intake follows an increase more than a decrease in activity induced changes in daily energy expenditure (Williams, 2008). Thus, it seems that the change from a physically active to a more sedentary routine does not induce an equivalent reduction in energy intake and generally results in weight gain. When looking at the effect of energy intake on physical activity, different patterns have been also found. An increase in energy intake does not result in an increase in energy expenditure through more body movement (Roberts *et al.*, 1990; Diaz *et al.*, 1992; Pasquet *et al.*, 1992; Levine *et al.*, 1999; Joosen *et al.*, 2005a; Siervo *et al.*, 2008). However, undereating seems to result in decreased habitual or voluntary activity and diet induced reduction of physical activity is difficult to overcome with exercise training (Taylor and Keys, 1950; Redman *et al.*, 2009).

Taking these results together, weight gain observed as a consequence of a change to a more sedentary routine can be explained by the absence of an equivalent reduction in energy intake. Moreover, a more sedentary lifestyle can give more opportunities to eat in our obesogenic environment. An example for this is the relation found between television viewing and fatness which was explained by increased food intake during viewing (Jackson *et al.*, 2009).

While our results shows that the effect of physical activity on energy balance is dependent on body mass and induced compensatory behaviours, the effect of physical activity on oxidative balance seems more enhanced when detrained is imposed. The next section discusses the effect of physical activity on metabolic flexibility and lipid metabolism.

2. PHYSICAL ACTIVITY LEVEL PREDICTS METABOLIC ALTERATIONS

2.1. Modulation of physical activity and metabolic flexibility

2.1.1. Physical activity level predicts metabolic flexibility

Metabolic flexibility is defined as the capacity to increase fat oxidation upon increased fatty acid availability and to switch between fat and glucose as the primary fuel (Corpeleijn *et al.*, 2009). In healthy individuals, during fasting, the body relies mainly on fatty acid oxidation while glucose is saved for glucose-dependent organs. Postprandially, lipolysis is rapidly suppressed by insulin and glucose is used as fuel. Our results show an overall effect of habitual physical activity on the daily postprandial NPRQ and insulin variances. We further showed that habitual PAL predicts MF in lean but not overweight subjects (**Chapter 3**), independent of any overlapping effect of recent exercise and energy balance. The stronger deterioration of MF observed after physical inactivity, when compared to the lack of significant improvement in MF after training at current recommendations in both lean and overweight subjects, highlights the importance of daily living activities.

In this line, during the shift to isoenergetic high-fat diet, Smith *et al.* (Smith *et al.*, 2000b) showed that the ability of lean men to adapt fat oxidation was related to physical fitness and fasting insulin concentration. We showed that both a reduction in spontaneous and structured physical activity and an extreme physical inactivity decreased metabolic flexibility. This statement is based on our observations being consistent with those from previous detraining and bed rest studies, which reported that a reduction in physical activity induces an increased reliance on carbohydrate as an energy substrate associated with a decrease in fat oxidation, an increase in plasma insulin concentration or a decrease in insulin action regardless of the energy balance conditions (Stein and Wade, 2005). Even changes in spontaneous physical activity, as low as a decrease from 6000 to 1500 in daily steps, significantly impairs insulin sensitivity in healthy lean men (Olsen *et al.*, 2008).

On the other hand, exercise training performed at current recommendations (Haskell *et al.*, 2007) provided mitigated results, as only trends were noted with both indexes. Although an obvious positive effect was observed in lean men, it clearly failed to improve metabolic flexibility in overweight subjects and as a consequence, the overall effect of this exercise on metabolic flexibility did not reach statistical significance. The observation, however, that training affected metabolic flexibility along the overall same linear relationship than detraining suggests that the training duration and intensity used in the present study, i.e. on the basis of the current recommendations developed originally for cardiovascular risks, were not enough to significantly affect metabolic flexibility. Moreover, the lack of effect of this exercise training on overweight insulin resistant subjects might simply reflect the fact that exercise training failed in this group to increase TEE.

We can also speculate that the intensity (50% VO_2peak), the duration (1h), the rate (4 times/week) of the sessions and/or the duration of our protocol were insufficient to positively impact metabolic flexibility. The improvement in insulin sensitivity after 7 days of exercise at 70% VO_{2peak}, but not at 50% VO_{2peak}, in obese subjects support such an hypothesis (Kang *et al.*, 1996). The fact that training following current recommendation affects metabolic flexibility along with the linear relationship we observed for habitual baseline physical activity levels supports the idea that a greater modulation in the exercise parameters may promote a higher improvement in metabolic flexibility. On the other hand, our tests took place at least 36hrs after the last bout of exercise to study the effect of training, and the delay might have been too long to highlight the effect of training. Moreover, the fact that the training performed at current recommendations only tend to, but not significantly, improve metabolic flexibility in both lean and overweight men may be simply due to the absence of associated body composition changes.

In conclusion, looking at the deleterious effects of physical activity on metabolic health together with the positive relation of physical activity level in free-living conditions with metabolic flexibility in our subjects, we can suggest that active individuals have a greater ability to switch from free fatty acid to glucose as the primary fuel between the fasting and the fed states than their inactive counterparts. Such a relationship suggests that an increase in physical activity should be associated with an improvement of metabolic flexibility and lipid metabolism whereas a reduction in physical activity level should decrease it. However, if enforced physical inactivity effectively impairs metabolic flexibility in lean active subjects, independent of energy balance, the effects of exercise training without weight loss on metabolic flexibility and total lipid metabolism appear less obvious in both lean and overweight sedentary individuals.

2.1.2. Body mass composition in relation with metabolic flexibility

As required by our protocol, our subjects succeeded in maintaining their body weight stable. However, in the detrained group, subjects lost FFM and increased FM while no changes were found in the trained sedentary group. Overweight subjects gained FFM. It is interesting to note that exercise induces differential mechanisms when combined or not with body weight loss. Combined with weight loss, exercise training improves insulin sensitivity (Mensink *et al.*, 2003), fasting fat oxidation and increases mitochondrial content (Goodpaster *et al.*, 2003). Training without weight loss (Duncan *et al.*, 2003) increases the activity of muscle oxidative enzymes and fat oxidation.

Several studies reported that exercise in absence of body mass loss has a relatively modest effect (Venables and Jeukendrup, 2008) or no effect (Dekker *et al.*, 2007) on insulin sensitivity. Only few studies (Houmard *et al.*, 1993; Duncan *et al.*, 2003) showed that exercise, without weight loss, increases insulin sensitivity in previously sedentary adults. In fact, we can argue that only one study (Houmard *et al.*, 1993) reported so far a beneficial effect of exercise *per se* on the response to insulin, since in the other study (Duncan *et al.*, 2003), body mass was effectively stable but body composition was modified by exercise with a reduction in fat mass and an increase in fat-free mass. Based on these evidences, we can assume that the combined resistive and aerobic exercise training performed during the bed rest

studies (Bergouignan *et al.*, 2006; Bergouignan *et al.*, 2009b) had a greater effect on metabolic flexibility than the exercise training at current recommendation because of a significant decrease in fat mass, indicating a negative energy balance. In support of that, the existence of a significant correlation between insulin sensitivity and adiposity is well established and several reports showed that combined exercise training and fat mass loss improves insulin sensitivity (Schenk *et al.*, 2009) and fasting fat oxidation (Goodpaster *et al.*, 2003).

Altogether, these results suggest that the prevention of metabolic inflexibility is related to the combined effect of exercise and the loss of fat mass rather than to the effect of exercise *per se*.

2.1.3. Innovative methodology to investigate metabolic flexibility

To investigate daily metabolic flexibility, we designed two novel statistic indexes derived from the intra-individual variances in insulin and NPRQ in response to two consecutive meals. This need to new indexes was drawn by the fact that the effects of physical activity training and detraining between healthy lean and insulin resistant subjects on metabolic flexibility did not appear significant when using common approaches from previous studies i.e. the change between fasting and post-prandial respiratory quotient (RQ) (Heilbronn *et al.*, 2007) and the area under the curve of RQ following meal ingestion (Corpeleijn *et al.*, 2008). When using indexes that take into account the overall daily responses to meal ingestion rather than RQ change in response to supraphysiological doses of insulin during a clamp or in response to high fat/carbohydrate meal or diets, we succeeded to elucidate variations in metabolic flexibility in lean active and sedentary lean and overweight healthy individuals, undetected by the classical indexes.

With such calculations any potentially confounding fasting contributions (Galgani *et al.*, 2008) is minimized for their weight becomes similar to that of any other data point collected over the day. The variance-derived indexes assumed a metabolically flexible state when the variance in insulin is low and the variance in NPRQ is high; and a metabolically inflexible state when the opposite is observed. We also calculated a complementary index of metabolic flexibility derived for each group being studied rather than individually estimated. In this approach, used to study the effect of training/detraining, we used the overall daily data from each group and the derived percentiles for both insulin and NPRQ. We assumed that the slope of the relationship between NPRQ and insulin percentiles gives us a quantitative insight of the effect of the physical activity intervention on metabolic flexibility. Thus, an increase in the slope reflects a higher shift in NPRQ associated with a lower insulin concentration, which is representative of improvements in metabolic flexibility.

2.2. Physical activity and lipid metabolism

2.2.1. Are current guidelines sufficient for improving total lipid metabolism?

In lean subjects trained according to the current guidelines of physical activity, we did not find significant improvements in fasting and total lipid oxidation (**Chapter 1**) and no correlation was found between AEE and fasting or postprandial lipid oxidation. However, training increased fasting fat oxidation in overweight subjects at rest, similar to other studies with matched energy balance (Poehlman *et al.*, 1994; Calles-Escandon *et al.*, 1996).

There are several reasons to postulate that physical activity might increase the capacity for fat oxidation. While exercise training at moderate intensity induces higher capacity for, and reliance upon, fat oxidation during the exercise session (Bergman *et al.*, 1999), chronic exercise increases the activity of oxidative enzymes and increases capillary density, thus facilitating fatty acid utilization also at rest (Goodpaster *et al.*, 2003). This improvement might also be attributed to an improvement in LPL (Coppack *et al.*, 1996) or alterations in muscle fibers (Kiens, 2006). Body composition might have an important role in enhancing fat oxidation. Indeed, overweight subjects increased their fat free mass and it was shown that changes in fat-free mass correlates with increases in fat oxidation (Poehlman *et al.*, 1994). Increases in RMR and RMR adjusted by FFM in overweight subjects, seem also a potential candidate explaining the improvement in fasting fat oxidation. Postprandial insulin concentration was reduced in overweight subjects suggesting improvement in insulin sensitivity. This might also have a role in improvement in fat oxidation. Insulin sensitivity is related to fat oxidation (Corpeleijn *et al.*, 2009) and particularly the augmentation of resting rates of fat oxidation is a specific metabolic correlate of the amplitude of improvement in insulin sensitivity (Goodpaster *et al.*, 2003). Insulin has a antilipolytic effect, and acts on LPL in adipose tissue to store TG and inhibits HSLP and NEFA liberation (Herd *et al.*, 2001). The efficacy of insulin in reducing lipolysis is much more powerful than in suppressing lipid oxidation (Golay *et al.*, 1986).

On the other hand, in lean subjects, training with no body composition changes, failed to increase fasting fat oxidation. Similarly, other studies found no improvement in fat oxidation in lean subjects at rest (Schrauwen *et al.*, 2002) but rather during exercise as response to chronic training (Sial *et al.*, 1998; Schrauwen *et al.*, 2002). Thus, it seems that fasting fat oxidation in lean subjects is less prone to changes than in overweight subjects when no changes in body weight occur. Combined with fat loss, exercise improves fasting fat oxidation (Corpeleijn *et al.*, 2009). It was shown that exercise, in the absence of weight loss or body mass composition, does not reduce fatty acid mobilization and uptake (Horowitz *et al.*, 2000).

Overall quantity of exercise seem to be primary factor influencing fat oxidation (Tsetsonis and Hardman, 1996b). We can speculate that modulation of exercise parameters such as longer period (>2months) or frequency (>4times/week) might affect more markedly fat metabolism and its related enzymes. However studies of longer duration (3-6months) and higher frequency (4-5 times/week), in

energy balance, found no changes in lipid profile i.e. TG concentration both in lean (Sunami *et al.*, 1999) and overweight (King *et al.*, 1991) subjects.

It should be noted that our subjects did not receive any instructions of increasing further physical activity outside the imposed structured exercise sessions. Thus, again, NEAEE might reinforce any metabolic improvements. In this line, current physical activity guidelines have been bound to several modifications which we resume in the following section.

2.2.2. Improvements of physical activity recommendations in the light of the importance of NEAEE

New improvements in the current guidelines emphasises the role of NEAEE (Table 1). Indeed, recent clarifications of the physical recommendations of The American College of Sports Medicine (ACSM) and the American Heart Association (AHA) (ACSM, 2007) released updated physical activity guidelines in 2007 and pointed to that being active throughout the whole day is beneficial in increasing the effect of the 30mn exercise recommended.

Although the 2007 recommendations are similar to the 1995 recommendations at the core, here we present briefly these modifications in the light of NEAEE importance.

- Moderate-intensity physical activity has been clarified. The 1995 document simply recommended that 30 minute of exercise should be practised preferably all days per week as the recommended frequency while the new recommendation identifies five days per week as the recommended minimum.

- The type of exercise has been specified. Vigorous-intensity physical activity has been explicitly incorporated into the recommendation. Indeed, the current recommendations acknowledge both the preferences of some adults for vigorous-intensity physical activity and the substantial beneficial effect of such exercise, science base related, and encourages the participation in either moderate- and/or vigorous-intensity physical activity. Moderate- and vigorous-intensity activities are complementary in the production of health benefits and that a variety of activities can be combined to meet the recommendation based on the amount (intensity x duration), and using the concept of METs (metabolic equivalents) to assign the intensity of the activity.

- The role of NEAEE has been specifically explicated in the current recommendations. Thus, the recommended structured exercise needed is in addition to routine activities of daily life. The updated recommendation now clearly states that the recommended amount of aerobic activity should be followed by an addition of light intensity routine activities of daily living such as self care, casual walking or grocery shopping, or less than 10 minutes of duration such as walking to the parking lot or taking out the trash. In the daily life, only some activities can be considered to have a moderate intensity for at least 10 minutes in duration. However, moderate- or vigorous-intensity activities conducted as a part of routinely daily activities (e.g., brisk walking to work, gardening with shovel, carpentry) realized in bouts of 10 minutes or more can be considered into the recommendation. Although implied, this concept was not effectively communicated in the original recommendation.

- "More is better". The new recommendation highlights the essential fact that physical activity above the recommended minimum amount procures more health benefits. Although the level that provides the optimal benefit for health has not been determined, it likely changes with genetic profile, age, sex, health status, body composition and other factors. Exceeding the minimum recommendation further decreases the risk of chronic diseases induced by an inactive lifestyle. Although the dose-response relation was acknowledged in the 1995 recommendation, this fact is now explicit.

- Short bouts of exercise of 10 minutes are now more explicitly clarified in the new recommendation. Although the original recommendation presented the concept of accumulating short bouts of physical activity toward the 30-minute goal, it was not completely clear there of how short these episodes could be.

- Muscle-strengthening activities have now been included into the physical activity recommendation. The 1995 recommendation introduced this type of exercise, however, it did not make specific declarations in this area.

- The current recommendations are now clearer in wording and minor changes have occurred to enhance clarity in communications. For example, the term "aerobic," or endurance, has been added to clarify the type of physical activity being recommended and to differentiate it from muscle-strengthening exercises.

While these recommendations are clearly beneficial to public health and proven helpful into preventing several diseases i.e. cardiovascular diseases, it is not clear if they are sufficient in preventing weight gain in pre-obese subjects as discussed in the Introduction. Based on our results and on these new recommendations with the emphasis of the role of NEAEE, we believe that future studies combining exercise with NEAEE are warranted to highlight the role of each component of activity energy expenditure to lipid metabolism and body weight regulation, and the total energy expenditure required to induce significant metabolic changes. However, potential benefice is ascertained in exogenous lipid metabolism and in a larger scale in the shift of the substrate use which defines the metabolic flexibility.

Table 1: Guidelines for healthy adults under age 65 - Basic recommendations from ACSM and AHA

All adults should avoid inactivity.
Some physical activity is better than none,
and adults who participate in any amount of physical activity gain some health benefits.

↓

For substantial health benefits,
adults should do at least 150 minutes (2 hours and 30 minutes) a week of moderate-intensity,
or 75 minutes (1 hour and 15 minutes) a week of vigorous-intensity aerobic physical activity,
or an equivalent combination of moderate- and vigorous intensity aerobic activity.
Aerobic activity should be performed in episodes of at least 10 minutes, and preferably,
it should be spread throughout the week.

↓

For additional and more extensive health benefits,
adults should increase their aerobic physical activity to 300 minutes (5 hours) a week of moderate intensity,
or 150 minutes a week of vigorous intensity aerobic physical activity,
or an equivalent combination of moderate- and vigorous-intensity activity.
Additional health benefits are gained by engaging in physical activity beyond this amount.

Adults should also do muscle-strengthening activities that are moderate or high intensity and involve all major muscle groups on 2 or more days a week, as these activities provide additional health benefits.

Moderate-intensity physical activity means working hard enough to raise your heart rate and break a sweat, yet still being able to carry on a conversation.
It should be noted that to lose weight or maintain weight loss, 60 to 90 minutes of physical activity may be necessary.
The 30-minute recommendation is for the average healthy adult to maintain health and reduce the risk for chronic disease.

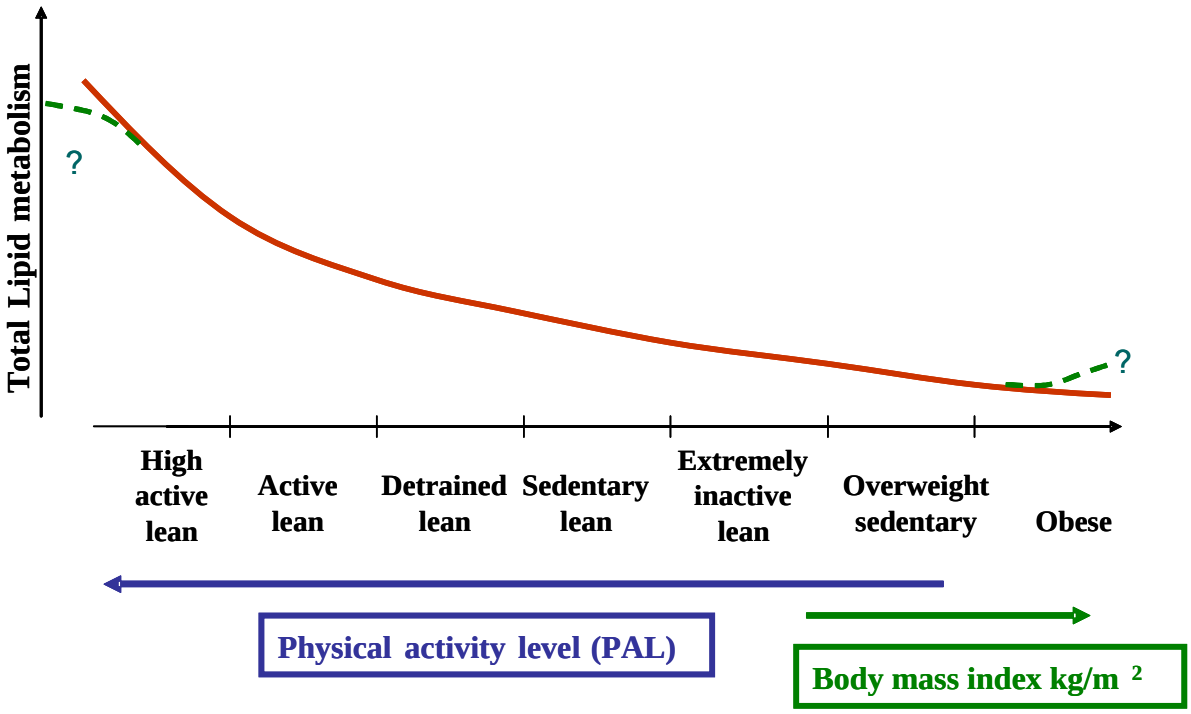


Figure 4: Hypothetical relationships between lipid metabolism, physical activity level and body mass index.

2.2.3. Chronic *vs.* acute effect of exercise on lipid metabolism

Our results reveal that while fasting and postprandial fat oxidation decreased in the detrained group and fasting fat oxidation increases with chronic training in overweight subjects, postprandial fat oxidation does not show significant improvement neither in lean nor overweight groups. In contrast to chronic training, prior exercise increases postprandial fat oxidation (Murphy *et al.*, 2000). Thus, chronic training effect on fat oxidation differs from the acute effect of exercise. Indeed, on the onset of exercise, there is a large increase in uptake and oxidation of long chain fatty acid in skeletal muscle. It is also well recognized that prolonged exercise for several hours at low intensity induces a gradual shift decrease in the respiration quotient and hence an enhanced lipid utilization at the expense of carbohydrates as energy fuel.

It was suggested that exercise has only short term beneficial effect on lipid metabolism (Schenk and Horowitz, 2007). Indeed, the favourable conditions of exercise are rapidly reversed in the absence of recent exercise, i.e. less than 16hr after the session of exercise (Gill and Hardman, 2003; Petitt *et al.*, 2003). De-training studies provide compelling evidence that exercise training may not markedly influence TG metabolism in the absence of recent exercise and that training induced changes in TG metabolism may be short-lived (Gill and Hardman, 2003). In our study, we did not find significant changes in fasting and postprandial NEFA and TG concentrations. Other studies have found no changes in NEFA after three months training nor in the expression of key genes of lipolytic pathway (Richterova *et al.*, 2004). Thus, exercise training in the absence of acute exercise does not appreciably influence postprandial lipemia and TG concentration, and the influence of moderate training on blood lipids is not supported with strong evidence (Tambalis *et al.*, 2009).

In conclusion, although endurance trained people exhibit low levels of postprandial lipemia, this favourable situation is rapidly reversed with de-training and it is likely that the triglyceride (TG) lowering effects of exercise are mainly the result of acute metabolic responses to recent exercise rather than long-term training adaptations. A large body of evidence suggests that postprandial lipemia can be attenuated following an individual exercise session, with the energy expended during exercise being an important determinant of the extent of TG lowering. However exercise training *per se* is likely to be beneficial to TG metabolism, as trained individuals are able to expend more energy during an exercise session than their untrained peers (Gill and Hardman, 2003). In this line, daily physical activity more likely impact lipid metabolism continuously. When looking at chronic training as formed by the accumulation of numerous bouts of exercise, we can rule out the beneficial of exercise to lipid oxidation.

2.3. Physical inactivity: a detrimental status for metabolic health

2.3.1. Detraining effect on metabolic physiology is more pronounced than physical activity

While minor effects appear on total lipid metabolism with training, the effects of detraining are more pronounced and engender deleterious effects on metabolism even though the duration of one month of detraining was less than the two months training (**Chapter 1**). Such findings highlight again the risk of the increased pattern of sedentarism in populations and the urgent need to find solution capable of encountering its effects, for it seems, based on our results, that it will take much longer to heal the metabolic consequences of being inactive than to prevent them. Indeed, with one month of detraining, we found a decrease in fasting, postprandial and exogenous fat oxidation. Detraining is known to be the partial or complete loss of adaptations due to training, in response to a lack of stimulus of training. The characteristics of detraining may differ depending on the duration of detraining or insufficient training (more or less than four weeks of detraining). This model can be used in interventional studies to investigate the effect of inactivity physiology which might resemble to those engendered by sedentarism.

It is well known that skeletal muscle is characterized by its ability to adapt dynamically to varying levels of functional demands. During periods of reduced stimulus to the physical training, a muscular detraining occurs. This detraining is characterized by a reduction in capillary density, which could take place within 2-3 weeks of inactivity (Mujika and Padilla, 2001b). The difference in arteriovenous oxygen decreases if the stop of the training continues beyond 3-8 weeks (Mujika and Padilla, 2001a). It is known that the duration of the depletion is correlated with mitochondrial density in skeletal muscle (Holloszy and Coyle, 1984). A key feature of muscular inactivity is a marked decrease in the oxidative capacity of skeletal muscle, as clearly shown by the reduced activity of mitochondrial enzymes (Mujika and Padilla, 2001b) and thus a rapid and progressive reduction of oxidative enzyme activities contributes to the reduction of mitochondrial production of ATP. The above changes are related to the reduction of VO_2max observed during a long-term detraining. These muscle characteristics remain above sedentary values in an athlete detraining, but generally return to baseline in recently trained subjects. The activities of glycolytic enzymes do not show changes during periods of detraining (Mujika and Padilla, 2001a). The distribution of muscle fibers remains unchanged during the first weeks of inactivity, but may reduce oxidative fibers in athletes of endurance sports and increase in strength sports in the eight weeks of detraining (Mujika and Padilla, 2001b). The performance, in general, is maintained for a maximum of four weeks of inactivity, but of eccentric forces and forces of a specific sport could drop significantly.

At the cellular level, inactive skeletal muscles have a reduced activity of AMPK (Winder and Hardie, 1996), of the mRNA of the nuclear regulator factor-1 (NRF-1) (Gordon *et al.*, 2001), the activity of the synthesis of aminolevulinic acid (ALA) (Holloszy and Winder, 1979), of the mRNA of the mitochondrial transcription factor A (mTFA) (Gordon *et al.*, 2001), and concentrations of protein cytochrome c (Holloszy, 1967) and reduction of mitochondrial density (Holloszy, 1967) than of the active muscles. Published data suggest a potential signalling pathway (caused by increased contractile activity): increased AMPK activity

generates an increase in NRF-1 protein, which in turn binds to promoters of the synthesis of ALA and mTFA genes, leading to an increase of cytochrome c and mitochondrial density (Xia *et al.*, 1997; Bergeron *et al.*, 2001b; Hood, 2001). Because not all promoters of the gene transcription of mitochondrial proteins have binding sites for NRF-1, other transcription factors may be involved in mitochondrial biogenesis modulated by the contractile activity.

The deleterious effects of physical inactivity are enhanced with longer duration. Thus, although metabolic alterations are triggered with short period of physical inactivity, as shown by our results, an intervention that takes longer time i.e. above 2 months or even a life style pattern of sedentarism might manifest increased metabolic alterations.

2.3.2. Effect of a short term detraining

In the short term (less than 4 weeks) and similar to our results in Chapter 1, cardiorespiratory detraining is characterized by a rapid decline in maximal oxygen uptake (VO2max) and blood volume (Mujika and Padilla, 2000). The heart rate increases but not sufficiently to offset the decline in stroke volume and cardiac output is reduced. The ventilation efficiency and performance in endurance are also compromised. These changes are more moderate in recently trained individuals. From a metabolic viewpoint, we found a reduction in fasting and postprandial fat oxidation which suggests that carbohydrate oxidation is favoured. However, our results show no effect of one month detraining on carbohydrate oxidation. Studies have shown, in the same line, that short term inactivity implies an increased dependency on carbohydrate metabolism during exercise, as evidenced by a high respiratory quotient during exercise (Moore *et al.*, 1987), and a reduction of the lipase activity, the content of GLUT-4, glycogen and lactate threshold (Coyle *et al.*, 1985; Mujika and Padilla, 2000). These changes may occur after 10 days of detraining (Mujika and Padilla, 2000). In muscle, capillary density and oxidative enzyme activities are reduced. Looking at the hormonal changes, we found an increase in both fasting and postprandial insulin concentration suggesting a reduced sensitivity to insulin; others studies have found a change in hormones that regulate the flow of electrolytes (Mujika and Padilla, 2001a). Based on the deleterious effect of physical inactivity, we believe that longer duration would have increased these alterations. This suggestion is supported by strong evidence from studies investigating the effect of extreme physical inactivity.

2.3.3. Effect of extreme physical inactivity

In humans, there is a longitudinal model of physical inactivity. This is the model of physical inactivity by prolonged bed rest at -6 ° head sloping used by space agencies to replicate the effects of space environment on the organism (Vernikos, 1996). After an inactivity of short term (7 days) and long term (42 days), several alterations were observed i.e. a development of resistance to effects of insulin, increased plasma triglyceride and fatty acids levels, muscle atrophy and a significant shift in the major substrate oxidation with a reduction of total fat oxidation (Blanc *et al.*, 1998; Blanc *et al.*, 2000). Inactivity reduced fasting and postprandial lipid

oxidation (Bergouignan *et al.*, 2006). However, this effect depends on the chemical nature of lipids (Bergouignan *et al.*, 2009a). Indeed, only the oxidation of saturated fat was reduced (-10%) while that of monounsaturated fats remain unaffected by the inactivity (Bergouignan *et al.*, 2009b).

The first consequence of prolonged bed rest is a net reduction of spontaneous and structured physical activity leading to a decrease in total energy expenditure (TEE) (Gretebeck *et al.*, 1995; Blanc *et al.*, 1998) which is primarily the result of the reduction of expenditure energy-related physical activity (33). A decrease in spontaneous food intake when food is available *ad libitum* (Blanc *et al.*, 1998) was observed ,with a preferential choice of foods rich in carbohydrate at the expense of those rich in lipids in parallel with an increase in leptin which could play a role in reducing spontaneous food intake. There is also a muscular atrophy associated with a greater muscle fatigue and decreased strength and exercise capacity characterized by a decline in aerobic capacity (VO_2max), which depends on the duration of bed rest and the initial level of VO_2max subjects (Blanc *et al.*, 1998; Paddon-Jones *et al.*, 2004; Bergouignan *et al.*, 2006). In addition to muscle atrophy, changes in the proportions of muscle fiber types were observed during long-term capital (Trappe *et al.*, 2004) with a decrease in the percentage of oxidative muscle fibers associated with an increased percentage of glycolytic fibers characterized by a lower insulin sensitivity.

At the metabolic level, there was a decrease in beta-oxidation (Barbe *et al.*, 1998), the activity of muscle lipoprotein lipase (Bey and Hamilton, 2003)), a decrease in gene expression carnityl palmitoyl transferase I (CPT-I), the protein responsible for the entry of long chain fatty acids into mitochondria (Stein *et al.*, 2002), increased glycolytic capacity (Stein *et al.*, 2005) and a reduced capacity to oxidize lipids via a decrease of gene expression of enzymes involved in beta-oxidation (Rimbert *et al.*, 2004). In contrast, no increase in glucose production has been demonstrated in patients in bed rest (Blanc et al., 2000). These alterations in lipid metabolic capacities, hyperlipemia and insulin resistance are similar to those found in healthy inactive subjects and obese or diabetic subjects and give further evidence that the defects in lipid metabolism considered conductive to weight gain might be secondary to the adoption of generalized sedentary behaviours in the general population.

Table 2: Comparison of the altered lipid metabolism observed in our healthy lean detrained subjects, in healthy lean extremely inactive subjects and in obese/diabetics individuals. Data of the extremely inactive subjects are obtained from Bergouignan *et al.* during bed rest studies both in men (Bergouignan *et al.*, 2006) and women (Bergouignan *et al.*, 2009b).

Parameters	Healthy detrained subjects	healthy extreme inactive subjects	Obese subjects and/or diabetic subjects
Body mass	stable	↓	↑↑↑
Fat mass	↓	stable	↑↑
Fat free mass	↓	↓↓	↑↑
Lipemia	stable	↑ in [TG], with a greater spillover of [NEFA] and putative increase in [VLDL]	↑ in [TG], [NEFA] and [VLDL]
Exogenous fatty acid retention in lipid fractions	↑	↑↑	↑↑↑
Insulinemia	↑	↑↑	↑↑
Total fat oxidation	↓	↓↓	↓↓
Exogenous fat oxidation	↓	↓↓	↓↓
Fatty acid transport in muscle :			
into myocyte FAT/CD36	stable	↓ in gene expression	no change reported
into mitochondria CPT1	stable	↓ in gene expression	↓ in gene expression and activity
Mitochondrial oxydative capacity	↓ (via mRNA PRKAA2 decrease)	↓↓ (via mRNA COX4 decrease)	↓↓
[IMTG]intramuscular triglyceride synthesis	↓ (via mRNA mtGPAT decrease)	↑	↑

TG, triglyceride; NEFA, non-esterified fatty acid; VLDL, very low density lipoprotein; CPT1, carnitine palmitoyl transferase1; FAT/CD36, fatty acid translocase CD36; PRKAA2, α2 isoform of the catalytic subunit of the adenosine monophosphate-activated protein kinase AMPK; COX4, citrate synthase.

2.4. Physical activity level predicts exogenous lipid metabolism

Since lipogenesis is negligible in humans, weight gain represents a consequence of a positive lipid balance mainly due to exogenous lipid partitioning towards storage rather than oxidation. Our major finding is that modulation of physical activity differentially alters both monounsaturated and saturated fatty acids. Indeed, PAL explained positively both dietary fatty acid oxidations in lean subjects but not in overweight subjects (**Chapter 1 and Chapter 2**). Training increased exogenous oleate oxidation and palmitate oxidation failed to reach statistically significant values in lean group, while detraining decreased both oleate and palmitate oxidation.

Studies mainly evaluated the total fat or endogenous trafficking with regards to physical activity level (Horowitz and Klein, 2000; Jeukendrup, 2002) and only a small number of studies such as Votruba *et al.* (Votruba *et al.*, 2002) were interested in dietary fat trafficking. Indeed, Votruba *et al.* (Votruba *et al.*, 2002) investigated the effect of different exercise intensity (low, moderate, intensive) on dietary fatty acid utilisation and showed a positive effect of exercise on dietary monounsaturated fatty acid oxidation (oleate) rather than saturated fatty acid (palmitate) and the increase in oleate oxidation was independent of exercise intensity.

On the other hand Bergouignan *et al.* investigated the effect of extreme physical inactivity on oleate and palmitate repartition in two bed rest studies both in men (Bergouignan *et al.*, 2006) and women (Bergouignan *et al.*, 2009b). They found that physical activity decreased palmitate oxidation but failed to show any changes in oleate oxidation. They concluded that while saturated and monounsaturated fats have similar plasma trafficking and clearance, physical inactivity affects the partitioning of saturated fats towards storage, likely leading to an accumulation of palmitate in muscle fat. These results suggested that Mediterranean diets should be recommended in sedentary subjects and recumbent patients.

However, we showed that oleate oxidation is decreased by detraining and the difference between our results and Bergouignan *et al.* (Bergouignan *et al.*, 2006; Bergouignan *et al.*, 2009b) might be due to the metabolic alterations induced by extreme physical activity such as muscle atrophy and changes in muscle fibres type, which did not occur with one month of detraining. Indeed, with extreme physical activity, there is a shift in the muscle fiber types characterized by a decrease in the oxidative twitch fibers (myosin heavy chain [MHC]-I and MHCIIa) and an increase in the glycolytic twitch fibers (MHCIIx) (Salanova *et al.*, 2008; Trappe *et al.*, 2008). Such a fibre type pattern with a higher proportion of glycolytic fibers in skeletal muscle was observed in obese and diabetic subjects (Tanner *et al.*, 2002). It might be that monounsaturated fatty acid are more oxidised in glycolytic fibres than saturated fatty acid, which compensate the decrease of its oxidation in oxidative fibres, when shift into more glycolytic fibers occur with detraining. Since one month of detraining does not induce such alterations in muscle fibre type, the decrease in oleate oxidation would be concomitant to the reduction in palmitate oxidation.

Our results show, that beyond any differential effect between the effects of modulation in physical activity according to the type of dietary fatty acid, both monounosaturated and saturated fatty acids would be modified with modulation of physical activity as shown by the positive relationship of both of these fats with

physical activity level (**Figure 5**). Nevertheless, it seems that saturated fatty acids are more affected by detraining. This result is interesting, considering that the saturated fatty acids are associated with an increased risk of obesity, diabetes (Gonzalez *et al.*, 2000; Votruba *et al.*, 2002; Petitt *et al.*, 2003), probably due to a reduced oxidation (Williams *et al.*, 2000) and storage in adipose tissue (DeLany *et al.*, 2000; Brunner *et al.*, 2001) and intermediate muscle lipids (Garaulet *et al.*, 2001; Sabin *et al.*, 2007), compared with monounsaturated fatty acids. In contrast, monounsaturated fatty acids are considered protective against the development of obesity and insulin resistance (Gonzalez *et al.*, 2000; Votruba *et al.*, 2002; Petitt *et al.*, 2003).

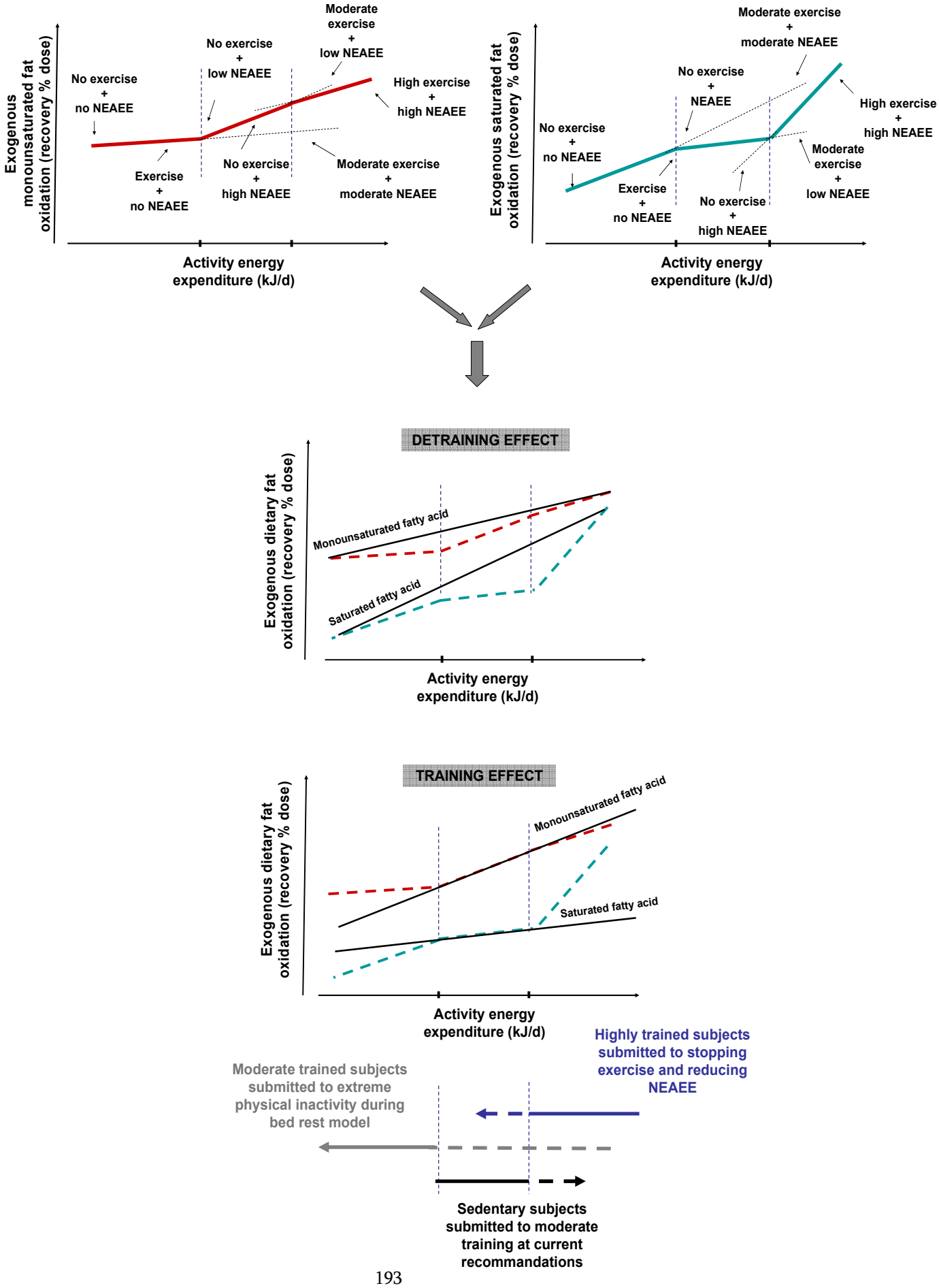
2.5. Applications of our results into public health

2.5.1. The relevance of the relationship of PAL with exogenous fatty acid oxidation into practical health implications

The positive relationship between exogenous fatty acid oxidation with PAL is of a particular importance for it suggests that improvement in exogenous fatty acid oxidations can be achieved with further modulations of physical level such as longer duration or frequency of the exercise applied. Moreover, we suggest that any increase in total energy expenditure by any intervention i.e. increases in NEAEE is of benefit for fat oxidation improvement. In other words, sedentary individuals may beneficially increase their fat oxidation by increasing any sort of activity without constraint of high physical activity imposed programs. However, we can also rule out the importance of the role of structured exercise *per se* and further investigations of a combined or separate structured and/or NEAEE are required. These findings reveal a potential new extent for physical activity guidelines and health outcomes in general population. While current recommendations did not achieve significant changes in total fat oxidation, the question remains how much physical activity would be sufficient to induce improvements in fat metabolism.

While the data investigating a causal role of physical activity in the physiopathology of obesity are unambiguous, our results provide key data in the current debate about the role of physical activity in the treatment of obesity and metabolic disorders associated with physical inactivity, demonstrating a positive relationship between the amount of energy expended during activities and the fate of dietary lipids. This suggests that research efforts on the types of physical activities to promote in different populations, are imperative in the context of treatment and prevention. Given the contrasting effects of the so-called spontaneous activities *versus* structured, it seems essential that these studies take into account the socio-ecological environments in which populations at risks evolve.

Figure 5: Relationships between Activity energy expenditure according to its two components: non exercise activity energy expenditure NEAEE and structured exercise with the exogenous fat oxidation of monounsaturated fatty acid and saturated fatty acid. The slopes of relationship on the down side of the figure represent the differential effect between training at the current recommendations, detraining and extreme physical inactivity. Training was performed in lean sedentary subjects according to the current recommendations of physical activity at 50%VO_2max, detraining was performed in highly active lean subjects by stopping structured exercise and reducing NEAEE, however active subjects maintained a high NEAEE as shown by their physical activity level, and extreme physical inactivity was performed in a moderate active subjects in a bed rest model combined or not with structured exercise. The results of the NO NEAEE at the left side of the graphs are adapted from bed rest studies of extreme physical activity (Bergouignan *et al.* (Bergouignan *et al.*, 2009b), Bergouignan *et al.* (Bergouignan *et al.*, 2006)). Both saturated and monounsaturated fatty acid oxidations are in a direct linear relation with activity energy expenditure. Slopes of relationship show that while monosaturated acid oxidation is more affected by physical activity, physical inactivity effects are enhanced on saturated fatty acid oxidation.


Exogenous monounsaturated fat oxidation (recovery % dose)
No exercise + no NEAEE
Exercise + no NEAEE
No exercise + low NEAEE
No exercise + high NEAEE
Moderate exercise + low NEAEE
Moderate exercise + moderate NEAEE
High exercise + high NEAEE
Activity energy expenditure (kJ/d)
Exogenous saturated fat oxidation (recovery % dose)
No exercise + no NEAEE
Exercise + no NEAEE
No exercise + NEAEE
No exercise + high NEAEE
Moderate exercise + moderate NEAEE
Moderate exercise + low NEAEE
High exercise + high NEAEE
Activity energy expenditure (kJ/d)
DETRAINING EFFECT
Exogenous dietary fat oxidation (recovery % dose)
Monounsaturated fatty acid
Saturated fatty acid
Activity energy expenditure (kJ/d)
TRAINING EFFECT
Exogenous dietary fat oxidation (recovery % dose)
Monounsaturated fatty acid
Saturated fatty acid
Activity energy expenditure (kJ/d)
Highly trained subjects submitted to stopping exercise and reducing NEAEE
Moderate trained subjects submitted to extreme physical inactivity during bed rest model
Sedentary subjects submitted to moderate training at current recommandations

2.5.2. Weight gain prevention: increases in physical activity or reduction in energy intake?

As discussed above, our results show no major improvement in total lipid metabolism with training at current guidelines of physical activity, when no changes in body weight occur. Moreover, it seems that weight gain prevention and treatment is more effectively altered when exercise is combined with weight loss (Corpeleijn *et al.*, 2009). Theoretically, there are two options for reversing the general population trend towards increasing body weight: reducing intake or increasing physical activity. In a recent study of Westerterp *et al.* (Westerterp, 2010), it was shown that overeating does not affect physical activity, while undereating decreases habitual or voluntary physical activity. Thus, it is easier to gain weight than to lose weight. An exercise-induced increase in energy requirement is typically compensated by increased energy intake, while a change to a more sedentary routine does not induce an equivalent reduction of intake and generally results in weight gain. They concluded that preventing overeating by eating less is likely to be the most effective strategy, despite the potential for a negative effect on physical activity when a negative energy balance is reached. There is evidence that physical activity is of importance for weight maintenance, especially for the prevention of weight regains after weight loss. Schoeller *et al.* (Schoeller *et al.*, 1997) assessed physical activity energy expenditure in weight-reduced women and found that lower activity levels were associated with greater weight gains at follow-up. Weinsier *et al.* (Weinsier *et al.*, 2002) compared total free-living activity energy expenditure and physical activity levels in women who were successful and unsuccessful at maintaining a normal body weight. Two groups were identified on the basis of extreme weight changes: weight maintainers, who had a weight gain of ≤2 kg/y, and weight gainers, who had a weight gain of ≥6 kg/y. Gainers had lower activity energy expenditures, lower physical activity levels, and less muscle strength. A lower activity energy expenditure in the gainers explained 77% of their greater weight gain after 1 year. The weight gain observed as a consequence of a change to a more sedentary routine can be explained by the absence of an equivalent reduction of energy intake. Additionally, a more sedentary lifestyle might give more opportunities to eat in our obesogenic environment. Increased television viewing in children has been found to be associated with elevated body fatness but not with lower total energy expenditure (Jackson *et al.*, 2009); the relationship between television viewing and fatness was explained by increased food intake during viewing.

However, with the positive relation of PAL and exogenous lipid oxidation, we can suggest that any increase in fat intake would be partially countered for with an active lifestyle. Thus, although that reduction in energy intake seems essential for the loss of weight or prevention, a healthy metabolic status conferred by an active lifestyle is of an importance even in face of an increased energy intake. Also, the benefits for health of a physically active lifestyle are well documented. In addition to orthopedic complications that are directly attributable to both the amount of physical activity and body mass, and a small proportion of people whose sensitivity to insulin or aerobic capacity do not improve in response to aerobic exercise, physical training has virtually no disadvantages (Teran-Garcia *et al.*, 2005). While most known effects of regular exercise are the control of body weight or, more simply, energy expenditure, it is not necessary for overweight people to reduce the mass of adipose tissue to improve their metabolic homeostasis (Bruce *et al.*, 2004; Troy *et al.*, 2008). Therefore, individuals may benefit from regular exercise through

in several adaptations, including: increased oxidative capacity of skeletal muscle, changes in intracellular proteins and lipids involved in cell signalling and cardiovascular adaptations improving insulin sensitivity of muscles and body in general and the distribution of substrates. All the above modifications lead to the prevention of metabolic diseases and obesity (Jennings *et al.*, 1986; Wojtaszewski and Richter, 2006).

2.5.3. The validity of our model

Our subjects were chosen from general population. We believe that choosing normal active subjects for detraining and sedentary lean subjects for training is representative of the habitual physical pattern accruing in life generally. In this line, although extreme physical inactivity models seems tempting to investigate the effect of reduction of both NEAEE and exercise, however this might induce differential effects such as muscle atrophy which is not observed in sedentary individuals. Moreover, to investigate the effect of training, choosing the current guidelines of physical activity is representative to the overall physical activity level of individuals without the interference of high oxidative capacities occurring when high active individuals train such as athletes. Therefore, studies like ours with moderate levels of inactivity/activity are more representative of habitual patterns, and any derived results can be extrapolated to overall recommendations and clinical implications in general populations. Thus we believe that future studies of a longer term for both moderate inactivity and activity models, with modulation in NEAEE would reveal the effect of physical activity and are adequately representative than extreme conditions such as extreme physical activity model or highly trained athletes who might exhibit high oxidative capacity and VO_2 max uncommon to general population characteristics.

Nevertheless, if we take in consideration the specific alterations and consequences of extreme physical inactivity and activity studies such as muscle atrophy or hypertrophy respectively, these studies would reveal the minimal and the optimal level of physical activity to meet metabolic changes and consequently health benefits.

3. PHYSICAL ACTIVITY MODULATION: MECHANISTIC PATTERNS

While modulation in physical activity level induces differential modification in energy balance as discussed above, we have also observed differential mechanistic patterns between an increase and a decrease in activity energy expenditure.

3.1. Physical inactivity versus physical activity

3.1.1. Physical inactivity versus physical activity: an inverse mirror of physical activity or a distinct metabolic feature?

Hamilton *et al.* (Hamilton *et al.*, 2007) highlighted the potential differences between the exercise physiology and the physical inactivity physiology by suggesting that inactivity initiated unique cellular processes that were qualitatively different from the exercise responses. Indeed, recent studies differentiate between the potentially unique molecular, physiologic and clinical effect of too much sitting (inactivity physiology) separate from the responses caused by structured exercise (exercise physiology). In theory, this may be in part because non exercise activity thermogenesis is generally a much greater component of total energy expenditure than exercise or because any type of brief, yet frequent, muscular contraction throughout the day maybe necessary to short-circuit unhealthy molecular signals causing metabolic diseases. A translational evidence for a molecular reason to maintain adequate levels of daily and frequent low-density activity came from the cellular regulation of skeletal muscle lipoprotein lipase, which is important for controlling plasma triglyceride catabolism (Goldberg *et al.*, 1988; Herd *et al.*, 2001, Bey and Hamilton, 2003), HDL cholesterol (Bey and Hamilton, 2003) and other metabolic risk factors (Komurcu-Bayrak *et al.*, 2007; Saiki *et al.*, 2007). Indeed, LPL regulation was more affected by voluntary reducing normal spontaneous standing and ambulatory time than adding vigorous exercise training (Seip *et al.*, 1997) on top of the normal level of non exercise activity (Hamilton *et al.*, 2007).

3.1.2. Differential mechanistic patterns of physical activity/inactivity effect through mRNA gene expressions

Our results show differential gene expression adaptation to modulation of physical activity level. We found higher expression of FAT/CD36, CPT-1, PGC-1α, SPTLC and DES1 gene expressions after chronic training. Detraining decreased significantly the expression of PRKAA2, mtGPAT and SREBP1 in the skeletal muscle.

- **Training effect**

Although no marked changes in total fat oxidation was found in both lean and overweight trained groups, training increased gene expressions of several enzymes implicated in fat metabolism and transport. This suggests that on a longer term, potential improvement in lipid oxidation might occur with training. Training increased FAT/CD36, CPT-1, PGC-1α, SPTLC and DES1 gene expressions. Other studies have shown that exercise enhances the gene expression of proteins involved in fat oxidative pathways (Tunstall *et al.*, 2002). Indeed, endurance exercise training is known to increase the protein expression and activity of factors associated with mitochondria and β oxidation (Holloszy, 1967; Coyle *et al.*, 1985) such as PGC-1α, PPAR-β in muscle. PGC1α is a co-activator involved in adaptive thermogenesis and fatty-acid oxidation (Meirhaeghe *et al.*, 2003). Thus, we might suggest that the increase in PGC1α might have led, partly to the increase in exogenous lipid oxidation in lean subjects and fasting fat oxidation in overweight subjects through a direct role in lipid oxidation in the mitochondria. This was facilitated by the increased availibity of fatty acid through transport into the cell and subsequently into the mitochondria through enhacement of gene expression of fatty acid transporters CD36 and CPT-1. CD36 has a major role in fatty acid transport across plasma membranes in muscle (Kiens, 2006) and CPT-1 acts as a shuttle transport of fatty acids into the mitochondria. Other studies indicate that exercise training alters the localization of FAT/CD36 and increases its association with CP-1, which may help augment fat oxidation (Schenk and Horowitz, 2006). Similarly, other studies found that endurance training in obese individuals improved CPT-1 activity and reduced sensitivity of CPT-1 for malonyl-CoA (Bruce *et al.*, 2006). Additionally, DES-1 and SPTLC2 were increased in lean group while SPTLC1 gene expression increased in the overweight group. DES1, SPTLC1 and SPTLC2 are responsible of the synthesis of ceramide, an intracellular signalling molecule.

However, studies have related ceramide concentration to insulin resistance and found that total ceramide content of skeletal muscle decreased in conjunction with enhanced insulin sensitivity in obese individuals after a short-term training program (Bruce *et al.*, 2006). On the other hand and in the line of our results, Helge *et al.* (Helge *et al.*, 2004) reported no difference in the ceramide content of muscle from trained and untrained individuals and found that prolonged exercise elevated the content of ceramide fatty acids both in trained and untrained men. Ceramide as a second messenger in regulation of different cell functions, could influence muscle cell adaptation to exercise (Helge *et al.*, 2004). Moreover, we can speculate that since transport of fatty acid into the cell and mitochondria is enhanced through CD36 and CPT-1 gene expression found in both groups, fatty acid availability is increased and exceeds further rate of oxidation improvement requiring longer period, thus fatty acids intermediates such as ceramide can accumulate within the cytosol. Gene expression improvement shown during two months is prior to future potential improvement in fatty acid which may occur later. The main genes affected were those regulating transport into the cell and mitochondria and genes responsible of ceramide synthesis. Consequently, the reduction in oleate and palmitate concentrations in NEFA fractions we found might be attributed to the improvement in transport enzymes inducing an enhanced clearance of exogenous fatty acid into the cell and further into mitochondria to be oxidised.

Altogether, our results show that moderate endurance training increased the expression of genes involved in fat metbolism, specifically, mitochondrial

biogenesis, transcriptional regulation, cytosolic and mitochondrial fatty acid transport and β-oxydation.

- **Detraining effect**

On the other hand, detraining did not induce significant changes in CD36, CPT-1 nor in PGC1-α mRNA gene expressions. However, it decreased genes responsible in IMTG formation and the activity of AMPK. Contrary to training, we found that PRKAA2, SREBP1 and mGPAT genes expressions were lower in the skeletal muscle of detrained subjects which might have led to a higher fatty acid turnover (Holloway *et al.*, 2009a; Holloway *et al.*, 2009b). The lower expression of catalytic subunit (PRKAA2) of AMPK as it was detected in other studies in detrained skeletal muscle would influence the reduction of dietary fatty acid oxidation (McGee *et al.*, 2003). AMPK was proposed to regulate the translocation of FAT/CD36 notably during muscle contraction and enhance fatty acid oxidation (Dzamko *et al.*, 2008). SREBP1 is responsible for sterol syntheses and recently have been advocated a role in the formation of IMTG in highly active athletes (Nadeau *et al.*, 2006). mtGPAT is present on mitochondria outer membrane and also participates in IMTG production and reduces accumulation of fatty acid metabolites within the skeletal muscle (Schenk and Horowitz, 2007). On the other hand, other studies found that endurance training increased the expression of SREBP-1 and mtGPAT (Kelley, 2002) and thus it is possible that detraining might have an opposite effect which was confirmed by our results. However, we did not observe significant changes in SREBP-1 and mtGPAT in our trained subjects. Moreover, the low expression of SREBP-1 found in the skeletal muscle of the detrained group might also reflect lower insulin sensitivity as a study by Guillet-Deniau et al. proposed that SREBP-1c expression can mimic the effect of insulin on lipogenic enzyme expression (Guillet-Deniau et al., 2002). In accordance, insulin concentrations were increased after detraining, suggesting a decrease in insulin sensitivity. These results are more interesting when looking at the potential relationship of IMTG and insulin resistance.

In conclusion, detraning decreased the expression of genes involved in the synthesis of intramuscular lipid and oxidative and transport capacity as reglulated by AMPK.

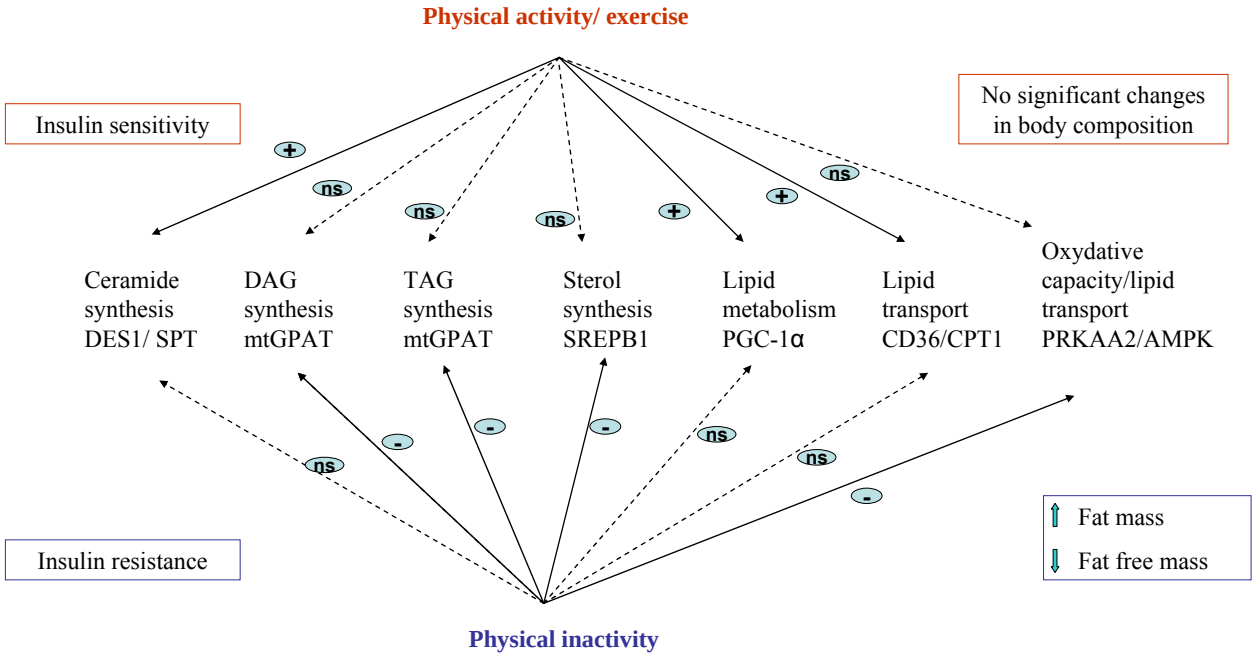


Figure 7: Effect of the physical activity level on lipid biosynthesis and oxidation in muscle cell via regulation of gene expressions, and the consequences on insulin responsiveness and body composition changes, independently of energy balance.
DES1, dihydroceramide desaturase; SPT, serine palmitoyl transferase; mtGPAT, mitochondrial glycerol-3-phosphate transferase; SREBP1, sterol regulatory element-binding protein 1; PGC1-α, peroxisome proliferator-activated receptor gamma coactivator 1-alpha; CD36, cluster of differentiation 36; CPT1, carnitine palmitoyl transferase1, PRKAA2, 5'-AMP-activated protein kinase catalytic subunit alpha-2; AMPK, 5' AMP-activated protein kinase, DAG, diacylglycerol; TAG, triacylglycerol; ns, non significant effect.

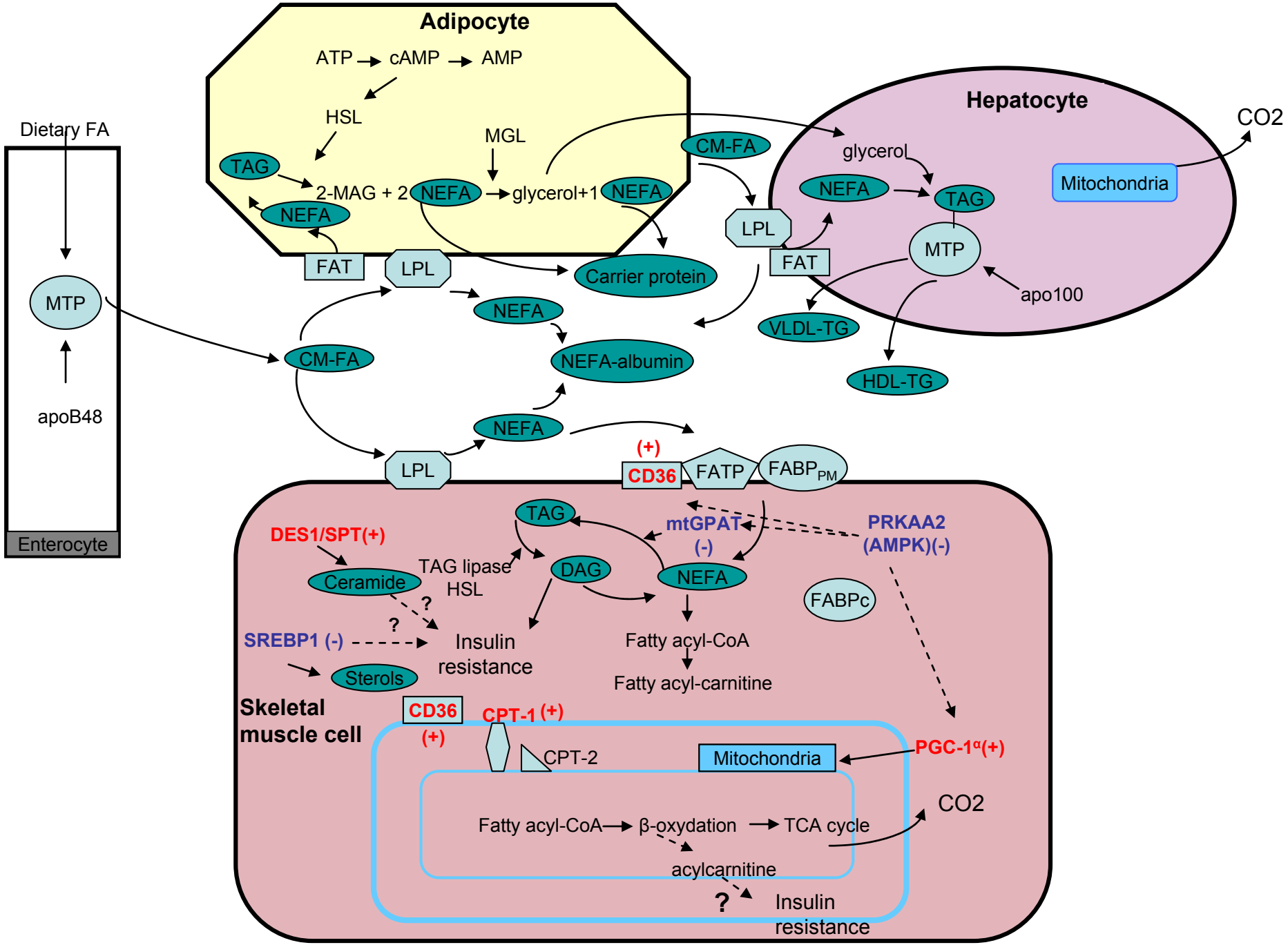

Adipocyte
ATP
cAMP
AMP
HSL
MGL
TAG
2-MAG + 2
NEFA
glycerol+1
NEFA
NEFA
FAT
LPL
Carrier protein
CM-FA
Hepatocyte
glycerol
NEFA
TAG
Mitochondria
CO2
LPL
FAT
MTP
apo100
VLDL-TG
HDL-TG
Dietary FA
MTP
apoB48
Enterocyte
CM-FA
NEFA
NEFA-albumin
NEFA
LPL
(+)
CD36
FATP
FABPPM
TAG
mtGPAT
(-)
PRKAA2
(AMPK)(-)
DES1/SPT(+)
Ceramide
TAG lipase
HSL
DAG
NEFA
FABPc
?
?
SREBP1 (-)
Insulin
resistance
Fatty acyl-CoA
Fatty acyl-carnitine
Sterols
Skeletal
muscle cell
CD36
(+)
CPT-1 (+)
CPT-2
Mitochondria
PGC-1α(+)
CO2
Fatty acyl-CoA
β-oxydation
TCA cycle
acylcarnitine
?
Insulin
resistance

Figure 6: Dietary fatty acids (FA) trafficking based on in vivo studies in relation with mRNA gene expression of enzymes involved in lipid trafficking and metabolism and modulation of physical activity level. The mRNA gene expressions that are decreased by detraining are highlighted in blue colour followed by (-). The mRNA gene expressions that are increased by physical activity are highlighted in red colour followed by a (+). After digestion and assimilation, dietary fatty acids FA are packaged into chylomicrons (CM), through the intestinal microsomal triacylglyceride transfer protein (MTP). The CM are hydrolyzed by lipoprotein lipase (LPL). The resultant non esterified fatty acids (NEFA) are then taken up by peripheral tissues via the fatty acid transporters. In the muscle, this process involves transport across the muscle endothelium, transport in the interstitial space as well as transport across cell membranes. There several fatty acid transport proteins including plasma membrane fatty-acid binding protein ($FABP_{PM}$), fatty acid translocase (FAT) and fatty-acid transporter protein (FATP). FAT/CD36 (CD36) present at the membrane cell and at the membrane of mitochondria increased after training enhancing fatty acid transport. Inside the muscle cell, several proteins (cytoplamic fatty –acid binding proteins (FABPc) are involved in trafficking NEFA to cytoplasmic lipid droplets for storage as intramuscular triacylglycerol (TAG) and diacylglycerol (DAG), a process regulated by Glycerol-3-phosphate acyltransferase of mitochondrial membrane (mtGPAT) which was reduced with in the detrained group, or as sterols regulated by SREBP1 which was also decreased with detraining. NEFA can also be stored as ceramide regulated by dihydroceramide desaturase (DES1) and serine palmitoyl transferase (SPT) genes; DES1 and SPTLC gene expressions were both increased with training. However, in the inner mitochondrial membrane, the NEFA, activated by acyl-CoAsynthtase to fatty acyl-CoA, necessitate the carnitine shuttles: carnitine palmitoyl transferase1 (CPT1) and carnitine palmitoyl transferase2 (CPT2). This transport was enhanced by physical activity. Then, they enter the β-oxidation and TCA to be oxidised. (PRKAA2), 5'-AMP-activated protein kinase catalytic subunit alpha-2 of (AMPK), 5' AMP-activated protein kinase, affects simultaneously other gene expressions and it is reduced by detraining while (PGC1-α), peroxisome proliferator-activated receptor gamma coactivator 1-alpha, which has a role in mitochondrial biogenesis is increased with training.

VLDL, very low density lipoprotein; HDL, high density lipoprotein, HSL, hormone sensitive lipase; ATP, adenosine triphosphate; AMP, adenosine monophosphate, cAMP, cyclic adenosine monophosphate; 2-MAG, 2-monoacylglycerol; MGL, monoacylglycerol lipase.

3.1.3. IMTG and insulin resistance

The result we found of the decrease in mGPAT and SREBP1 is interesting when looking at the high level of IMTG observed in the athletes in the literature (Russell, 2004), for it suggests eventually a future decrease in IMTG when detraining. We also found a parallele increase in insulin concentration suggesting reduction in insulin sensitivity. The potential lipotoxic effect of intramyocellular triglyceride (IMTG) accumulation has been suggested to be a major component in the developpement of insulin resistance. The accumulation of intramuscular triglycerides IMTG in sedentary individuals is associated with reduced sensitivity to insulin, whereas among athletes, it is rather related to the availability of energy storage and increased insulin sensitivity, which is called the "paradox of athletes." Increased levels of IMTGs correlate with insulin resistance in both obese and diabetic patients, but this relationship does not exist in endurance trained subjects (Russell, 2004). This may be, in part, related to differences in the gene expression and activities of key enzymes involved in fatty acid transport and oxidation as well as in the peroxidation status of IMTGs in obese/diabetic as compared with trained individuals. In fact, the degree to which elevated fatty acid availability contributes to an increase in ectopic storage of fatty acids in muscle and if that storage could lead to insulin resistance, depends on the quality of the handling of fatty acids in muscle skeletal.

However, when comparing the divergent conditions of obesity and trained subjects, an apparent consistency exists in the relationship between IMTG and insulin sensitivity. It has been suggested by Goodpaster *et al.* (Goodpaster *et al.*, 2001) that this should not be seen as a contradiction, but more as an indication that the relationship between IMTG and insulin resistance is associated with the fatty acid oxidative capacity of the muscle. This suggests that IMTG may not directly affect insulin resistance but instead provide a source for other toxic lipid metabolites. Indeed, as athletes are clearly sensitive to insulin, despite a high content of IMTG (Thamer *et al.*, 2003), it was suggested that the balance between mobilization and oxidation of IMTG-derived fatty acids is a major factor that reduces lipid intermediates and dissociates IMTG storage of muscle insulin resistance (van Loon, 2004). This proposal was supported by a strong increase in the activity of DAG acyltransferase of diacylglycerols in skeletal muscle during exercise, which converts DAG into TAG triacylglycerols. Consequently, transport of fatty acids to storage reduced the levels of DAG and ceramides, increased levels of IMTG and prevented the insulin resistance induced by lipids (Liu *et al.*, 2007; Schenk and Horowitz, 2007).

However, further evidence suggests a strong relationship between fatty acid induced insulin resistance in human skeletal muscle and alterations in the DAG signalling pathways but not ceramide (Russell, 2004). The finding that ceramide might not be linked to insulin resistance might explain the increase in DES1 and SPTLC1 mRNA gene expressions we found after training, when considering that training ameliorated insulin sensitivity in our overweight subjects. Thus, the accumulation of IMTG in muscle is an important risk marker of insulin sensitivity, via lipid intermediates that interfere with insulin signalling. In addition, it is possible that when the accumulation of IMTG is established and lipid intermediates accumulate, they can interfere with the detection and selection of substrate. In situations where demand for energy does not dispute the oxidative capacity of skeletal muscle to fatty acids, the dynamics of lipid oxidation (metabolic flexibility)

with fatty acid uptake and storage in IMTG may be very important to limit the accumulation of lipid intermediates. Thus, the greater IMTG storage in the trained athlete represents an adaptive response to endurance training, allowing a greater contribution of the IMTG pool as a substrate source during exercise. In contrast, elevated IMTG stores in the obese and/or type 2 diabetes patient seem to be secondary to a structural imbalance between plasma free fatty acid availability, fatty acid (FA) storage and oxidation. Therefore, the reported correlation between IMTG content and insulin resistance does not represent a functional relationship, as it is strongly influenced by training status and/or habitual physical activity (van Loon and Goodpaster, 2006).

In conclusion, gene expressions are differentially modified by modulation of physical activity level, reinforcing the idea that physical inactivity patterns are not merely inverse mirror of physical activity.

3.1.4. Gene expressions in overweight subjects

Interestingly, looking at values of several gene expression, while CD36 ranged in the same order between lean and overweight subjects, other gene expressions such as CPT-1 and ACC enzymes responsible for fat oxidation, were divided by two fold or more in overweight subjects. ACC catalyses the carboxylation of acetyl-CoA to form malonyl-CoA, an intermediate that inhibits CPT-1. CPT-1 catalyses the rate limiting step in the transfer of fatty acyl-CoA into mitochondria, where they undergo oxidation (Kiens, 2006). Some studies have shown a reduced activity of CPT1 in muscle of obese and insulin resistant (Kelley *et al.*, 1999). This suggests that fat uptake facilitated by CD36 is similar (Kelley *et al.*, 1999) between lean and overweight subjects, and reduction in fat oxidation is more related to the reduction in fat oxidation enzymes than uptake (Russell, 2004). In other words, fatty acid oxidation is limited by factors inside the muscle cell, rather than by limitations in transmembrane transport (Kiens, 2006). However, a comparison of lean and obese rat muscles revealed that fatty acid transport, esterification, and oxidation are upregulated in muscles of obese Zucker rats (Holloway *et al.*, 2009a). In addition, it was shown recently, that mitochondrial content, rather than mitochondrial capacity is responsible for the reductions in fat oxidation in obesity (Holloway *et al.*, 2009b). In **Chapter 4**, we propose a novel mechanistic pattern to explain the impaired metabolic capacities observed in overweight subjects.

3.2. A novel mechanistic pattern: reduction in TCA cycle to explain impaired fatty acid oxidation

Chapter 4 explicits an interesting finding of the present work explaining a possible mechanistic pattern for the reduction in oxidative capacity in overweight subjects.

The acetate recovery factor (ARF) was proposed to correct tracer derived (^{13}C)-fat oxidation for the loss of label to sequestration within the bicarbonate pool and small metabolites that exchange with the tricarboxylic acid cycle intermediates (TCA cycle) (Sidossis *et al.*, 1995a, 1995b, Pouteau *et al.*, 1998). ARF can be considered as representative of TCA flux. Indeed, acetate is immediately converted into acetyl coenzyme A (acetyl-CoA), and it is assumed that almost 100% of the labelled [^{13}C] acetyl-CoA will enter the TCA cycle (Mion *et al.*, 1964). Under normal resting conditions, only a fraction of the infused [^{13}C]acetate is recovered as $^{13}CO_2$ in breath, indicating that part of the ^{13}C label is lost in the TCA cycle i.e. formation of glutamate and glutamine and in lower quantity in phospho*enol*pyruvate(PEP), and eventually glucose and lactate, in exchange for an unlabeled oxaloacetate (OAA) from gluconeogenic precursors (Sidossis *et al.*, 1995b), or buffered within the bicarbonate pool.

Thus, the extent of underestimation of the true rate of oxidation depends on the rate of the TCA cycle in relation to the rate of exchange reactions (Sidossis *et al.*, 1995b) and the dilution in the bicarbonate pool. In our study, we compiled data from five distinct studies, in which we measured the exogenous [1-^{13}C]ARF, and we found an average dARF of 45.3 ± 1.5% in overweight subjects that was significantly lower than the 50.6 ± 0.6% observed in lean subjects.

The reduction in dARF in overweight subjects is likely due to enhanced metabolic pathways where the labelled carbon can be lost due to either a greater dispersion into the bicarbonate pool or lost via isotopic exchange reactions in the TCA cycle. The flux of the TCA cycle was shown to be influenced by metabolic disorders due to obesity and diabetes (Schrauwen *et al.*, 2000; Schrauwen and Hesselink, 2008). Schrauwen *et al.* (Schrauwen and Hesselink, 2008) hypothesized that a reduced TCA flux occurs in type 2 diabetes mellitus. Further, when skeletal muscle mitochondrial TCA activity was accelerated during exercise, iARF was found to be the highest in lean healthy individuals (Schrauwen *et al.*, 2000), followed by obese than type 2 diabetic patients (Mensink *et al.*, 2001).

At rest, Blaak *et al.* (Blaak *et al.*, 2000b) found that iARF was significantly lower in obese type 2 diabetic muscle compared to obese healthy controls muscle. Furthermore, the ß-adrenergic infusion stimulation-induced increase in iARF was significantly blunted in obese type 2 diabetes. In addition, Koves *et al.* (Koves *et al.*, 2008) found a mismatch between the rate of β-oxidation showed by increased levels of acyl-carnitines (β-oxidation intermediates) (Koves *et al.*, 2008) and the rate of TCA explaining the incomplete oxidation in obese and diabetic myotubes (Gaster, 2009a).

Recently, using ^{13}C magnetic resonance, Befroy *et al.* (Befroy *et al.*, 2007) assessed rates of substrates oxidation in muscle between lean insulin-resistant offspring of type 2 diabetic patients (n=7) and insulin sensitive control subjects (n=12) by monitoring the incorporation of ^{13}C label into C_4 glutamate during [2-^{13}C]acetate infusion. They found that rates of muscle mitochondrial substrate oxidation were decreased in lean, insulin-resistant offspring subjects. Taking these results together and the lower dARF we found in our overweight insulin resistant subjects, we can extend the hypothesis of Schrauwen *et al.* (Schrauwen and Hesselink, 2008) for reduction in TCA flux in type 2 diabetes mellitus to a reduction in TCA flux in overweight insulin-resistant subjects, leading to reduction in lipid metabolism.

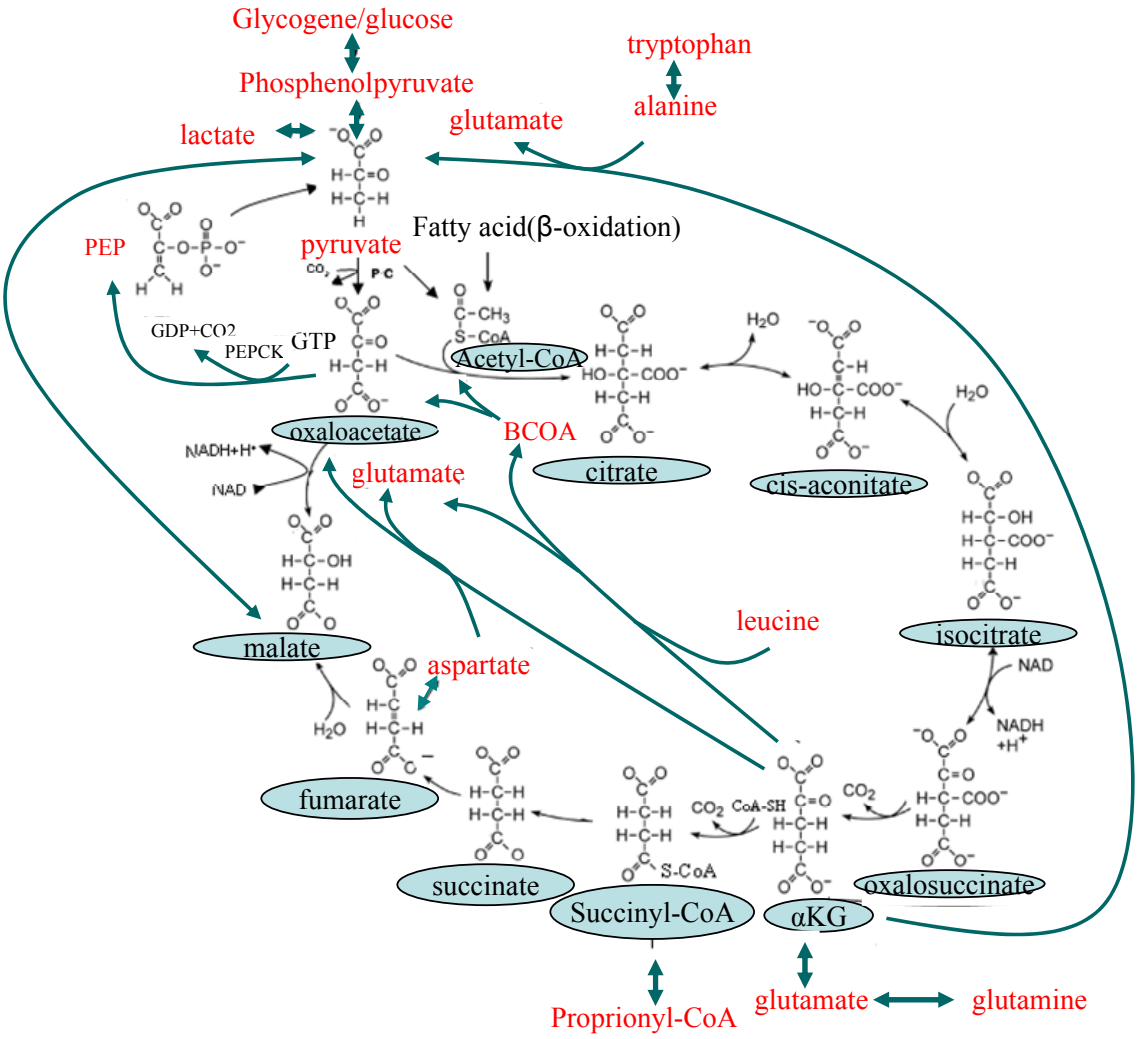


Figure 8: Tricarboxylic acid cycle TCA and potential exchange reactions where label can be lost away from recovery in CO_2. The encircled green parameters represent the main products of TCA. Red parameters are potential products from exchange reactions of TCA we found in the literature van Hall *et al.* (Van Hall *et al.*, 1999), Sorokina *et al.* (Sorokina *et al.*, 2007), Pound *et al.* (Pound *et al.*, 2009), Gaster *et al.* (Gaster, 2009b), Befroy et al. (Befroy *et al.*, 2007), Gibala *et al.* (Gibala *et al.*, 2000), Dioguardi *et al.* (Dioguardi, 2004)).

3.3. Exercise mimicking effect molecules

Our results show a positive effect on genes involved in lipid metabolism. Thus, it would be beneficial for public health to further investigate such molecular variations in order to reproduce them pharmacologically. Through evolution, populations have been genetically programmed to reduce energy expenditure and in the modern world this aspect induced a largely sedentary population (thrifty gene theory: as discussed previously p166). Combined with an energy-rich intake, the result has been an exponential increase in obesity and chronic disease. Although public efforts and initiatives to increase physical activity, participation and adherence rates are low, particularly once obesity and chronic disease are established (Goodyear, 2008; Wing, 1999). These issues point out the need for alternative approaches to decrease the rate obesity and minimize its consequences, while keeping a focus on the molecular targets linked to the beneficial effects of exercise.

Chronic and regular exercise is well known for its beneficial effects and a better knowledge of the molecular mechanisms leading to these health promoting effects is a good basis for initiating new therapeutic targets to fight metabolic and vascular disease. The investigation of metabolic pathways responsive to exercise in various tissues, and in particular skeletal muscle, gave the idea of the promising concept of exercise mimetic drugs. Indeed, the ability to pharmacologically enhance muscular strength is an essential pursuit for many disease states, including metabolic diseases such as type 2 diabetes, and the recent research for 'exercise mimetic' drugs for the treatment of obesity-related metabolic disease has mostly revolved around the endurance exercise capacity in parallele with increasing of energy expenditure and metabolic homeostasis. From an obesity perspective, a pivotal aim would be a development of components that enhance energy expenditure while concomitantly decreasing body fat and increasing metabolic homeostasis.

3.3.1. Potential exercise-related targets

We showed that exercise increase the expression of several genes: FAT/CD36, PGC-1α and CPT1, both in lean and overweight individuals, and thus, it would be beneficial to reproduce pharmacologically such beneficial modifications in these specific genes. Similarly, other studies have shown a positive effect of acute exercise training in modulating the actions, in skeletal muscle, of enzymes, transcription factors, transporters and chaperones thought to control the long-term adaptive responses to chronic training and include: 5'AMP-activated protein kinase (AMPK) (Richter and Ruderman, 2009), p38-mitogen-activated protein kinase (MAPK) (Goodyear *et al.*, 1996), heat shock proteins (Hernando and Manso, 1997; Walsh *et al.*, 2001), peroxisome proliferator-activated receptor (PPAR)-γ coactivator-1α (PGC-1α) (Goto *et al.*, 2000, Baar *et al.*, 2002), nuclear factor κB (Kramer *et al.*, 2007), GLUT4 (Rodnick *et al.*, 1992), fatty acid translocase/CD36 (Bonen *et al.*, 1999), calcium/calmodulin-dependent protein kinase II (CAMK) (Fluck *et al.*, 2000; Rose *et al.*, 2007), certain protein kinase C isoforms (Rockl *et al.*, 2008; Rose *et al.*, 2004), c-Jun N-terminal kinase (Goodyear *et al.*, 1996) and myokines (e.g. IL-6) (Pedersen and Febbraio, 2008).

In general, these adaptations to physical activity have been related with health benefits. Importantly, for some of these factors, e.g. AMPK (Hayashi *et al.*, 1998; Bergeron *et al.*, 2001a; Jorgensen *et al.*, 2005; Jorgensen *et al.*, 2007), heat shock protein 72 (Chung *et al.*, 2008), GLUT4 (Rodnick *et al.*, 1992), PGC-1α (Handschin *et al.*, 2007), protein kinase C-λ (Farese *et al.*, 2007; Rockl *et al.*, 2008) and calcium signalling (Chin, 2005), transgenic, knockout and/or pharmacological activation studies in experimental animals have concluded that manipulation of the relevant pathways in a pattern mimicking exercise may result in beneficial metabolic responses.

In some examples, interestingly, the opposite is true: mouse lines in which signalling through c-Jun N-terminal kinase (Hirosumi *et al.*, 2002) and nuclear factor κB (Kim *et al.*, 2001) modified clearly indicated that a reduction in activity improved insulin resistance, in contrast to the majority of data available, which, as reviewed (Kramer and Goodyear, 2007), suggest that acute exercise activates these pathways. It is likely that such discordance arises from the fact that these processes do not accurately model the episodic nature of exercise. This highlights an important consideration with respect to mimicking the effects of exercise, namely that, in some instances, the level of activity of factors during rest periods in the trained state should be mimicked, rather than the activity of these same factors during an acute exercise bout. This highlights the complexity of metabolic homeostasis and the need for comprehensive characterisation of exercise signalling pathways and their manipulation.

A number of approaches have been used recently to modulate the activity of certain molecules linked to exercise-related signalling processes. Promising results have been shown with respect to their ability to improve both exercise capacity and metabolic health. These target molecules are discussed below, in the light of our results.

PPARδ

We did not find any effect of physical activity training or detraining in PPARδ gene expression. PPARδ is present in rodent and human skeletal muscle, and has a role in muscle fatty acid and glucose oxidation, as well as oxidative gene expression (Muoio *et al.*, 2002; Kramer *et al.*, 2007). Pparδ overexpression has been advocated in the prevention of obesity (Wang *et al.*, 2003). Moreover, it has been shown in mice, that when Pparδ is overexpressed in skeletal muscle, it results in a transition towards increased type 1 (oxidative) muscle fibres and enhanced endurance exercise capacity (Wang *et al.*, 2004). Narkar *et al.* (Narkar et al., 2008) treated mice with a specific PPARδ agonist (GW1516) for 4 weeks and found that while the PPARδ agonist increased the expression of multiple skeletal muscle genes putatively responsible for increasing endurance exercise capacity, however, it failed to increase the proportion of type 1 muscle fibres, and the exercise capacity. When combined with exercise, GW1516 treatment enhanced the effect of the endurance exercise capacity in parallel with a transition to oxidative fibres greater than what was shown with exercise training alone (Narkar *et al.*, 2008). Thus, it is not clear whether the exercise activates a different signalling cascade interacting with PPARδ stimulation in response to training. While obese persons poorly adhere to exercise training programmes, this approach is thus limited, but could accelerate the adaptive response to training if used clinically in conjunction with an exercise programme.

AMPK

We found a lower expression of the muscle-specific catalytic subunit (PRKAA2) of AMPK in detrained skeletal muscle, after one month of detraining. AMPK is central to regulation of cellular metabolism and is activated when the energy charge of the cell is low, e.g. during energy deprivation and exercise (Steinberg and Jorgensen, 2007). AMPK has also a role in regulation of exercise metabolism, including modulation of fibre type shift, gene expression and adaptation to training (Richter and Ruderman, 2009). An activator of AMPK, 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR; also known as Z-riboside or AICA riboside), has a potential role of increasing exercise capacity in mice (Narkar *et al.*, 2008) in the absence of exercise training. This study also provided evidence to suggest interaction between the AMPK and PPARδ pathways. Narkar *et al.* (Narkar *et al.*, 2008) have validated the hypothesis that chronic AMPK activation can copy some of the endurance-enhancing effects of exercise training and, importantly, that it is possible to pharmacologically enhance endurance and muscle oxidative capacity without the need for a simultaneous training programme. Although chronic AICAR treatment of obese rats did not reduce bodyweight and obesity, however it was associated with increased skeletal muscle insulin-sensitivity and prevention of obesity-related complications, thus validating its efficacy (Buhl *et al.*, 2002). Clinically, a part from its exercise mimicking effect, AICAR has clinically beneficial effect. As an example, an acute (2 h) intravenous infusion of AICAR in patients with type 2 diabetes resulted in favourable modulation of plasma glucose and NEFA concentrations (Boon *et al.*, 2008). A medicinal chemistry approach is therefore warranted, in the context of developing suitable compounds for trials of this potential application.

SIRT1

Resveratrol which is a naturally derived polyphenolic compound, found most commonly in the skin of certain grape varieties and in red wine, has a role in protecting mammals from cardio-vascular complications and it is known for its life-extending properties in lower organisms (Baur and Sinclair, 2006). The mammalian homologue SIRT1 was identified as putative primary target for resveratrol. Moreover, resveratrol also induces AMPK activation (Hou *et al.*, 2008). After 3months treatment with resveratrol, Lagouge *et al.* (Lagouge *et al.*, 2006) found an enhanced exercise capacity, and a prevention of protected obesity in mice. Preliminary results in our laboratory showed that this molecule prevent the loss of mass and strength in tail-suspended rat model simulating weightlessness (Momken, personal communication). Lan *et al.* (Lan et al., 2008) established strong molecular links between SIRT1, AMPK, PGC-1α and PPARδ. SIRT1-activating compounds target multiple tissues (Lagouge *et al.*, 2006; Feige *et al.*, 2008), raising the possibility that such agents have the potential to mimic multiple beneficial aspects of the training response rather than inducing a muscle-specific target.

PGC-1α

We have shown that two months training increases the gene expression of PGC-1α both in lean and overweight subjects. The potential role of PGC-1α activation as a molecular target for metabolic diseases has been emphasized, because it is integrated in coordinating the downstream signals from some beneficial effects of several factors such AMPK and PPAR. Moreover, PGC-1α is present in skeletal muscle of humans, and is highly inducible by exercise (Pilegaard *et al.*, 2003; Mathai

et al., 2008). Polymorphisms in this gene have been identified and correlate, independently (Fanelli *et al.*, 2005) or with junction with PPARδ (Andrulionyte *et al.*, 2006; Stefan *et al.*, 2007) with insulin resistance, obesity and the capacity of adaptation to exercise. Recently, Handschin *et al.* (Handschin and Spiegelman, 2008) have highlighted PGC-1α as a potential pharmaceutical target.

A reduced energy intake and elevated energy expenditure through physical activity remain the most effective approach to fight the current obesity epidemic on a society level. An interest in encouraging active life style habits and maintaining them through the adult life is essential. However, a specific category of individuals such as subjects with morbidly obesity, do not have the ability to engage into exercise and will require another approach to produce beneficial health effect. An understanding of the signalling pathways activated by exercise in metabolically relevant tissue has already proven its efficacy and thus opens a new possibility of drug targets to fight obesity. While no single agent will ever mimic the broad range of exercise-related health benefits (Goodyear, 2008; Richter *et al.*, 2008, Church and Blair, 2009; Hawley and Holloszy, 2009), it is of a particular interest in targeting specific aspects of the exercise response. This domain has only been recently investigated in clinical development. Although several targets have been determined, difficulties yield in identifying the complexity of mimicking the overall exercise effect and to develop it pharmacologically.

4. CONCLUSION & PERSPECTIVES

Physical inactivity, regardless of measurable effects on energy balance, decreases total and exogenous fat oxidation, and has a lowering effect on insulin sensitivity. The reduction in total and exogenous fat oxidation is probably due in part to decreased plasma clearance of exogenous fatty acids and a decreased expression of enzymes involved in regulating lipid metabolism. Such effects were reversed with exercise at the current recommendations, however not to a similar extent, suggesting that there was no main effect of current recommendations on lipid metabolism. It seems that only a physical training that increases significantly energy expenditure is capable of counterbalancing the effects of physical inactivity on the distribution of dietary lipids and the involved cellular mechanisms. This conclusion is reinforced by the observation that physical activity level is closely related to exogenous fatty acid oxidation in lean subjects. Thus, while inactivity reduces the oxidation of saturated fatty acids (palmitate) and that of monounsaturated fatty acids (oleate), physical activity, in return improves the oxidation of oleic acid in lean subjects, when energy expenditure is maintained at a sufficient level. Since oleic and palmitic acids appear to have similar absorption, transport and uptake into peripheral tissues, the saturated fatty acids are more oriented toward storage in muscle and adipose tissue, except when sufficient expenditure energy, and mono-unsaturated acids are more directed to be oxidized.

In conclusion, our results show that habitual physical activity level, regardless of quantifiable effects on the energy balance, predicts the oxidation of exogenous lipids and that physical activity level differentially affect the direction of exogenous lipid in favour of oxidation. While data demonstrating a causal role of physical activity in the pathophysiology of obesity are unambiguous, our results provide key data in the current debate about the role of physical activity in the treatment of obesity and metabolic disorders associated with physical inactivity, demonstrating a positive relationship between the amount of energy expended during activities and fate of dietary lipids. This suggests that research efforts on the types of physical activities to promote in different populations are imperative in the context of treatment and prevention. Given the contrasting effects of so-called spontaneous activities versus structured, it seems essential that these studies take into account the socio-ecological risks to which populations evolve. Indeed, it will take much more integrated and international approach to have significant impact on the obesity problem. We must accept that obesity is not just a disease, but a symptom of a much larger global problem- the effect on human health of environment and lifestyle changes. It may not be too late to develop highly integrated policies for education and intervention. Sedentarism and its dramatic consequences, is likely to remain a huge threat to public health in the years to come. In the absence of effective interventions to promote physical activity at all types through exercise and non exercise physical activity, and with reduction of compensatory behaviours to the energy expenditure due to physical activity, the frequency of obesity is likely to escalate worldwide, with the main impact being seen in the population at risk. Thus, prevention of sedentarism and its complications should be essential component of future public health strategies for all nations. An urgent priority is the establishment

of a multidisciplinary international task force representing all parties that can help reverse the underlying socioeconomic causes of the problem and address the issues that have led to the obesity epidemic.

References

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Achten J, Gleeson M, Jeukendrup AE. 2002. Determination of the exercise intensity that elicits maximal fat oxidation. Med Sci Sports Exerc 34:92-97.

ACSM. 2007. The American College of Sports Medicine. Physical activity guidelines. In.

Alpert SS. 2007. The cross-sectional and longitudinal dependence of the resting metabolic rate on the fat-free mass. Metabolism 56:363-372.

Andersson A, Sjodin A, Hedman A, Olsson R, Vessby B. 2000. Fatty acid profile of skeletal muscle phospholipids in trained and untrained young men. Am J Physiol Endocrinol Metab 279:E744-751.

Andersson A, Sjodin A, Olsson R, Vessby B. 1998. Effects of physical exercise on phospholipid fatty acid composition in skeletal muscle. Am J Physiol 274:E432-438.

Andrulionyte L, Peltola P, Chiasson JL, Laakso M. 2006. Single nucleotide polymorphisms of PPARD in combination with the Gly482Ser substitution of PGC-1A and the Pro12Ala substitution of PPARG2 predict the conversion from impaired glucose tolerance to type 2 diabetes: the STOP-NIDDM trial. Diabetes 55:2148-2152.

Arner P. 2005. Human fat cell lipolysis: biochemistry, regulation and clinical role. Best Pract Res Clin Endocrinol Metab 19:471-482.

Astrup A, Buemann B, Western P, Toubro S, Raben A, Christensen NJ. 1994. Obesity as an adaptation to a high-fat diet: evidence from a cross-sectional study. Am J Clin Nutr 59:350-355.

Astrup A, Ryan L, Grunwald GK, Storgaard M, Saris W, Melanson E, Hill JO. 2000. The role of dietary fat in body fatness: evidence from a preliminary meta-analysis of ad libitum low-fat dietary intervention studies. Br J Nutr 83 Suppl 1:S25-32.

Baar K, Wende AR, Jones TE, Marison M, Nolte LA, Chen M, Kelly DP, Holloszy JO. 2002. Adaptations of skeletal muscle to exercise: rapid increase in the transcriptional coactivator PGC-1. Faseb J 16:1879-1886.

Baba T, Kanda T, Yoshida A, Tsukui S, Nara M, Inukai T, Umeda T, Tamura J, Kobayashi I. 2000. Reciprocal changes in leptin and tumor necrosis factor-alpha with exercise in insulin resistant rats. Res Commun Mol Pathol Pharmacol 108:133-143.

Barbe P, Galitzky J, De Glisezinski I, Riviere D, Thalamas C, Senard JM, Crampes F, Lafontan M, Berlan M. 1998. Simulated microgravity increases beta-adrenergic lipolysis in human adipose tissue. J Clin Endocrinol Metab 83:619-625.

Baur JA, Sinclair DA. 2006. Therapeutic potential of resveratrol: the in vivo evidence. Nat Rev Drug Discov 5:493-506.

Befroy DE, Petersen KF, Dufour S, Mason GF, de Graaf RA, Rothman DL, Shulman GI. 2007. Impaired mitochondrial substrate oxidation in muscle of insulin-resistant offspring of type 2 diabetic patients. Diabetes 56:1376-1381.

Bergeron R, Previs SF, Cline GW, Perret P, Russell RR, 3rd, Young LH, Shulman GI. 2001a. Effect of 5-aminoimidazole-4-carboxamide-1-beta-D-ribofuranoside infusion on in vivo glucose and lipid metabolism in lean and obese Zucker rats. Diabetes 50:1076-1082.

Bergeron R, Ren JM, Cadman KS, Moore IK, Perret P, Pypaert M, Young LH, Semenkovich CF, Shulman GI. 2001b. Chronic activation of AMP kinase results in NRF-1 activation and mitochondrial biogenesis. Am J Physiol Endocrinol Metab 281:E1340-1346.

Bergman BC, Butterfield GE, Wolfel EE, Casazza GA, Lopaschuk GD, Brooks GA. 1999. Evaluation of exercise and training on muscle lipid metabolism. Am J Physiol 276:E106-117.

Bergouignan A, Momken I, Schoeller DA, Normand S, Zahariev A, Lescure B, Simon C, Blanc S. 2010. Regulation of energy balance during long-term physical inactivity induced by bed rest with and without exercise training. J Clin Endocrinol Metab 95:1045-1053.

Bergouignan A, Momken I, Schoeller DA, Simon C, Blanc S. 2009a. Metabolic fate of saturated and monounsaturated dietary fats: the Mediterranean diet revisited from epidemiological evidence to cellular mechanisms. Prog Lipid Res 48:128-147.

Bergouignan A, Schoeller DA, Normand S, Gauquelin-Koch G, Laville M, Shriver T, Desage M, Le Maho Y, Ohshima H, Gharib C, Blanc S. 2006. Effect of physical inactivity on the oxidation of saturated and monounsaturated dietary Fatty acids: results of a randomized trial. PLoS Clin Trials 1:e27.

Bergouignan A, Trudel G, Simon C, Chopard A, Schoeller DA, Momken I, Votruba SB, Desage M, Burdge GC, Gauquelin-Koch G, Normand S, Blanc S. 2009b. Physical inactivity differentially alters dietary oleate and palmitate trafficking. Diabetes 58:367-376.

Bessesen DH, Rupp CL, Eckel RH. 1995. Dietary fat is shunted away from oxidation, toward storage in obese Zucker rats. Obes Res 3:179-189.

Bey L, Hamilton MT. 2003. Suppression of skeletal muscle lipoprotein lipase activity during physical inactivity: a molecular reason to maintain daily low-intensity activity. J Physiol 551:673-682.

Biddle SJ, Fox KR. 1998. Motivation for physical activity and weight management. Int J Obes Relat Metab Disord 22 Suppl 2:S39-47.

Bingham SA, Goldberg GR, Coward WA, Prentice AM, Cummings JH. 1989. The effect of exercise and improved physical fitness on basal metabolic rate. Br J Nutr 61:155-173.

Binnert C, Pachiaudi C, Beylot M, Hans D, Vandermander J, Chantre P, Riou JP, Laville M. 1998. Influence of human obesity on the metabolic fate of dietary long- and medium-chain triacylglycerols. Am J Clin Nutr 67:595-601.

Blaak EE, Hul G, Verdich C, Stich V, Martinez A, Petersen M, Feskens EF, Patel K, Oppert JM, Barbe P, Toubro S, Anderson I, Polak J, Astrup A, Macdonald IA, Langin D, Holst C, Sorensen TI, Saris WH. 2006. Fat oxidation before and after a high fat load in the obese insulin-resistant state. J Clin Endocrinol Metab 91:1462-1469.

Blaak EE, van Aggel-Leijssen DP, Wagenmakers AJ, Saris WH, van Baak MA. 2000a. Impaired oxidation of plasma-derived fatty acids in type 2 diabetic subjects during moderate-intensity exercise. Diabetes 49:2102-2107.

Blaak EE, Van Baak MA, Kemerink GJ, Pakbiers MT, Heidendal GA, Saris WH. 1994a. Beta-adrenergic stimulation of energy expenditure and forearm skeletal muscle metabolism in lean and obese men. Am J Physiol 267:E306-315.

Blaak EE, Van Baak MA, Kemerink GJ, Pakbiers MT, Heidendal GA, Saris WH. 1994b. beta-Adrenergic stimulation of skeletal muscle metabolism in relation to weight reduction in obese men. Am J Physiol 267:E316-322.

Blaak EE, Wagenmakers AJ, Glatz JF, Wolffenbuttel BH, Kemerink GJ, Langenberg CJ, Heidendal GA, Saris WH. 2000b. Plasma FFA utilization and fatty acid-binding protein content are diminished in type 2 diabetic muscle. Am J Physiol Endocrinol Metab 279:E146-154.

Blaak EE, Westerterp KR, Bar-Or O, Wouters LJ, Saris WH. 1992. Total energy expenditure and spontaneous activity in relation to training in obese boys. Am J Clin Nutr 55:777-782.

Black AE, Cole TJ. 2000. Within- and between-subject variation in energy expenditure measured by the doubly-labelled water technique: implications for validating reported dietary energy intake. Eur J Clin Nutr 54:386-394.

Black AE, Coward WA, Cole TJ, Prentice AM. 1996. Human energy expenditure in affluent societies: an analysis of 574 doubly-labelled water measurements. Eur J Clin Nutr 50:72-92.

Blair SN, Kohl HW, 3rd, Paffenbarger RS, Jr., Clark DG, Cooper KH, Gibbons LW. 1989. Physical fitness and all-cause mortality. A prospective study of healthy men and women. Jama 262:2395-2401.

Blanc S, Normand S, Pachiaudi C, Fortrat JO, Laville M, Gharib C. 2000. Fuel homeostasis during physical inactivity induced by bed rest. J Clin Endocrinol Metab 85:2223-2233.

Blanc S, Normand S, Ritz P, Pachiaudi C, Vico L, Gharib C, Gauquelin-Koch G. 1998. Energy and water metabolism, body composition, and hormonal changes induced by 42 days of enforced inactivity and simulated weightlessness. J Clin Endocrinol Metab 83:4289-4297.

Blundell JE, Cooling J, King NA. 2002. Differences in postprandial responses to fat and carbohydrate loads in habitual high and low fat consumers (phenotypes). Br J Nutr 88:125-132.

Blundell JE, King NA. 1996. Overconsumption as a cause of weight gain: behavioural-physiological interactions in the control of food intake (appetite). Ciba Found Symp 201:138-154; discussion 154-138, 188-193.

Blundell JE, King NA. 1999. Physical activity and regulation of food intake: current evidence. Med Sci Sports Exerc 31:S573-583.

Blundell JE, Stubbs RJ, Hughes DA, Whybrow S, King NA. 2003. Cross talk between physical activity and appetite control: does physical activity stimulate appetite? Proc Nutr Soc 62:651-661.

Bonen A, Dyck DJ, Ibrahimi A, Abumrad NA. 1999. Muscle contractile activity increases fatty acid metabolism and transport and FAT/CD36. Am J Physiol 276:E642-649.

Bonen A, Tandon NN, Glatz JF, Luiken JJ, Heigenhauser GJ. 2006. The fatty acid transporter FAT/CD36 is upregulated in subcutaneous and visceral adipose tissues in human obesity and type 2 diabetes. Int J Obes (Lond) 30:877-883.

Boon H, Bosselaar M, Praet SF, Blaak EE, Saris WH, Wagenmakers AJ, McGee SL, Tack CJ, Smits P, Hargreaves M, van Loon LJ. 2008. Intravenous AICAR administration reduces hepatic glucose output and inhibits whole body lipolysis in type 2 diabetic patients. Diabetologia 51:1893-1900.

Booth FW, Chakravarthy MV, Gordon SE, Spangenburg EE. 2002. Waging war on physical inactivity: using modern molecular ammunition against an ancient enemy. J Appl Physiol 93:3-30.

Borsheim E, Bahr R. 2003. Effect of exercise intensity, duration and mode on post-exercise oxygen consumption. Sports Med 33:1037-1060.

Bouchard C, Perusse L. 1993. Genetic aspects of obesity. Ann N Y Acad Sci 699:26-35.

Bouchard C, Tremblay A, Despres JP, Nadeau A, Lupien PJ, Theriault G, Dussault J, Moorjani S, Pinault S, Fournier G. 1990. The response to long-term overfeeding in identical twins. N Engl J Med 322:1477-1482.

Bray GA, Popkin BM. 1998. Dietary fat intake does affect obesity! Am J Clin Nutr 68:1157-1173.

Brooks GA, Mercier J. 1994. Balance of carbohydrate and lipid utilization during exercise: the "crossover" concept. J Appl Physiol 76:2253-2261.

Bruce CR, Kriketos AD, Cooney GJ, Hawley JA. 2004. Disassociation of muscle triglyceride content and insulin sensitivity after exercise training in patients with Type 2 diabetes. Diabetologia 47:23-30.

Bruce CR, Thrush AB, Mertz VA, Bezaire V, Chabowski A, Heigenhauser GJ, Dyck DJ. 2006. Endurance training in obese humans improves glucose tolerance and mitochondrial fatty acid oxidation and alters muscle lipid content. Am J Physiol Endocrinol Metab 291:E99-E107.

Brunner EJ, Wunsch H, Marmot MG. 2001. What is an optimal diet? Relationship of macronutrient intake to obesity, glucose tolerance, lipoprotein cholesterol levels and the metabolic syndrome in the Whitehall II study. Int J Obes Relat Metab Disord 25:45-53.

Buhl ES, Jessen N, Pold R, Ledet T, Flyvbjerg A, Pedersen SB, Pedersen O, Schmitz O, Lund S. 2002. Long-term AICAR administration reduces metabolic disturbances and lowers blood pressure in rats displaying features of the insulin resistance syndrome. Diabetes 51:2199-2206.

Burstein R, Coward AW, Askew WE, Carmel K, Irving C, Shpilberg O, Moran D, Pikarsky A, Ginot G, Sawyer M, Golan R, Epstein Y. 1996. Energy expenditure variations in soldiers performing military activities under cold and hot climate conditions. Mil Med 161:750-754.

Calles-Escandon J, Goran MI, O'Connell M, Nair KS, Danforth E, Jr. 1996. Exercise increases fat oxidation at rest unrelated to changes in energy balance or lipolysis. Am J Physiol 270:E1009-1014.

Campfield LA, Smith FJ, Burn P. 1998. Strategies and potential molecular targets for obesity treatment. Science 280:1383-1387.

Carlsson S, Andersson T, Lichtenstein P, Michaelsson K, Ahlbom A. 2006. Genetic effects on physical activity: results from the Swedish Twin Registry. Med Sci Sports Exerc 38:1396-1401.

Caspersen CJ, Powell KE, Christenson GM. 1985. Physical activity, exercise, and physical fitness: definitions and distinctions for health-related research. Public Health Rep 100:126-131.

Chin ER. 2005. Role of Ca2+/calmodulin-dependent kinases in skeletal muscle plasticity. J Appl Physiol 99:414-423.

Chung J, Nguyen AK, Henstridge DC, Holmes AG, Chan MH, Mesa JL, Lancaster GI, Southgate RJ, Bruce CR, Duffy SJ, Horvath I, Mestril R, Watt MJ, Hooper PL, Kingwell BA, Vigh L, Hevener A, Febbraio MA. 2008. HSP72 protects against obesity-induced insulin resistance. Proc Natl Acad Sci U S A 105:1739-1744.

Church TS, Blair SN. 2009. When will we treat physical activity as a legitimate medical therapy...even though it does not come in a pill? Br J Sports Med 43:80-81.

Clement K, Ferre P. 2003. Genetics and the pathophysiology of obesity. Pediatr Res 53:721-725.

Clement K, Viguerie N, Poitou C, Carette C, Pelloux V, Curat CA, Sicard A, Rome S, Benis A, Zucker JD, Vidal H, Laville M, Barsh GS, Basdevant A, Stich V, Cancello R, Langin D. 2004. Weight loss regulates inflammation-related genes in white adipose tissue of obese subjects. Faseb J 18:1657-1669.

Clore JN, Li J, Gill R, Gupta S, Spencer R, Azzam A, Zuelzer W, Rizzo WB, Blackard WG. 1998. Skeletal muscle phosphatidylcholine fatty acids and insulin sensitivity in normal humans. Am J Physiol 275:E665-670.

Cockram CS. 2000. The epidemiology of diabetes mellitus in the Asia-Pacific region. Hong Kong Med J 6:43-52.

Colberg SR, Simoneau JA, Thaete FL, Kelley DE. 1995. Skeletal muscle utilization of free fatty acids in women with visceral obesity. J Clin Invest 95:1846-1853.

Collier GR, McMillan JS, Windmill K, Walder K, Tenne-Brown J, de Silva A, Trevaskis J, Jones S, Morton GJ, Lee S, Augert G, Civitarese A, Zimmet PZ. 2000. Beacon: a novel gene involved in the regulation of energy balance. Diabetes 49:1766-1771.

Coppack SW, Yost TJ, Fisher RM, Eckel RH, Miles JM. 1996. Periprandial systemic and regional lipase activity in normal humans. Am J Physiol 270:E718-722.

Cordain L, Gotshall RW, Eaton SB, Eaton SB, 3rd. 1998. Physical activity, energy expenditure and fitness: an evolutionary perspective. Int J Sports Med 19:328-335.

Corpeleijn E, Mensink M, Kooi ME, Roekaerts PM, Saris WH, Blaak EE. 2008. Impaired skeletal muscle substrate oxidation in glucose-intolerant men improves after weight loss. Obesity (Silver Spring) 16:1025-1032.

Corpeleijn E, Saris WH, Blaak EE. 2009. Metabolic flexibility in the development of insulin resistance and type 2 diabetes: effects of lifestyle. Obes Rev 10:178-193.

Couet C, Delarue J, Ritz P, Antoine JM, Lamisse F. 1997. Effect of dietary fish oil on body fat mass and basal fat oxidation in healthy adults. Int J Obes Relat Metab Disord 21:637-643.

Coyle EF, Martin WH, 3rd, Bloomfield SA, Lowry OH, Holloszy JO. 1985. Effects of detraining on responses to submaximal exercise. J Appl Physiol 59:853-859.

De Glisezinski I, Marion-Latard F, Crampes F, Berlan M, Hejnova J, Cottet-Emard JM, Stich V, Riviere D. 2001. Lack of alpha(2)-adrenergic antilipolytic effect during exercise in subcutaneous adipose tissue of trained men. J Appl Physiol 91:1760-1765.

Dekker MJ, Lee S, Hudson R, Kilpatrick K, Graham TE, Ross R, Robinson LE. 2007. An exercise intervention without weight loss decreases circulating interleukin-6 in lean and obese men with and without type 2 diabetes mellitus. Metabolism 56:332-338.

DeLany JP, Schoeller DA, Hoyt RW, Askew EW, Sharp MA. 1989. Field use of D2 18O to measure energy expenditure of soldiers at different energy intakes. J Appl Physiol 67:1922-1929.

DeLany JP, Windhauser MM, Champagne CM, Bray GA. 2000. Differential oxidation of individual dietary fatty acids in humans. Am J Clin Nutr 72:905-911.

Diaz EO, Prentice AM, Goldberg GR, Murgatroyd PR, Coward WA. 1992. Metabolic response to experimental overfeeding in lean and overweight healthy volunteers. Am J Clin Nutr 56:641-655.

Dioguardi FS. 2004. Wasting and the substrate-to-energy controlled pathway: a role for insulin resistance and amino acids. Am J Cardiol 93:6A-12A.

DiPietro L, Seeman TE, Stachenfeld NS, Katz LD, Nadel ER. 1998. Moderate-intensity aerobic training improves glucose tolerance in aging independent of abdominal adiposity. J Am Geriatr Soc 46:875-879.

Doucet E, Almeras N, White MD, Despres JP, Bouchard C, Tremblay A. 1998. Dietary fat composition and human adiposity. Eur J Clin Nutr 52:2-6.

Dovey SM, Reeder AI, Chalmers DJ. 1998. Continuity and change in sporting and leisure time physical activities during adolescence. Br J Sports Med 32:53-57.

Dowse G, Zimmet P. 1993. The thrifty genotype in non-insulin dependent diabetes. Bmj 306:532-533.

Drake JW. 1999. The distribution of rates of spontaneous mutation over viruses, prokaryotes, and eukaryotes. Ann N Y Acad Sci 870:100-107.

Dreon DM, Frey-Hewitt B, Ellsworth N, Williams PT, Terry RB, Wood PD. 1988. Dietary fat:carbohydrate ratio and obesity in middle-aged men. Am J Clin Nutr 47:995-1000.

Duncan GE, Perri MG, Theriaque DW, Hutson AD, Eckel RH, Stacpoole PW. 2003. Exercise training, without weight loss, increases insulin sensitivity and postheparin plasma lipase activity in previously sedentary adults. Diabetes Care 26:557-562.

Dzamko NL, Schertzer JD, Ryall J, Steel R, Macaulay SL, Wee S, Chen ZP, Michell BJ, Oakhill JS, Watt MJ, Jorgensen SB, Lynch GS, Kemp BE, Steinberg GR. 2008. AMPK independent pathways regulate skeletal muscle fatty acid oxidation. J Physiol.

Eaton SB, Konner M, Shostak M. 1988. Stone agers in the fast lane: chronic degenerative diseases in evolutionary perspective. Am J Med 84:739-749.

Egger GJ, Vogels N, Westerterp KR. 2001. Estimating historical changes in physical activity levels. Med J Aust 175:635-636.

Ekelund U, Aman J, Yngve A, Renman C, Westerterp K, Sjostrom M. 2002. Physical activity but not energy expenditure is reduced in obese adolescents: a case-control study. Am J Clin Nutr 76:935-941.

Fanelli M, Filippi E, Sentinelli F, Romeo S, Fallarino M, Buzzetti R, Leonetti F, Baroni MG. 2005. The Gly482Ser missense mutation of the peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC-1 alpha) gene associates with reduced insulin sensitivity in normal and glucose-intolerant obese subjects. Dis Markers 21:175-180.

Farese RV, Sajan MP, Yang H, Li P, Mastorides S, Gower WR, Jr., Nimal S, Choi CS, Kim S, Shulman GI, Kahn CR, Braun U, Leitges M. 2007. Muscle-specific knockout of PKC-lambda impairs glucose transport and induces metabolic and diabetic syndromes. J Clin Invest 117:2289-2301.

Feige JN, Lagouge M, Canto C, Strehle A, Houten SM, Milne JC, Lambert PD, Mataki C, Elliott PJ, Auwerx J. 2008. Specific SIRT1 activation mimics low energy levels and protects against diet-induced metabolic disorders by enhancing fat oxidation. Cell Metab 8:347-358.

Fernandez-Real JM, Ricart W. 1999. Insulin resistance and inflammation in an evolutionary perspective: the contribution of cytokine genotype/phenotype to thriftiness. Diabetologia 42:1367-1374.

Feskens EJ, van Dam RM. 1999. Dietary fat and the etiology of type 2 diabetes: an epidemiological perspective. Nutr Metab Cardiovasc Dis 9:87-95.

Flatt JP. 1988. Importance of nutrient balance in body weight regulation. Diabetes Metab Rev 4:571-581.

Flatt JP. 1995a. Body composition, respiratory quotient, and weight maintenance. Am J Clin Nutr 62:1107S-1117S.

Flatt JP. 1995b. Use and storage of carbohydrate and fat. Am J Clin Nutr 61:952S-959S.

Flatt JP, Ravussin E, Acheson KJ, Jequier E. 1985. Effects of dietary fat on postprandial substrate oxidation and on carbohydrate and fat balances. J Clin Invest 76:1019-1024.

Flegal KM, Carroll MD, Ogden CL, Johnson CL. 2002. Prevalence and trends in obesity among US adults, 1999-2000. Jama 288:1723-1727.

Fluck M, Waxham MN, Hamilton MT, Booth FW. 2000. Skeletal muscle Ca(2+)-independent kinase activity increases during either hypertrophy or running. J Appl Physiol 88:352-358.

Forbes-Ewan CH, Morrissey BL, Gregg GC, Waters DR. 1989. Use of doubly labeled water technique in soldiers training for jungle warfare. J Appl Physiol 67:14-18.

Friedlander AL, Casazza GA, Horning MA, Buddinger TF, Brooks GA. 1998. Effects of exercise intensity and training on lipid metabolism in young women. Am J Physiol 275:E853-863.

Galgani JE, Moro C, Ravussin E. 2008. Metabolic flexibility and insulin resistance. Am J Physiol Endocrinol Metab 295:E1009-1017.

Gan SK, Kriketos AD, Ellis BA, Thompson CH, Kraegen EW, Chisholm DJ. 2003. Changes in aerobic capacity and visceral fat but not myocyte lipid levels predict increased insulin action after exercise in overweight and obese men. Diabetes Care 26:1706-1713.

Garaulet M, Perez-Llamas F, Perez-Ayala M, Martinez P, de Medina FS, Tebar FJ, Zamora S. 2001. Site-specific differences in the fatty acid composition of abdominal adipose tissue in an obese

population from a Mediterranean area: relation with dietary fatty acids, plasma lipid profile, serum insulin, and central obesity. Am J Clin Nutr 74:585-591.
Gardner DS, Rhodes P. 2009. Developmental origins of obesity: programming of food intake or physical activity? Adv Exp Med Biol 646:83-93.
Gaster M. 2009a. Reduced lipid oxidation in myotubes established from obese and type 2 diabetic subjects. Biochem Biophys Res Commun 382:766-770.
Gaster M. 2009b. Reduced TCA flux in diabetic myotubes: A governing influence on the diabetic phenotype? Biochem Biophys Res Commun 387:651-655.
Gaster M, Rustan AC, Beck-Nielsen H. 2005. Differential utilization of saturated palmitate and unsaturated oleate: evidence from cultured myotubes. Diabetes 54:648-656.
Geisbrecht BV, Gould SJ. 1999. The human PICD gene encodes a cytoplasmic and peroxisomal NADP(+)-dependent isocitrate dehydrogenase. J Biol Chem 274:30527-30533.
Gibala MJ, Young ME, Taegtmeyer H. 2000. Anaplerosis of the citric acid cycle: role in energy metabolism of heart and skeletal muscle. Acta Physiol Scand 168:657-665.
Gill JM, Hardman AE. 2003. Exercise and postprandial lipid metabolism: an update on potential mechanisms and interactions with high-carbohydrate diets (review). J Nutr Biochem 14:122-132.
Golay A, Chen YD, Reaven GM. 1986. Effect of differences in glucose tolerance on insulin's ability to regulate carbohydrate and free fatty acid metabolism in obese individuals. J Clin Endocrinol Metab 62:1081-1088.
Goldberg IJ, Le NA, Ginsberg HN, Krauss RM, Lindgren FT. 1988. Lipoprotein metabolism during acute inhibition of lipoprotein lipase in the cynomolgus monkey. J Clin Invest 81:561-568.
Gonzalez CA, Pera G, Quiros JR, Lasheras C, Tormo MJ, Rodriguez M, Navarro C, Martinez C, Dorronsoro M, Chirlaque MD, Beguiristain JM, Barricarte A, Amiano P, Agudo A. 2000. Types of fat intake and body mass index in a Mediterranean country. Public Health Nutr 3:329-336.
Goodpaster BH, He J, Watkins S, Kelley DE. 2001. Skeletal muscle lipid content and insulin resistance: evidence for a paradox in endurance-trained athletes. J Clin Endocrinol Metab 86:5755-5761.
Goodpaster BH, Katsiaras A, Kelley DE. 2003. Enhanced fat oxidation through physical activity is associated with improvements in insulin sensitivity in obesity. Diabetes 52:2191-2197.
Goodyear LJ. 2008. The exercise pill--too good to be true? N Engl J Med 359:1842-1844.
Goodyear LJ, Chang PY, Sherwood DJ, Dufresne SD, Moller DE. 1996. Effects of exercise and insulin on mitogen-activated protein kinase signaling pathways in rat skeletal muscle. Am J Physiol 271:E403-408.
Gordon JW, Rungi AA, Inagaki H, Hood DA. 2001. Effects of contractile activity on mitochondrial transcription factor A expression in skeletal muscle. J Appl Physiol 90:389-396.
Goto M, Terada S, Kato M, Katoh M, Yokozeki T, Tabata I, Shimokawa T. 2000. cDNA Cloning and mRNA analysis of PGC-1 in epitrochlearis muscle in swimming-exercised rats. Biochem Biophys Res Commun 274:350-354.
Gretebeck RJ, Schoeller DA, Gibson EK, Lane HW. 1995. Energy expenditure during antiorthostatic bed rest (simulated microgravity). J Appl Physiol 78:2207-2211.
Guillet-Deniau I, Mieulet V, Le Lay S, Achouri Y, Carre D, Girard J, Foufelle F, Ferre P. 2002. Sterol regulatory element binding protein-1c expression and action in rat muscles: insulin-like effects on the control of glycolytic and lipogenic enzymes and UCP3 gene expression. Diabetes 51:1722-1728.
Gura T. 1998. Uncoupling proteins provide new clue to obesity's causes. Science 280:1369-1370.
Gustafson SL, Rhodes RE. 2006. Parental correlates of physical activity in children and early adolescents. Sports Med 36:79-97.
Haapanen N, Miilunpalo S, Pasanen M, Oja P, Vuori I. 1997a. Association between leisure time physical activity and 10-year body mass change among working-aged men and women. Int J Obes Relat Metab Disord 21:288-296.
Haapanen N, Miilunpalo S, Vuori I, Oja P, Pasanen M. 1997b. Association of leisure time physical activity with the risk of coronary heart disease, hypertension and diabetes in middle-aged men and women. Int J Epidemiol 26:739-747.
Hamilton MT, Hamilton DG, Zderic TW. 2007. Role of low energy expenditure and sitting in obesity, metabolic syndrome, type 2 diabetes, and cardiovascular disease. Diabetes 56:2655-2667.
Handschin C, Chin S, Li P, Liu F, Maratos-Flier E, Lebrasseur NK, Yan Z, Spiegelman BM. 2007. Skeletal muscle fiber-type switching, exercise intolerance, and myopathy in PGC-1alpha muscle-specific knock-out animals. J Biol Chem 282:30014-30021.
Handschin C, Spiegelman BM. 2008. The role of exercise and PGC1alpha in inflammation and chronic disease. Nature 454:463-469.
Hansen BC. 1999. The metabolic syndrome X. Ann N Y Acad Sci 892:1-24.
Hansen KC, Zhang Z, Gomez T, Adams AK, Schoeller DA. 2007. Exercise increases the proportion of fat utilization during short-term consumption of a high-fat diet. Am J Clin Nutr 85:109-116.
Haskell WL, Lee IM, Pate RR, Powell KE, Blair SN, Franklin BA, Macera CA, Heath GW, Thompson PD, Bauman A. 2007. Physical activity and public health: updated recommendation for adults from

the American College of Sports Medicine and the American Heart Association. Med Sci Sports Exerc 39:1423-1434.
Hawley JA, Holloszy JO. 2009. Exercise: it's the real thing! Nutr Rev 67:172-178.
Hayashi T, Hirshman MF, Kurth EJ, Winder WW, Goodyear LJ. 1998. Evidence for 5' AMP-activated protein kinase mediation of the effect of muscle contraction on glucose transport. Diabetes 47:1369-1373.
Hayes M, Chustek M, Heshka S, Wang Z, Pietrobelli A, Heymsfield SB. 2005. Low physical activity levels of modern Homo sapiens among free-ranging mammals. Int J Obes (Lond) 29:151-156.
Heilbronn LK, Gregersen S, Shirkhedkar D, Hu D, Campbell LV. 2007. Impaired fat oxidation after a single high-fat meal in insulin-sensitive nondiabetic individuals with a family history of type 2 diabetes. Diabetes 56:2046-2053.
Helge JW, Ayre KJ, Hulbert AJ, Kiens B, Storlien LH. 1999. Regular exercise modulates muscle membrane phospholipid profile in rats. J Nutr 129:1636-1642.
Helge JW, Dobrzyn A, Saltin B, Gorski J. 2004. Exercise and training effects on ceramide metabolism in human skeletal muscle. Exp Physiol 89:119-127.
Herd SL, Kiens B, Boobis LH, Hardman AE. 2001. Moderate exercise, postprandial lipemia, and skeletal muscle lipoprotein lipase activity. Metabolism 50:756-762.
Hernando R, Manso R. 1997. Muscle fibre stress in response to exercise: synthesis, accumulation and isoform transitions of 70-kDa heat-shock proteins. Eur J Biochem 243:460-467.
Hill JO, Peters JC. 1998. Environmental contributions to the obesity epidemic. Science 280:1371-1374.
Hirosumi J, Tuncman G, Chang L, Gorgun CZ, Uysal KT, Maeda K, Karin M, Hotamisligil GS. 2002. A central role for JNK in obesity and insulin resistance. Nature 420:333-336.
Holloszy JO. 1967. Biochemical adaptations in muscle. Effects of exercise on mitochondrial oxygen uptake and respiratory enzyme activity in skeletal muscle. J Biol Chem 242:2278-2282.
Holloszy JO, Coyle EF. 1984. Adaptations of skeletal muscle to endurance exercise and their metabolic consequences. J Appl Physiol 56:831-838.
Holloszy JO, Winder WW. 1979. Induction of delta-aminolevulinic acid synthetase in muscle by exercise or thyroxine. Am J Physiol 236:R180-183.
Holloway GP, Benton CR, Mullen KL, Yoshida Y, Snook LA, Han XX, Glatz JF, Luiken JJ, Lally J, Dyck DJ, Bonen A. 2009a. In obese rat muscle transport of palmitate is increased and is channeled to triacylglycerol storage despite an increase in mitochondrial palmitate oxidation. Am J Physiol Endocrinol Metab 296:E738-747.
Holloway GP, Bonen A, Spriet LL. 2009b. Regulation of skeletal muscle mitochondrial fatty acid metabolism in lean and obese individuals. Am J Clin Nutr 89:455S-462S.
Hood DA. 2001. Invited Review: contractile activity-induced mitochondrial biogenesis in skeletal muscle. J Appl Physiol 90:1137-1157.
Horowitz JF, Klein S. 2000. Lipid metabolism during endurance exercise. Am J Clin Nutr 72:558S-563S.
Horowitz JF, Leone TC, Feng W, Kelly DP, Klein S. 2000. Effect of endurance training on lipid metabolism in women: a potential role for PPARalpha in the metabolic response to training. Am J Physiol Endocrinol Metab 279:E348-355.
Horton TJ, Drougas H, Brachey A, Reed GW, Peters JC, Hill JO. 1995. Fat and carbohydrate overfeeding in humans: different effects on energy storage. Am J Clin Nutr 62:19-29.
Horton TJ, Pagliassotti MJ, Hobbs K, Hill JO. 1998. Fuel metabolism in men and women during and after long-duration exercise. J Appl Physiol 85:1823-1832.
Hou X, Xu S, Maitland-Toolan KA, Sato K, Jiang B, Ido Y, Lan F, Walsh K, Wierzbicki M, Verbeuren TJ, Cohen RA, Zang M. 2008. SIRT1 regulates hepatocyte lipid metabolism through activating AMP-activated protein kinase. J Biol Chem 283:20015-20026.
Houmard JA, Shinebarger MH, Dolan PL, Leggett-Frazier N, Bruner RK, McCammon MR, Israel RG, Dohm GL. 1993. Exercise training increases GLUT-4 protein concentration in previously sedentary middle-aged men. Am J Physiol 264:E896-901.
Hoyt RW, Jones TE, Stein TP, McAninch GW, Lieberman HR, Askew EW, Cymerman A. 1991. Doubly labeled water measurement of human energy expenditure during strenuous exercise. J Appl Physiol 71:16-22.
Hu FB, Li TY, Colditz GA, Willett WC, Manson JE. 2003. Television watching and other sedentary behaviors in relation to risk of obesity and type 2 diabetes mellitus in women. Jama 289:1785-1791.
Hube F, Hauner H. 1999. The role of TNF-alpha in human adipose tissue: prevention of weight gain at the expense of insulin resistance? Horm Metab Res 31:626-631.
Hunter GR, Kekes-Szabo T, Snyder SW, Nicholson C, Nyikos I, Berland L. 1997. Fat distribution, physical activity, and cardiovascular risk factors. Med Sci Sports Exerc 29:362-369.
Hunter GR, Kekes-Szabo T, Treuth MS, Williams MJ, Goran M, Pichon C. 1996. Intra-abdominal adipose tissue, physical activity and cardiovascular risk in pre- and post-menopausal women. Int J Obes Relat Metab Disord 20:860-865.
Jackson DM, Djafarian K, Stewart J, Speakman JR. 2009. Increased television viewing is associated with elevated body fatness but not with lower total energy expenditure in children. Am J Clin Nutr 89:1031-1036.

Jebb SA, Prentice AM, Goldberg GR, Murgatroyd PR, Black AE, Coward WA. 1996. Changes in macronutrient balance during over- and underfeeding assessed by 12-d continuous whole-body calorimetry. Am J Clin Nutr 64:259-266.

Jennings G, Nelson L, Nestel P, Esler M, Korner P, Burton D, Bazelmans J. 1986. The effects of changes in physical activity on major cardiovascular risk factors, hemodynamics, sympathetic function, and glucose utilization in man: a controlled study of four levels of activity. Circulation 73:30-40.

Jeukendrup AE. 2002. Regulation of fat metabolism in skeletal muscle. Ann N Y Acad Sci 967:217-235.

Jones PJ, Jacobs I, Morris A, Ducharme MB. 1993. Adequacy of food rations in soldiers during an arctic exercise measured by doubly labeled water. J Appl Physiol 75:1790-1797.

Joosen AM, Bakker AH, Westerterp KR. 2005a. Metabolic efficiency and energy expenditure during short-term overfeeding. Physiol Behav 85:593-597.

Joosen AM, Gielen M, Vlietinck R, Westerterp KR. 2005b. Genetic analysis of physical activity in twins. Am J Clin Nutr 82:1253-1259.

Jorgensen SB, Treebak JT, Viollet B, Schjerling P, Vaulont S, Wojtaszewski JF, Richter EA. 2007. Role of AMPKalpha2 in basal, training-, and AICAR-induced GLUT4, hexokinase II, and mitochondrial protein expression in mouse muscle. Am J Physiol Endocrinol Metab 292:E331-339.

Jorgensen SB, Wojtaszewski JF, Viollet B, Andreelli F, Birk JB, Hellsten Y, Schjerling P, Vaulont S, Neufer PD, Richter EA, Pilegaard H. 2005. Effects of alpha-AMPK knockout on exercise-induced gene activation in mouse skeletal muscle. Faseb J 19:1146-1148.

Jump DB, Clarke SD. 1999. Regulation of gene expression by dietary fat. Annu Rev Nutr 19:63-90.

Kang J, Robertson RJ, Hagberg JM, Kelley DE, Goss FL, DaSilva SG, Suminski RR, Utter AC. 1996. Effect of exercise intensity on glucose and insulin metabolism in obese individuals and obese NIDDM patients. Diabetes Care 19:341-349.

Kant AK, Graubard BI. 2006. Secular trends in patterns of self-reported food consumption of adult Americans: NHANES 1971-1975 to NHANES 1999-2002. Am J Clin Nutr 84:1215-1223.

Kantomaa MT, Tammelin TH, Nayha S, Taanila AM. 2007. Adolescents' physical activity in relation to family income and parents' education. Prev Med 44:410-415.

Kelley DE. 2002. Skeletal muscle triglycerides: an aspect of regional adiposity and insulin resistance. Ann N Y Acad Sci 967:135-145.

Kelley DE, Goodpaster B, Wing RR, Simoneau JA. 1999. Skeletal muscle fatty acid metabolism in association with insulin resistance, obesity, and weight loss. Am J Physiol 277:E1130-1141.

Kelley DE, Goodpaster BH. 1999. Effects of physical activity on insulin action and glucose tolerance in obesity. Med Sci Sports Exerc 31:S619-623.

Kelley DE, Williams KV, Price JC, McKolanis TM, Goodpaster BH, Thaete FL. 2001. Plasma fatty acids, adiposity, and variance of skeletal muscle insulin resistance in type 2 diabetes mellitus. J Clin Endocrinol Metab 86:5412-5419.

Kempen KP, Saris WH, Westerterp KR. 1995. Energy balance during an 8-wk energy-restricted diet with and without exercise in obese women. Am J Clin Nutr 62:722-729.

Kendall A, Levitsky DA, Strupp BJ, Lissner L. 1991. Weight loss on a low-fat diet: consequence of the imprecision of the control of food intake in humans. Am J Clin Nutr 53:1124-1129.

Kesaniemi YK, Danforth E, Jr., Jensen MD, Kopelman PG, Lefebvre P, Reeder BA. 2001. Dose-response issues concerning physical activity and health: an evidence-based symposium. Med Sci Sports Exerc 33:S351-358.

Kien CL, Bunn JY, Ugrasbul F. 2005. Increasing dietary palmitic acid decreases fat oxidation and daily energy expenditure. Am J Clin Nutr 82:320-326.

Kiens B. 2006. Skeletal muscle lipid metabolism in exercise and insulin resistance. Physiol Rev 86:205-243.

Kim JK, Kim YJ, Fillmore JJ, Chen Y, Moore I, Lee J, Yuan M, Li ZW, Karin M, Perret P, Shoelson SE, Shulman GI. 2001. Prevention of fat-induced insulin resistance by salicylate. J Clin Invest 108:437-446.

King AC, Haskell WL, Taylor CB, Kraemer HC, DeBusk RF. 1991. Group- vs home-based exercise training in healthy older men and women. A community-based clinical trial. Jama 266:1535-1542.

Kjaer M. 1998. Adrenal medulla and exercise training. Eur J Appl Physiol Occup Physiol 77:195-199.

Komurcu-Bayrak E, Onat A, Poda M, Humphries SE, Acharya J, Hergenc G, Coban N, Can G, Erginel-Unaltuna N. 2007. The S447X variant of lipoprotein lipase gene is associated with metabolic syndrome and lipid levels among Turks. Clin Chim Acta 383:110-115.

Kopecky J, Clarke G, Enerback S, Spiegelman B, Kozak LP. 1995. Expression of the mitochondrial uncoupling protein gene from the aP2 gene promoter prevents genetic obesity. J Clin Invest 96:2914-2923.

Kotz CM, Levine JA. 2005. Role of nonexercise activity thermogenesis (NEAT) in obesity. Minn Med 88:54-57.

Koves TR, Ussher JR, Noland RC, Slentz D, Mosedale M, Ilkayeva O, Bain J, Stevens R, Dyck JR, Newgard CB, Lopaschuk GD, Muoio DM. 2008. Mitochondrial overload and incomplete fatty acid oxidation contribute to skeletal muscle insulin resistance. Cell Metab 7:45-56.

Kramer DK, Al-Khalili L, Guigas B, Leng Y, Garcia-Roves PM, Krook A. 2007. Role of AMP kinase and PPARdelta in the regulation of lipid and glucose metabolism in human skeletal muscle. J Biol Chem 282:19313-19320.

Kramer HF, Goodyear LJ. 2007. Exercise, MAPK, and NF-kappaB signaling in skeletal muscle. J Appl Physiol 103:388-395.

Kriketos AD, Pan DA, Sutton JR, Hoh JF, Baur LA, Cooney GJ, Jenkins AB, Storlien LH. 1995. Relationships between muscle membrane lipids, fiber type, and enzyme activities in sedentary and exercised rats. Am J Physiol 269:R1154-1162.

Kruger J, Yore MM, Kohl HW, 3rd. 2007. Leisure-time physical activity patterns by weight control status: 1999-2002 NHANES. Med Sci Sports Exerc 39:788-795.

Kuhl JE, Ruderman NB, Musi N, Goodyear LJ, Patti ME, Crunkhorn S, Dronamraju D, Thorell A, Nygren J, Ljungkvist O, Degerblad M, Stahle A, Brismar TB, Andersen KL, Saha AK, Efendic S, Bavenholm PN. 2006. Exercise training decreases the concentration of malonyl-CoA and increases the expression and activity of malonyl-CoA decarboxylase in human muscle. Am J Physiol Endocrinol Metab 290:E1296-1303.

Lagouge M, Argmann C, Gerhart-Hines Z, Meziane H, Lerin C, Daussin F, Messadeq N, Milne J, Lambert P, Elliott P, Geny B, Laakso M, Puigserver P, Auwerx J. 2006. Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1alpha. Cell 127:1109-1122.

Lan F, Cacicedo JM, Ruderman N, Ido Y. 2008. SIRT1 modulation of the acetylation status, cytosolic localization, and activity of LKB1. Possible role in AMP-activated protein kinase activation. J Biol Chem 283:27628-27635.

Lees SJ, Booth FW. 2004. Sedentary death syndrome. Can J Appl Physiol 29:447-460; discussion 444-446.

Leitzmann MF, Park Y, Blair A, Ballard-Barbash R, Mouw T, Hollenbeck AR, Schatzkin A. 2007. Physical activity recommendations and decreased risk of mortality. Arch Intern Med 167:2453-2460.

Levine JA. 2007. Nonexercise activity thermogenesis--liberating the life-force. J Intern Med 262:273-287.

Levine JA, Eberhardt NL, Jensen MD. 1999. Role of nonexercise activity thermogenesis in resistance to fat gain in humans. Science 283:212-214.

Levine JA, Kotz CM. 2005. NEAT--non-exercise activity thermogenesis--egocentric & geocentric environmental factors *vs.* biological regulation. Acta Physiol Scand 184:309-318.

Lissner L, Heitmann BL. 1995. Dietary fat and obesity: evidence from epidemiology. Eur J Clin Nutr 49:79-90.

Lissner L, Heitmann BL, Bengtsson C. 1997. Low-fat diets may prevent weight gain in sedentary women: prospective observations from the population study of women in Gothenburg, Sweden. Obes Res 5:43-48.

Liu L, Zhang Y, Chen N, Shi X, Tsang B, Yu YH. 2007. Upregulation of myocellular DGAT1 augments triglyceride synthesis in skeletal muscle and protects against fat-induced insulin resistance. J Clin Invest 117:1679-1689.

Mathai AS, Bonen A, Benton CR, Robinson DL, Graham TE. 2008. Rapid exercise-induced changes in PGC-1alpha mRNA and protein in human skeletal muscle. J Appl Physiol 105:1098-1105.

Mayer J, Marshall NB, Vitale JJ, Christensen JH, Mashayekhi MB, Stare FJ. 1954. Exercise, food intake and body weight in normal rats and genetically obese adult mice. Am J Physiol 177:544-548.

Mayer J, Roy P, Mitra KP. 1956. Relation between caloric intake, body weight, and physical work: studies in an industrial male population in West Bengal. Am J Clin Nutr 4:169-175.

McGee SL, Howlett KF, Starkie RL, Cameron-Smith D, Kemp BE, Hargreaves M. 2003. Exercise increases nuclear AMPK alpha2 in human skeletal muscle. Diabetes 52:926-928.

Meirhaeghe A, Crowley V, Lenaghan C, Lelliott C, Green K, Stewart A, Hart K, Schinner S, Sethi JK, Yeo G, Brand MD, Cortright RN, O'Rahilly S, Montague C, Vidal-Puig AJ. 2003. Characterization of the human, mouse and rat PGC1 beta (peroxisome-proliferator-activated receptor-gamma co-activator 1 beta) gene in vitro and in vivo. Biochem J 373:155-165.

Mensink M, Blaak EE, van Baak MA, Wagenmakers AJ, Saris WH. 2001. Plasma free Fatty Acid uptake and oxidation are already diminished in subjects at high risk for developing type 2 diabetes. Diabetes 50:2548-2554.

Mensink M, Blaak EE, Vidal H, De Bruin TW, Glatz JF, Saris WH. 2003. Lifestyle changes and lipid metabolism gene expression and protein content in skeletal muscle of subjects with impaired glucose tolerance. Diabetologia 46:1082-1089.

Mensink M, Blaak EE, Wagenmakers AJ, Saris WH. 2005. Lifestyle intervention and fatty acid metabolism in glucose-intolerant subjects. Obes Res 13:1354-1362.

Miller WC, Koceja DM, Hamilton EJ. 1997. A meta-analysis of the past 25 years of weight loss research using diet, exercise or diet plus exercise intervention. Int J Obes Relat Metab Disord 21:941-947.

Miller WC, Lindeman AK, Wallace J, Niederpruem M. 1990. Diet composition, energy intake, and exercise in relation to body fat in men and women. Am J Clin Nutr 52:426-430.

Mion CM, Hegstrom RM, Boen ST, Scribner BH. 1964. Substitution of sodium acetate for sodium bicarbonate in the bath fluid for hemodialysis. Trans Am Soc Artif Intern Organs 10:110-115.
Moore RL, Thacker EM, Kelley GA, Musch TI, Sinoway LI, Foster VL, Dickinson AL. 1987. Effect of training/detraining on submaximal exercise responses in humans. J Appl Physiol 63:1719-1724.
Morgan E. 1993. Bipedalism. Nutr Health 9:193-203.
Mu J, Brozinick JT, Jr., Valladares O, Bucan M, Birnbaum MJ. 2001. A role for AMP-activated protein kinase in contraction- and hypoxia-regulated glucose transport in skeletal muscle. Mol Cell 7:1085-1094.
Mujika I, Padilla S. 2000. Detraining: loss of training-induced physiological and performance adaptations. Part I: short term insufficient training stimulus. Sports Med 30:79-87.
Mujika I, Padilla S. 2001a. Cardiorespiratory and metabolic characteristics of detraining in humans. Med Sci Sports Exerc 33:413-421.
Mujika I, Padilla S. 2001b. Muscular characteristics of detraining in humans. Med Sci Sports Exerc 33:1297-1303.
Muoio DM, MacLean PS, Lang DB, Li S, Houmard JA, Way JM, Winegar DA, Corton JC, Dohm GL, Kraus WE. 2002. Fatty acid homeostasis and induction of lipid regulatory genes in skeletal muscles of peroxisome proliferator-activated receptor (PPAR) alpha knock-out mice. Evidence for compensatory regulation by PPAR delta. J Biol Chem 277:26089-26097.
Murgatroyd PR, Goldberg GR, Leahy FE, Gilsenan MB, Prentice AM. 1999. Effects of inactivity and diet composition on human energy balance. Int J Obes Relat Metab Disord 23:1269-1275.
Murphy MH, Nevill AM, Hardman AE. 2000. Different patterns of brisk walking are equally effective in decreasing postprandial lipaemia. Int J Obes Relat Metab Disord 24:1303-1309.
Mutch DM, Clement K. 2006. Genetics of human obesity. Best Pract Res Clin Endocrinol Metab 20:647-664.
Nadeau KJ, Ehlers LB, Aguirre LE, Moore RL, Jew KN, Ortmeyer HK, Hansen BC, Reusch JE, Draznin B. 2006. Exercise training and calorie restriction increase SREBP-1 expression and intramuscular triglyceride in skeletal muscle. Am J Physiol Endocrinol Metab 291:E90-98.
Nakamura MT, Nara TY. 2004. Structure, function, and dietary regulation of delta6, delta5, and delta9 desaturases. Annu Rev Nutr 24:345-376.
Nara M, Kanda T, Tsukui S, Inukai T, Umeda T, Inoue S, Kobayashi I. 1999. Reduction of leptin precedes fat loss from running exercise in insulin-resistant rats. Exp Clin Endocrinol Diabetes 107:431-434.
Narkar VA, Downes M, Yu RT, Embler E, Wang YX, Banayo E, Mihaylova MM, Nelson MC, Zou Y, Juguilon H, Kang H, Shaw RJ, Evans RM. 2008. AMPK and PPARdelta agonists are exercise mimetics. Cell 134:405-415.
Neel JV. 1962. Diabetes mellitus: a "thrifty" genotype rendered detrimental by "progress"? Am J Hum Genet 14:353-362.
Neel JV. 1999. Diabetes mellitus: a "thrifty" genotype rendered detrimental by "progress"? 1962. Bull World Health Organ 77:694-703; discussion 692-693.
Nishida Y, Higaki Y, Tokuyama K, Fujimi K, Kiyonaga A, Shindo M, Sato Y, Tanaka H. 2001. Effect of mild exercise training on glucose effectiveness in healthy men. Diabetes Care 24:1008-1013.
Oberbach A, Bossenz Y, Lehmann S, Niebauer J, Adams V, Paschke R, Schon MR, Bluher M, Punkt K. 2006. Altered fiber distribution and fiber-specific glycolytic and oxidative enzyme activity in skeletal muscle of patients with type 2 diabetes. Diabetes Care 29:895-900.
Ogden CL, Carroll MD, Curtin LR, McDowell MA, Tabak CJ, Flegal KM. 2006. Prevalence of overweight and obesity in the United States, 1999-2004. Jama 295:1549-1555.
Oguma Y, Sesso HD, Paffenbarger RS, Jr., Lee IM. 2002. Physical activity and all cause mortality in women: a review of the evidence. Br J Sports Med 36:162-172.
Olsen RH, Krogh-Madsen R, Thomsen C, Booth FW, Pedersen BK. 2008. Metabolic responses to reduced daily steps in healthy nonexercising men. Jama 299:1261-1263.
Oshida Y, Yamanouchi K, Hayamizu S, Sato Y. 1989. Long-term mild jogging increases insulin action despite no influence on body mass index or VO2 max. J Appl Physiol 66:2206-2210.
Paddon-Jones D, Sheffield-Moore M, Urban RJ, Sanford AP, Aarsland A, Wolfe RR, Ferrando AA. 2004. Essential amino acid and carbohydrate supplementation ameliorates muscle protein loss in humans during 28 days bedrest. J Clin Endocrinol Metab 89:4351-4358.
Padilla S, Mujika I, Orbananos J, Santisteban J, Angulo F, Jose Goiriena J. 2001. Exercise intensity and load during mass-start stage races in professional road cycling. Med Sci Sports Exerc 33:796-802.
Paeratakul S, Popkin BM, Keyou G, Adair LS, Stevens J. 1998. Changes in diet and physical activity affect the body mass index of Chinese adults. Int J Obes Relat Metab Disord 22:424-431.
Pasquet P, Brigant L, Froment A, Koppert GA, Bard D, de Garine I, Apfelbaum M. 1992. Massive overfeeding and energy balance in men: the Guru Walla model. Am J Clin Nutr 56:483-490.
Pate RR, Pratt M, Blair SN, Haskell WL, Macera CA, Bouchard C, Buchner D, Ettinger W, Heath GW, King AC, *et al.* 1995. Physical activity and public health. A recommendation from the Centers for Disease Control and Prevention and the American College of Sports Medicine. Jama 273:402-407.

Pedersen BK, Febbraio MA. 2008. Muscle as an endocrine organ: focus on muscle-derived interleukin-6. Physiol Rev 88:1379-1406.

Petitt DS, Arngrimsson SA, Cureton KJ. 2003. Effect of resistance exercise on postprandial lipemia. J Appl Physiol 94:694-700.

Piers LS, Walker KZ, Stoney RM, Soares MJ, O'Dea K. 2003. Substitution of saturated with monounsaturated fat in a 4-week diet affects body weight and composition of overweight and obese men. Br J Nutr 90:717-727.

Pilegaard H, Saltin B, Neufer PD. 2003. Exercise induces transient transcriptional activation of the PGC-1alpha gene in human skeletal muscle. J Physiol 546:851-858.

Poehlman ET, Gardner AW, Arciero PJ, Goran MI, Calles-Escandon J. 1994. Effects of endurance training on total fat oxidation in elderly persons. J Appl Physiol 76:2281-2287.

Popkin BM. 2006. Global nutrition dynamics: the world is shifting rapidly toward a diet linked with noncommunicable diseases. Am J Clin Nutr 84:289-298.

Popkin BM, Paeratakul S, Zhai F, Ge K. 1995. Dietary and environmental correlates of obesity in a population study in China. Obes Res 3 Suppl 2:135s-143s.

Pound KM, Sorokina N, Ballal K, Berkich DA, Fasano M, Lanoue KF, Taegtmeyer H, O'Donnell JM, Lewandowski ED. 2009. Substrate-enzyme competition attenuates upregulated anaplerotic flux through malic enzyme in hypertrophied rat heart and restores triacylglyceride content: attenuating upregulated anaplerosis in hypertrophy. Circ Res 104:805-812.

Pouteau E, Maugere P, Darmaun D, Marchini JS, Piloquet H, Dumon H, Nguyen P, Krempf M. 1998. Role of glucose and glutamine synthesis in the differential recovery of 13CO2 from infused [2-13C] versus [1-13C] acetate. Metabolism 47:549-554.

Prentice AM, Jebb SA. 2003. Fast foods, energy density and obesity: a possible mechanistic link. Obes Rev 4:187-194.

Prewitt TE, Schmeisser D, Bowen PE, Aye P, Dolecek TA, Langenberg P, Cole T, Brace L. 1991. Changes in body weight, body composition, and energy intake in women fed high- and low-fat diets. Am J Clin Nutr 54:304-310.

Raitakari OT, Porkka KV, Taimela S, Telama R, Rasanen L, Viikari JS. 1994. Effects of persistent physical activity and inactivity on coronary risk factors in children and young adults. The Cardiovascular Risk in Young Finns Study. Am J Epidemiol 140:195-205.

Redman LM, Heilbronn LK, Martin CK, de Jonge L, Williamson DA, Delany JP, Ravussin E. 2009. Metabolic and behavioral compensations in response to caloric restriction: implications for the maintenance of weight loss. PLoS One 4:e4377.

Richter EA, Kiens B, Wojtaszewski JF. 2008. Can exercise mimetics substitute for exercise? Cell Metab 8:96-98.

Richter EA, Ruderman NB. 2009. AMPK and the biochemistry of exercise: implications for human health and disease. Biochem J 418:261-275.

Richterova B, Stich V, Moro C, Polak J, Klimcakova E, Majercik M, Harant I, Viguerie N, Crampes F, Langin D, Lafontan M, Berlan M. 2004. Effect of endurance training on adrenergic control of lipolysis in adipose tissue of obese women. J Clin Endocrinol Metab 89:1325-1331.

Rimbert V, Boirie Y, Bedu M, Hocquette JF, Ritz P, Morio B. 2004. Muscle fat oxidative capacity is not impaired by age but by physical inactivity: association with insulin sensitivity. Faseb J 18:737-739.

Ritz P, Acheson KJ, Gachon P, Vico L, Bernard JJ, Alexandre C, Beaufrere B. 1998. Energy and substrate metabolism during a 42-day bed-rest in a head-down tilt position in humans. Eur J Appl Physiol Occup Physiol 78:308-314.

Roberts SB, Young VR, Fuss P, Fiatarone MA, Richard B, Rasmussen H, Wagner D, Joseph L, Holehouse E, Evans WJ. 1990. Energy expenditure and subsequent nutrient intakes in overfed young men. Am J Physiol 259:R461-469.

Rockl KS, Witczak CA, Goodyear LJ. 2008. Signaling mechanisms in skeletal muscle: acute responses and chronic adaptations to exercise. IUBMB Life 60:145-153.

Rodearmel SJ, Wyatt HR, Barry MJ, Dong F, Pan D, Israel RG, Cho SS, McBurney MI, Hill JO. 2006. A family-based approach to preventing excessive weight gain. Obesity (Silver Spring) 14:1392-1401.

Rodnick KJ, Henriksen EJ, James DE, Holloszy JO. 1992. Exercise training, glucose transporters, and glucose transport in rat skeletal muscles. Am J Physiol 262:C9-14.

Roepstorff C, Halberg N, Hillig T, Saha AK, Ruderman NB, Wojtaszewski JF, Richter EA, Kiens B. 2005. Malonyl-CoA and carnitine in regulation of fat oxidation in human skeletal muscle during exercise. Am J Physiol Endocrinol Metab 288:E133-142.

Rose AJ, Frosig C, Kiens B, Wojtaszewski JF, Richter EA. 2007. Effect of endurance exercise training on Ca2+ calmodulin-dependent protein kinase II expression and signalling in skeletal muscle of humans. J Physiol 583:785-795.

Rose AJ, Michell BJ, Kemp BE, Hargreaves M. 2004. Effect of exercise on protein kinase C activity and localization in human skeletal muscle. J Physiol 561:861-870.

Russell AP. 2004. Lipotoxicity: the obese and endurance-trained paradox. Int J Obes Relat Metab Disord 28 Suppl 4:S66-71.

Sabin MA, Crowne EC, Stewart CE, Hunt LP, Turner SJ, Welsh GI, Grohmann MJ, Holly JM, Shield JP. 2007. Depot-specific effects of fatty acids on lipid accumulation in children's adipocytes. Biochem Biophys Res Commun 361:356-361.

Saiki A, Oyama T, Endo K, Ebisuno M, Ohira M, Koide N, Murano T, Miyashita Y, Shirai K. 2007. Preheparin serum lipoprotein lipase mass might be a biomarker of metabolic syndrome. Diabetes Res Clin Pract 76:93-101.

Salanova M, Schiffl G, Puttmann B, Schoser BG, Blottner D. 2008. Molecular biomarkers monitoring human skeletal muscle fibres and microvasculature following long-term bed rest with and without countermeasures. J Anat 212:306-318.

Salbe AD, Weyer C, Harper I, Lindsay RS, Ravussin E, Tataranni PA. 2003. Relation between physical activity and obesity. Am J Clin Nutr 78:193-194; author reply 194-195.

Saris WH, Blair SN, van Baak MA, Eaton SB, Davies PS, Di Pietro L, Fogelholm M, Rissanen A, Schoeller D, Swinburn B, Tremblay A, Westerterp KR, Wyatt H. 2003. How much physical activity is enough to prevent unhealthy weight gain? Outcome of the IASO 1st Stock Conference and consensus statement. Obes Rev 4:101-114.

Schenk S, Harber MP, Shrivastava CR, Burant CF, Horowitz JF. 2009. Improved insulin sensitivity after weight loss and exercise training is mediated by a reduction in plasma fatty acid mobilization, not enhanced oxidative capacity. J Physiol.

Schenk S, Horowitz JF. 2006. Coimmunoprecipitation of FAT/CD36 and CPT I in skeletal muscle increases proportionally with fat oxidation after endurance exercise training. Am J Physiol Endocrinol Metab 291:E254-260.

Schenk S, Horowitz JF. 2007. Acute exercise increases triglyceride synthesis in skeletal muscle and prevents fatty acid-induced insulin resistance. J Clin Invest 117:1690-1698.

Schmitz KH, Jacobs DR, Jr., Leon AS, Schreiner PJ, Sternfeld B. 2000. Physical activity and body weight: associations over ten years in the CARDIA study. Coronary Artery Risk Development in Young Adults. Int J Obes Relat Metab Disord 24:1475-1487.

Schoeller DA, Shay K, Kushner RF. 1997. How much physical activity is needed to minimize weight gain in previously obese women? Am J Clin Nutr 66:551-556.

Schrauwen P. 2007. High-fat diet, muscular lipotoxicity and insulin resistance. Proc Nutr Soc 66:33-41.

Schrauwen P, Blaak EE, Van Aggel-Leijssen DP, Borghouts LB, Wagenmakers AJ. 2000. Determinants of the acetate recovery factor: implications for estimation of [13C]substrate oxidation. Clin Sci (Lond) 98:587-592.

Schrauwen P, Hesselink MK. 2008. Reduced tricarboxylic acid cycle flux in type 2 diabetes mellitus? Diabetologia 51:1694-1697.

Schrauwen P, Lichtenbelt WD, Saris WH, Westerterp KR. 1998. Fat balance in obese subjects: role of glycogen stores. Am J Physiol 274:E1027-1033.

Schrauwen P, van Aggel-Leijssen DP, Hul G, Wagenmakers AJ, Vidal H, Saris WH, van Baak MA. 2002. The effect of a 3-month low-intensity endurance training program on fat oxidation and acetyl-CoA carboxylase-2 expression. Diabetes 51:2220-2226.

Schutz Y. 1995. Macronutrients and energy balance in obesity. Metabolism 44:7-11.

Schutz Y, Flatt JP, Jequier E. 1989. Failure of dietary fat intake to promote fat oxidation: a factor favoring the development of obesity. Am J Clin Nutr 50:307-314.

Seip RL, Mair K, Cole TG, Semenkovich CF. 1997. Induction of human skeletal muscle lipoprotein lipase gene expression by short-term exercise is transient. Am J Physiol 272:E255-261.

Shepard TY, Weil KM, Sharp TA, Grunwald GK, Bell ML, Hill JO, Eckel RH. 2001. Occasional physical inactivity combined with a high-fat diet may be important in the development and maintenance of obesity in human subjects. Am J Clin Nutr 73:703-708.

Sheppard L, Kristal AR, Kushi LH. 1991. Weight loss in women participating in a randomized trial of low-fat diets. Am J Clin Nutr 54:821-828.

Shetty PS. 1999. Adaptation to low energy intakes: the responses and limits to low intakes in infants, children and adults. Eur J Clin Nutr 53 Suppl 1:S14-33.

Shetty PS, Prentice AM, Goldberg GR, Murgatroyd PR, McKenna AP, Stubbs RJ, Volschenk PA. 1994. Alterations in fuel selection and voluntary food intake in response to isoenergetic manipulation of glycogen stores in humans. Am J Clin Nutr 60:534-543.

Sial S, Coggan AR, Hickner RC, Klein S. 1998. Training-induced alterations in fat and carbohydrate metabolism during exercise in elderly subjects. Am J Physiol 274:E785-790.

Sidossis LS, Coggan AR, Gastaldelli A, Wolfe RR. 1995a. A new correction factor for use in tracer estimations of plasma fatty acid oxidation. Am J Physiol 269:E649-656.

Sidossis LS, Coggan AR, Gastaldelli A, Wolfe RR. 1995b. Pathway of free fatty acid oxidation in human subjects. Implications for tracer studies. J Clin Invest 95:278-284.

Siervo M, Fruhbeck G, Dixon A, Goldberg GR, Coward WA, Murgatroyd PR, Prentice AM, Jebb SA. 2008. Efficiency of autoregulatory homeostatic responses to imposed caloric excess in lean men. Am J Physiol Endocrinol Metab 294:E416-424.

Smith SR, de Jonge L, Zachwieja JJ, Roy H, Nguyen T, Rood J, Windhauser M, Volaufova J, Bray GA. 2000a. Concurrent physical activity increases fat oxidation during the shift to a high-fat diet. Am J Clin Nutr 72:131-138.

Smith SR, de Jonge L, Zachwieja JJ, Roy H, Nguyen T, Rood JC, Windhauser MM, Bray GA. 2000b. Fat and carbohydrate balances during adaptation to a high-fat. Am J Clin Nutr 71:450-457.

Sorokina N, O'Donnell JM, McKinney RD, Pound KM, Woldegiorgis G, LaNoue KF, Ballal K, Taegtmeyer H, Buttrick PM, Lewandowski ED. 2007. Recruitment of compensatory pathways to sustain oxidative flux with reduced carnitine palmitoyltransferase I activity characterizes inefficiency in energy metabolism in hypertrophied hearts. Circulation 115:2033-2041.

Speakman JR. 2007. A nonadaptive scenario explaining the genetic predisposition to obesity: the "predation release" hypothesis. Cell Metab 6:5-12.

Speakman JR. 2008. Thrifty genes for obesity, an attractive but flawed idea, and an alternative perspective: the 'drifty gene' hypothesis. Int J Obes (Lond) 32:1611-1617.

Stefan N, Thamer C, Staiger H, Machicao F, Machann J, Schick F, Venter C, Niess A, Laakso M, Fritsche A, Haring HU. 2007. Genetic variations in PPARD and PPARGC1A determine mitochondrial function and change in aerobic physical fitness and insulin sensitivity during lifestyle intervention. J Clin Endocrinol Metab 92:1827-1833.

Stein T, Schluter M, Galante A, Soteropoulos P, Tolias P, Grindeland R, Moran M, Wang T, Polansky M, Wade C. 2002. Energy metabolism pathways in rat muscle under conditions of simulated microgravity. J Nutr Biochem 13:471.

Stein TP, Schluter MD, Galante AT, Soteropoulos P, Ramirez M, Bigbee A, Grindeland RE, Wade CE. 2005. Effect of hind limb muscle unloading on liver metabolism of rats. J Nutr Biochem 16:9-16.

Stein TP, Wade CE. 2005. Metabolic consequences of muscle disuse atrophy. J Nutr 135:1824S-1828S.

Steinberg GR, Jorgensen SB. 2007. The AMP-activated protein kinase: role in regulation of skeletal muscle metabolism and insulin sensitivity. Mini Rev Med Chem 7:519-526.

Stubbs RJ, Harbron CG, Murgatroyd PR, Prentice AM. 1995a. Covert manipulation of dietary fat and energy density: effect on substrate flux and food intake in men eating ad libitum. Am J Clin Nutr 62:316-329.

Stubbs RJ, Hughes DA, Johnstone AM, Horgan GW, King N, Blundell JE. 2004. A decrease in physical activity affects appetite, energy, and nutrient balance in lean men feeding ad libitum. Am J Clin Nutr 79:62-69.

Stubbs RJ, Ritz P, Coward WA, Prentice AM. 1995b. Covert manipulation of the ratio of dietary fat to carbohydrate and energy density: effect on food intake and energy balance in free-living men eating ad libitum. Am J Clin Nutr 62:330-337.

Sunami Y, Motoyama M, Kinoshita F, Mizooka Y, Sueta K, Matsunaga A, Sasaki J, Tanaka H, Shindo M. 1999. Effects of low-intensity aerobic training on the high-density lipoprotein cholesterol concentration in healthy elderly subjects. Metabolism 48:984-988.

Swan G. 2004. Findings from the latest National Diet and Nutrition Survey. Proc Nutr Soc 63:505-512.

Tambalis K, Panagiotakos DB, Kavouras SA, Sidossis LS. 2009. Responses of blood lipids to aerobic, resistance, and combined aerobic with resistance exercise training: a systematic review of current evidence. Angiology 60:614-632.

Tanner CJ, Barakat HA, Dohm GL, Pories WJ, MacDonald KG, Cunningham PR, Swanson MS, Houmard JA. 2002. Muscle fiber type is associated with obesity and weight loss. Am J Physiol Endocrinol Metab 282:E1191-1196.

Tanskanen M, Uusitalo AL, Hakkinen K, Nissila J, Santtila M, Westerterp KR, Kyrolainen H. 2008. Aerobic fitness, energy balance, and body mass index are associated with training load assessed by activity energy expenditure. Scand J Med Sci Sports.

Taylor HL, Keys A. 1950. Adaptation to caloric restriction. Science 112:215-218.

Taylor HL, Klepetar E, Keys A, Parlin W, Blackburn H, Puchner T. 1962. Death rates among physically active and sedentary employees of the railroad industry. Am J Public Health Nations Health 52:1697-1707.

Telama R, Yang X. 2000. Decline of physical activity from youth to young adulthood in Finland. Med Sci Sports Exerc 32:1617-1622.

Teran-Garcia M, Rankinen T, Koza RA, Rao DC, Bouchard C. 2005. Endurance training-induced changes in insulin sensitivity and gene expression. Am J Physiol Endocrinol Metab 288:E1168-1178.

Thamer C, Machann J, Bachmann O, Haap M, Dahl D, Wietek B, Tschritter O, Niess A, Brechtel K, Fritsche A, Claussen C, Jacob S, Schick F, Haring HU, Stumvoll M. 2003. Intramyocellular lipids: anthropometric determinants and relationships with maximal aerobic capacity and insulin sensitivity. J Clin Endocrinol Metab 88:1785-1791.

Thomas CD, Peters JC, Reed GW, Abumrad NN, Sun M, Hill JO. 1992. Nutrient balance and energy expenditure during ad libitum feeding of high-fat and high-carbohydrate diets in humans. Am J Clin Nutr 55:934-942.

Thomas TR, Londeree BR, Gerhardt KO, Gehrke CW. 1977. Fatty acid profile and cholesterol in skeletal muscle of trained and untrained men. J Appl Physiol 43:709-713.

Toledo FG, Menshikova EV, Azuma K, Radikova Z, Kelley CA, Ritov VB, Kelley DE. 2008. Mitochondrial capacity in skeletal muscle is not stimulated by weight loss despite increases in insulin action and decreases in intramyocellular lipid content. Diabetes 57:987-994.

Trappe S, Creer A, Minchev K, Slivka D, Louis E, Luden N, Trappe T. 2008. Human soleus single muscle fiber function with exercise or nutrition countermeasures during 60 days of bed rest. Am J Physiol Regul Integr Comp Physiol 294:R939-947.

Trappe S, Trappe T, Gallagher P, Harber M, Alkner B, Tesch P. 2004. Human single muscle fibre function with 84 day bed-rest and resistance exercise. J Physiol 557:501-513.

Tremblay A, Lavallee N, Almeras N, Allard L, Despres JP, Bouchard C. 1991. Nutritional determinants of the increase in energy intake associated with a high-fat diet. Am J Clin Nutr 53:1134-1137.

Troy S, Soty M, Ribeiro L, Laval L, Migrenne S, Fioramonti X, Pillot B, Fauveau V, Aubert R, Viollet B, Foretz M, Leclerc J, Duchampt A, Zitoun C, Thorens B, Magnan C, Mithieux G, Andreelli F. 2008. Intestinal gluconeogenesis is a key factor for early metabolic changes after gastric bypass but not after gastric lap-band in mice. Cell Metab 8:201-211.

Tryon WW, Goldberg JL, Morrison DF. 1992. Activity decreases as percentage overweight increases. Int J Obes Relat Metab Disord 16:591-595.

Tsao TS, Li J, Chang KS, Stenbit AE, Galuska D, Anderson JE, Zierath JR, McCarter RJ, Charron MJ. 2001. Metabolic adaptations in skeletal muscle overexpressing GLUT4: effects on muscle and physical activity. Faseb J 15:958-969.

Tsetsonis NV, Hardman AE. 1996a. Effects of low and moderate intensity treadmill walking on postprandial lipaemia in healthy young adults. Eur J Appl Physiol Occup Physiol 73:419-426.

Tsetsonis NV, Hardman AE. 1996b. Reduction in postprandial lipemia after walking: influence of exercise intensity. Med Sci Sports Exerc 28:1235-1242.

Tucker LA, Kano MJ. 1992. Dietary fat and body fat: a multivariate study of 205 adult females. Am J Clin Nutr 56:616-622.

Tunstall RJ, Mehan KA, Wadley GD, Collier GR, Bonen A, Hargreaves M, Cameron-Smith D. 2002. Exercise training increases lipid metabolism gene expression in human skeletal muscle. Am J Physiol Endocrinol Metab 283:E66-72.

Ukropcova B, Sereda O, de Jonge L, Bogacka I, Nguyen T, Xie H, Bray GA, Smith SR. 2007. Family history of diabetes links impaired substrate switching and reduced mitochondrial content in skeletal muscle. Diabetes 56:720-727.

van Aggel-Leijssen DP, Saris WH, Wagenmakers AJ, Senden JM, van Baak MA. 2002. Effect of exercise training at different intensities on fat metabolism of obese men. J Appl Physiol 92:1300-1309.

Van Etten LM, Westerterp KR, Verstappen FT, Boon BJ, Saris WH. 1997. Effect of an 18-wk weight-training program on energy expenditure and physical activity. J Appl Physiol 82:298-304.

Van Hall G, Saltin B, Wagenmakers AJ. 1999. Muscle protein degradation and amino acid metabolism during prolonged knee-extensor exercise in humans. Clin Sci (Lond) 97:557-567.

van Loon LJ. 2004. Use of intramuscular triacylglycerol as a substrate source during exercise in humans. J Appl Physiol 97:1170-1187.

van Loon LJ, Goodpaster BH. 2006. Increased intramuscular lipid storage in the insulin-resistant and endurance-trained state. Pflugers Arch 451:606-616.

Venables MC, Jeukendrup AE. 2008. Endurance training and obesity: effect on substrate metabolism and insulin sensitivity. Med Sci Sports Exerc 40:495-502.

Vernikos J. 1996. Human physiology in space. Bioessays 18:1029-1037.

Vessby B, Tengblad S, Lithell H. 1994. Insulin sensitivity is related to the fatty acid composition of serum lipids and skeletal muscle phospholipids in 70-year-old men. Diabetologia 37:1044-1050.

Votruba SB, Atkinson RL, Hirvonen MD, Schoeller DA. 2002. Prior exercise increases subsequent utilization of dietary fat. Med Sci Sports Exerc 34:1757-1765.

Votruba SB, Atkinson RL, Schoeller DA. 2003. Prior exercise increases dietary oleate, but not palmitate oxidation. Obes Res 11:1509-1518.

Wade AJ, Marbut MM, Round JM. 1990. Muscle fibre type and aetiology of obesity. Lancet 335:805-808.

Walsh RC, Koukoulas I, Garnham A, Moseley PL, Hargreaves M, Febbraio MA. 2001. Exercise increases serum Hsp72 in humans. Cell Stress Chaperones 6:386-393.

Wang YX, Lee CH, Tiep S, Yu RT, Ham J, Kang H, Evans RM. 2003. Peroxisome-proliferator-activated receptor delta activates fat metabolism to prevent obesity. Cell 113:159-170.

Wang YX, Zhang CL, Yu RT, Cho HK, Nelson MC, Bayuga-Ocampo CR, Ham J, Kang H, Evans RM. 2004. Regulation of muscle fiber type and running endurance by PPARdelta. PLoS Biol 2:e294.

Weinsier RL, Hunter GR, Desmond RA, Byrne NM, Zuckerman PA, Darnell BE. 2002. Free-living activity energy expenditure in women successful and unsuccessful at maintaining a normal body weight. Am J Clin Nutr 75:499-504.

Weinsier RL, Hunter GR, Heini AF, Goran MI, Sell SM. 1998. The etiology of obesity: relative contribution of metabolic factors, diet, and physical activity. Am J Med 105:145-150.

Wendorf M, Goldfine ID. 1991. Archaeology of NIDDM. Excavation of the "thrifty" genotype. Diabetes 40:161-165.

Westerterp KR. 1998. Alterations in energy balance with exercise. Am J Clin Nutr 68:970S-974S.

Westerterp KR. 1999. Obesity and physical activity. Int J Obes Relat Metab Disord 23 Suppl 1:59-64.

Westerterp KR. 2010. Physical activity, food intake, and body weight regulation: insights from doubly labeled water studies. Nutr Rev 68:148-154.

Westerterp KR, Meijer GA, Janssen EM, Saris WH, Ten Hoor F. 1992. Long-term effect of physical activity on energy balance and body composition. Br J Nutr 68:21-30.

Westerterp KR, Plasqui G. 2004. Physical activity and human energy expenditure. Curr Opin Clin Nutr Metab Care 7:607-613.

Whybrow S, Hughes DA, Ritz P, Johnstone AM, Horgan GW, King N, Blundell JE, Stubbs RJ. 2008. The effect of an incremental increase in exercise on appetite, eating behaviour and energy balance in lean men and women feeding ad libitum. Br J Nutr 100:1109-1115.

Williams DE, Prevost AT, Whichelow MJ, Cox BD, Day NE, Wareham NJ. 2000. A cross-sectional study of dietary patterns with glucose intolerance and other features of the metabolic syndrome. Br J Nutr 83:257-266.

Williams PT. 2008. Asymmetric weight gain and loss from increasing and decreasing exercise. Med Sci Sports Exerc 40:296-302.

Williamson DF. 1996. Dietary intake and physical activity as "predictors" of weight gain in observational, prospective studies of adults. Nutr Rev 54:S101-109.

Williamson DF, Madans J, Anda RF, Kleinman JC, Kahn HS, Byers T. 1993. Recreational physical activity and ten-year weight change in a US national cohort. Int J Obes Relat Metab Disord 17:279-286.

Winder WW, Hardie DG. 1996. Inactivation of acetyl-CoA carboxylase and activation of AMP-activated protein kinase in muscle during exercise. Am J Physiol 270:E299-304.

Wing RR. 1999. Physical activity in the treatment of the adulthood overweight and obesity: current evidence and research issues. Med Sci Sports Exerc 31:S547-552.

Wojtaszewski JF, Richter EA. 2006. Effects of acute exercise and training on insulin action and sensitivity: focus on molecular mechanisms in muscle. Essays Biochem 42:31-46.

Wu T, Gao X, Chen M, van Dam RM. 2009. Long-term effectiveness of diet-plus-exercise interventions *vs.* diet-only interventions for weight loss: a meta-analysis. Obes Rev 10:313-323.

Xia Y, Buja LM, Scarpulla RC, McMillin JB. 1997. Electrical stimulation of neonatal cardiomyocytes results in the sequential activation of nuclear genes governing mitochondrial proliferation and differentiation. Proc Natl Acad Sci U S A 94:11399-11404.

Yang X, Telama R, Leino M, Viikari J. 1999. Factors explaining the physical activity of young adults: the importance of early socialization. Scand J Med Sci Sports 9:120-127.

Zimmet PZ. 1999. Diabetes epidemiology as a tool to trigger diabetes research and care. Diabetologia 42:499-518.

Zurlo F, Lillioja S, Esposito-Del Puente A, Nyomba BL, Raz I, Saad MF, Swinburn BA, Knowler WC, Bogardus C, Ravussin E. 1990. Low ratio of fat to carbohydrate oxidation as predictor of weight gain: study of 24-h RQ. Am J Physiol 259:E650-657.

Annexes

Two hollidaymakers take things easy in their beach chairs de Proessdorf

ANNEXE 1

1. L'OBESITE HUMAINE

1.1. L'obésité : une maladie chronique et évolutive

Le mot obésité vient du latin «obesitas», qui signifie la graisse. « Esus » est le participe passé du verbe edere (à manger), avec « ob » qui signifie plus ou encore (Harper, 2008). Le dictionnaire anglais d'Oxford a documenté sa première utilisation en 1611 par Randle Cotgrave dans le dictionnaire des langues française et anglaise. En 1997, l'organisation mondiale de la santé (OMS) a défini l'obésité comme une maladie. L'obésité témoigne de l'incapacité du système réglant les réserves énergétiques à faire face aux évolutions des modes de vie (Basdevant A, 2004; Friedman, 2004). C'est une maladie chronique évolutive liée à l'environnement et aboutissant à une pathologie d'organe. Maladie, car l'inflation de masse grasse entraîne des conséquences sur le bien-être physique, psychologique et social (WHO, 1997). Chronique, car, pour entraîner des conséquences pathologiques, cette situation d'excès de poids doit être durable. Evolutive, car on peut identifier plusieurs phases successives (Basdevant A, 2004). L'obésité devient une pathologie d'organe liée au dysfonctionnement d'une structure complexe, le tissu adipeux (Clement *et al.*, 2004). À un certain stade, la réversibilité de la maladie est mise en cause par des mécanismes biologiques et par la persistance de la pression environnementale.

1.2. Quand est-on obèse ?

L'obésité est une maladie dans laquelle l'hypertrophie de la masse adipeuse se traduit par un excès de poids et présente un effet néfaste sur la santé. Elle est définie par l'indice de masse corporelle (IMC) et s'évalue ultérieurement en termes de répartition des graisses par l'intermédiaire du rapport tour de taille/tour de hanches et par la détermination des facteurs de risque cardiovasculaire. L'IMC est fortement lié à la fois au pourcentage de masse grasse et à la masse grasse totale (Gray and Fujioka, 1991) .

1.2.1. L'indice de masse corporelle IMC

L'indice de masse corporelle ou IMC est une méthode simple et largement utilisée pour estimer la masse grasse corporelle (Mei *et al.*, 2002). L'IMC a été développée au 19ème siècle par le statisticien et anthropologue belge Adolphe Quetelet (Quetelet, (1871)). L'IMC est un reflet représentatif du pourcentage de la masse grasse dans la majorité de la population adulte. Il est toutefois moins précis pour certaines personnes telles les femmes enceintes ou les sportifs avec une masse musculaire importante. Une formule combinant l'IMC, l'âge et le sexe, peut être utilisée pour estimer pourcentage de masse grasse d'une personne avec une précision

de 4%. Pour les adultes, l'indice de masse corporelle est égal à la masse (exprimée en kilogrammes) divisée par le carré de la taille de la personne(en mètres) :

IMC = masse / (taille en m) ^{2}

Les définitions les plus utilisées pour l'obésité, établies par l'OMS en 1997 et publiées en 2000, donnent les valeurs affichées dans le tableau.1.

IMC	Classification
< 18.5	Sous poids
18.5–24.9	Poids normal
25.0–29.9	Surpoids
30.0–34.9	Obésité classe I modérée/commune
35.0–39.9	Obésité classe II sévère
> 40.0	Obésité classe III massive/morbide

Tableau .1.

Certaines modifications des définitions de l'OMS ont été faites par certains sujets. La littérature chirurgicale divise la classe III de l'obésité en d'autres catégories (Uwaifo, 2006).Comme les populations asiatiques développent des conséquences négatives sur la santé à un IMC plus faible que les Caucasiens, certains pays ont redéfini l'obésité. Les Japonais ont défini l'obésité à partir d'un IMC supérieur à 25 (Kanazawa *et al.*, 2005) tandis que la Chine utilise un IMC supérieur à 28 (Bei-Fan, 2002).

1.2.2. Tour de taille et rapport tour de taille/tour de hanches: Obésité abdominale

Un tour de taille (>102 cm pour les hommes et >88 cm pour les femmes) et un rapport tour de taille /tour de hanches (le tour de taille divisée par celui des hanches >0.9 pour les hommes et >0.85 pour les femmes) sont tous les deux utilisés comme mesures de l'obésité abdominale. Pour les personnes ayant un IMC de moins de 35, la masse grasse corporelle intra-abdominale est liée à des conséquences négatives sur la santé indépendantes de la masse grasse corporelle totale (U.S, 2000). La masse grasse intra-abdominale ou graisse viscérale est particulièrement en forte corrélation avec les maladies cardiovasculaires. Dans une étude menée sur 15.000 personnes, le tour de taille avait également une meilleure corrélation avec le syndrome métabolique que l'IMC (Janssen *et al.*, 2004). Les femmes avec une obésité abdominale ont un risque cardiovasculaire similaire à celui des hommes (Larsson *et al.*, 1992). Chez les personnes avec un IMC de plus de 35, la mesure de tour de la taille ajoute cependant peu au pouvoir prédictif de l'IMC puisque la plupart des individus avec un tel IMC ont des valeurs de tour de taille anormales.

1.2.3. Le pourcentage de masse grasse

Le pourcentage de masse grasse est la masse grasse totale exprimée en pourcentage de la masse corporelle totale. Il est généralement admis que les hommes de plus de 25% de masse grasse et les femmes de plus de 33% de masse grasse sont

obèses (Schwarz). Le pourcentage de masse grasse peut être estimé à partir d'un IMC de la personne par la formule suivante :

% de masse grasse = (1,2 * IMC) + (0,23 * âge) - 5,4 - (10,8 * sexe)
où le sexe est de 0 pour les femmes et 1 pour les hommes.

Cette formule prend en compte le fait que le pourcentage de masse grasse est de 10 points en pourcentage plus élevé chez les femmes que chez les hommes pour un même IMC. Elle reconnaît aussi que le pourcentage de masse grasse augmente avec l'âge, même si le poids reste constant. Les résultats de cette formule ont une précision de 4%.

Il existe de nombreuses autres méthodes utilisées pour déterminer le pourcentage de graisse corporelle. La pesée hydrostatique, l'une des plus précises méthodes de calcul de la graisse corporelle, consiste à peser la personne sous l'eau. Deux autres méthodes plus simples mais moins précises ont été utilisées historiquement, mais ne sont plus recommandées actuellement. La première méthode est le test des plis, par laquelle une pincée de la peau est mesurée afin de déterminer l'épaisseur de la couche de graisse sous-cutanée (Wells, 2005). La seconde méthode d'analyse est l'impédancimétrie bioélectrique qui utilise la résistance électrique. Néanmoins, l'impédancimétrie bioélectrique n'a pas fourni un avantage par rapport à l'IMC (NICE, 2006). Les techniques de mesure du pourcentage de masse grasse principalement utilisées pour la recherche incluent la tomodensitométrie (CT scan), l'imagerie par résonance magnétique (IRM), et l'absorptiométrie à rayons X en double d'énergie (DEXA). Ces techniques fournissent des mesures très précises, mais sont difficilement utilisées chez les personnes sévèrement obèses en raison de limites de poids de la plupart des équipements et l'insuffisance de diamètre de nombreux scanneurs de CT ou IRM (Wells, 2005).

1.3. Obésité infantile

Chez l'enfant, en raison de la croissance, les valeurs seuils d'indice de masse corporelle de définition du surpoids et de l'obésité varient en fonction de l'âge et du sexe de l'enfant. L'obésité chez les enfants et les adolescents est définie par un IMC au-delà du 95ème percentile d'une référence internationale d'une population donnée . Les données de référence sur lesquelles les percentiles sont basés sont issues des années 1963 à 1994 et ne sont donc pas affectées par l'augmentation récente de la prévalence de l'obésité (Flegal *et al.*, 2001). L'obésité infantile a atteint des proportions épidémiques au XXè siècle avec l'augmentation de la prévalence à la fois dans les pays développés et en voie de développement. Le taux de l'obésité chez les garçons canadiens a augmenté de 11% en 1980 à plus de 30% dans les années 1990, alors que pendant cette même période, le taux est passé de 4 à 14% chez les enfants brésiliens de (Flynn *et al.*, 2006). Tout comme dans l'obésité chez les adultes, de nombreux facteurs contribuent à l'augmentation des taux d'obésité chez les enfants. Les régimes alimentaires élevés en énergie ou en matière grasse et la diminution de l'activité physique sont considérés comme les deux plus importantes causes de l'augmentation récente de la prévalence de l'obésité. Les auto-activités liées au transport, l'éducation physique à l'école, et les sports organisés ont baissé dans de nombreux pays. Les traitements utilisés contre l'obésité infantile portent essentiellement sur des interventions sur le mode de vie et des techniques

comportementales. Les médicaments ne sont pas approuvé par la FDA pour être utilisé dans ce groupe d'âge (Flynn *et al.*, 2006).

1.4. Pourquoi l'obésité est-elle considérée comme un problème ?

Les maladies chroniques découlant de l'obésité continueront à croître au cours des prochaines décennies. Le coût associé est mesuré non seulement sur le plan personnel et la santé de la population, mais aussi sur une échelle économique mondiale. Actuellement, ~ 8% de la population est obèse et ~ 25% sont en surpoids, et on s'attend à ce que les chiffres subissent une hausse supplémentaire de 50% en 10 ans. En outre, dans les régions développées (par exemple l'Amérique du Nord, l'Europe de l'Ouest, l'Australie), l'obésité approche déjà 25% (James, 2008). Au Royaume-Uni, les estimations actuelles suggèrent que, en 2050, les coûts associés aux maladies liées à l'obésité vont augmenter de > 15% (James, 2008).

1.4.1. Epidémiologie

Durant des milliers d'années, l'obésité a été rarement vue (Haslam, 2007). Ce n'est qu'à partir du 20ème siècle que la prévalence de l'obésité a augmenté, si bien que, en 1997, l'organisation mondiale de la santé (OMS) a officiellement reconnu l'obésité comme une épidémie mondiale (Caballero, 2007). À partir de 2005, l'OMS estime que, au moins 400 millions d'adultes (9,8%) sont obèses, avec des taux plus élevés chez les femmes que chez les hommes. Le taux d'obésité augmente avec l'âge, au moins jusqu'à 50 ou 60 ans (Peter G. Kopelman, 2005). Un temps considérée comme un problème des pays développés, l'obésité est en augmentation dans le monde entier. Cette augmentation a été ressentie de façon spectaculaire dans la plupart des zones urbaines. La seule région du monde où l'obésité n'est pas commune, c'est l'Afrique subsaharienne (Haslam, 2007).
Les statistiques des années 2005-2007 révèlent qu'en France, on estime que la moitié (46.4%) des adultes français sont en surpoids, dont 14.5% sont obèses (ObEpi Roche 2009) (**figure 1**). Ces estimations sont inférieures à celles rapportées pour les adultes dans les Etats-Unis (31.1% obésité chez les hommes et 32.2% chez les femmes) (Ogden *et al.*, 2006), le Canada (22.9% chez les hommes et 23.2% chez les femmes) (Hopman *et al.*, 2007) et l'Angleterre (22.7% chez les hommes et 32.2% chez les femmes) (Wardle and Boniface, 2008). Cependant, ces estimations pour la population française étaient proches de celles de Norvège (15.5% obésité chez les hommes et 21.0% chez les femmes) *(Droyvold et al.*, 2006), l'Espagne (56.2% en surpoids ou obésité chez les hommes et 40.9% chez les femmes) (Rodriguez Artalejo *et al.*, 2002) et l'Italie (31.3% en surpoids 8.2% obésité)(Gallus *et al.*, 2006), bien que les estimations pour l'Espagne et l'Italie basées sur une autoévaluation rapportée aient été sous-estimées. Ces données confirment que la France, comme plusieurs de ses pays voisins d'Europe occidentale, est moins fortement touchée par le surpoids et l'obésité que le Royaume-Uni et les pays d'Europe de l'Est (Gallus *et al.*, 2006) (**figure 2, 3**).

Au niveau mondial, les données épidémiologiques deviennent préoccupantes : l'OMS estime à 400 millions le nombre de personnes obèses dans le monde, soit 7% de la population mondiale, ce chiffre devant atteindre 12% en 2020 si les tendances évolutives actuelles se confirment (Misra and Khurana, 2008). En France, les études Obepi/Inserm situent la prévalence de l'obésité à 8,7% de la population en 1997 et à 12,4% en 2006 (Charles *et al.*, 2008). L'obésité est un

problème de santé publique et de politique en raison de sa prévalence, les coûts associés et les effets sur la santé (Satcher, 2001). Les efforts de la santé publique visent à comprendre et à corriger les facteurs environnementaux responsables de l'augmentation de la prévalence de l'obésité dans la population. Les solutions visent à modifier les facteurs qui provoquent l'excès d'apport calorique et la consommation et inhibent l'activité physique.

1.4.2. Relation de l'obésité avec d'autres maladies

L'obésité est associée à de nombreuses maladies, en particulier les maladies cardiaques, le diabète de type 2, des difficultés respiratoires durant le sommeil, certains types de cancer, et de l'arthrose (Haslam, 2007). Autre constat de santé publique : l'obésité s'avère jouer un rôle central dans le développement d'une série de maladies chroniques, avant le diabète non insulino-dépendant (80% des diabètes sont liés à l'obésité) l'hypertension artérielle et des maladies cardiovasculaires mais aussi certains cancers et des maladies respiratoires et articulaires sources de handicap. S'ajoute le retentissement psychologique et social, le tout générant des coûts indirects conséquents Les dépenses liées à l'obésité représenteraient 7% de l'objectif national des dépenses d'assurance maladie (Ondam) pour 2008 en France. Au rythme actuel de la progression de l'obésité, ce coût pourrait doubler d'ici 2020 et représenter 14% de l'Ondam.

1.4.3. Mortalité

L'obésité est l'une des principales causes évitables de mortalité dans le monde (Allison *et al.*, 1999; Mokdad *et al.*, 2004; Barness *et al.*, 2007). Des études américaines et européennes à grande échelle ont constaté que le risque de mortalité varie avec l'IMC, le risque le plus faible se trouve à un IMC de 22.5-25 kg / m^2 (Whitlock *et al.*, 2009) chez les non-fumeurs et à un IMC de 24-27 kg / m^2 chez les fumeurs et augmente avec les changements dans les deux sens (Calle *et al.*, 1999; Pischon *et al.*, 2008). L'obésité accroît le risque de décès chez les actuels et anciens fumeurs, ainsi que chez ceux qui n'ont jamais fumé (Pischon *et al.*, 2008). Un IMC de plus de 32 a été associée à un taux de mortalité doublé chez les femmes pour une période de plus de 16 ans (Manson *et al.*, 1995) et l'obésité est estimée responsable d'un excès de 111.909 à 365.000 décès par an aux Etats-Unis (Allison *et al.*, 1999). L'obésité réduit l'espérance de vie moyenne de six à sept ans (Peeters *et al.*, 2003). Un IMC de 30-35 réduit l'espérance de vie de deux à quatre ans (Whitlock *et al.*, 2009), tandis que l'obésité sévère (IMC> 40) réduit l'espérance de vie de 20 ans pour les hommes et de cinq ans pour les femmes (Schwarz *et al.*, 2007). Des modélisations récentes prévoient qu'au rythme actuel de progression de l'obésité et du fait de sa précocité croissante, la perspective d'un raccourcissement de la durée de vie est envisageable pour les générations actuelles (Olshansky *et al.*, 2005).

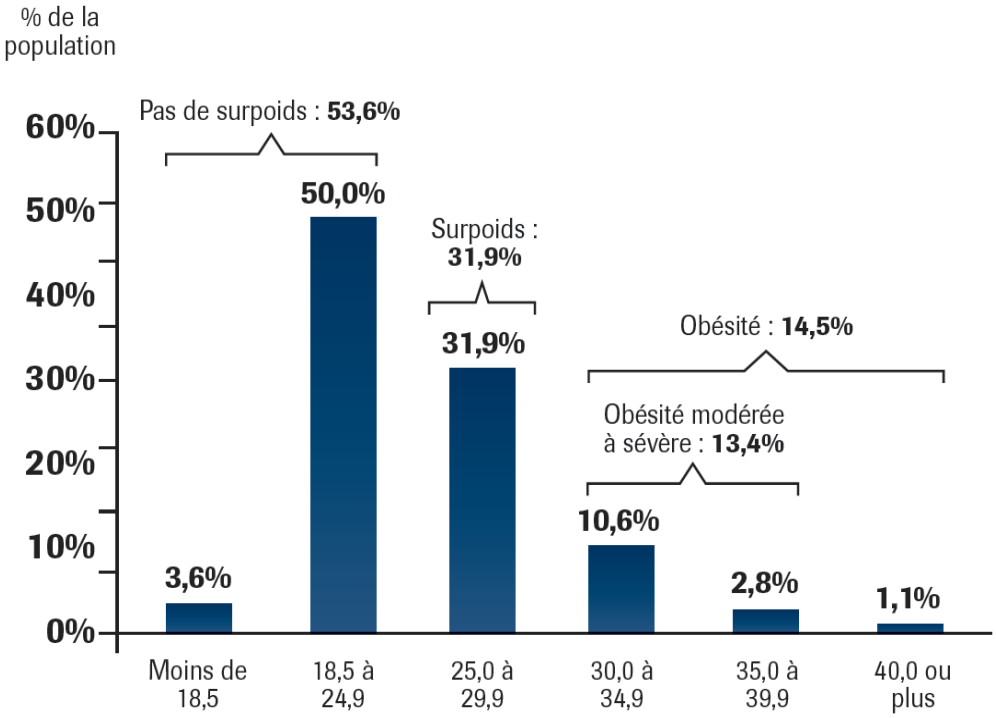

Figure 1 : Répartition de la population française en fonction de son indice de masse corporelle (d'après le rapport d'ObEpi Roche 2009)

1.4.4. Morbidité

Des conséquences multiples sont associées à l'obésité : 1) des conséquences métaboliques et cardiovasculaires comme le diabète, la dyslipidémie et l'hypertension artérielle, 2) des conséquences mécaniques comme les atteintes ostéo-articulaires, l'incontinence urinaire. 3) L'association obésité-cancer, longtemps négligée, est actuellement devenue une source de préoccupation en particulier pour le système digestif et la prostate. 4) et les maladies liées ou aggravées par l'inflammation. Ces comorbidités sont reflétées principalement dans le syndrome métabolique (Haslam, 2007). Le syndrome métabolique est un ensemble de troubles médicaux, dont le diabète de type 2, l'hypertension artérielle, l'hypercholestérolémie, et les niveaux élevés de triglycérides.

Les complications sont causées, soit directement par l'obésité, soit indirectement par le biais de mécanismes de partage d'une cause commune comme une mauvaise alimentation ou un mode de vie sédentaire. La force du lien entre l'obésité et les conditions spécifiques varie. L'un des plus important est le lien avec le diabète de type 2. L'excès de la masse grasse est responsable de 64% des cas de diabète chez les hommes et 77% des cas chez les femmes. Les conséquences de l'obésité sur la santé peuvent être classées par les effets de l'augmentation de la masse grasse (l'arthrose, l'apnée obstructive du sommeil, la stigmatisation sociale) ou par l'augmentation du nombre de cellules graisseuses (diabète, cancer, maladies cardiovasculaires, la stéatose hépatique) (Bray, 2004; Haslam, 2007). L'augmentation de la masse grasse dans le corps modifie la réponse de l'organisme à l'insuline, ce qui pourrait conduire à la résistance à l'insuline. Aussi, l'augmentation de la masse grasse crée aussi un état pro-inflammatoire, ce qui augmente le risque de thrombose (Bray, 2004).

1.4.5. Les coûts économiques

En plus de ses effets sur la santé, l'obésité entraîne de nombreux problèmes, y compris les inconvénients en matière d'emploi (Puhl *et al.*, 2008) et l'augmentation des coûts du travail. Ces effets sont ressentis par tous les niveaux de la société en allant des individus aux sociétés et aux gouvernements. Selon les estimations, 2 à 8 % du coût total des soins dans les pays occidentalisés seraient afférents à l'obésité. En France, la fourchette serait comprise entre 3 et 4 %. Aux Etats-Unis, le coût médical direct de l'obésité (traitements, visites médicales, opérations..) est de 51 milliards de dollars par an, auxquels s'ajoutent 49 milliards de coût indirect, liés à la perte de productivité (Egger, 2009). Aussi, l'estimation des dépenses annuelles sur la gamme des produits diététiques est de $ 40 milliards à 100 milliards de dollars dans les seuls Etats-Unis (Volpp *et al.*, 2008).

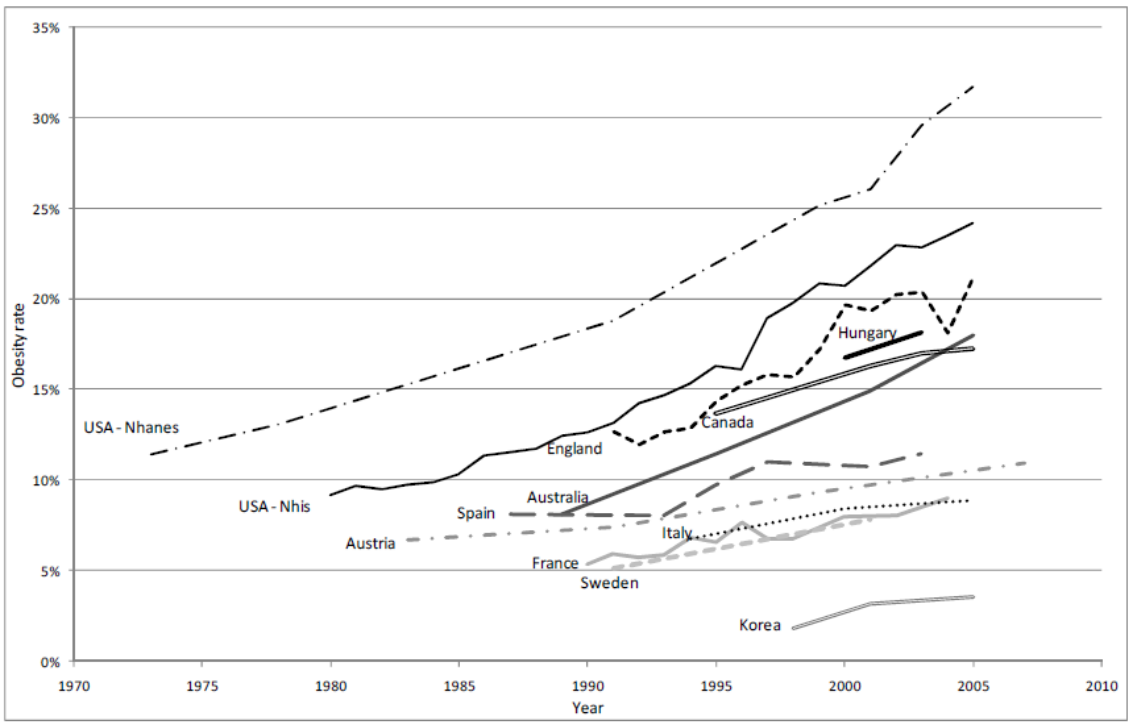

Figure 2: Pourcentages d'obésité chez des hommes âgés de 15-64 ans (standardisés par l'âge). Nhanes, National Health and Nutrition Examination Survey (IMC mesuré); Nhis, National Health Interview Survey (IMC auto-évalué) (d'après DELSA/HEA/WD/HWP (2009)).

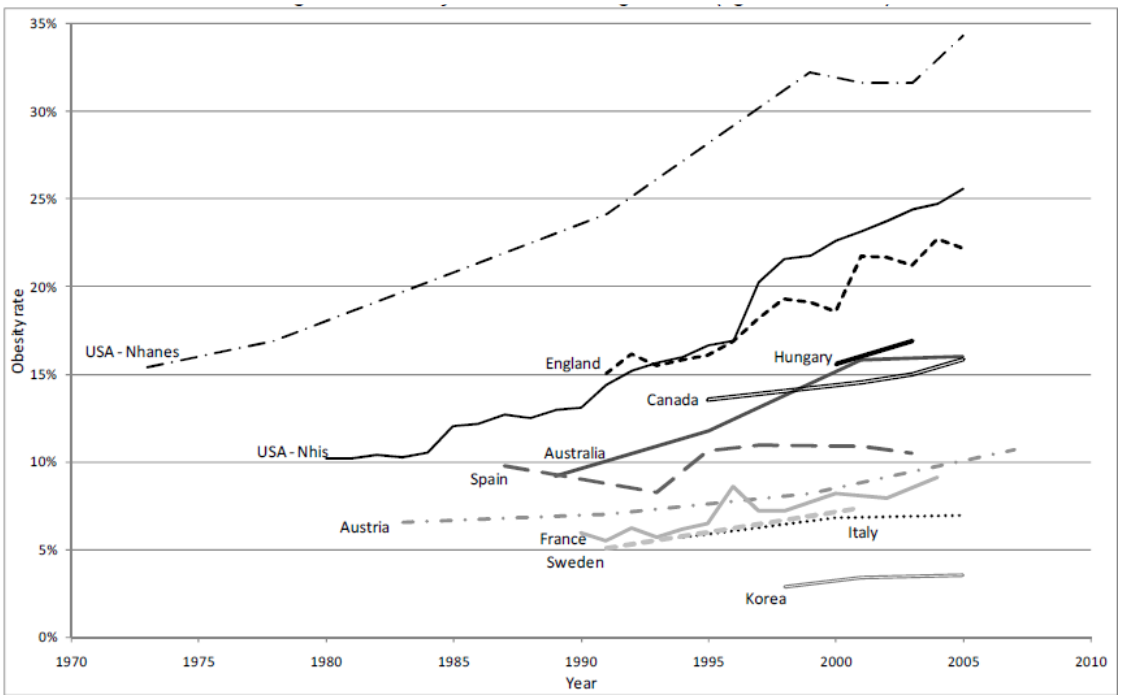

Figure 3: Pourcentages d'obésité chez des femmes âgées de 15-64 ans (standardisés par l'âge) Nhanes, National Health and Nutrition Examination Survey (IMC mesuré); Nhis, National Health Interview Survey (IMC auto-évalué) (d'après DELSA/HEA/WD/HWP (2009)).

REFERENCES

Healthy Weight: Assessing Your Weight: BMI: About BMI for Children and Teens. In: Center for disease control and prevention.

Allison DB, Fontaine KR, Manson JE, Stevens J, VanItallie TB. 1999. Annual deaths attributable to obesity in the United States. Jama 282:1530-1538.

Barness LA, Opitz JM, Gilbert-Barness E. 2007. Obesity: genetic, molecular, and environmental aspects. Am J Med Genet A 143A:3016-3034.

Basdevant A GGB, editor. 2004. Médecine de l'obésité. Paris.

Bei-Fan Z. 2002. Predictive values of body mass index and waist circumference for risk factors of certain related diseases in Chinese adults: study on optimal cut-off points of body mass index and waist circumference in Chinese adults. Asia Pac J Clin Nutr 11 Suppl 8:S685-693.

Bray GA. 2004. Medical consequences of obesity. J Clin Endocrinol Metab 89:2583-2589.

Caballero B. 2007. The global epidemic of obesity: an overview. Epidemiol Rev 29:1-5.

Calle EE, Thun MJ, Petrelli JM, Rodriguez C, Heath CW, Jr. 1999. Body-mass index and mortality in a prospective cohort of U.S. adults. N Engl J Med 341:1097-1105.

Charles MA, Eschwege E, Basdevant A. 2008. Monitoring the obesity epidemic in France: the Obepi surveys 1997-2006. Obesity (Silver Spring) 16:2182-2186.

Clement K, Viguerie N, Poitou C, Carette C, Pelloux V, Curat CA, Sicard A, Rome S, Benis A, Zucker JD, Vidal H, Laville M, Barsh GS, Basdevant A, Stich V, Cancello R, Langin D. 2004. Weight loss regulates inflammation-related genes in white adipose tissue of obese subjects. Faseb J 18:1657-1669.

Droyvold WB, Nilsen TI, Kruger O, Holmen TL, Krokstad S, Midthjell K, Holmen J. 2006. Change in height, weight and body mass index: Longitudinal data from the HUNT Study in Norway. Int J Obes (Lond) 30:935-939.

Egger G. 2009. Health, "illth," and economic growth: medicine, environment, and economics at the crossroads. Am J Prev Med 37:78-83.

Flegal KM, Ogden CL, Wei R, Kuczmarski RL, Johnson CL. 2001. Prevalence of overweight in US children: comparison of US growth charts from the Centers for Disease Control and Prevention with other reference values for body mass index. Am J Clin Nutr 73:1086-1093.

Flynn MA, McNeil DA, Maloff B, Mutasingwa D, Wu M, Ford C, Tough SC. 2006. Reducing obesity and related chronic disease risk in children and youth: a synthesis of evidence with 'best practice' recommendations. Obes Rev 7 Suppl 1:7-66.

Friedman JM. 2004. Modern science versus the stigma of obesity. Nat Med 10:563-569.

Gallus S, Colombo P, Scarpino V, Zuccaro P, Negri E, Apolone G, La Vecchia C. 2006. Overweight and obesity in Italian adults 2004, and an overview of trends since 1983. Eur J Clin Nutr 60:1174-1179.

Gray DS, Fujioka K. 1991. Use of relative weight and Body Mass Index for the determination of adiposity. J Clin Epidemiol 44:545-550.

Harper D. 2008. In: Online Etymology Dictionary: Obesity.

Haslam D. 2007. Obesity: a medical history. Obes Rev 8 Suppl 1:31-36.

Hopman WM, Leroux C, Berger C, Joseph L, Barr SI, Prior JC, Harrison M, Poliquin S, Towheed T, Anastassiades T, Goltzman D. 2007. Changes in body mass index in Canadians over a five-year period: results of a prospective, population-based study. BMC Public Health 7:150.

James WP. 2008. The epidemiology of obesity: the size of the problem. J Intern Med 263:336-352.

Janssen I, Katzmarzyk PT, Ross R. 2004. Waist circumference and not body mass index explains obesity-related health risk. Am J Clin Nutr 79:379-384.

Kanazawa M, Yoshiike N, Osaka T, Numba Y, Zimmet P, Inoue S. 2005. Criteria and classification of obesity in Japan and Asia-Oceania. World Rev Nutr Diet 94:1-12.

Larsson B, Bengtsson C, Bjorntorp P, Lapidus L, Sjostrom L, Svardsudd K, Tibblin G, Wedel H, Welin L, Wilhelmsen L. 1992. Is abdominal body fat distribution a major explanation for the sex difference in the incidence of myocardial infarction? The study of men born in 1913 and the study of women, Goteborg, Sweden. Am J Epidemiol 135:266-273.

Manson JE, Willett WC, Stampfer MJ, Colditz GA, Hunter DJ, Hankinson SE, Hennekens CH, Speizer FE. 1995. Body weight and mortality among women. N Engl J Med 333:677-685.

Mei Z, Grummer-Strawn LM, Pietrobelli A, Goulding A, Goran MI, Dietz WH. 2002. Validity of body mass index compared with other body-composition screening indexes for the assessment of body fatness in children and adolescents. Am J Clin Nutr 75:978-985.

Misra A, Khurana L. 2008. Obesity and the metabolic syndrome in developing countries. J Clin Endocrinol Metab 93:S9-30.

Mokdad AH, Marks JS, Stroup DF, Gerberding JL. 2004. Actual causes of death in the United States, 2000. Jama 291:1238-1245.

NICE. 2006. In. p 36.

Ogden CL, Carroll MD, Curtin LR, McDowell MA, Tabak CJ, Flegal KM. 2006. Prevalence of overweight and obesity in the United States, 1999-2004. Jama 295:1549-1555.

Olshansky SJ, Passaro DJ, Hershow RC, Layden J, Carnes BA, Brody J, Hayflick L, Butler RN, Allison DB, Ludwig DS. 2005. A potential decline in life expectancy in the United States in the 21st century. N Engl J Med 352:1138-1145.

Peeters A, Barendregt JJ, Willekens F, Mackenbach JP, Al Mamun A, Bonneux L. 2003. Obesity in adulthood and its consequences for life expectancy: a life-table analysis. Ann Intern Med 138:24-32.

Peter G. Kopelman IDC, Michael J. Stock, William H. Dietz 2005. Clinical obesity in adults and children: In Adults and Children. In: Publishing B, editor. p 493.

Pischon T, Boeing H, Hoffmann K, Bergmann M, Schulze MB, Overvad K, van der Schouw YT, Spencer E, Moons KG, Tjonneland A, Halkjaer J, Jensen MK, Stegger J, Clavel-Chapelon F, Boutron-Ruault MC, Chajes V, Linseisen J, Kaaks R, Trichopoulou A, Trichopoulos D, Bamia C, Sieri S, Palli D, Tumino R, Vineis P, Panico S, Peeters PH, May AM, Bueno-de-Mesquita HB, van Duijnhoven FJ, Hallmans G, Weinehall L, Manjer J, Hedblad B, Lund E, Agudo A, Arriola L, Barricarte A, Navarro C, Martinez C, Quiros JR, Key T, Bingham S, Khaw KT, Boffetta P, Jenab M, Ferrari P, Riboli E. 2008. General and abdominal adiposity and risk of death in Europe. N Engl J Med 359:2105-2120.

Puhl RM, Moss-Racusin CA, Schwartz MB, Brownell KD. 2008. Weight stigmatization and bias reduction: perspectives of overweight and obese adults. Health Educ Res 23:347-358.

Quetelet, editor. (1871). LAJ.Antropométrie ou Mesure des Différences Facultés de l'Homme. Brussels.

Rodriguez Artalejo F, Lopez Garcia E, Gutierrez-Fisac JL, Banegas Banegas JR, Lafuente Urdinguio PJ, Dominguez Rojas V. 2002. Changes in the prevalence of overweight and obesity and their risk factors in Spain, 1987-1997. Prev Med 34:72-81.

Satcher D. 2001. Remarks from David Satcher, M.D., Ph.D., Assistant Secretary for Health and Surgeon General. Nutr Rev 59:S7-9.

Schwarz PE, Reimann M, Li J, Bergmann A, Licinio J, Wong ML, Bornstein SR. 2007. The Metabolic Syndrome - a global challenge for prevention. Horm Metab Res 39:777-780.

Schwarz S. In: emedicine.com, editor. Obesity.

U.S. 2000. Preventive Services Task Force Evidence Syntheses In: HSTAT: Guide to Clinical Preventive Services rERaSER, Guide to Community Preventive Services., editor.

Uwaifo GI. 2006. In: Obesity: emedicine.com.

Volpp KG, John LK, Troxel AB, Norton L, Fassbender J, Loewenstein G. 2008. Financial incentive-based approaches for weight loss: a randomized trial. Jama 300:2631-2637.

Wardle J, Boniface D. 2008. Changes in the distributions of body mass index and waist circumference in English adults, 1993/1994 to 2002/2003. Int J Obes (Lond) 32:527-532.

Wells Ja. 2005. In. p 16.

Whitlock G, Lewington S, Sherliker P, Clarke R, Emberson J, Halsey J, Qizilbash N, Collins R, Peto R. 2009. Body-mass index and cause-specific mortality in 900 000 adults: collaborative analyses of 57 prospective studies. Lancet 373:1083-1096.

WHO. 1997. Obesity: preventing and managing the global epidemic. Report of a WHO Consultation on Obesity. In: World Health Organization. Geneva. p 3-5.

ANNEXE 2

L'OXYDATION LIPIDIQUE

La balance lipidique, c'est-à-dire l'équilibre entre ingestion et oxydation lipidique est le déterminant majeur de la balance énergétique. Un ensemble de voies métaboliques énergétiques permet la phosphorylation de l'ADP en ATP, grâce à l'oxydation des lipides. Elle comprend plusieurs voies métaboliques : la béta-oxydation, cycle de Krebs ou cycle TCA et la chaîne respiratoire mitochondriale.

1. β-Oxydation

Dans l'organisme, l'oxydation des acides gras se produit dans la mitochondrie et aboutit à la production d'acétyl-COA au cours d'un processus nommé béta-oxydation. Ce processus se fait sur le carbone β et se poursuit aussi longtemps que la molécule d'acides gras n'est pas totalement dégradée en acétyl-COA. Ce dernier rejoint le cycle TCA ou cycle de Krebs. Les atomes d'hydrogène libérés sont oxydés dans la chaîne respiratoire. La béta-oxydation est directement liée à la consommation d'O_2.
La première étape est l'activation des acides gras par liaison avec le Coenzyme A (en utilisant l'énergie de l'ATP, aboutissant à un acyl-CoA. Celui–ci est transporté dans la matrice mitochondrie par une petite molécule : la carnitine.
Un acyl-CoA saturé est dégradé par une séquence récurrente de 4 réactions:1) Oxydation par le FAD, 2) hydratation, 3) oxydation par le NAD+, 4) thiolyse par le CoA. Le résultat pour chaque cycle est la formation d'un acyl-CoA plus court de 2 carbones et la formation du FADH2, NADH et acétyl-CoA. L'oléate $C_{18}H_{34}O_2$ va passer 8fois dans la β-oxydation, tandis que le palmitate $C_{16}H_{32}O_2$ fera 7 tours.

2. Cycle TCA

Le cycle de Krebs ou cycle des acides tricarboxyliques ou encore cycle de l'acide citrique (citrate) est une série de 8 réactions enzymatiques dont la finalité est de produire des intermédiaires énergétiques qui serviront à la production d'ATP dans la chaîne respiratoire. A chaque tour de cycle, une molécule d'acétyl-CoA (2 carbones) réagit avec une molécule d'oxaloacétate (4 carbones) pour donner du citrate, molécules à 6 carbones. Au cours des réactions suivantes, 2 carbones du citrate sont éliminés sous forme de CO_2, assurant ainsi la régénération de l'oxaloacétate (4 carbones). Ce dernier pourra alors de nouveau recevoir un acétyl et recommencer le cycle. Le cycle de Krebs se déroule dans la matrice de la mitochondrie, en aérobiose (présence d'oxygène). Avec la chaîne respiratoire qui réoxyde les coenzymes NADH et $CoQH_2$ produits par le cycle, le cycle de Krebs est le processus ultime de dégradation des différents métabolites qui seront dégradés en CO_2 et eau. En 1 tour du cycle on a :
Acetyl-CoA + oxaloacetate + 3 NAD+ + GDP + Pi +FAD --> oxaloacetate + 2 CO_2 + $FADH_2$ + 3 NADH + 3 H+ + GTP

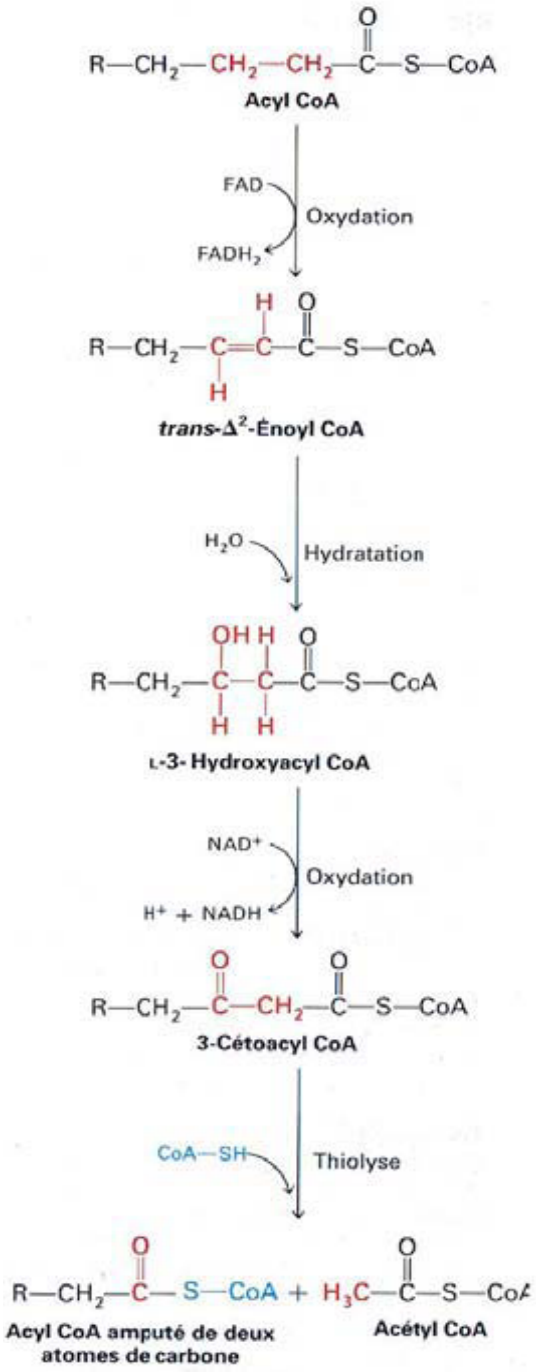

Figure 1 : β-Oxydation

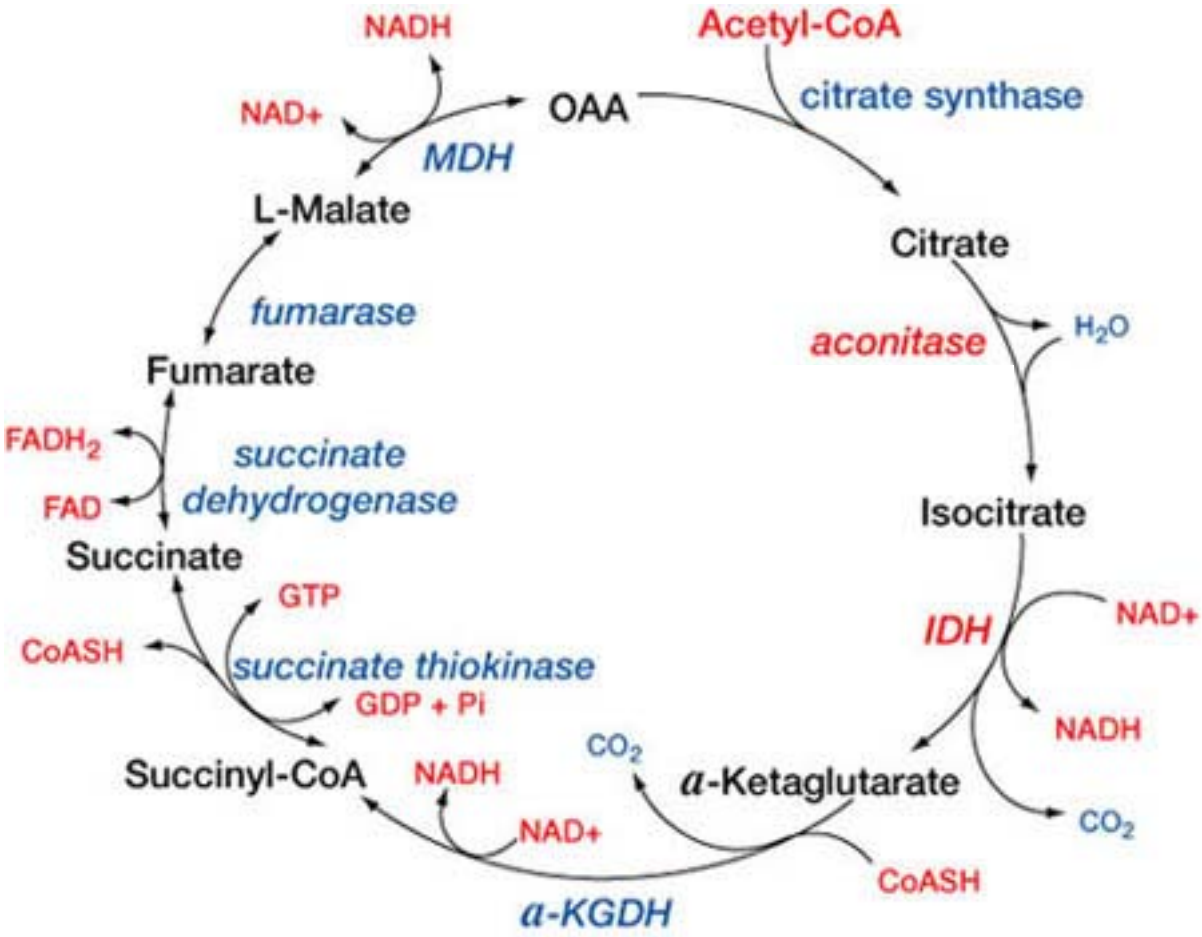

Figure 2 : Le cycle tricarboxylique TCA ou cycle de krebs

ANNEXE 3

Exercice réalisé d'avril à juin 2009 par
Edwina ANTOUN

École doctorale : ED414 Sciences de la Vie et de la Santé
Domaine: Physiologie

Université de rattachement : Université de Strasbourg

Nom du mentor : Bernard CHAPUS

L'obésité: inactivité physique ou lipides alimentaires? Étude des relations entre l'activité physique, la nature des lipides alimentaires et la régulation pondérale.

Présentation de NCT : 1 Juillet 2009

Sujet académique : Rôle du niveau d'activité physique dans la régulation de la balance oxydative des lipides exogènes - Inférences dans la physiopathologie de l'obésité.

Nom du directeur de thèse : Pr. Chantal SIMON – Dr. Yvon LEMAHO

Equipe : Epidémiologie des Maladies Cardiovasculaires et des Cancers- EA 1801- Faculté de médecine - Université de Strasbourg-11 rue Humann, 67000 Strasbourg

SOMMAIRE

Introduction

La rédaction de ce document est la concrétisation de la formation « Valorisation des compétences », proposée par l'Association Bernard Gregory. L'objectif principal est de mettre en évidence les compétences acquises et les potentiels professionnels développés au cours la thèse. Le nouveau chapitre de thèse est rédigé pour être présenté à un public de non spécialistes du sujet et constitue une analyse de la gestion du projet de thèse.

Cadre général et enjeux de la thèse

Présentation du projet et enjeux

La fréquence de l'obésité n'a cessé d'augmenter ces dernières années. Les conséquences en termes de santé publique sont inquiétantes. L'obésité est caractérisée par une diminution de la capacité à utiliser les graisses alimentaires comme source d'énergie. L'augmentation du nombre de sujets obèses pourrait refléter le fait que l'activité physique de la population est aujourd'hui trop faible et ne permet pas d'obtenir une régulation satisfaisante de l'équilibre lipidique. Une démonstration claire de ces hypothèses reste cependant à établir. Des données récentes suggèrent une interaction entre l'activité physique et le type de graisses alimentaires ingérées, telles que l'huile d'olive et les graisses d'origine animale.

L'objectif de ma thèse est d'étudier si des niveaux faibles ou élevés d'activité physique affectent la capacité de l'organisme humain à brûler les graisses d'origine alimentaires et si cet effet dépend du type de graisses ingérées.

L'approche choisie est d'étudier le métabolisme des graisses alimentaires chez des sujets minces ou obèses, actifs ou sédentaires, avant et après une intervention sur l'activité physique (entraînement *vs.* désentraînement).

L'**enjeu scientifique** de ce travail de recherche est de contribuer à la compréhension des mécanismes complexes qui expliquent les changements de l'utilisation des graisses observés quand le niveau d'activité physique est modifié.

Les pouvoirs publics français se sont engagés ces dernières années dans un programme national Nutrition-Santé. L'**enjeu sociétal** de mon projet s'intègre dans l'objectif général de ce programme qui est d'améliorer l'état de santé de l'ensemble de la population en agissant sur l'un de ses déterminants majeurs qu'est l'activité physique.

L'**enjeu économique** est de mieux comprendre l'impact de l'activité physique à la lumière des recommandations actuelles afin d'établir des stratégies préventives de l'obésité. Cette pathologie, responsable d'accidents cardio-vasculaires, de diabètes, de maladies respiratoires et de cancers, représente un coût sanitaire. En dehors de leurs conséquences sur le plan l'humain, l'impact économique est considérable. Dans l'union européenne, les soins consacrés à l'obésité absorbent chaque année l'équivalent de 7% des budgets nationaux de santé. Aux Etats-Unis, l'obésité constitue à elle seule, une facture de 118 milliards de dollars par an en coût direct et indirect.

Mots-clés : obésité- activité physique- oxydation des lipides.

Contexte de la thèse

Ce travail a été réalisé au sein de 1) l'équipe de « Epidémiologie des Maladies Cardiovasculaires et des Cancers. Influence de la Nutrition et de la Sédentarité » dirigé par le Pr. Chantal Simon, et en collaboration avec 2) l'équipe « Physiologie énergétique » sous la direction de Stéphane Blanc au DEPE CNRS. Les tests ont été réalisés dans 3) le département de Médecine Interne et Nutrition de l'Hôpital de Hautepierre (C Simon, CHU Strasbourg). Cette structure bénéficie de l'agrément pour la recherche chez le sujet sain et malade.
Les deux équipes participent à plusieurs projets dont :
ICAPS : étude de l'influence de l'activité physique aux seins des collèges en Alsace sur 800 élèves.
Le projet Monalisa : enquête sur le comportement alimentaire de 1200 personnes en Alsace
L'équipe « physiologie énergétique » a participé dans les études de BEDREST effectués par l'agence spatiale européenne et a étudié l'effet de l'inactivité physique extrême sur l'oxydation des lipides.
L'équipe d'accueil (EA) 180 est une unité de recherche de la faculté de médecine de l'université de Strasbourg.
L'équipe « physiologie énergétique » fait partie du Département d'Ecologie, Physiologie et Ethologie DEPE de l'institut pluridisciplinaire Hubert Curien IPHC et est une unité mixte de recherche de l'université de Strasbourg et le centre national de recherche scientifique CNRS (UMR CNRS ULP 7178). Cette équipe fait partie intégrante du DEPE.

Choix du sujet de thèse

Originaire de Liban, je suis venue en France pour pouvoir poursuivre mon cursus universitaire dans le domaine de Sciences de la Vie et de Biologie. J'ai poursuivi un Master2 de recherche en physiologie et conditions extrêmes à l'université Claude Bernard à Lyon. Cette année comprenait aussi un aspect pratique avec un stage que j'ai effectué dans les hôpitaux universitaires à Tours sous la direction de Pr. Arbeille en physiologie spatiale sur l'étude des réponses hémodynamiques en réaction à un tilt test chez les humains. J'ai été tout de suite passionnée par la recherche clinique qu'offrait ce stage. Cette maîtrise m'a apporté beaucoup au niveau de mon intérêt pour la recherche clinique. Après le master, j'ai postulé pour une bourse de thèse au sein de l'équipe 1801 qui proposait un sujet de thèse répondant parfaitement à mes attentes. Le sujet se positionnait en effet au cœur des problèmes de santé publique (sédentarité, obésité) et est aux frontières de nombreux domaines (nutrition, biochimie et physiologie) et m'offrait une formation assez intéressante au niveau des techniques expérimentales disponibles.

Tout bien considéré, la thèse permet d'acquérir des compétences très intéressantes pour l'insertion dans la vie active, que ce soit dans l'univers de l'entreprise ou dans celui de l'enseignement supérieur et de la recherche académique. Ace jour, je ne peux qu'être satisfaite de la richesse des activités que j'ai eu à mener et des compétences que j'ai eu développer ou acquérir et les premiers éléments d'une logique de recherche ; de la définition d'une problématique à sa résolution pratique conduisant à la réponse.

-Rôle dans la définition et la programmation du sujet

Je ne suis pas intervenue dans le choix du sujet initial, défini aux préalables par ma directrice Pr. Chantal Simon (Professeur de nutrition, EA 1801, Faculté de Médecine, Strasbourg) et mon co-directeur Stéphane Blanc (Directeur du Département d'Ecologie, Physiologie & Ethologie - Institut Pluridisciplinaire Hubert Curien - UMR CNRS ULP 7178).

Déroulement, gestion et coût du projet

Lorsque j'ai intégrée l'équipe, le projet a été déjà lancé avec la réalisation de 17 sujets parmi les 36 sujets à recruter. L'aide des post doc et des précédentes doctorantes a été précieuse dans l'apprentissage des méthodologies et l'intégration des travaux déjà effectués.

La **première partie** de la thèse a été consacrée au recrutement de 28 volontaires parmi les 263 entretiens que j'ai effectué, et à la préparation et la réalisation de 159 jours d'expérimentation sur 28 volontaires sains.

La **deuxième partie** était consacrée à la rédaction de quatre articles. J'ai apprécié la confiance de mes co-directeurs de thèse, qui m'a permise de mener d'une façon quasi-autonome le déroulement des tests. D'autre part, le sujet m'a permis d'approfondir mes connaissances dans le domaine de la recherche fondamentale et appliquée (protocole clinique).

Préparation et cadrage du projet

-Evaluation des facteurs de succès et de risques

Travailler sur l'humain est directement lié à un facteur de risque de l'abandon volontaire recruté. A partir des 47 sujets recrutés, 9 ont décidé d'arrêter volontairement après le début des tests. En plus, Il existe une grande variabilité entre individus. Aussi, la nécessité de travailler avec plusieurs personnes (volontaires, chirurgiens, infirmières, éducateur sportif, techniciens) pouvait entraîner quelques difficultés dans la gestion des plannings.

Cependant, l'équipe bénéficie d'un bon support technique et budgétaire.

-Choix des partenaires

La réalisation de ce projet m'a offert l'opportunité d'être en interface entre 3 équipes (EA US 1801 Faculté de médecine Strasbourg, UMR CNRS US 7178 institut pluridisciplinaire Hubert Curien, département Ecologie, Physiologie et Etologie, Strasbourg et le département de nutrition du centre hospitalier universitaire de Strasbourg), et de collaborer nationalement avec une 4ème équipe du centre de biologie CBS CRNH-Rhône Alpes (Lyon) où j'ai fait des analyses pendant deux mois.

-Gestions des aspects contractuels

Comme allocataire de recherche ministérielle, j'ai signé « la charte des thèses », contrat de travail à dure déterminée (CDD) de trois ans. Ce contrat précise les droits et les devoirs du doctorant pendant la thèse. A titre d'exemple, le doctorant, chercheur non permanent en formation, bénéficie de l'accès aux structures de son unité d'accueil au même titre que les titulaires, d'un sujet défini et de l'encadrement personnalisé de son directeur de thèse. Le doctorant s'engage à suivre des formations complémentaires et à participer à la vie de l'établissement.

Conduite du projet :

Périodicité des bilans d'avancement.

La durée de référence d'une préparation de thèse est de trois ans. Pour se conformer à la durée prévue, le doctorant et le directeur de thèse doivent se respecter leurs engagements relatifs au temps de travail nécessaire.

a) La réunion du comité de thèse a eu lieu en moitié de l'exercice. Parmi les membres du comité, figuraient ma directrice de thèse, mon co-directeur, un membre extérieur Pr. Laville directrice du département de nutrition au CNRH Lyon) et un chercheur de l'unité (spécialiste des mitochondries). Cette réunion a permis de faire le point sur l'avancement des travaux, les difficultés rencontrés, et d'apporter des suggestions et des critiques très enrichissantes et constructives pour la poursuite du travail.

b) Ma directrice et mon directeur de thèse ont fait preuve de disponibilité, contribuant au bon déroulement de mes activités. Des bilas d'avancement mensuels nous ont permis de discuter des problèmes éventuels, des résultats, des méthodes d'analyses et de l'organisation de poursuite du travail.

-Gestion du projet sur la durée et au quotidien

Au quotidien, il m'a fallu gérer la logistique associée au projet (recrutement des sujets : 417 appels téléphoniques, 263 entretiens, préparation et réalisation de 159 jours d'expérimentation, suivi des commandes des matériaux : étiquetage et rangement de plus de 7 500 tubes, d'achats et pesages de régimes alimentaires complets de 340 journées, gérance de 36 dossiers de rapports médicaux, assurer le planning et le suivi des 504 séances d'entraînements avec 3 éducateurs sportifs, organiser 50 tests d'effort avec l'hôpital civil et les sujets, organiser les plannings des tests avec les sujets, les médecins collaborateurs(1chirurgien et 2médecins), 2infirmières, 3 diététiciennes,2 techniciens, sans interférence d'un sujet à l'autre. Ces fonctions impliquent une prise de contacts avec de nombreux partenaires et une part de temps important consacré aux appels téléphoniques, courriers, réunions, achats et surtout la réalisation. Aussi, une organisation rigoureuse, une réactivité et surtout l'adaptabilité aux imprévus sont indispensables.

-Problèmes rencontrés et solutions apportées

Déménagement : dans la deuxième année de thèse, ma directrice a du déménager et libérer les locaux de l'hôpital ou se faisait les expériences pour des causes professionnelles. Donc j'ai du recruter les derniers 10 sujets et caser 70 jours d'expérimentation en un lapse de 5 mois, un travail qui prenait normalement une année à réaliser.

Démission : suite à cet imprévu et le travail surchargé par conséquent, notre éducateur sportif principal a été surmené et a présenté sa démission. J'ai négocié avec lui et je lui ai réorganisé et réduit son travail pour qu'il se sente plus à l'aise. Il a accepté de rester.

Retrait du consentement : après le début des tests, j'ai réussi à convaincre deux sujets à continuer les deux mois d'entraînement car ils étaient fatigués et les séances leur prenaient beaucoup de temps mais deux autres étaient catégoriques et sont partis (un à cause d'être embauché dans un nouvel travail et l'autre pour des raisons personnelles)

Matériel : il a fallu prévoir plusieurs mois en avance les commandes des matériels car les facturations à la faculté et par suite la réception étaient retardées au niveau administration de l'université.

Gestion du temps : Il a fallu optimiser la gestion du temps, puisque il y a eu plusieurs imprévus au niveau des plannings (urgence pour le médecin, imprévu personnel pour les sujets, maladie d'un personnel) qu'il fallait réagir tout de suite et replannifier avec l'accord de tous les concernés, les jours des tests. Pour cela, il fallait être disponible à chaque instant, avoir une vue globale de tout le projet et être en bon terme avec tous les sujets et associés pour faciliter les changements de dates.

Evaluation et prise en charge du coût du projet

Estimation du coût consolidé de la thèse

Montants en euros TTC

	Nature de la dépense	Détails *		Coûts totaux (euros TTC)			
				Nombre d'unités/ mois	Coût unitaire moyen	Quote-part utilisation	Total
1	**Ressources Humaines**						
1.1	Doctorant	1658.25	0.3	36	2155.73	1.00	77606
1.2	Encadrant 1	5000	0.3	36	6500.00	0.15	35100
1.3	Prime Encadrement						
1.4	Encadrant 2	3500	0.3	36	4550.00	0.20	32760
1.5	Prime Encadrement						
1.6	Autres personnels	8300	0.3	36	10790.00	0.50	194220
1.7	Sous-traitance						
	Sous-total Ressources Humaines						**339686**
2	**Consommables**						
2.1	Fournitures expérimentales			36		1.00	371 000
2.2	Fournitures de bureau	30		36		0.20	216
	Sous-total Consommables						**371 216**
3	**Infrastructures**						
3.1	Entretien, gardiennage, secrétariat	2000	0.3	36	600	0.10	2160
3.2	Loyers des locaux/charges locatives	5000	4000	36		0.05	16200
3.4	Autres						
	Sous-total Infrastructures						**18360**
4	**Matériel (amortissements)**						
4.2	Ordinateur de bureau	0.33	1200			1.00	396
4.3	Logiciels de bureau	0.33	300			1.00	99
	Sous-total Matériel						**495**
5	**Déplacements**						
5.1	Missions en France	150	2000			1.00	2150
	Sous-total Déplacements						**2150**
6	**Formation**						
6.2	Autres frais (Inscription à l'Université, Sécurité Sociale étudiante, etc.)			3	355	1.00	1065
	Sous-total Formation						**1065**
7	**Documentation et communication**						
7.1	Affranchissements, Internet, téléphone	50		36		0.10	180
7.2	Publicité, communication, impressions	1000				0.25	250
7.3	Documentation (périodiques, livres, bases de données, bibliothèque, etc.)	300					300
	Sous-total Documentation et communication						**730**
8	**Charges financières (intérêts des emprunts)**						
	Sous-total Charges financières						
9	**Charges exceptionnelles**						
	Sous-total Charges exceptionnelles						
10	**TOTAL**						**733 702**

Détails des ressources humaines et consommables (sans encadrants)

		Coût (Keuros)/36mois
Ressources humaines	Doctorante	66
	Post doc	135
	Infirmière	42
	Technicien	96
Matériels	Rotors	30
	Tubes	35
	Isotopes	96
	Génomic	30
Analyse	Isotopes	67
	Génomic	30
	Protéomic	45
Indemnisation	48volontaires	38
Total		**710**

Compétences, savoir faire et qualités professionnelles

Domaines d'expertise : Métabolisme énergétique, Nutrition, Santé publique, Recherche et développement, Physiologie humaine, Recherche clinique, Communication

Compétences scientifiques et techniques

Compétences scientifiques : élargissement de la culture scientifique dans plusieurs domaines : biologie, physiologie, nutrition, biochimie et médecine. Connaissance de la réglementation dans le domaine de l'éthique et la propriété intellectuelle, application de la démarche qualité, aspect législatif et contractuel de la recherche.
Compétences techniques : analyse des données, utilisation des logiciels bureautiques et statistiques, analyses biologiques et chimiques, expérimentation et physiologie humaine, calorimétrie, différents instruments de mesure de la dépense énergétique, techniques d'isotopes stables et la méthode de l'eau doublement marquée.

Compétences de gestion

Gestion du projet : organisation et plannings des tests**,** administration, aspects législatifs, rapports d'activité, adaptation des plannings des sujets avec les membres des l'équipe et les séances d'entraînement d'où la nécessité d'être organisé afin de répartir dans le temps les différentes taches à accomplir. Tenir aux échéances et s'adapter aux impératifs et contretemps de chacun et réadapter tout le planning. Etre capable d'avoir une vision d'ensemble du projet et prendre de recul.
Gestion du personnel : Interlocuteur des techniciens, volontaires et collaborateurs. Gestion avec négociation et diplomatie de la communication entre les équipes. Travail de recherche individuel combiné à des situations collectives d'où l'apprentissage du travail d'équipe.

Compétences en communication

Langues : communications et publications en anglais, avec l'obtention du Toeic (score 945/990)

ADDAL : membre actif de l'association des docteurs et doctorants d'alsace – équipe organisatrice des rencontres entre entreprises et doctorants. Ma participation à l'organisation de rencontres au sein de Addal et la participation au forum BioTechno (rencontres doctorant / entreprises) m'ont permis de tisser un réseau de connaissances et de confronter un certain nombre d'expériences qui me seront certainement utiles dans la suite de ma vie professionnelle.

Compétences d'encadrement

Encadrement de deux techniciens dans le cadre de leur travail sur le projet.

Savoir faire administratif et rédactionnel

Rédaction des fiches méthodologiques, de cahier d'observations d'étude clinique, des dossiers pour demande de bourse (FRM) et poste d'attaché temporaire à l'enseignement et la recherche (campagne 2009-2010).

Savoir-être et qualités personnelles

La thèse est un excellent outil pour développer ses aptitudes à la communication ainsi que les qualités d'écoute, de rigueur, d'adaptabilité, de créativité et de persévérance.
L'ensemble de ces compétences acquises au cours du projet est transférable à bien d'autres domaines.

Résultats, impact de la thèse

Résultats attendus : Au niveau **scientifique**, cette étude montrera si l'inactivité physique, indépendamment de ses effets sur la balance énergétique, induit des situations métaboliques supposées causales dans l'étiologie de l'obésité. L'étude évaluera l'efficacité de différents niveaux d'activité physique sur l'orientation des lipides exogènes au profit de l'oxydation. Enfin, la génomique et la protéomique fonctionnelles fourniront une approche mécanistique et pourraient ouvrir de nouvelles voies de traitements thérapeutiques. Au niveau **personnel**, j'ai désormais la capacité de mettre en place et de gérer un projet en fonction de nombreuses contraintes humaines ou techniques. De plus, mes contacts avec les différentes équipes et les volontaires m'ont permis d'élargir mes compétences à plusieurs niveaux : relationnels, administratifs et scientifique au sujet de l'obésité et de tous les phénomènes impliqués dans cette maladie et m'a ainsi permis d'avoir une vision plus globale et synthétique de cette pathologie. De plus, mon champ de connaissances sur l'obésité et ses conséquences est applicable au sujet de nombreuses autres maladies, très étudiées également ce qui me permet d'être confiante quant à ma capacité à m'intégrer dans une équipe de recherche dans l'industrie.

Projet professionnel

Le bilan de compétences m'a permis d'éclaircir mes choix sur les pistes professionnels que j'aimerais envisager dans le futur. La recherche clinique s'est avérée un domaine qui me passionne et donc j'ai du élargir mon réseau professionnel

avec des représentants de certaines boites pharmaceutiques leader dans le domaine de recherche clinique comme Roche, Novartis et Lilly. Des postes comme attaché de recherche clinique, associé de recherche clinique CRA, chargé de mission scientifique ou chercheuse dans recherche et développement R&D me sont favorables. J'ai aussi découvert une passion pour le contrôle qualité et un poste dans ce domaine m'intéresse aussi. Finalement, mes compétences de bonne transmission de savoir et de données que j'ai développées dans les différentes communications m'ouvrent le domaine d'enseignement comme maître de conférences.

CONCLUSION

Les compétences scientifiques, techniques, gestionnelles et personnelles que j'ai acquises durant les quatres années de travail de thèse ont renforcé ma motivation de travailler dans le domaine de recherche clinique. Ce plaisir de travailler dans le domaine de santé publique est complété aussi par ma formation supplémentaire d'investigateur de recherche clinique. Ma formation et mon expérience sont pluridisciplinaires et pourront donc certainement intéresser de nombreux acteurs de la santé publique. De même, un doctorat en physiologie offre un profil recherché dans l'industrie pharmaceutique. J'ai également découvert que la gestion des personnels (techniciens, infirmières, éducateur physique, chirurgiens, collègues, intervenants externes, commerciaux en visite) ainsi que la gestion des ressources matérielles sont deux compétences que j'apprécie. Je pense que le management d'une équipe de recherche clinique et de projets scientifiques pourrait me permettre d'appliquer l'expérience que j'ai acquise en associant à la fois le domaine de la science avec le monde de gestion. Ainsi, c'est dans cette perspective que j'aimerais avancer, et ces points représentent pour moi des objectifs que j'aimerais atteindre dans ma carrière.

Coquillages en pleine lumière de Lynn Fecteau

RÉSUMÉ

L'activité physique, en tant que composante la plus modulable de la dépense énergétique totale, pourrait jouer un rôle clef dans le devenir des lipides. Toutefois, hormis les données existantes sur les effets aigus de l'activité physique sur la lipémie, nous disposons de peu de données concernant l'effet de la modulation du niveau d'activité physique habituel sur l'oxydation et la répartition des lipides alimentaires. Le but de ce travail de thèse a été d'étudier, chez l'homme normopondéré et en surpoids, les mécanismes par lesquels une dépense énergétique liée à l'activité physique, basse ou élevée, module l'oxydation des lipides alimentaires en portant une attention toute particulière à l'évaluation des recommandations actuelles sur le niveau d'activité physique. Nous avons soumis deux groupes de sujets sédentaires différant par leur masse corporelle (surpoids *vs.* normopondérés) à 2 mois d'entraînement basé sur les recommandations actuelles et un troisième groupe d'hommes actifs normopondérés à un mois de désentraînement, avec un maintien d'un poids stable tout au cours de l'étude. Le devenir métabolique des acides gras exogènes (saturés ou monoinsaturés) était évalué par des molécules marquées par isotopes stables. Après le désentraînement, les oxydations lipidiques à jeun, totale et exogène diminuent. A l´inverse, un entraînement selon les recommandations actuelles, induit une modeste augmentation de l'oxydation lipidique qui n´apparaît significative que pour les acides gras monoinsaturés. Ainsi, l´inactivité physique a des effets plus marqués sur le métabolisme lipidique que l´entraînement. Toutefois, le niveau de l´activité habituelle, indépendamment d´effets quantifiables sur la balance énergétique, prédit le niveau d´oxydation des lipides exogènes. Nos résultats apportent des données clefs dans le débat actuel quant au rôle de l'activité physique dans le traitement de l'obésité et des troubles métaboliques associés à la sédentarité, en démontrant une relation positive entre la quantité d'énergie dépensée lors d'activités et le devenir des lipides alimentaires. Cependant, l´entraînement n´a pas modifié la dépense énergétique liée à l´activité physique chez les sujets en surpoids. Ceci s´explique par une réduction spontanée des activités de la vie de tous les jours. Ces résultats pourraient en partie expliquer les résultats médiocres obtenus au long cours dans le traitement de l´obésité par l´exercice physique. En conséquence, nos résultats suggèrent que de plus amples études sont nécessaires pour déterminer le niveau d´activité physique nécessaire et suffisant pour augmenter l´oxydation lipidique si l´on souhaite que cette activité physique prévienne la prise de poids. Un effort particulier en recherche doit être mis sur la nature de l´activité physique à promouvoir. Il semble sur la base de nos résultats que des exercices de type structurés ne sont pas suffisants. Une approche socio-écologique qui prend en compte l´environnement dans lequel évolue les individus est impérative, s´il l´on veut éviter que l´activité de la vie de tout les jours devienne le tampon économisant l´énergie dépensée dans les activités structurées.
Mots-clés: oxydation lipidique, activité physique, isotopes stables, obésité.

ABSTRACT

Physical activity, being the most adjustable component of total energy expenditure, may play a key role in the fate of lipids. However, a part from the effects of acute exercise little is known on the effect of modulating habitual physical activity level on lipid oxidation and trafficking. The objective of our theses was to investigate, in lean and overweight men, the mechanisms by which low or moderate physical activity energy expenditure, modulates exogenous lipid oxidation, with particularly evaluating physical activity current recommendations.

We submitted two groups of sedentary men (lean vs. overweight) to two months of training based on current recommendations, and a third group to one month of detraining, with body maintenance throughout the interventions. The fate of exogenous fatty acids (saturated: palmitate; monounsaturated: oleate) was evaluated using stable isotopes. After detraining, fasting, total and exogenous lipid oxidation decreased. Conversely, training based on current recommendations induced a modest increase of lipid oxidation which was only significant for monounsaturated fatty acids. Thus, physical inactivity has more marked effects than training. Nevertheless, habitual physical level, independently of measurable effects on energy balance, predicts the level of exogenous lipid oxidation. Our results bring key answers in the actual debate of the role of physical activity in the treatment of obesity and metabolic alterations associated with sedentarism, by demonstrating a positive relationship between the energy expended during activities and the becoming of lipids. However, training did not increase activity energy expenditure in overweight men. This is explained by the spontaneous reduction of everyday activities. These results can partly explain the deceiving results obtained all along in the treatment of obesity by physical activity. Consequently, our results suggest that further studies are warranted to determine physical activity level required and sufficient to increase lipid oxidation if we wish that this physical activity prevents weight gain. A particular effort should be put on the type of physical activity to be encouraged. It seems, based on our results, that structured physical activity is not sufficient. A socio-ecological approach that takes in account the environment, in which individuals evolve, is imperative if we want to avoid that everyday activities buffer the energy expended in structured activities.
Key words: lipid oxidation, physical activity, stable isotopes, obesity.