U N I V E R S I T É  L O U I S  P A S T E U R – S T R A S B O U R G

Numéro d'ordre n° 5722

**THÈSE**

Pour obtenir le grade de

**DOCTEUR DE L'UNIVERSITÉ LOUIS PASTEUR**

Discipline: Sciences du Vivant
Domaine: Physiologie et biologie des organismes

Présentée et soutenue publiquement par

**Audrey BERGOUIGNAN**

Le 29 juillet 2008

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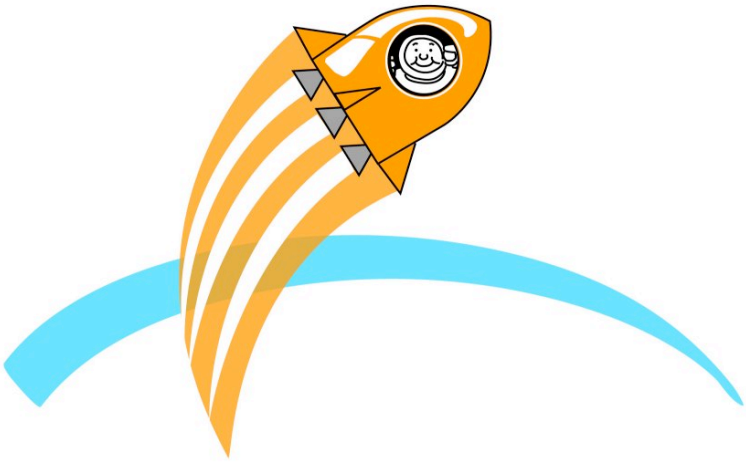

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**Ce travail de thèse a fait l'objet des articles, communications orales et affichées suivants :**

## Articles originaux et revues

- **Bergouignan A.**, Momken I., Schoeller D., Normand S., Lescure B., Hargens A., Guilland JC., Simon C., Blanc S. Balancing appetite, energy intake and energy expenditure during physical inactivity induced by 60 days of bed rest. Am J Clin Nutr, soumis.

- **Bergouignan A.**, Blanc S., Whitesell LF., Buck J., Nickles RJ., Stone CK., Schoeller D. Bio-distribution of an oral load of 14(R,S)-[$^{18}$F]fluoro-6-thia-heptadecanoic acid in pigs. Applied Radiation & Isotopes, soumis.

- **Bergouignan A**., Schoeller D., Momken I., Simon C., Blanc S. Metabolic fate of saturated and mono-unsaturated dietary fats: the Mediterranean diet revisited from epidemiological evidences to cellular mechanisms points towards a central role for activity energy expenditure. Progress in Lipid Research, en révision.

- **Bergouignan A.,** Trudel G., Simon C., Schoeller D., Chopard A., Desage M., Burdge B., Gauquelin-Koch G., Normand S., Blanc S. Enforced physical inactivity differentially alters dietary oleate and palmitate trafficking. Diabetes, sous presse.

- **Bergouignan A.**, Schoeller D., Votruba S., Simon C., Blanc S. The acetate recovery factor to correct tracer derived dietary fat oxidation in humans. American Journal of Physiology Endocinology and Metabolism. Am J Physiol Endocrinol Metab. 2008 ; 294(4) :E645-53.

- **Bergouignan A.**, Schoeller D., Normand S., Gauquelin-Koch G., Laville M., Shriver T., Desage M., Maho YL., Ohshima H., Gharib C., Blanc S. Effect of Physical Inactivity on the Oxidation of Saturated and Monounsaturated Dietary Fatty Acids: Results of a Randomized Trial. PLoS Clin Trials, 1(5), e. 2006.

- Zahariev A., **Bergouignan A.**, Caloin M., Normand S., Gauquelin-Koch G., Gharib C., 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(4), p. 344-50, 2005.

## Articles sans comité de lecture

- **Bergouignan A.,** Blanc S. Le métabolisme énergétique s'adapte à l'inactivité… Diabète & Obésité, 2(8), p. 106-112, April 2007.

- **Bergouignan A.**, Blanc S. Energy and oxidative balances in response to the space environment. COSPAR, 36$^{th}$ Scientific Assembly, p.102. 16-23, July 2006.
- **Bergouignan A.**, Blanc S. Énergétique de l'obésité. J Soc Biol, 200(1), p.29-35, 2006.

- Blanc S., **Bergouignan A.** La dépense énergétique : composantes et déterminants. Nutrition & Facteurs de risque, 3, p. 19-22, Janvier 2005.

## Communications orales :

- **Bergouignan A.,** Trudel G., Simon C., Schoeller D., Chopard A., Desage M., Burdge B., Gauquelin-Koch G., Normand S., Blanc S. Simulated microgravity differentially alters dietary oleate and palmitate trafficking.
  29th Annual ISGP Meeting, Life in Space for Life on Earth, 22-27 June 2008 in Angers, France.

- **Bergouignan A.**, Schoeller D., Votruba S., Simon C., Blanc S. The acetate recovery factor to correct tracer derived dietary fat oxidation in humans.
  Recent Advances and Controversies in the Measurement of Energy Metabolism, 5-7 February 2008 in Denver, Colorado, USA.

- **Bergouignan A**., Blanc S. Effect of physical inactivity on feeding behaviour
  18th Conference of Neurex, 18-19 June 2007, Strasbourg, France.

- **Bergouignan A.**, Normand S., Schoeller D., Gharib C., Gauquelin-Koch G., Blanc S. Gender differences in lipid metabolism responses to long-term bed-rest.
  57th International Astronautical Congress, 2-6 October 2006 in Valencia, Spain.

## Communications affichées:

- **Bergouignan A.**, Normand S., Schoeller D., Blanc S. Effect of physical inactivity on energy and oxidative balances in women under simulated weightlessness
  6th Meeting of French Space Agency (CNES) Young Researchers, 17-19 October 2006 in Toulouse, France.

- **Bergouignan A.**, Zahariev A., Caloin M., Normand S., Gauquelin-Koch G., Gharib C., 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.
  5th Humans in Space Symposium, 22-26 May 2005 in Graz, Austria.
  .

*« Manger seul ne gardera pas un homme en bonne santé ; il doit également faire de l'exercice. L'alimentation et l'exercice, bien que possédant des qualités opposées, travaillent ensemble à une bonne santé. La nature de l'exercice est de consommer les substrats fournis par la nourriture et la boisson pour créer de bons déficits.»*

***Hippocrate, 5ème siècle avant JC***

# SOMMAIRE

# PRÉAMBULE

*La Vénus de Willendorf*
*(Paléolithique supérieur)*

Comme l'ont soulevé Trevathan et collègues (traduit de (Trevathan & Smith, 1999), « *une meilleure compréhension de nombreux problèmes de santé modernes émergera lorsque nous prendrons en compte le fait que la plus grande part de l'évolution humaine a eu lieu alors que nos ancêtres étaient des chasseurs-cueilleurs* ». En effet, 95% de la biologie humaine, et probablement des comportements humains, ont été naturellement sélectionnés au moment de l'émergence du génome humain moderne avec l'apparition de l'*Homo sapiens sapiens,* il y a environ 45000 ans (Trevathan & Smith, 1999). Depuis, notre patrimoine génétique aurait subi peu de mutations et serait resté relativement stable. 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. Néanmoins, l'un des points sur lequel nous différons notablement de l'*Homo sapiens sapiens* est notre mode de vie qui a considérablement changé au cours du dernier siècle.

À l'ère Paléolithique, une activité physique quotidienne faisait partie intégrante de l'existence de nos ancêtres du fait de leur vie nomade et de leur mode d'alimentation. En effet, l'activité physique représentait leur unique moyen, à travers la pratique de la chasse et de la cueillette, d'avoir accès à la nourriture. Le régime alimentaire était alors basé sur une consommation élevée de graines, de fruits et légumes frais et de viande provenant d'espèces sauvages. Avec le développement agricole, notre régime alimentaire s'est diversifié (lait, poissons, féculents…), puis avec la révolution industrielle sont apparus les produits raffinés, les plats préparés ou encore la 'junk food'. À travers les âges, notre alimentation s'est ainsi progressivement enrichie en lipides pour atteindre aujourd'hui une proportion de 41% de l'énergie ingérée totale dans les pays occidentaux (Matthys *et al.*, 2003) comparée aux 35% estimés de l'ère Paléolithique (Eaton, 2006). Au-delà de ces changements de types quantitatifs, la nature des acides gras a aussi été modifiée avec une proportion d'acides gras saturés qui est passée de 7,5% (Eaton, 2006) à 15,4% (Matthys *et al.*, 2003). Sachant que les acides gras sont différemment utilisés par l'organisme suivant leur nature, cette modification dans la composition des acides gras du régime alimentaire est également un changement environnemental en soi important à noter.

Parallèlement, les innovations technologiques nous ont permis d'avoir accès à la nourriture ou de nous déplacer sans dépenser une trop grande énergie et ont causé l'émergence de loisirs passifs comme la télévision ou les jeux vidéos. Tous ces changements sociétaux ont favorisé l'adoption de comportements sédentaires dans nos populations modernes. En supposant que les hommes de l'âge de pierre et ceux de l'ère spatiale puissent être comparés, il a été estimé que la dépense énergétique totale des humains modernes représentait seulement 65% de celles de l'*Homo sapiens sapiens* (Cordain *et al.*, 1998). Basés sur cette observation, Cordain et collègues ont écrit que le niveau d'activité physique actuel est « *sûrement, en dessous du niveau d'activité physique pour lequel notre physiologie et notre biologie ont été génétiquement programmé au cours de l'évolution* » (Cordain *et al.*, 1998). Il semble donc exister un important décalage entre le mode de vie contemporain, notamment dans les pays occidentaux, et celui de nos ancêtres biologiquement sélectionnés à l'ère Paléolithique. C'est dans un environnement caractérisé par une alimentation modérée en graisses et une activité physique élevée que le fonctionnement de notre organisme est susceptible d'être optimal. Le cas contraire pourrait alors induire des dysfonctionnements de l'organisme.

De récentes données épidémiologiques ont associé les comportements sédentaires adoptés par les sociétés occidentales à de nombreuses maladies chroniques dont l'obésité. Bien que l'on parle actuellement d'un 'Sedentary Death Syndrom' (Lees, 2004), il est surprenant de constater à quel point notre connaissance de la physiologie de l'inactivité physique est limitée. En effet, les données sur les effets délétères de l'inactivité physique restent indirectes et reposent principalement sur des études épidémiologiques ou sur les effets

bénéfiques de l'exercice physique. Face à la croissance alarmante de l'obésité au niveau mondial, il apparaît urgent de mettre en place des études longitudinales et mécanistiques permettant d'étudier les relations de cause à effets entre l'inactivité physique et le développement de l'obésité. De telles études permettront de démasquer les mécanismes impliqués dans la régulation du poids qui sont altérés par l'inactivité physique et ainsi mettre en avant certains leviers sur lesquels des méthodes de traitement ou de prévention pourraient être appliquées.

La description des manifestations cliniques de l'obésité remonte à l'époque gréco-romaine, mais ce n'est qu'au XXe siècle que l'on s'est intéressé à la physiopathologie de la maladie. La majorité des études du siècle dernier ont cherché des causes biologiques dans la genèse de la maladie en négligeant l'impact des changements environnementaux, comme l'énergie ingérée et le niveau d'activité physique. L'obésité est une maladie du stockage des lipides et une vision classique de son étiologie repose sur une incapacité à utiliser les lipides qui serait causale dans la prise de poids. Or l'observation relativement récente d'un alignement étroit de la balance lipidique sur la balance énergétique suggère fortement que le niveau d'activité physique, en tant qu'élément le plus modulable de la dépense énergétique totale, pourrait jouer un rôle clef dans le devenir des lipides alimentaires. Le corollaire direct de cette observation est que l'incapacité à utiliser les lipides en tant que substrat, observée chez les sujets obèses et post-obèses, pourrait être secondaire à l'adoption généralisée d'un mode de vie sédentaire.

Au cours de cette thèse, nous nous sommes intéressés aux effets directs de l'inactivité physique sur la régulation des balances énergétique et lipidique et plus spécifiquement, sur le devenir métabolique des lipides exogènes. Pour cela nous avons soumis des sujets sains à un niveau d'inactivité physique extrême pour la population générale en utilisant le modèle de l'alitement prolongé tête déclive à -6° (ou décubitus) utilisé par les Agences Spatiales pour simuler les effets de la microgravité sur l'organisme.

Dans l'**introduction**, le **Chapitre 1** présente l'énergétique de l'obésité à travers les concepts de balance énergétique et de balance oxydative des substrats puis s'attache à mettre en avant les lipides alimentaires et l'inactivité physique en tant que facteurs environnementaux majeurs dans les troubles métaboliques associés à l'obésité. Le **Chapitre 2** présente l'énergétique de l'inactivité physique en résumant les conséquences de l'alitement prolongé sur le métabolisme énergétique et oxydatif. À la suite de cette revue bibliographique, le **Chapitre 3** expose les objectifs et les hypothèses de ce travail de thèse.

La **partie expérimentale** est composée de deux grandes parties :

- **Une partie fondamentale :** Dans le **Chapitre 4**, nous nous sommes tout d'abord intéressés aux effets directs de l'inactivité physique sur la balance énergétique, c'est-à-dire sur les entrées énergétiques, y compris le comportement alimentaire, et sur les sorties en déterminant la dépense énergétique totale et ses composantes, avec un effort pour déterminer les mécanismes hormonaux sous-jacents. Nous nous sommes ensuite attachés à déterminer les effets directs de l'inactivité physique sur le métabolisme post-prandial des lipides alimentaires en fonction de leur nature. Plus spécifiquement, nous présentons l'impact de l'inactivité physique sur l'oxydation des acides gras exogènes saturés et monoinsaturés chez l'homme (**Chapitre 5**) et la femme (**Chapitre 6**). Nous avons aussi cherché à caractériser l'influence de l'inactivité physique sur le devenir

métabolique de ces acides gras saturés et monoinsaturés alimentaires et sur les mécanismes impliqués dans le métabolisme des lipides.

- **Une partie méthodologique :** Les protocoles classiquement utilisés pour l'étude du métabolisme lipidique exogène sont essentiellement basés sur l'utilisation des isotopes stables et sont donc lourds, invasifs et très coûteux. Dans cette partie méthodologique, nous avons testé de nouvelles méthodes en vue de nous affranchir de ces contraintes. Ainsi, le **Chapitre 7** valide par comparaison avec la méthode de référence de la dilution isotopique, l'utilisation de la méthode des plis cutanés pour déterminer de manière non invasive et peu coûteuse les changements de la balance énergétique à travers les changements de masse grasse. En combinant des résultats obtenus dans les Chapitres 2 et 3 de la partie fondamentale avec ceux d'études précédentes, le **Chapitre 8** propose une méthode qui permet d'alléger considérablement les protocoles employés pour mesurer l'oxydation lipidique exogène. Le **Chapitre 9** est une étude préliminaire au cours de laquelle une nouvelle méthode non invasive a été testée en vue d'étudier le devenir des lipides alimentaires dans l'organisme à l'aide de la tomographie par émission de positrons.

Enfin nous présenterons les principales conclusions de ce travail de thèse dans une discussion générale afin d'envisager les perspectives.

# INTRODUCTION

*Bacchus de Rubens (1638)*

# CHAPITRE 1

# Énergétique de l'obésité humaine

Adapté de 'Energétique de l'obésité'

Audrey Bergouignan & Stéphane Blanc

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# 1. L'OBESITE HUMAINE

Au cours des 30 dernières années, la prévalence de la surcharge pondérale et de l'obésité n'a cessé de croître pour atteindre des proportions alarmantes. Au début du 21ème siècle, le nombre de personnes en surcharge pondérale a même dépassé celui des personnes sous-nutries. On pensait ce problème limité aux pays industrialisés, mais des rapports récents de l'Organisation Mondiale de la Santé montrent que les pays en développement ne sont pas épargnés (OMS, 2003). Aux Etats-Unis, l'étude NHANES III a révélé que 54% des Américains présentent une surcharge pondérale (indice de masse corporelle (IMC) > 25 $kg.m^{-2}$) et 22% sont cliniquement obèses (IMC > 30 $kg.m^{-2}$) avec une proportion plus importante chez les femmes (25%) que chez les hommes (20%) (Flegal *et al.*, 1998). Les données provenant d'études nationales laissent à penser que la prévalence de l'obésité dans les pays européens se situe actuellement entre 10 et 20% chez l'homme et entre 10 et 25% chez la femme (OMS, 2003). En France, les enquêtes les plus récentes indiquent que 29.2 % des adultes présentent un excès de poids et que 12.4 % sont obèses (ObEpi, 2003), avec une fréquence plus élevée de l'obésité dans le Nord (18.1%) et l'Est (14.1%) par rapport à d'autres régions (Simon *et al.*, 1997; ObEpi, 2006). Ces mêmes études montrent que l'obésité a augmenté de 17 % entre 1997 et 2001, contre 5,9% aux États-Unis lors de la dernière décade. Si la tendance observée en France n'est pas inversée, nous aurons rejoint le niveau actuel des Etats-Unis en 2020. Encore plus alarmant est le taux croissant de surcharge pondérale chez les enfants. Une étude menée en 2001 chez 4326 élèves de 6ème du Bas-Rhin indique que 22,7% d'entre eux présentent un excès de poids (Klein-Platat *et al.*, 2003). Il a été estimé que 70% des adolescents touchés deviendront obèse à l'âge adulte (Rossner, 1999).

Si l'obésité doit être considérée comme une maladie à part entière, elle est également un des principaux facteurs de risque d'autres maladies chroniques telles que la cardiopathie coronarienne, l'hypertension, l'accident vasculaire cérébrale, la résistance à l'insuline, le diabète de type 2 ou encore certains cancers. Par exemple, en 2000, 175 millions de personnes dans le monde étaient atteintes de diabète de type 2 dont 80% étaient en surpoids (IMC > 25 $kg.m^{-2}$). Si les prédictions se révèlent justes, 300 millions de personnes seront touchées par cette pathologie en 2025 (King *et al.*, 1998).

Une relation positive entre l'IMC et le risque de mortalité a été établie (**Figure 1**). Une faible augmentation de l'IMC, par exemple de 28 à 29 $kg.m^{-2}$, correspond à une élévation du risque de mortalité de 10% (Manson *et al.*, 1995). Les données épidémiologiques montrent, en effet, que l'obésité cause directement ou indirectement la mort prématurée de 300 000 personnes chaque année rien qu'aux États-Unis. Les conséquences économiques de l'obésité sont tout aussi considérables avec un coût estimé entre 2 et 7% du budget de la santé dans de nombreux pays développés (voir exemples dans le **Tableau 1**).

Au-delà d'un défi scientifique, l'obésité représente donc un véritable défi de santé publique et la mise en place de stratégies efficaces de prévention et de traitement de cette pathologie se révèle d'une extrême urgence. Dans ce contexte, la compréhension approfondie des causes susceptibles d'intervenir dans le développement de l'obésité est un indispensable pré-requis. L'hypothèse la plus consensuelle suggère que l'obésité résulte d'une prédisposition génétique à être obèse, s'exprimant dans des conditions environnementales particulières telles qu'une alimentation riche en lipides et un mode de vie sédentaire. Alors qu'il est largement accepté que ces facteurs ne peuvent influencer la prise de poids qu'en favorisant une balance énergétique positive, la contribution respective de la

génétique, de l'alimentation et de l'activité physique reste un sujet de controverse dans la littérature.

En se basant sur les données de la littérature, l'objectif de ce chapitre est de démontrer le rôle central des facteurs environnementaux, et en particulier du niveau de l'activité physique, dans les processus biologiques de régulation/dérégulation du poids. Au préalable, les concepts de balances énergétique et oxydative des substrats, concepts fondamentaux de ces processus de régulation, sont discutés.

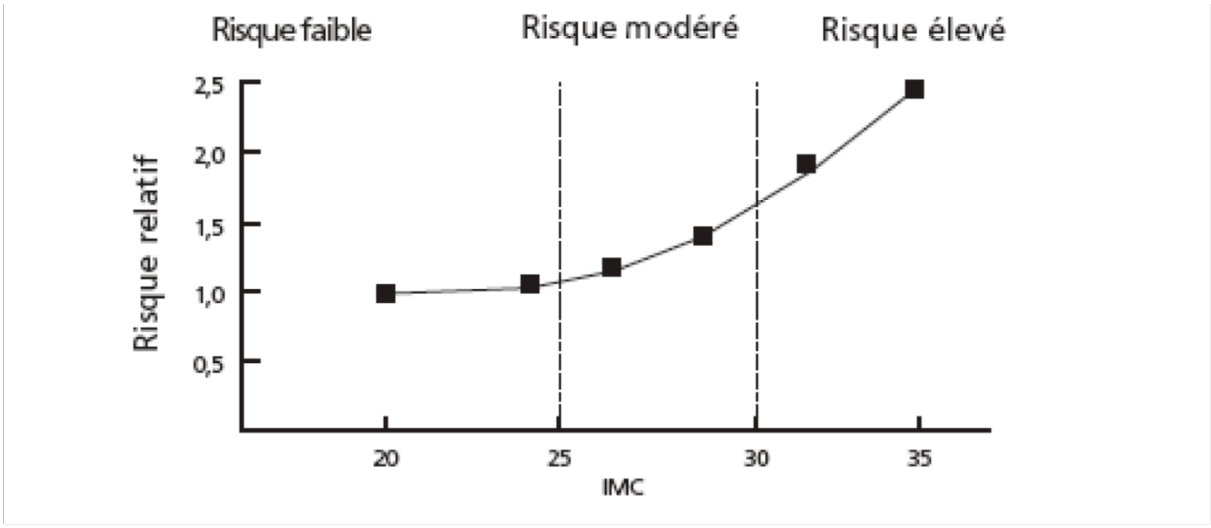


**Figure 1 :** Relation entre l'indice de masse corporelle (IMC en $kg/m^{-2}$) et le risque relatif de mortalité (d'après (Manson *et al.*, 1995).

**Tableau 1: Coûts économiques de l'obésité**

| Pays | Année | Étude | IMC | Coûts directs estimés | Coûts de santé nationaux |
|---|---|---|---|---|---|
| Australie | 1989-1990 | NHMRC (1999) | >30 | A $464 millions | >2% |
| Etats-Unis d'Amérique | 1994 | Wolf & Colditz (1994) | >29 | US $45,8 milliards | 6,80% |
| France | 1992 | Lévy et coll. (1995) | >27 | FF 12 miliards | 2% |
| Pays-Bas | 1981-1989 | Seidell & Deerenberg (1994) | >25 | NLG 1 milliard | 4% |

# 2. ASPECTS ENERGETIQUES DE LA REGULATION DU POIDS CORPOREL

## 2.1. Le concept de balance énergétique

On trouvera à la **Figure 2** les principales influences qui s'exercent sur l'équilibre énergétique de la prise de poids.

Tout être vivant étant soumis aux lois de la thermodynamique, il répond au principe de conservation de l'énergie. L'organisme peut être considéré comme une boîte noire et toute variation de masse ne s'explique que par la différence entre les sorties (dépense énergétique totale) et les entrées (énergie ingérée). Il s'agit du concept de balance énergétique selon lequel tout gain de masse ne peut résulter que d'une augmentation des apports caloriques et/ou d'une réduction de la dépense énergétique totale. Les conséquences pondérales de tels déséquilibres énergétiques sont communément acceptés.

### 2.1.1. Apport énergétique

L'apport énergétique total représente l'ensemble de l'énergie ingérée consommée sous forme d'aliments et de boissons pouvant être métabolisé par l'organisme. Les macronutriments ont des teneurs énergétiques différentes. Tandis que 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).

### 2.1.2. Dépense énergétique totale

Le second volet de l'équation qui permet le calcul de la balance énergétique, à savoir la dépense énergétique totale, est classiquement décomposée en trois principaux composants : le métabolisme de repos, la thermogenèse post-prandiale et le coût de l'activité physique.

**Le métabolisme de repos**

Le métabolisme de repos correspond à l'énergie dépensée en post-absorptif i.e. après une nuit à jeun (minimum 12 à 14 heures), au repos sans mouvement, allongé, éveillé, et à thermoneutralité. Le métabolisme de repos correspond à une situation où exercice physique et alimentation exercent une influence minimale sur le métabolisme. En d'autres termes, cette dépense représente l'énergie requise pour maintenir *a minima* l'activité métabolique des tissus, l'énergie nécessaire pour assurer la circulation du sang, la respiration et les fonctions gastro-intestinales et rénales. D'un point de vue plus holistique, le métabolisme de repos comprend l'énergie dépensée lors du sommeil et le coût des processus cognitifs. La masse maigre, qui comprend les tissus métaboliquement actifs de l'organisme, est le principal déterminant du métabolisme basal comme de repos. 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). Ajustée pour les différences de masse maigre, la variabilité interindividuelle du métabolisme de repos varie entre 3 et 8 %.

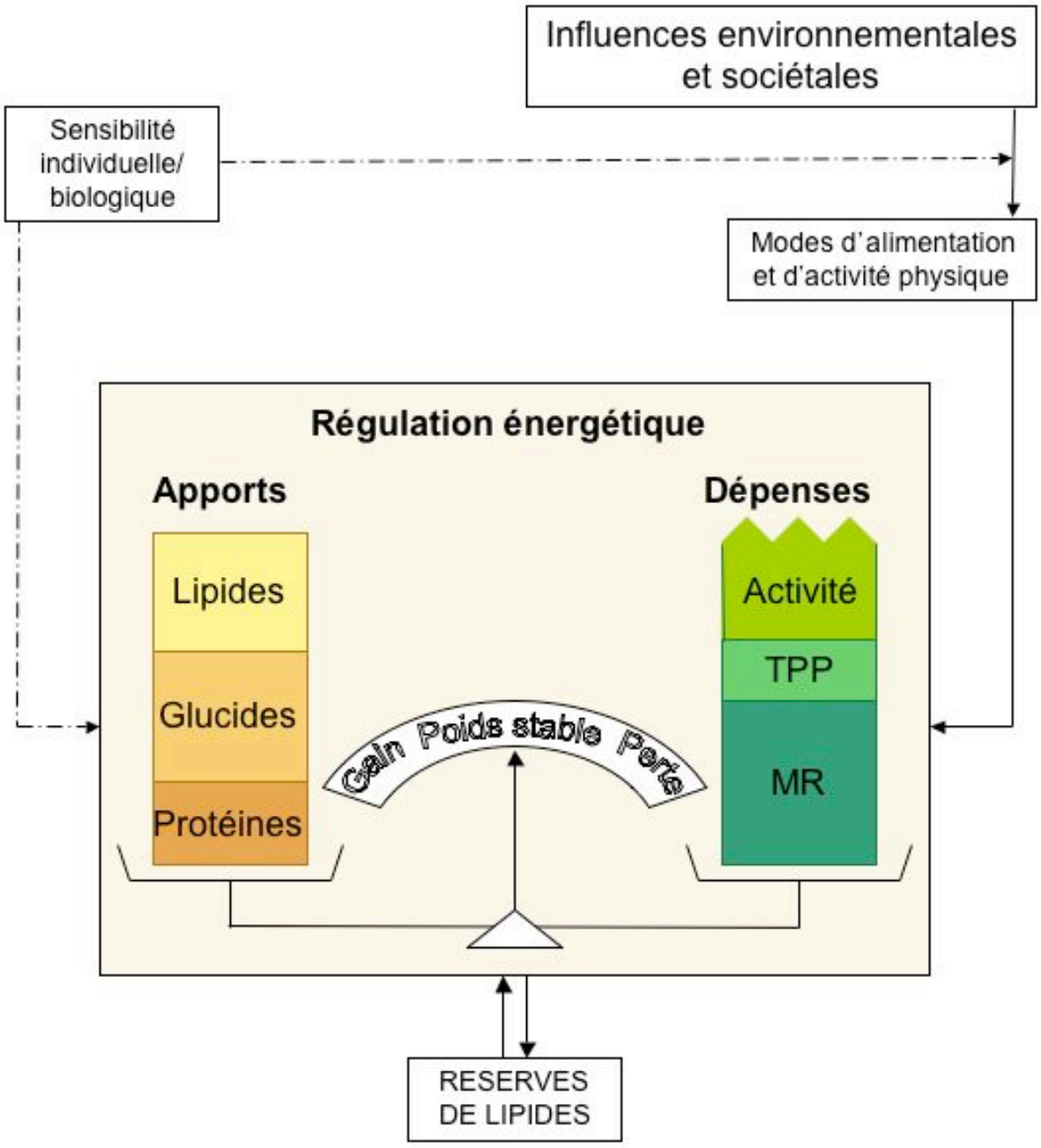


**Figure 2 :** Influences s'exerçant sur la balance énergétique et la prise de poids

**La thermogenèse postprandiale**

Toute consommation de nourriture entraîne une augmentation de la dépense énergétique de repos dont l'intensité et la durée sont fonction de la ration alimentaire et de la composition en macronutriment. Cette dépense représente le coût de la digestion, du transport et du stockage des nutriments et à un plus faible niveau, l'activation du système nerveux sympathique lors de l'ingestion de glucides. Lors d'un repas mixte standard, la valeur de la thermogenèse post-prandiale est stable aux alentours de 10% de l'énergie ingérée et n'est observée que quelques heures suivant un repas.

**La dépense énergétique liée à l'activité physique**

La dépense énergétique liée à l'activité physique est l'élément le plus variable de la dépense de 24 h. 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. 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. Bien que ce rapport soit extrêmement pragmatique, il n'est pas tout à fait satisfaisant puisque la majorité des dépenses liées à l'activité sont fonction de la masse alors que le métabolisme de repos est proportionnel à la masse$^{0,75}$. Ce PAL a permis de classifier les individus. Ainsi, 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).

## 2.2. Régulation physiologique du poids corporel

Des facteurs sociétaux tels que les traditions ou le cycle saisonnier du travail mais aussi la sensibilité individuelle à l'obésité pour des raisons génétiques et physiologiques peuvent jouer un rôle dans le contrôle du poids. Toutefois, les processus physiologiques sont les principaux responsables de la régulation du poids de l'organisme. On pense que l'organisme se défend mieux contre la sous-nutrition et la perte de poids qu'il ne le fait contre la surconsommation et la prise de poids (Blundell & King, 1996). Les mécanismes physiologiques responsables de la régulation du poids ne sont pas complètement élucidés. Cependant, des signaux au niveau de l'intestin, du tissu adipeux et du cerveau et peut-être dans d'autres tissus, qui détectent l'arrivée d'éléments nutritifs, leur distribution et/ou leur stockage (Valassi *et al.*, 2008) ont été mis en évidence. Ces modifications sont coordonnées dans le cerveau (Neary *et al.*, 2004; Valassi *et al.*, 2008) et entraînent des modifications au niveau de l'alimentation, de l'activité physique et du métabolisme de façon à réguler les réserves énergétiques de l'organisme (Valassi *et al.*, 2008) ; (Novak & Levine, 2007). Parmi les principales hormones intervenant dans cette régulation de la balance énergétique, on peut mentionner la leptine sécrétée par les adipocytes en fonction de leurs réserves en triglycérides et qui se fixe à des récepteurs situés au niveau de l'hypothalamus et les hormones intestinales satiétogènes comme le petide pancréatique (PP), l'orexyne, la cholecystokinine, le peptide YY, le neurpeptide Y, le GLP1 (glucagon-like peptide 1), etc.. ; ou encore la ghréline seule hormone stimulant la prise alimentaire mise en évidence à ce jour.

## 2.3. Dynamique de la prise de poids

Malgré la régulation physiologique importante qui s'exerce sur le poids pour faire face aux variations de la balance énergétique, une balance énergétique positive maintenue sur le long terme peut mener à une prise de poids. Une balance énergétique positive chronique débute par un apport énergétique supérieur aux besoins lié à une augmentation de l'apport énergétique total, d'une diminution de la dépense énergétique totale ou d'une combinaison des deux. Schutz (Schutz, 1995) a modélisé l'effet d'un apport énergétique supérieur aux besoins sur la balance énergétique et le poids corporel (**Figure 3**). Il a ainsi distingué trois phases dans le processus de prise de poids. La première phase est la 'phase pré-obèse statique' au cours de laquelle un individu est en balance énergétique positive mais présente un poids constant. Ensuite arrive la 'phase dynamique' au cours de laquelle le sujet prend du poids suite à une balance énergétique positive sur une période prolongée. Cette phase peut durer plusieurs années. L'écart entre apport énergétique et dépense énergétique s'amoindrie au cours du temps à cause d'une augmentation du métabolisme de repos liée à une masse maigre plus importante et à une dépense énergétique liée à l'activité physique supplémentaire due au coût plus élevé de déplacer un poids plus important pour l'organisme. Lorsque la balance énergétique est rétablie mais avec un poids désormais plus élevé, l'individu est en 'phase obèse statique'.

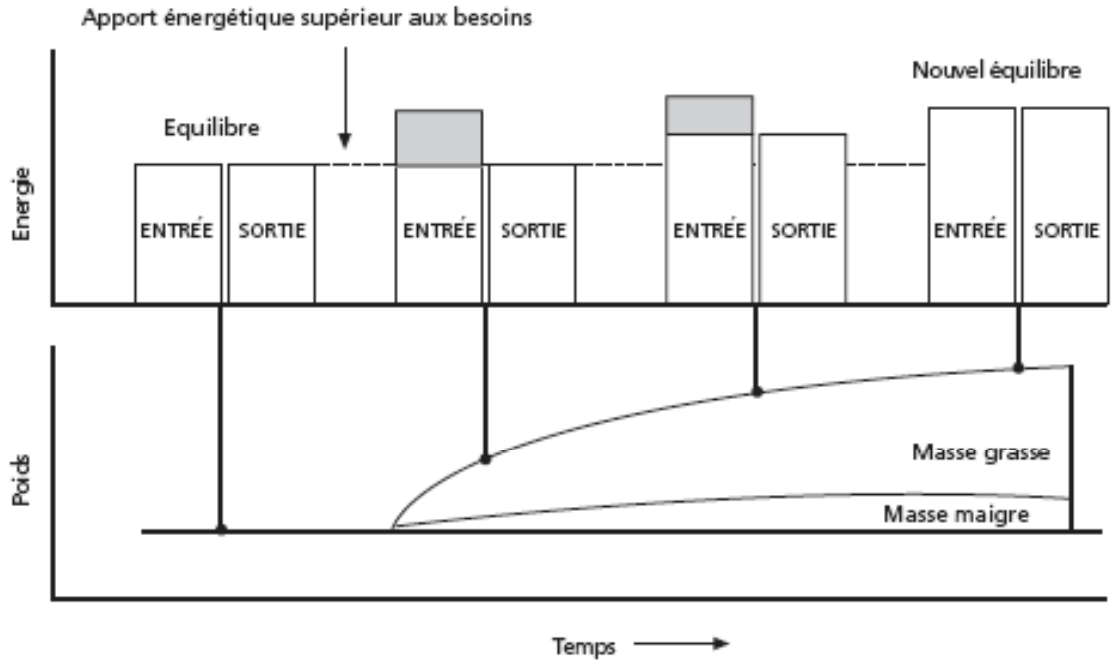


**Figure 3 :** Effet d'un apport énergétique supérieur aux besoins sur la dépense énergétique, la balance énergétique et le poids corporel (d'après (Schutz, 1995), avec l'aimable autorisation de l'auteur)

## 2.4. Les causes d'une balance énergétique positive

L'obésité est communément considérée comme une maladie multifactorielle complexe ; c'est une affection qui résulte d'un mode de vie favorisant une balance énergétique positive, mais c'est également une maladie qui se manifeste plus facilement chez les personnes ayant une prédisposition génétique à la balance énergétique positive. Il a été estimé que 40 à 70% de la variabilité interindividuelle dans la prévalence de l'obésité est d'ordre génétique (Bouchard & Perusse, 1993). Toutefois, les facteurs génétiques ne peuvent à eux seuls expliquer la croissance galopante de l'obésité. En effet, le génome de l'homme moderne est resté relativement stable au cours des 10 000 dernières années (Macaulay *et al.*, 1999) tandis que l'obésité ne s'est manifestée de manière exponentielle qu'au cours des dernières décades. On estime plutôt actuellement 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 (**Figure 4**). Ainsi selon Roland Wiensier : « *Our genes permit us to become obese, the environment determines if we become obese* » (Weinsier *et al.*, 1998).

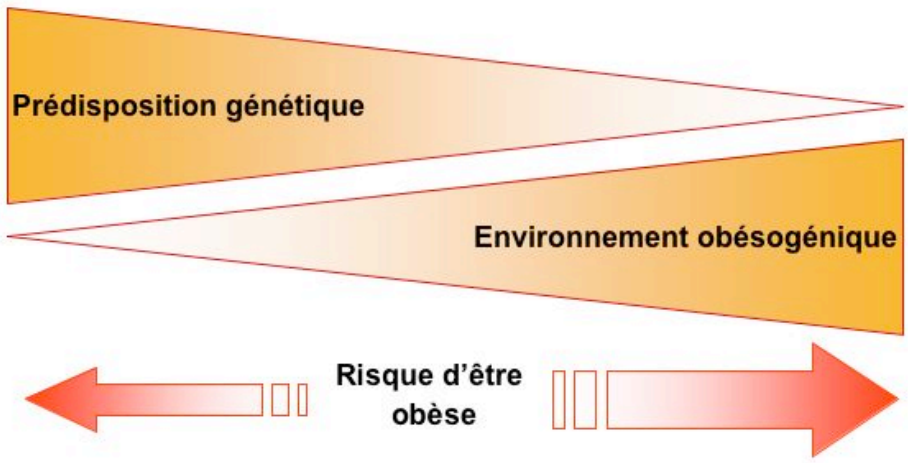


**Figure 4 :** Interaction gènes-environnement dans le risque à être obèse

# 3. LE CONCEPT DE BALANCE OXYDATIVE

Il existe une seconde condition pour assurer la stabilité du poids. Cette condition, plus subtile, mais tout aussi importante, à trait à la notion de balance oxydative des substrats (Flatt, 1988). En d'autres termes la proportion de substrats oxydés au niveau cellulaire e.g. protides, lipides et glucides, doit représenter la composition en macronutriments de la prise alimentaire.

## 3.1. Apport en macronutriments

Comme nous l'avons précisé précédemment, les aliments sont constitués de trois principaux macronutriments : les glucides, protides et les lipides, ces derniers présentant la densité énergétique la plus importante. Elle est en grande partie responsable de l'effet d'hyperphagie, ou surconsommation passive, que montrent de nombreux sujets exposés à des aliments riches en graisse (Blundell & King, 1996). L'apport énergétique total est aussi influencé par la sensation agréable que procurent les aliments au niveau buccal, aussi appelée palatabilité. Le plaisir que procure les aliments joue ainsi un rôle important sur le comportement alimentaire (Blundell & 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. Il a été montré que la consommation de lipides à travers le plaisir gustatif qu'ils procurent et leur faible effet sur la satiation tend à faire pencher la balance en faveur d'un bilan énergétique positif.

Bien que les glucides semblent avoir un impact plus marqué sur le système de satiété de l'organisme (Blundell & King, 1996), le sucré est l'un des goût les plus puissants et qui procurent le plus de plaisir. L'industrie agro-alimentaire a largement tiré parti de ces observations et a élaboré en proportions croissantes des aliments sucrés riches en graisses de manière à accroître leur sapidité et leur consommation.

## 3.2. Capacité d'oxydation et de stockage de l'organisme

La composition du régime alimentaire en macronutriments agit également sur la quantité d'énergie en excès qui va être stocké dans l'organisme. En effet, 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, 1995). Ceux ayant une faible capacité de stockage sont préférentiellement oxydés lorsque les apports dépassent les besoins. La capacité de stockage des protéines, bien qu'importante, est très coûteuse puisque la mobilisation des protéines ne peut se faire qu'à travers une perte de masse maigre et celle des glucides est restreinte et limitée au niveau des stocks de glycogène. En revanche, la capacité de stockage des lipides est considérée comme quasi illimitée. Ainsi, 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. Par conséquent, 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. Cela est vrai tant qu'un nouvel équilibre entre lipides ingérés et oxydés n'est pas établi. En réalité, cet équilibre sera atteint lorsque la masse grasse de l'individu aura augmenté entraînant par effet de masse une augmentation de la masse maigre métaboliquement active ainsi qu'une oxydation lipidique correspondant à la proportion contenue dans l'alimentation (**Figure 2**). L'individu aura alors atteint la phase 'obèse statique' précédemment expliquée. Par conséquent, il existe une relation positive entre la balance lipidique et la balance énergétique (Schrauwen *et al.*, 1998) (**Figure 5**).

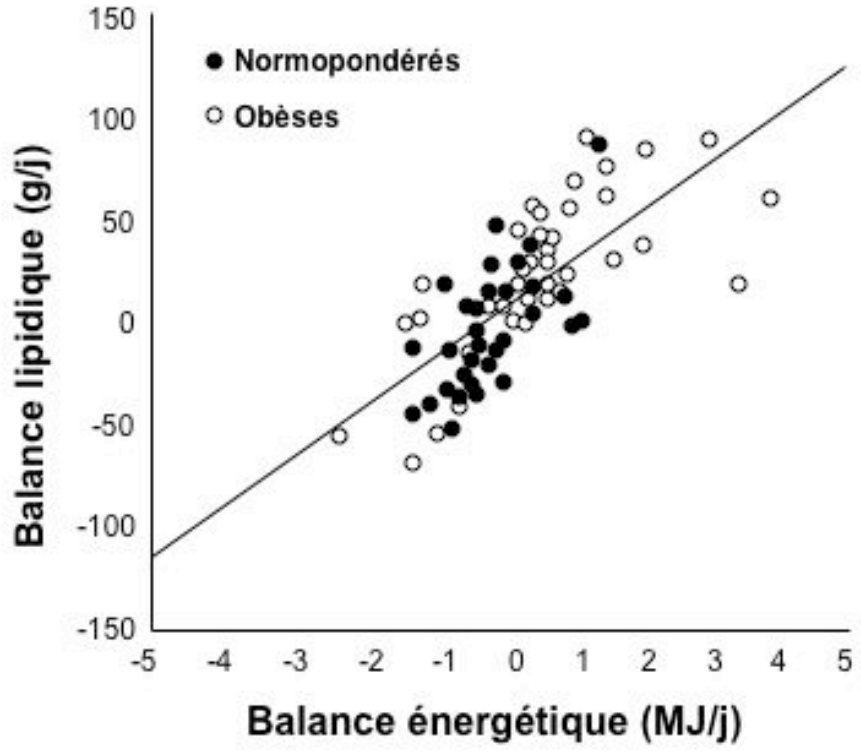


**Figure 5 :** Relation entre la balance énergétique de 24h et la balance lipidique de 24h chez des sujets obèses et des sujets minces (Schrauwen *et al.*, 1998)

## 3.3. Le modèle à double compartiment de Flatt

Le maintien d'un poids stable dépend donc essentiellement de la balance lipidique qui elle-même est influencée par la balance glucidique et sujette aux variations des apports énergétiques et de la dépense énergétique. Ceci est parfaitement représenté par le modèle à double compartiment de Jean-Pierre Flatt (Flatt, 1995) (**Figure 6**).

Les réservoirs de petite et de grande taille représentent les capacités de stockage respectives de l'organisme pour le glycogène et les lipides (exprimées en termes de contenu énergétique). La petite turbine correspond à l'utilisation exclusive du glucose par le cerveau. Les proportions relatives de glucose et d'acides gras utilisées par le reste du corps, correspondant à la grande turbine, sont supposées être influencées par la disponibilité en glucose et acides gras, qui est considérée comme proportionnelle à la taille des deux réservoirs. Le remplissage des stocks de glycogène et de lipides se fait de manière quasi instantanée en fonction de l'apport énergétique, c'est-à-dire de la quantité et de la qualité des repas. Les stocks peuvent aussi être ajustés par des mécanismes de régulation physiologique tels que la glyconéogenèse en cas d'hypoglycémies (flux du grand au petit réservoir), et de la *de novo* lipogenèse lors d'hyperglycémies (flux du petit au grand réservoir). 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 sur le niveau du gros réservoir et ne sont donc pas suivis par des modifications équivalentes de l'oxydation lipidique.

Différents paramètres règlent les flux entrants et sortants des réservoirs de glycogène et de lipides. Alors que les flux entrants dépendent de la quantité et de la qualité des repas, à leur tour influencées par la disponibilité, la diversité et la palatabilité de l'alimentation, les flux sortants ne peuvent être modulés que par la pratique d'un exercice physique. En effet, l'exercice physique intense qui utilise prioritairement les réserves glycogéniques augmente l'oxydation lipidique (Schrauwen *et al.*, 1997). Le rôle de l'exercice physique dans la régulation de l'ajustement de la balance lipidique est un aspect central de la régulation de la masse corporelle.

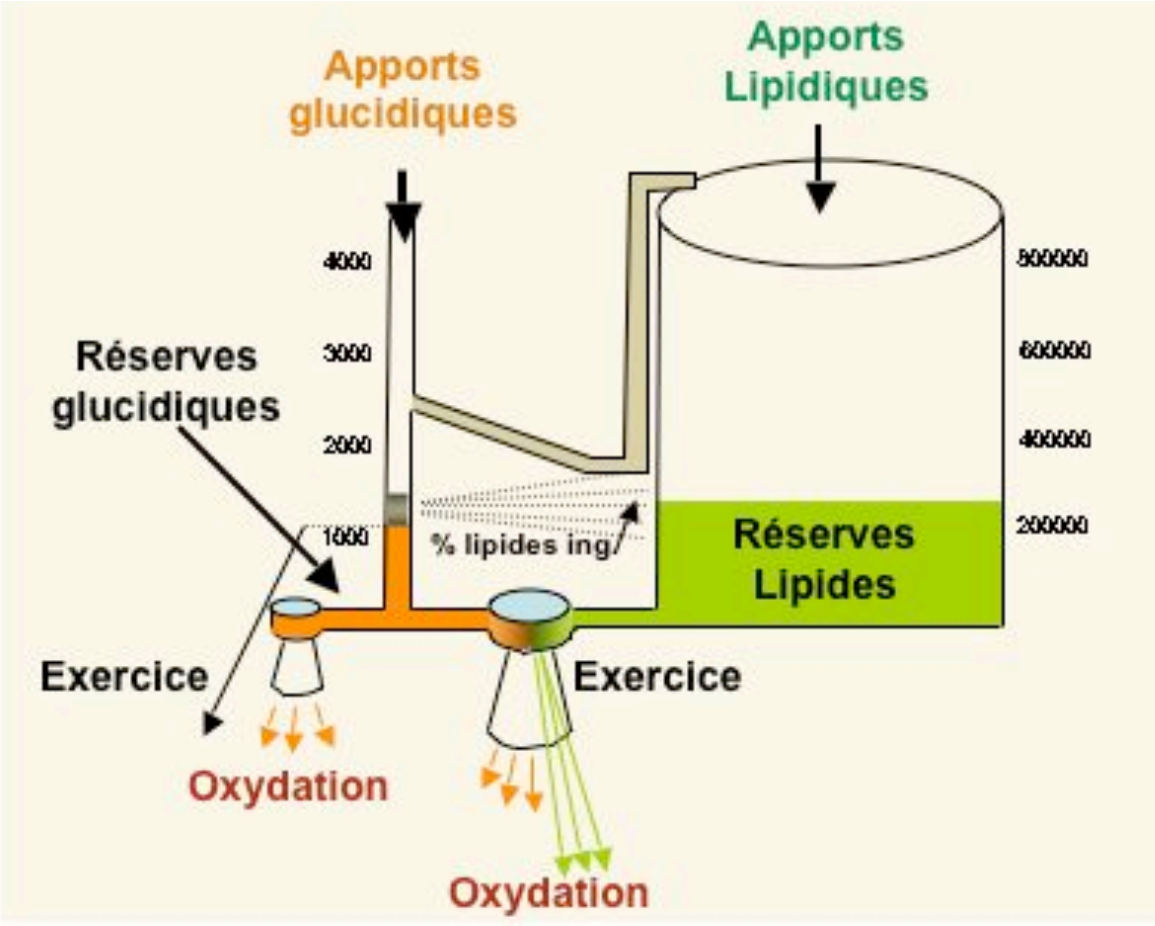


**Figure 6** : Le modèle à deux compartiments (Flatt, 1995), avec l'aimable autorisation de l'auteur)

## 3.4. L'obésité : un problème de balance lipidique positive

Du point de vue énergétique, l'obésité est définie comme le résultat d'une balance énergétique positive chronique. Basée sur les différences d'ajustement des balances oxydatives, l'obésité peut aussi être définie comme un échec dans la régulation de la balance lipidique. La lipogenèse étant négligeable chez l'homme, cet 'échec' pourrait représenter plus spécifiquement une perturbation de la répartition des lipides alimentaires entre oxydation et stockage.

### 3.4.1. Une capacité réduite à oxyder les lipides

De nombreuses données montrent que l'obésité s'accompagne d'une incapacité à utiliser les lipides comme substrat énergétique (Schutz *et al.*, 1989). Une altération de la capacité du muscle à oxyder les acides gras libres (AGL) a été rapportée en situation post-absorptive (Colberg, 1995) et post-prandiale (Binnert, 1998; Giacco *et al.*, 2004) et au cours d'un exercice (Blaak, 2004). 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 AGL, 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, 1998). De même, chez les rats non-obèses, les lipides exogènes sont orientées 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).

### 3.4.2. Quels sont les mécanismes potentiels ?

Les mécanismes biologiques responsables de cette capacité réduite à utiliser les lipides caractéristiques des individus obèses mais aussi observés chez des sujets diabétiques (Blaak *et al.*, 2000) sont encore mal connus, mais restent bien mieux caractérisés que leurs causes. Quelques pistes peuvent être évoquées. L'oxydation lipidique réduite observée chez les obèses suggère que cette incapacité à utiliser les lipides en tant que substrats serait dû à une dérégulation du métabolisme lipidique caractérisée 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. Toutefois, à ce jour aucune étude n'a, à notre connaissance, comparé le profil de la LPL entre individus minces et obèses. Néanmoins, 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. Par ailleurs, 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 (Oberbach *et al.*, 2006). Puisque la capacité oxydative musculaire dépend en grande partie du type de fibre musculaire, un tel profil pourrait expliquer en partie cette capacité réduite à oxyder les acides gras. Il est intéressant de noter que certains auteurs ont constaté une relation inverse entre le pourcentage de masse grasse et le pourcentage de fibres lentes (Wade *et al.*, 1990). Enfin, une autre étape qui pourrait être limitante dans l'oxydation des acides gras à chaîne longue est l'entrée dans la mitochondrie, organite dans lequel a lieu la bêta-oxydation et le cycle tricarboxylique. Ce

transport s'effectue grâce à la carnitine palmitoyl-transférase 1 (CPT1). Certaines études ont montré une activité réduite de la CPT1 au niveau des muscles de sujets obèses et insulino-résistants (Kelley *et al.*, 1999). Par ailleurs, le déséquilibre entre l'entrée et l'oxydation des acides gras dans le myocyte semble résulter en une accumulation de triglycérides intra-musculaires, lesquels ont été fortement associés à l'insulino-résistance au niveau du muscle (Kelley & Goodpaster, 2001; Kelley *et al.*, 2002). Ainsi, la capacité réduite à oxyder les lipides serait une des causes du développement de l'insulino-résistance et expliquerait en partie pourquoi l'obésité est un facteur de risque majeur de l'insulino-résistance et du diabète de type 2.

#### 3.4.3. L'incapacité à oxyder les lipides causes ou conséquence de l'obésité ?

Il est intéressant de noter qu'aucune amélioration dans l'oxydation lipidique n'est observée après retour à un poids normal (Blaak *et al.*, 1994; Kelley *et al.*, 1999). Certains auteurs ont alors suggéré que cette incapacité à oxyder les lipides serait causale dans l'étiologie de l'obésité, plutôt qu'adaptative. Par conséquent, la détermination des causes de cette altération métabolique représente un pré requis indispensable à la mise en place de stratégies préventives et thérapeutiques.

Il est communément accepté que la génétique joue un rôle dans la capacité à oxyder les lipides de chaque individu. En effet, l'oxydation lipidique a été définie comme un trait familial avec une héritabilité de 30% (Bouchard & Perusse, 1993). Néanmoins, les importantes altérations environnementales qu'ont subies nos sociétés modernes au cours du siècle dernier peuvent être vues comme l'élément déclencheur de la croissance galopante de l'obésité observée sur la même échelle de temps. Nous avons vu précédemment à l'aide du modèle à double compartiment de Flatt, que les facteurs diététiques, en particuliers les lipides, et l'activité physique ont une forte répercussion sur la régulation de la balance lipidique. Par ailleurs, ils semblent être les facteurs modifiables impliqués dans les balances énergétique et oxydative les plus influencés par les facteurs externes, tels que l'environnement.

# 4. LES LIPIDES ET L'ACTIVITE PHYSIQUE : DEUX REGULATEURS MAJEURS DU POIDS

## 4.1. L'apport lipidique

### 4.1.1. Les lipides alimentaires

La quantité de lipides du régime alimentaire a été associée à la prévalence de l'obésité dans une étude cross-sectionnelle de Bray et collègues (Bray & Popkin, 1998) basées sur des enquêtes alimentaires nationales. Celle-ci met en évidence une corrélation positive entre le pourcentage de lipides dans l'alimentation et le pourcentage d'individus en surpoids dans les populations de 20 pays différents (**Figure 7**). Les observations faites à partir de l'impact des migrations de certaines populations d'un environnement à un autre amènent des évidences supplémentaires. Par exemple, dans l'étude de la migration du Ni-Hon-San (Curb & Marcus, 1991), 8006 hommes japonais vivant à Honolulu ont été comparés à

2183 hommes vivant à Hiroshima et Nagasaki. Tandis que l'énergie ingérée était légèrement supérieure à Honolulu qu'à Hiroshima et Nagasaki, le pourcentage provenant des lipides était deux fois plus élevé à Honolulu. L'IMC moyen était aussi plus élevé à Honolulu où deux fois plus d'hommes étaient obèses. Une étude longitudinale en Chine suggère aussi qu'une augmentation dans la quantité lipidique consommée mène à une élévation du poids (Popkin *et al.*, 1995; Paeratakul *et al.*, 1998). Dans ces études, les paramètres confondants potentiels de la relation entre la quantité de lipides dans le régime alimentaire et le poids, tels que l'âge, le sexe, l'activité physique et la consommation de tabac mais aussi la proportion de macronutriments non lipidiques (protides et glucides) et l'énergie ingérée totale, ont été pris en compte. Un effet significatif de la quantité de lipides dans l'alimentation sur l'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 de 100kcal de glucides et de protides combinés 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.

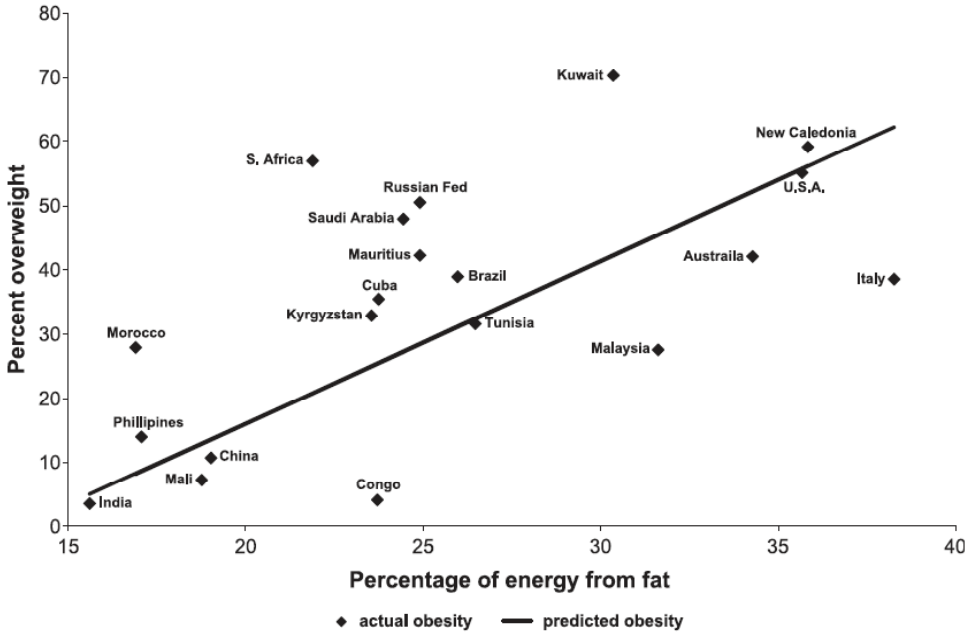


**Figure 7 :** Relation entre le pourcentage de lipides alimentaires et de personnes en surpoids dans les populations (Bray & Popkin, 1998)

### 4.1.2. L'influence de la nature des acides gras

Une seconde dimension est à considérer quant aux apports lipidiques. Les acides gras varient en fonction de deux paramètres : la longueur de la chaîne carbonée et le degré d'insaturation. Suivant leur nombre de double liaison, les acides gras sont classés en trois grandes catégories : les acides gras saturés (sans double liaison), les monoinsaturés (une seule double liaison) et les polyinsaturés (plusieurs doubles liaisons). Il a été montré que le pourcentage de lipides saturés, monoinsaturés ou polyinsaturés dans le régime alimentaire peut influencer la prise de poids. Par exemple, un lien entre l'apport alimentaire en acides gras saturés et le statut pondéral a été observé dans plusieurs études épidémiologiques (Storlien *et al.*, 1998; Storlien *et al.*, 2000). Dans un groupe de 128 hommes de l'étude Québec Family, un pourcentage élevé en graisses saturées dans l'alimentation est associé à un tour de taille plus élevé (Doucet *et al.*, 1998). Quelques études d'intervention menées sur de petits échantillons indiquent 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).

Ces résultats peuvent s'expliquer par l'influence de la nature des acides gras sur les balances énergétique et lipidique et de manière ultime, sur la régulation de la masse et de la composition corporelle. À l'aide d'un marquage isotopique, DeLany et collaborateurs (DeLany, 2000) ont montré chez 6 hommes sains qu'à la suite d'un repas standardisé, les acides gras poly- et mono-insaturés sont plus oxydés que les acides gras saturés et que plus la chaîne carbonée est longue et moins les acides gras sont oxydés. Basée sur ces résultats, une étude d'intervention (Kien, 2005) a proposé à des sujets sains de suivre pendant 28 jours un régime alimentaire riche en acide palmitique ou en acide oléique et a montré une réduction de l'oxydation lipidique post-prandiale et de la dépense énergétique totale à la suite du régime riche en acide palmitique et aucun changement après le régime riche en acide oléique. En substituant les acides gras saturés (crème) par des acides gras mono-insaturés (huile d'olive) dans le régime alimentaire pendant 4 semaines de sujets en surpoids, Piers et collègues (Piers, 2003)ont mis en évidence une perte significative de masse corporelle et de masse grasse (-1,6 ± 1,1 kg et -1,1 ± 0,7 kg, respectivement) sans aucun changement de l'apport énergétique et lipidique. En conséquence, au-delà des apports lipidiques totaux, la nature des acides gras est un élément de l'alimentation important à prendre en compte.

Alors que les lipides apparaissent comme l'élément ayant le plus grand impact sur la régulation du poids du côté des entrées énergétiques, l'activité physique se révèle être l'élément le plus variable des sorties énergétiques (ou dépense énergétique totale).

## 4.2. L'activité physique

Les données transversales révèlent souvent un rapport inverse entre IMC ou l'adiposité et le PAL (Physical Activity Level) et ce, aussi bien chez l'enfant, l'adolescent que l'adulte ; bien que d'autres études n'aient pas mis en évidence de telles relations (Hunter *et al.*, 1996; Hunter *et al.*, 1997; Salbe *et al.*, 1997; Ekelund *et al.*, 2002). Ces données indiquent que les sujets obèses ou présentant un surpoids sont moins actifs que leurs homologues minces. Par ailleurs, ces corrélations ne mettent pas en évidence une relation de cause à effet. Il est donc difficile de savoir avec certitude si les sujets obèses sont moins actifs du fait de leur obésité ou si c'est une faible activité qui est à l'origine de leur obésité. Quoi qu'il en soit, les résultats d'autres types d'études laissent à penser que des degrés d'activité faibles ou en diminution sont les premiers responsables de l'obésité ; par exemple, il n'y a pas d'obésité chez les athlètes de haut niveau, alors que ceux qui abandonnent le

sport enregistrent fréquemment une prise de poids et une augmentation de l'adiposité (Williamson, 1996; Haapanen *et al.*, 1997). De plus, quelques 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.*, 1997; Schmitz *et al.*, 2000). Résumées en termes simples, ces études suggèrent 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 fait, si la relation PAL/adiposité est difficile à mettre en évidence, c'est en partie due à l'hétérogénéité de ce composant de la dépense énergétique totale. La dépense énergétique liée à l'activité physique peut en effet à son tour être décomposée en activité structurée et spontanée. L'activité structurée comprend l'activité volontaire réalisée lors d'exercices physiques tels que le jogging, le tennis, la natation, etc… L'activité spontanée comprend toutes les actions de la vie quotidienne telles que monter les escaliers, se lever de sa chaise, balayer, marcher, etc… Les interrelations existantes entre ces composantes de l'activité physique sont complexes et il a été montré chez la personne âgée que toute intervention visant à améliorer la capacité à l'exercice par participation à un programme d'entraînement se traduit par une réduction parallèle de l'activité spontanée (Morio *et al.*, 1998). *In fine*, l'énergie totale dépensée dans l'activité physique n'est que très peu modifiée par l'intervention.

L'importance de l'activité spontanée dans la régulation pondérale a récemment été mise en évidence grâce au développement d'accéléromètres complexes capables de la quantifier. L'équipe de Lévine à la Clinique Mayo aux USA a récemment démontré une relation très significative entre l'activité physique spontanée et l'adiposité de sujets minces et obèses (Levine & Kotz, 2005). Alors que ces deux groupes de sujets passent le même temps couché, les sujets obèses restent 2,5 fois plus de temps assis que les sujets minces. Ce résultat n'est pas modifié après une perte de masse les ramenant à un IMC normal, ce qui suggère une prédisposition génétique quant à ce type d'activité physique. Les mêmes auteurs 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 2 mois à une surnutrition de 1000 kcal/j sont ceux qui augmentent le plus leur activité spontanée.

Ces données suggèrent que le niveau d'activité physique est l'un des principaux modulateurs de la balance énergétique, et à terme du poids corporel. Il est intéressant de noter néanmoins que les effets de l'activité physique ne semblent pas se limiter pas à la régulation de la balance énergétique. En effet, l'activité physique, et en particuliers l'exercice, se révèle aussi être un modulateur important du second élément régulateur du poids corporel : la balance lipidique.

## 4.3. L'exercice physique comme modulateur du devenir des lipides alimentaires

Des études chez l'homme et l'animal suggèrent en effet que l'exercice physique pourrait favoriser la redistribution des lipides alimentaires au profit des muscles pour y être oxydés (ou pour un stockage transitoire dans les myocytes en vue d'une oxydation ultérieure), par rapport au tissu adipeux (stockage) (Calles-Escandon *et al.*, 1996; Friedlander, 1998).

Comme nous l'avons précédemment décrit, l'obésité est caractérisée par une oxydation lipidique réduite et par conséquent, par une incapacité à s'adapter à des régimes riches en lipides (Astrup *et al.*, 1994). Cela suggère que la surcharge pondérale résulte d'une incapacité à ajuster l'oxydation lipidique assez rapidement en réponse à une surconsommation lipidique. D'après le modèle à double compartiment de Flatt, cette maladaptation serait due à de faibles variations dans les stocks de glycogène. Ainsi, l'exercice pourrait moduler l'oxydation lipidique suite à une diminution de la réserve glycogénique.

Sur la base de ce modèle, Schrauwen et collègues (Schrauwen *et al.*, 1998) ont montré chez 10 sujets obèses homme et femme que le passage d'un régime alimentaire modéré en lipides (30% de l'énergie totale) à un régime hyperlipidique (60% de l'énergie totale) entraîne une balance lipidique positive. Cependant, 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 temps plus rapide leur balance énergétique et lipidique (**Figure 8**).

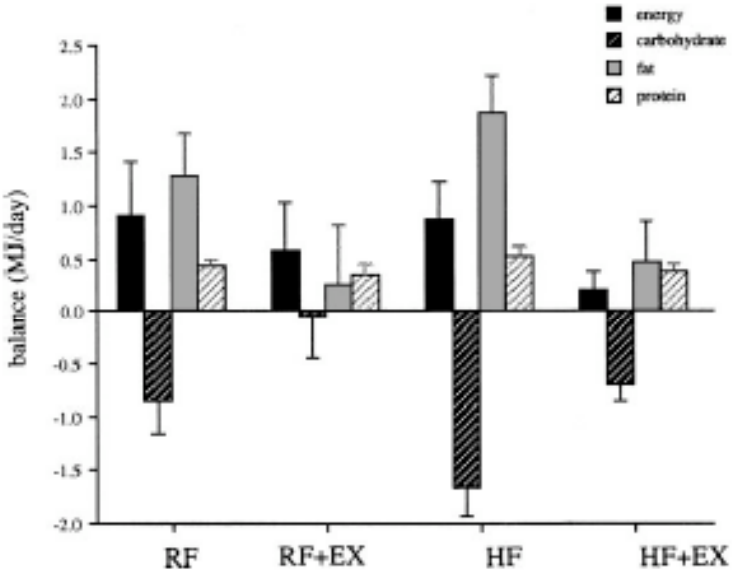


**Figure 8 :** Balances énergétique et oxydative des substrats sur 24h mesurée dans une chambre calorimétrique après 3 jours d'un régime alimentaire réduit en lipides (RF) ou après 3 jours d'un régime hyperlipidique (HF) accompagné ou non d'un exercice physique visant à épuiser les stocks de glycogènes (RF+EX ou HF+EX). L'exercice physique est réalisé à la fin du 3ème jour (Schrauwen *et al.*, 1998).

Dans la continuité, Steven Smith et collaborateurs du Pennington Biomedical Research Center aux USA ont conduit une série d'études visant à déterminer le rôle de l'activité physique dans la vitesse d'ajustement de la balance lipidique. Après 5 jours de régime contrôle (37% de l'énergie totale provenant des lipides), ils ont soumis six individus minces à un régime eucalorique hyperlipidique (50% de l'énergie totale provenant des lipides, pas de modifications de l'apport calorique global) pendant quatre jours confinés, donc inactifs, dans une chambre calorimétrique (Smith, 2000a). Ils ont suivi le temps nécessaire pour que

l'oxydation lipidique des individus reflète la proportion de lipides contenus dans l'alimentation. 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 corrèle négativement avec la balance lipidique et serait ainsi en relation avec la capacité des individus à ajuster leur balance lipidique en réponse à une surcharge en lipides. Ils ont donc répété cette expérience dans une seconde étude (Smith, 2000b) au cours de laquelle les volontaires pratiquent un exercice physique dans une chambre calorimétrique afin d'atteindre un niveau d'activité physique de 1,8. Pour cela, ils ont marché d'un bon pas pendant 2h30 en moyenne par jour sur un tapis roulant contre 30 minutes lorsqu'ils étaient dans des conditions d'inactivité. 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 (**Figure 9**). 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 i.e. la 'tolérance' lipidique est diminuée chez des sujets sédentaires. 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. L'ensemble de ces résultats expérimentaux pourraient expliquer le fait qu'une association entre le gain de poids et l'apport en lipides n'ait été observée que chez des sujets présentant un niveau d'activité physique faible dans une étude de cohorte réalisée sur 6 ans (Lissner & Heitmann, 1995).

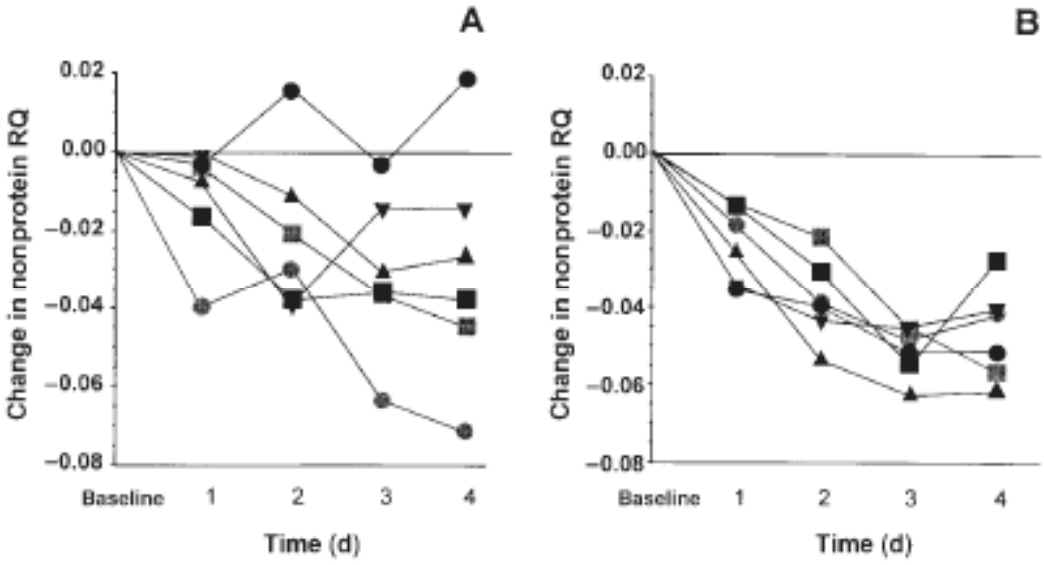


**Figure 9 :** Valeurs individuelles des changements du quotient respiratoire non protéique lors d'une activité physique faible (PAL = 1.4 ; A) et d'une activité physique élevée (PAL = 1.8 ; B). Chaque individu est représenté par un symbole différent (n=6) (Smith, 2000b).

# 5. CONCLUSION : VERS UN ROLE CENTRAL DE LA SEDENTARITE ?

Bien qu'une vision de l'étiologie de l'obésité repose sur des troubles de l'oxydation lipidique, les données de la littérature résumées dans les paragraphes précédents suggèrent un rôle central de l'activité physique dans la régulation de la balance énergétique mais aussi lipidique. Le siècle dernier a été marqué par la révolution industrielle et le progrès technologique qui ont entraîné une diminution du coût (énergétique) de la vie. Sur la même échelle de temps, la proportion de personnes atteintes d'obésité a été exponentielle. Dans ce contexte, nous nous demandons si l'augmentation de la prévalence de l'obésité pourrait simplement refléter le fait que le niveau d'activité physique de la population générale est passé en dessous d'un seuil requis pour assurer une régulation optimale des balances énergétique et lipidique. Ainsi l'incapacité des obèses à utiliser les lipides pourrait être secondaire à une vie sédentaire et non pas en soit causale dans le développement de la prise de poids. Ceci suggère qu'à l'hypothèse classiquement avancée de prédisposition génétique à l'obésité, nous pouvons opposer la notion de prédisposition génétique à être physiquement actifs.

Bien que certaines données épidémiologiques suggèrent que la prévalence de la surcharge pondérale est plus importante chez les sujets sédentaires que chez les sujets actifs, il convient de noter qu'à ce jour, une relation causale entre mécanismes de régulation du poids et inactivité n'est pas démontrée. Comparée à la connaissance étendue que nous avons de la physiologie de l'exercice, notre compréhension de l'effet de l'inactivité physique sur les réponses physiologiques et les mécanismes cellulaires ou encore sur son rôle dans le développement des maladies chroniques n'en est qu'à ses prémices. En fait, les effets préjudiciables de l'inactivité dérivent essentiellement des effets positifs de l'entraînement. Cette approche est en soi critiquable puisqu'elle ne représente pas directement la réalité, c'est-à-dire l'adoption générale d'un comportement sédentaire dans nos sociétés modernes. Si la sédentarité est l'état physiologique anormal, les sujets des groupes contrôles devraient être des individus physiquement actifs et non pas inactifs comme c'est le cas dans la plupart des études expérimentales de ces dernières années. Toutefois, une raison majeure à ce manque de données est l'absence de modèles expérimentaux pour étudier avec une approche longitudinale et mécanistique la physiologie de l'inactivité physique.

Quelques modèles ont toutefois étaient développés et les résultats obtenus sont particulièrement significatifs. Par exemple, Wisloff et collaborateurs (Wisloff *et al.*, 2005) ont génétiquement sélectionné deux populations de rats : une active et une sédentaire. À la 11ème génération il y avait une différence de 347% entre les distances parcourues jusqu'à épuisement par ces deux populations. Les animaux sédentaires ont également une adiposité plus importante que les actifs et présentent de nombreux facteurs de risques cardiovasculaires. Le point important est que ce bilan négatif peut être inversé par un entraînement progressif des rats sédentaires. Il existe aussi le paradigme d'inactivité par suspension du train postérieur chez le rat. En utilisant ce modèle, certains auteurs ont montré clairement le rôle de l'inactivité sur l'inhibition de l'activité de la lipoprotéine lipase musculaire (Bey & Hamilton, 2003). Ces auteurs ont montré que 10 heures jusqu'à 11 jours d'inactivité entraînait une réduction de 80% de l'activité de cette enzyme clef de la répartition tissulaire et du devenir métabolique des acides gras.

Chez l'Homme, il existe un modèle longitudinal d'inactivité physique. Il s'agit du modèle d'inactivité physique par alitement prolongé tête déclive à -6°

utilisé par les Agences Spatiales pour reproduire les conséquences de l'environnement spatial sur l'organisme (Vernikos, 1996). Les sujets soumis à un tel protocole ont un PAL de 1,2-1,4 ce qui permet d'étudier les conséquences physiologiques directs de l'inactivité chez des sujets sains actifs sans aucune prédisposition génétique pour l'obésité ou le diabète et qui récupéreront (Blanc *et al.*, 1998).

Dans le chapitre suivant, après avoir résumé les données existantes sur l'évolution de la sédentarité, nous présentons les différentes adaptations des balances énergétique et oxydative des substrats obtenus lors de protocoles d'alitements prolongés utilisés en tant que modèle d'inactivité.

# CHAPITRE 2

# Énergétique de l'inactivité physique

Adapté de :

'L'alitement prolongé: Un modèle d'inactivité physique'

Audrey Bergouignan & Stéphane Blanc

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# 1. L'INACTIVITE PHYSIQUE

## 1.1. L'hypothèse de Jean Mayer : l'existence d'un niveau seuil d'activité physique

L'ensemble des données du **Chapitre 1** suggère une relation entre l'inactivité physique et la prise de poids. Cette hypothèse en soi n'est pas nouvelle. Elle avait déjà été avancée dans les années 1950 par Jean Mayer chez le rat (Mayer *et al.*, 1954) puis chez l'homme (Mayer, 1956) (**Figure 1**). Elle a tout d'abord montré que les rats actifs (Mayer, 1954) qui réalisent 1 à 6h d'activité physique par jour en courant sur un tapis roulant sont minces. Cette zone d'activité physique a été nommée « zone compensatoire » puisque la prise alimentaire augmente en fonction de la durée de l'exercice physique et le poids est maintenu stable. À des niveaux d'activité physique plus élevés, la prise alimentaire cesse d'augmenter et les rats extrêmement actifs perdent du poids. Au contraire, les rats qui passent moins d'une heure par jour à courir, augmentent leur prise alimentaire ce qui résulte en une prise de poids comparés aux rats de la zone compensatoire. Ce résultat a été interprété comme un dérèglement dans la régulation de la prise alimentaire qui conduit à un gain d'adiposité. Dans une seconde étude cross-sectionnelle, Mayer et collègues (Mayer, 1956) ont utilisé une méthode indirecte pour classer des Indiens adultes du Bengale en fonction de leur niveau d'activité physique en se basant sur leurs catégories professionnelles. Les auteurs 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. Ces derniers ont une prise alimentaire cependant supérieure à celle des hommes ayant un travail physique important et présentent un poids plus élevé, ce qui est en accord avec le déséquilibre énergétique observés au préalable chez les rats. Bien que l'hypothèse de Mayer soit attractive, cela reste une étude transversale dans laquelle les hommes inactifs en surpoids étaient probablement en balance énergétique stable. Le rôle direct de l'inactivité 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 être démontré. Toutefois, Schoeller et collaborateurs (Schoeller *et al.*, 1997) mais également Wiensier et collaborateurs (Weinsier *et al.*, 2002) ont démontré au cours d'études longitudinales que des femmes post-obèses étaient capables de maintenir leurs IMC normaux si et seulement si elles conservaient un PAL d'environ 1,70-1,75.

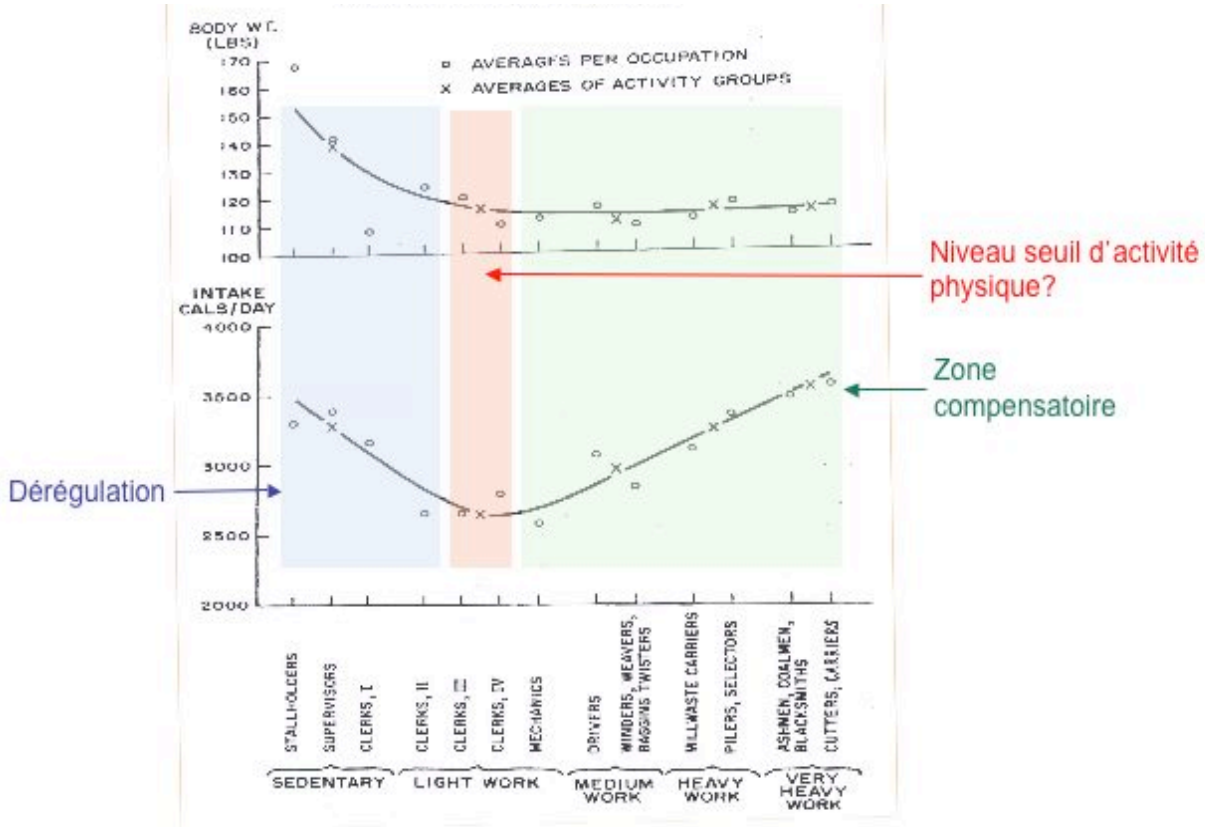


**Figure 1 :** Relation entre l'apport alimentaire, le poids corporel et le niveau d'activité physique chez les Indiens du Bengale (Mayer, 1956)

## 1.2. L'évolution de la sédentarité

Les données concernant l'évolution de notre niveau d'activité physique au cours du siècle dernier sont rares. Une étude originale a estimé que des individus vivants dans les conditions environnementales que connaissaient nos aïeuls il y a 150 ans, dépensaient 2,8 fois plus d'énergie que nos contemporains ; cette différence représentant 16km/j de marche supplémentaire (Egger *et al.*, 2001). Cordain et collaborateurs ont également montré que des populations modernes dont l'approvisionnement en nourriture est principalement basé sur la pratique de la chasse et de la cueillette ont une activité physique supérieure de 72kJ/j comparé aux adultes représentatifs de la population américaine, ce qui représente une dépense énergétique équivalente à 19-33 kilomètres de marche par jour pour un adulte de 70 kg (Cordain *et al.*, 1998). Par conséquent, du fait des avancées technologiques qui ont permis d'avoir un accès quasiment illimité à la nourriture, de se déplacer ou encore d'avoir des loisirs sans dépenser d'énergie, notre niveau d'activité actuel est très faible. Par exemple, il a été estimé que 60% de la population américaine ne participe pas à une activité physique régulière tandis que 25% est quasiment totalement sédentaire (Stein & Colditz, 2004). De 1950 à 2000, le temps passé par jour devant la télévision a quasiment doublé pour atteindre environ 8 h/j au début du siècle (Brownson *et al.*, 2005). Dietz & Gortmaker (Dietz & Gortmaker, 1993) ont montré que chez les jeunes enfants le temps passé devant la télévision est un facteur prédictif de l'IMC quelques années plus tard, tandis que Rissanen et collaborateurs (Rissanen *et al.*, 1991) ont montré qu'un faible degré d'activité physique pendant les périodes de loisir est, chez l'adulte, un facteur prédictif d'une prise de poids importante (≥5 kg) dans les 5 ans qui suivent. Le degré d'obésité de la population américaine corrèle également bien avec les ventes annuelles de voiture, de lave-linge et de lave-vaisselle (Levine & Kotz, 2005).

Les conséquences délétères de l'inactivité ont été chiffrées. En 2000, le nombre de morts liées à une mauvaise alimentation et à l'inactivité étaient de 400000, soit une augmentation de 25% par rapport aux estimations de 1990 (Mokdad *et al.*, 2004). Ce chiffre talonne de peu la mortalité liée au tabac. Certains auteurs parlent même d'un 'Sedentary Death Syndrom' (Lees, 2004).

Alors que l'ensemble des données résumées précédemment suggère une importante augmentation de la sédentarité au cours du siècle dernier, associée à des problèmes majeurs de santé publique, notre connaissance des mécanismes physiologiques sous-tendant les effets délétères de l'inactivité reste limitée. Chez l'Homme, les données les plus complètes sont celles obtenues lors d'alitement prolongé utilisé comme modèle de simulation de la microgravité.

# 2. LE MODELE D'INACTIVITE PHYSIQUE PAR ALITEMENT PROLONGE

Les effets de l'alitement ont été mis en évidence dès la Seconde Guerre Mondiale chez des patients allongés suite à des fractures osseuses de la jambe. Ces malades présentaient des taux anormalement élevés d'excrétions calciques et azotées. Don Whedon et ses collègues (Dietrick *et al.*, 1948) eut alors l'idée d'étudier des individus sains alités pour la même période de temps et observa une augmentation

similaire de l'excrétion de ces deux paramètres. Ce phénomène n'était donc pas la conséquence des fractures comme il le pensait à l'origine, mais de l'immobilisation prolongée. Par la suite, d'autres études ont abouti aux mêmes conclusions concernant les problèmes cardio-vasculaires, musculaires et neurosensoriels dont sont atteintes les personnes alitées pour de longues durées suite à de graves maladies mais aussi les personnes âgées.

Depuis l'alitement prolongé tête déclive à -6° (ou décubitus ; **Figure 2**) a été largement utilisé par les Agences Spatiales en vue de simuler les conséquences de l'environnement spatial sur l'organisme comme le déconditionnement cardio-vasculaire, l'atrophie musculaire, la déminéralisation osseuse, la perte de fluides électrolytiques ou encore l'altération du métabolisme intermédiaire. Selon les normes établies par l'Organisation Mondiale de la Santé en 1997, le PAL des sujets sédentaires est défini à 1,4. Le PAL des sujets classiquement observé lors d'alitement prolongé étant de 1,2 à 1,4 (Blanc, 1998), ce modèle permet donc d'étudier les conséquences physiologiques de l'inactivité chez des sujets sains qui récupéreront.

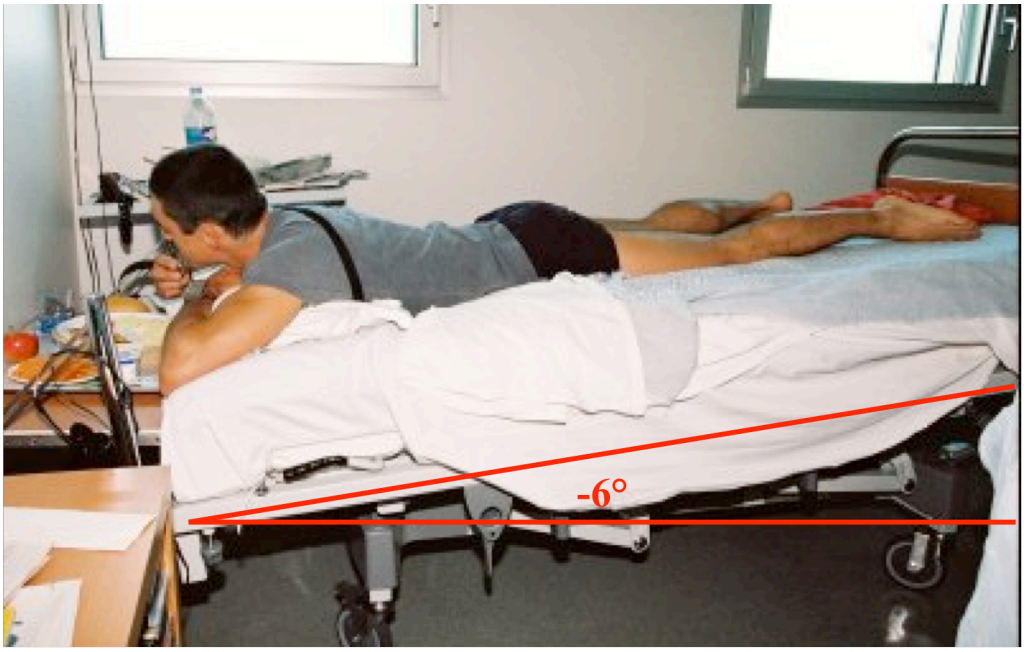


**Figure 2 :** L'alitement prolongé tête déclive à -6° ou décubitus.

# 3. EFFET DE L'INACTIVITE PHYSIQUE SUR LA BALANCE ENERGETIQUE

## 3.1. Dépense énergétique totale et ses composantes

La première conséquence de l'alitement prolongé est une nette réduction de l'activité physique spontanée et structurée qui se traduit par une baisse de la dépense énergétique totale (DET). Comparées aux valeurs obtenues avant l'alitement, des diminutions de 22% et de 20% ont été mesurées au cours de décubitus respectivement de 10 jours (Gretebeck *et al.*, 1995) et de 42 jours (Blanc, 1998). Pour comprendre l'origine de cette réduction de la dépense énergétique, il faut analyser l'évolution de chacune de ses composantes. Le métabolisme de repos (MR) qui correspond à l'énergie requise pour maintenir une activité *a minima* des tissus, est la composante principale de la DET et sa variabilité est expliquée pour 70 à 80% par la masse maigre. Par conséquent, la diminution du MR d'environ 6% (Blanc, 1998) est principalement induite par la réduction de la masse métaboliquement active observée lors des protocoles de décubitus. La thermogenèse post-prandiale (TPP) qui correspond à l'énergie allouée aux processus gastro-intestinaux de la digestion et au stockage des aliments ne présente pas de changement (Blanc, 1998) et reste autour des 10% classiquement observés. Les faibles variations de ces deux composantes de la DET : MR et TPP suggèrent donc que la diminution de la DET est principalement la conséquence de la baisse de la dépense énergétique liée à l'activité physique, elle-même évaluée à 39% (Blanc, 1998).

## 3.2. Énergie et macronutriments ingérés

### 3.2.1. L'adaptation du comportement alimentaire

Une adaptation du comportement alimentaire s'observe lors d'une telle réduction de la DET. En effet, lors des études d'alitement prolongé, les sujets volontaires diminuent spontanément leur prise alimentaire lorsque la nourriture est mise à disposition de manière *ad libitum*. Une baisse de 17% de l'énergie ingérée a été observée en parallèle de la diminution de 20% de la DET mesurée lors du décubitus de 42 jours (Blanc, 1998). À cela s'ajoute des changements dans les proportions de macronutriments ingérés avec un choix préférentiel des aliments riches en glucides au détriment de ceux riches en lipides. En revanche, l'ingestion des protéines n'est pas modifiée par l'alitement. Lorsque les sujets reprennent une activité normale, ils augmentent spontanément leur prise alimentaire jusqu'à atteindre des valeurs de pré-alitement et les proportions de macronutriments ingérés sont rétablies. La balance énergétique résultant de cette diminution de l'énergie ingérée lors de longues périodes d'inactivité reste néanmoins à déterminer.

### 3.2.2. Régulation hormonale

Ces résultats suggèrent une mise en place de mécanismes adaptatifs en réponse à l'inactivité physique. La leptine, hormone satiétogène, pourrait jouer un rôle dans la réduction de la prise alimentaire spontanée. En effet, en réponse à un alitement

de 7 jours, une augmentation de la leptine est notée indépendamment des changements de masse grasse (Blanc, 2000b). Les changements de cette hormone au cours d'une longue période d'inactivité demeure cependant mal connue.

### 3.2.3. Biais potentiels

Bien que des mécanismes physiologiques de la régulation du comportement alimentaire semblent être déclenchés par le passage de l'état actif à l'état inactif, des effets confondants comme la monotonie des repas proposés ou leur aspect peu appétissant sont à prendre en compte. Vu les similitudes entre les repas préparés au cours des expériences de décubitus et ceux qui sont proposés en milieux hospitaliers, ces observations sont à prendre en compte pour les malades effectuant de longs séjours à l'hôpital. Par conséquent, des études complémentaires seraient nécessaires afin de mieux comprendre les mécanismes de régulation du comportement alimentaire en fonction du niveau d'activité physique via les modulations des taux de leptine plasmatique mais aussi d'autres hormones sécrétées par le tube digestif qui pourraient influencer préférentiellement les proportions en macronutriments.

# 4. EFFET DE L'INACTIVITE PHYSIQUE SUR LA BALANCE OXYDATIVE DES SUBSTRATS

## 4.1. Métabolisme protéique

### 4.1.1. L'atrophie musculaire

Malgré le maintien d'une balance énergétique stable, c'est-à-dire entrées énergétiques (énergie ingérée) égales aux sorties énergétiques (DET), l'alitement prolongé entraîne une diminution de la masse corporelle. Ce phénomène s'explique essentiellement par une importante atrophie musculaire systématiquement observée au cours des études d'alitement. Par exemple, des décubitus d'une durée de 28 jours à 3 mois ont entraîné une perte moyenne de poids de 2,8 à 2,9 kg dont 2,0 à 2,7 kg de masse maigre (Blanc, 1998; Paddon-Jones *et al.*, 2004; Bergouignan *et al.*, 2006). Par ailleurs, à cette atrophie musculaire est associée une plus grande fatigabilité des muscles ainsi qu'une diminution de la force et de la capacité à l'exercice caractérisée par une baisse de la capacité aérobique ($VO_2max$) qui dépend de la durée de l'alitement et du niveau initial de la $VO_2max$ des sujets. Cette diminution de la capacité à l'exercice fait partie du syndrome de déconditionnement cardiovasculaire.

### 4.1.2. Une balance protéique négative

En conditions physiologiques normales, le contenu musculaire de l'organisme est finement réglé par les variations des taux de synthèse et de dégradation protéiques. Le déclin de la masse musculaire induit par l'inactivité physique implique donc une balance protéique négative qui s'explique essentiellement par une diminution de l'anabolisme plutôt que par une augmentation du catabolisme des protéines.

### 4.1.3. Des contre-mesures physiques souvent incompatibles

Pour faire face à cette atrophie musculaire délétère pour l'organisme, des contre-mesures ont été mises en place. La première mesure testée et la plus évidente a été l'application d'une activité physique. Celle-ci a un effet bénéfique clairement démontré qui dépend néanmoins du type d'exercice physique pratiqué. En effet, l'exercice de type résistif semble plus efficace dans la prévention de la perte de masse maigre que l'exercice de type aérobique. Cependant, le volume à pratiquer pour observer un effet est très important et souvent incompatible avec l'état des patients alités suite à de graves maladies ou à des accidents corporels. Des mesures alternatives d'ordre alimentaire ont donc été testées.

### 4.1.4. Des contre-mesures alimentaires

Aucune variation du renouvellement protéique n'a été mise en évidence à l'état post-absorptif. Par conséquent, les changements du métabolisme protéique liés à l'alitement s'effectueraient en phase post-prandiale caractérisée par une plus grande disponibilité en acides aminés et par une élévation des taux d'insuline plasmatique, hormone anabolique qui stimule la synthèse protéique et en inhibe la dégradation. À partir de cette hypothèse, une étude a montré qu'une prise alimentaire protéique de 1,0g/kg masse corporelle/jour au lieu des 0,6g/kg masse corporelle/jour recommandés par l'OMS permet de prévenir la baisse de la synthèse protéique lors d'un alitement prolongé (Stuart *et al.*, 1990). Les effets bénéfiques de cette supplémentation protéique sur l'atrophie musculaire proviendraient essentiellement du pourcentage élevé d'acides aminés branchés comme l'isoleucine, la leucine et la valine (Stein *et al.*, 2003). Bien que cette supplémentation protéique permette de maintenir la balance azotée, elle ne prévient pas nécessairement la perte de masse musculaire. En combinant les effets d'un régime riche en protéines assurant une grande disponibilité en précurseurs protéiques avec un régime hyperglucidique qui stimule la sécrétion d'insuline, Paddon-Jones et collaborateurs (Paddon-Jones *et al.*, 2004) ont ainsi prévenu la perte de masse et de force musculaires. Puisqu'un régime riche en glucides ou l'hyperinsulémie résultante activent la synthèse protéique, on s'attendrait à ce que la diminution de la sensibilité à l'insuline classiquement observée au niveau des muscles atrophiés l'inhibe. Cependant, aucun résultat n'a encore clairement confirmé cette hypothèse et la contribution de l'insulino-résistance dans la perte de masse maigre reste encore incertaine.

Bien que cette approche soit bénéfique, un des contre effets inattendus de celle-ci est une hypercalciurie et une résorption osseuse probablement liées à des modifications du pH. Des recherches supplémentaires sont donc nécessaires afin d'estimer la quantité minimale de protéines permettant de prévenir l'atrophie musculaire sans aggraver la déminéralisation osseuse déjà induite par l'alitement.

### 4.1.5. Insulino-résistance

Les effets délétères de l'inactivité physique sur la capacité de l'insuline à stimuler la captation du glucose par le muscle squelettique ont clairement été montrés à l'aide de la technique du clamp euglycémique-hyperinsulémique (Stuart *et al.*, 1988). Cependant, un test oral de tolérance au glucose couplé à l'utilisation d'isotopes stables a montré que la résistance aux effets de l'insuline semblait certes principalement localisée au niveau musculaire chez les hommes mais aussi au niveau du foie chez les femmes (Blanc, 2000a). L'inactivité semble donc être associée au développement d'une insulino-résistance chez des sujets sains mais aussi d'une hyperlipémie. Ces résultats suggèrent donc que l'inactivité *per se* entraîne chez des sujets sains des profils métaboliques proches de ce que l'on observe chez des personnes atteintes de diabète de type 2.

Le degré d'hyperinsulémie semble être dépendant de la quantité d'énergie dépensée au cours d'un exercice physique ce qui suppose que le développement de l'insulino-résistance induit par l'alitement peut être prévenu par la pratique quotidienne d'une activité physique. En effet, une étude ancienne a montré qu'une dépense énergétique de 1000 kcal/jour, à la suite d'une heure d'exercice physique de type isotonique, permet de rétablir la réponse du glucose et de l'insuline à son niveau basal lors d'une charge orale en glucose (Dolkas & Greenleaf, 1977).

## 4.2. Changements dans l'utilisation des substrats

Dans ce contexte physiologique, un changement dans le profil d'oxydation caractérisé par une diminution de l'oxydation lipidique et une augmentation de l'oxydation glucidique est observé à jeun et ce, dans toutes les études d'alitement (Blanc, 2000a; Stein & Wade, 2005). Ces changements métaboliques sont indépendants du statut énergétique des sujets (Stein & Wade, 2005).

## 4.3. Mécanismes sous-jacents

### 4.3.1. Changements structuraux des muscles

Les mécanismes impliqués dans ce changement d'utilisation des substrats sont mal connus, toutefois quelques hypothèses peuvent être avancées. Ce phénomène peut en partie s'expliquer par des changements structuraux au niveau musculo-squelettique. En effet, en plus de l'atrophie musculaire, des variations dans les proportions des types de fibres musculaires ont été observées au cours d'immobilisation de longue durée (Trappe, 2004). Cela se traduit par une diminution du pourcentage des fibres musculaires oxydatives associée à une augmentation du pourcentage des fibres glycolytiques caractérisées par une sensibilité à l'insuline plus faible.

### 4.3.2. Diminution de la bêta-oxydation

La diminution de la bêta-oxydation peut aussi être la conséquence soit d'une moindre disponibilité en acides gras, soit d'une réduction de la capacité musculaire à oxyder les lipides. Bien qu'une plus grande sensibilité des récepteurs bêta-adrénergiques soit induite par l'alitement, la baisse de l'activité du système nerveux sympathique précédemment mentionnée, ne permet pas l'activation de la lipolyse (Barbe *et al.*, 1998). L'augmentation des taux d'acides gras libres observée au niveau plasmatique suppose donc une altération de la clairance de ces métabolites.

### 4.3.3. Transport des acides gras

Des études chez le rat utilisant un paradigme d'inactivité par suspension du train postérieur (Bey, 2003) ont montré que 11 heures à 11 jours d'inactivité entraînent une diminution de 80% de l'activité de la lipoprotéine lipase musculaire, enzyme clef de la répartition tissulaire et du devenir métabolique des acides gras. Une autre étude (Stein *et al.*, 2002) basée sur ce même modèle a aussi mis en évidence une diminution dans l'expression génique de la carnityl palmitoyl transférase I (CPT-I), protéine responsable de l'entrée des acides gras à longues chaînes dans la mitochondrie.

### 4.3.4. Réorganisation des voies métaboliques

Au-delà des altérations observées au niveau du transport des acides gras dans le mycocyte et la mitochondrie, une réorganisation dans les voies métaboliques serait en cause. En effet, Stein et collaborateurs (Stein *et al.*, 2002) ont montré chez le rat suspendu une augmentation des capacités glycolytiques au niveau des muscles atrophiés à travers l'augmentation significative de l'expression de trois enzymes clefs de la glycolyse : l'hexokinase, la phosphofructokinase et la pyruvate kinase. Inversement, une réduction de la capacité à oxyder les lipides via une diminution de l'expression génique des enzymes impliquées dans la bêta-oxydation a été mise en évidence. Il est intéressant de souligner qu'une réduction similaire de l'activité des enzymes mitochondriales a été notée chez 16 hommes sains sédentaires comparée aux résultats obtenus chez 16 hommes actifs (Rimbert, 2004).

### 4.3.5. Augmentation des capacités glucogéniques du foie

En parallèle des altérations des voies glucidique et lipidique, une étude complémentaire chez le rat inactif (Stein *et al.*, 2005) a montré une augmentation des capacités glucogéniques du foie qui expliquerait la plus grande utilisation en glucose par les muscles. Basés sur cette étude, les auteurs ont suggéré que l'augmentation de glucose entraînerait une augmentation des taux de malonyl-CoA qui, par inhibition de la CPT-I, diminuerait l'entrée des acides gras dans la mitochondrie. Ces acides gras non oxydés resteraient dans le cytosol du myocyte et s'y accumuleraient sous forme de gouttelettes lipidiques stimulant le développement de l'insulino-résistance au niveau musculaire. En revanche, aucune augmentation de la production de glucose n'a encore été mise en évidence chez des sujets alités (Blanc, 2000a). Des études complémentaires sont donc à mettre en place, en particuliers en vue d'établir des contre-mesures efficaces afin de prévenir ces dysfonctionnements métaboliques induits par l'inactivité.

# 5. CONCLUSION

En conclusion, le modèle d'alitement prolongé constitue un modèle unique pour étudier la physiologie de l'inactivité physique. L'inactivité physique induite par alitement prolongé entraîne, indépendamment de l'état de la balance énergétique, des altérations métaboliques similaires à celles observées chez les sujets obèses (réduction de l'oxydation des lipides totaux, hyperlipémie et hyperinsulémie en conditions post-absorptives) ou diabétiques (insulino-résistance) ou chez les personnes hospitalisées (perte de masse et force musculaires). Les mécanismes sous jacents restent largement méconnus.

# CHAPITRE 3

## Objectifs & Hypothèses

# 1. BUT DE L'ETUDE

Alors que le bilan humain et économique de l'adoption générale des comportements sédentaires dans nos sociétés modernes est alarmant, nous disposons à ce jour de peu de données concernant la physiologie de l'inactivité physique. **Le but majeur de ce travail de thèse a été de déterminer chez l'homme et la femme normo-pondérés si l'inactivité physique altère la régulation des balances énergétique et oxydative des lipides impliquées dans la régulation du poids et de caractériser les mécanismes sous-jacents.** Il est important de rappeler que l'obésité représente **un échec dans la répartition des lipides alimentaires** entre oxydation et stockage. Alors que les précédentes études d'alitement étaient de courtes ou moyennes durées et se sont limitées à l'étude du métabolisme lipidique en conditions post-absorptives, nous nous sommes particulièrement intéressés dans cette thèse à l'impact d'un niveau d'inactivité physique extrême induit par un alitement **de longue durée** chez l'**homme** et la **femme** sur le **métabolisme post-prandial des lipides exogènes.**

*A contrario*, l'exercice physique peut être considéré comme un traitement préventif et thérapeutique de l'obésité. On peut alors imaginer une démarche similaire à celle utilisée dans la prescription des doses de médicaments : donner une quantité minimale pour éviter les contre-effets, mais tout en maintenant les effets bénéfiques. Dans le cas de l'obésité, le but est d'établir des recommandations d'activité physique efficaces, tout en évitant de démotiver les populations sédentaires et obèses qui pourraient facilement ne pas se sentir capables de les respecter. **L'objectif secondaire de notre étude est donc de tester l'efficacité de 2 types de protocoles d'entraînement physique à limiter les effets de l'inactivité physique sur les balances énergétique et oxydative des lipides.**

# 2. HYPOTHESES ET OBJECTIFS SPECIFIQUES

D'un point de vue général, ce travail de thèse repose principalement sur l'hypothèse établie par Jean Mayer dans les années 1950 à partir d'études transversales chez le rat et l'humain. Selon elle, **il existerait un seuil d'activité physique en dessous duquel les mécanismes de régulation du poids sont inopérants.** Avant de déterminer ce seuil, il est nécessaire de déterminer de manière longitudinale les effets directs de l'inactivité physique dans la régulation du poids. Pour cela, nous avons soumis des sujets homme et femme sains sans aucune prédisposition génétique à l'obésité à une longue période d'inactivité physique en utilisant le modèle de l'alitement prolongé.

Ce travail est issu de deux alitements prolongés organisés conjointement par les Agences Spatiales Internationales (l'ESA, la NASA, la JAXA, la DLR, la CSA et le CNES) respectivement de 3 mois chez l'homme (the Long Term Toulouse Bed Rest study) et de 2 mois chez la femme (the Women International Space Simulation for Exploration).

**Dans la première partie de ce travail**, nous avons caractérisé les conséquences de **l'inactivité physique sur les mécanismes de régulation de la balance énergétique. L'hypothèse sous-jacente est que la sédentarité altère de manière non proportionnelle les différentes composantes de la balance énergétique à savoir les apports caloriques et le comportement alimentaire, et les différents postes de la dépense énergétique.**

Les objectifs spécifiques sont de déterminer les effets de l'inactivité sur :

- La dépense énergétique totale, le métabolisme de repos, le thermogenèse post-prandiale et la dépense énergétique liée à l'activité physique.
- Le comportement alimentaire.
- Les mécanismes hormonaux potentiellement impliqués dans ces changements.

**Dans la seconde partie de ce travail de thèse**, nous avons **dans un premier temps** testé l'effet direct de l'inactivité physique sur l'oxydation des lipides totaux et exogènes. Plus spécifiquement, nous avons cherché à déterminer l'effet de l'inactivité 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 l'inactivité sur :

- La composition corporelle et l'évolution des lipides intramusculaires.
- La lipémie et des index de sensibilité à l'insuline.
- L'oxydation des lipides totaux en conditions post-absorptive et post-prandiale.
- 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.

**Dans un second temps**, nous avons **testé l'efficacité de deux protocoles d'entraînement physique sur le maintien de la balance lipidique lors d'alitements prolongés.** Le premier était un entraînement d'exercice physique de type résistif qui avait pour but de maintenir la masse maigre et le métabolisme de repos des sujets sans modification majeure de la dépense énergétique totale. Le second était un entraînement associant un exercice physique de type résistif et aérobique qui avait pour but supplémentaire d'augmenter significativement la dépense énergétique totale.

Les objectifs spécifiques ont été de déterminer l'effet préventif :

- D'un protocole d'entraînement en exercice résistif sur l'oxydation des lipides totaux et exogènes en condition d'inactivité physique extrême.
- D'un protocole d'entraînement combinant exercices de type résistif et aérobique sur l'oxydation des lipides totaux et exogènes et sur le devenir des acides gras exogènes en condition d'inactivité physique extrême.

# RESULTATS
## PARTIE FONDAMENTALE

*Les trois grâces de Rubens (1639)*

# CHAPITRE 4

# Balancing appetite, energy intake and energy expenditure during physical inactivity induced by 60 days of bed rest

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# Résumé

## Introduction

Au cours du siècle dernier, la révolution industrielle et le progrès technologique ont engendré une diminution de l'activité physique tandis que l'énergie ingérée a augmenté. Il a été supposé que ces changements environnementaux agiraient de manière synergique et encourageraient une prise de poids. Quelques études d'intervention ont montré en effet que l'inactivité physique engendre une diminution de la dépense énergétique totale qui n'est pourtant pas suivie par une baisse des apports énergétiques. Ainsi, la sédentarité pourrait être à l'origine d'une balance énergétique positive et d'une prise de poids sur le long terme. Néanmoins, les effets d'une longue période d'inactivité sur la balance énergétique restent à ce jour inconnus.

## Objectifs

Dans cette étude, nous nous proposons :

- d'étudier les effets à long terme de l'inactivité physique, induite par 60 jours d'alitement, sur l'appétit, l'énergie ingérée spontanée, la balance énergétique chez la femme de poids normal,
- de déterminer la contribution de la pratique d'un entraînement physique combinant exercices de type résistif et aérobic sur l'ajustement de l'énergie ingérée à la dépense énergétique totale
- d'appréhender les mécanismes hormonaux impliqués dans la régulation de la balance énergétique.

## Matériel et Méthodes

Après une période ambulatoire de 20 jours, 16 femmes minces étaient divisées en deux groupes : un groupe contrôle (n=8) qui est resté allongé pendant deux mois et un groupe exercice (n=8) qui a effectué, simultanément à l'alitement, un protocole d'exercice physique de type résistif et aérobic. L'apport énergétique fournit aux volontaires étaient estimés pour couvrir leurs besoins énergétiques. Toutefois, elles pouvaient adapter spontanément leur énergie ingérée en demandant plus de nourriture ou en en laissant sur le plateau. Pendant toute la durée de l'expérience, la composition corporelle (par DEXA), l'énergie ingérée, la dépense énergétique totale (par la méthode de l'eau doublement marquée) et ses composantes (par calorimétrie indirecte), et les hormones intestinales plasmatiques à jeun et la leptine étaient mesurés.

## Résultats

Au cours de l'alitement, la dépense énergétique totale était plus élevée dans le groupe exercice (8.85±1.14MJ/d) que dans le groupe contrôle (7.12±0.78MJ/d, $p=0.004$) et l'énergie ingérée a significativement diminué dans les deux groupes ($p<0.0001$). La balance énergétique résultante de ces changements était de -0.60±0.99MJ/d dans le groupe contrôle (p=NS vs zéro) et de -1.36±0.75MJ/d dans le groupe exercice (p=0.0013 vs zéro). L'énergie ingérée spontanée était négativement associée à une augmentation de la concentration plasmatique de la leptine ($R^2=0.46$, $p=0.006$. La consommation alimentaire envisagée par les volontaires diminua dans les deux groupes (p=0,025) et corrélait avec la

concentration du GLP1 ($R^2$=0.29, p=0.04)., une hormone intestinale satiétogène. Néanmoins, les taux plasmatiques de GLP1 à jeun augmentaient seulement dans le groupe exercice (p=0.0076).

## Discussion

Ainsi, sur de longues périodes d'inactivité physique, une balance énergétique stable peut être atteinte. En revanche, au lieu d'optimiser la régulation de la balance énergétique, l'entraînement physique induit une sensation de satiété, médié du moins en partie par la leptine et le GLP1, qui entraîne une énergie ingérée inférieure aux besoins énergétiques et donc une balance énergétique négative. Bien que l'inactivité physique ne semble pas altérer la balance énergétique sur le long terme, on peut se demander de ce qu'il en est au niveau de la balance oxydative des substrats.

# 1. ABSTRACT

**Background:** Short and moderate periods of physical inactivity do not induce a compensatory reduction of energy intake (EI) and leads to a positive energy balance (EB). Long-term effects are unknown.

**Objective:** To examine the long-term effect of physical inactivity (60d of bed-rest) and the resulting decrease in total energy expenditure (TEE) on spontaneous EI, EB, reported hunger and related hormonal determinants in women with and without added exercise.

**Design:** After a 20d-ambulatory period, 16 healthy lean women were divided in two groups (n=8, each): a control group restricted to bed-rest and an exercise group subjected to a combined aerobic and resistive exercise training while also restricted to bed-rest. Volunteers were initially provided a base diet estimated to equal their TEE but were allowed to request changes in the amount of the base diet as well as *ad libitum* snacks. Body composition (DXA), EI, TEE (doubly labeled water) and its components (indirect calorimetry), and fasting gut hormones and leptin were measured.

**Results:** During bed rest, TEE was greater in the exercise group (8.85±1.14MJ/d) than in the control group (7.12±0.78MJ/d, $p=0.004$) and EI decreased in both groups compared to the ambulatory period ($p<0.0001$). EB was -0.60±0.99MJ/d in the control group ($p=0.13$ vs zero) and -1.36±0.75MJ/d in the exercise group ($p=0.001$ vs zero). EI was negatively associated with fasting leptin levels adjusted for fat mass ($R^2=0.46$, $p=0.006$). Fasting GLP1 concentration increased only in the exercise group ($p=0.008$). Desire to consume food decreased in both groups ($p=0.025$) and was weakly related to GLP1 ($R^2=0.29$, $p=0.04$).

**Conclusions:** EB can be achieved during long-term extreme physical inactivity conditions. The added exercise training induced satiety and negative EB by mechanisms involving, at least in part, a deficiency in non-exercise activity and GLP-1.

# 2. INTRODUCTION

The agricultural and technological revolutions of the late 20th century have influenced both of the discretionary components of the energy balance equation, namely energy intake (EI) and physical activity. Epidemiological data suggest that during the last decades, energy (and fat) intake have increased (1) and that physical activity has decreased (2). These changes have been considered to act synergistically in the direction of encouraging weight gain in a susceptible genotype. Some studies (3-5) have indeed suggested that energy intake poorly tracks changes in energy expenditure and results in positive energy balance during conditions of reduced activity. These observations have been used as proof of a defect in the mechanisms of energy balance regulation conducive to weight gain when individuals are inactive.

This hypothesis is not new. In the early 1950s, Mayer et al. (6) indirectly assessed physical activity in Indian men based on job classification and compared this with questionnaire based estimates for EI. They observed that for physically demanding jobs, that the more the job required high level of physical activity the more the men increased their EI. For men employed in sedentary jobs, however, EI increased as the physical demands decreased and body weight was more than that of men who were employed in active jobs. Based on these observations, Mayer hypothesized a breakdown of the regulation of the EI below a physical activity level threshold, which leads to an increased body weight; however, the difficulties in accurately measuring total energy expenditure (TEE) rendered the conclusion elusive.

The development of the doubly labeled water (DLW) method and improved whole room calorimeters have obviated this problem. Based on a review of cross-sectional data from DLW studies in adults, Schoeller (7) provided support for that hypothesis in men, but not in women. In addition, intervention studies investigated the effect of physical inactivity on appetite and EB. Murgatroyd et al. (3) noted that on a given diet (high-fat vs. low-fat diets), subjects (active vs. sedentary) consumed the same level of energy regardless of the level of TEE. Similarly, Shepard et al. (4) observed that the decrease in activity associated with the sedentary environment induced by 1d spent in a calorimeter room generated a positive EB in both lean and obese subjects. Stubbs et al. (5) performed a medium-term study of 7 days in which TEE was clamped at approximately 1.4 and 1.8×resting metabolic rate (RMR) in a whole-body indirect calorimeter and subjects were fed *ad libitum*. By day 7 cumulative EB was 26.3 and 11.1 MJ, respectively, and most of the excess energy was stored as fat. Consequently, as suggested from the Mayer Hypothesis, short- and medium-term studies showed no tendency for EI to begin to decrease in line with a consistent reduction in TEE due to inactivity. Thus, an increase in sedentariness has considerable scope to decrease TEE without any compensatory EI, which generate a positive EB.

There is growing evidence that longer studies are needed and that it may take 2-4 weeks for EI to adjust to changes in TEE (8). During long-term bed rest induced-severe inactivity, we reported a decrease in EI gradually to match energy requirements (9). Consequently, it is possible that studies focusing only on short/medium-term effects may be searching for evidence of regulation over too short of a timescale. Whether or not physical inactivity affects EB and feeding behavior in the long-term, and to what extent, remain to be established.

The purpose of the present study was to examine the effect of physical inactivity induced by 60 days of bed rest on the regulation of EB in 8 healthy lean

women. The approach was different from previous studies (3-5) that aimed to investigate the rate and extent to which individual energy intake adapts from offered meal sizes equivalent to the active conditions. In our study, however, we employed a paradigm that discourages the initial well observed passive overeating. The base diet provided a diet estimated to equal the energy requirements of the physically inactive period, but participants were offered snacks and could change the amount of the base diet upon request. The underlying objective was to study the extent to which long-term physical inactivity *per se* affects reported hunger, spontaneous energy intake, and related hormonal determinants. The secondary aim was to determine if an exercise training protocol performed concomitantly with bed rest affected the adjustment of EI to EE changes.

# 3. MATERIAL & METHODS

## 3.1. Participants

Sixteen healthy women volunteered for a 60-day bed rest sponsored by the European, French, Canadian and American Space Agencies, and organized at the Institute of Space Medicine (Toulouse, France). **Table 1** shows their baseline characteristics. Inclusion criteria included age between 25 and 45 years old, non-smokers, no regular consumption of alcohol and participation in at least 30 minutes of moderate activity per day, this being achieved either with structured exercise or with activities of daily living. Exclusion criteria included familial history of clinical or biomedical diseases, regular high volume physical activities, acute diseases requiring medications during the 3 months prior to the study, sleep disorders, chronic back pain, history of thrombophlebitis, special food diets and tendon tears or bone fractures. The participants were asked to stop birth control pills three months before the study. They were thoroughly briefed about the experimental procedures and signed a consent form approved by the Institutional Review Board of Midi-Pyrénées I (France). All subjects tolerated the experiments well.

## 3.2. Study design

The three month experiment was divided into three periods: a 20-day ambulatory control period, 60 days of bed-rest in head-down tilt position (-6°), and a 20-day recovery period. The 20 days of the control and recovery periods were required to complete baseline data collection and their recovery, respectively. During the control and recovery periods, the subjects were confined to the Institute, but were asked, under professional trainers supervision, to maintain a minimal amount of exercise. During the bed rest, all activities of daily living i.e. shower, rest room, eating, etc, were performed in supine position. Standing or sitting were forbidden. Cameras were used at night to ensure compliance of the subjects to these conditions. During the bed rest, the subjects were divided into two groups (n=8, each): A control group that with no added activity, and an exercise group subjected to a specified supine resistance and aerobic exercise training protocol.

**TABLE 1.** Characteristics of the participants in ambulatory period at baseline during bed rest and one year after the end of the experiment

| | | Baseline | | 60 days after bed rest | | After one year | |
|---|---|---|---|---|---|---|---|
| | **Units** | **Control** | **Exercise** | **Control** | **Exercise** | **Control** | **Exercise** |
| **n** | | 8 | 8 | 8 | 8 | 6 | 8 |
| **Age** | years | 34 ± 4 | 33 ± 4 | 34 ± 4 | 33 ± 4 | 35 ± 3 | 34 ± 4 |
| **Height** | m | 1.63 ± 0.06 | 1.65 ± 0.07 | 1.62 ± 0.06 | 1.65 ± 0.07 | 1.62 ± 0.06 | 1.65 ± 0.07 |
| **Body mass** | kg | 55.6 ± 3.8 | 58.4 ± 6.5 | 52.3 ± 3.8* | 54.9 ± 6.1* | 54.8 ± 4.8 | 58.7 ± 6.3 |
| **BMI** | kg/m² | 21.3 ± 1.4 | 21.7 ± 1.4 | 19.7 ± 1.2* | 20.2 ± 1.5* | 21.3 ± 0.9 | 21.3 ± 1.5 |
| **Fat free mass** | kg | 40.8 ± 3.1 | 43.8 ± 5.8 | 38.0 ± 3.0* | 42.5 ± 5.7* | 40.6 ± 3.4 | 44.0 ± 6.0 |
| **Fat mass** | kg | 14.8 ± 3.7 | 14.5 ± 3.2 | 14.3 ± 3.5* | 12.4 ± 3.6* | 14.2 ± 4.1 | 14.7 ± 2.6 |
| | % | 26.4 ± 5.5 | 25.0 ± 5.0 | 27.1 ± 5.5 | 22.6 ± 6.2* | 25.7 ± 5.8 | 25.1 ± 4.5 |
| **$VO_2$peak** | l/min | 1.9 ± 0.4 | 2.1 ± 0.4 | | | | |
| | ml/kg/min | 34.5 ± 7.7 | 35.1 ± 4.4 | | | | |

Values are mean ± SD

BMI: body mass index; $VO_2$peak, peak $O_2$ consumption.

Paired t-test: *$p<0.05$ vs. Baseline period; no between-group differences were noted.

One year after the end of the study (R+360), the participants were asked to come back to the Institute of Space Medicine to complete post-intervention tests. Two volunteers from the control group decided to withdraw from the study at that time.

## 3.3. Exercise training protocol

The resistance exercise training program was performed on an inertial flywheel ergometer (10). This device allows subjects to perform maximal concentric and eccentric actions in the supine squat and calf press. A total of 19 sessions were scheduled for each subject approximately every third day and lasted 35 min. Ten minutes of light supine cycling and submaximal supine squat and calf press repetitions were completed as warmup. The supine squat exercise consisted of four sets of seven maximal concentric and eccentric repetitions with 2 min resting time between each session.

The aerobic training protocol was performed three to four times per week using a supine lower body negative pressure (LBNP) treadmill. In principle, the device consists of a vacuum chamber that provides an attractive force between subject and a vertical treadmill to substitute for the effects of gravity (e.g. axial load of about 60-65% of their body weigth) in upright exercise (11). The negative pressure required to produce 1.0 body weight (BW) and to normalize heart rate to upright exercise in 1g was approximately 48-55 mmHg. Subjects performed 40min of dynamic treadmill exercise, followed by 10min of resting LBNP. This protocol was similar to that which successfully preserved upright exercise capacity during 30 days of bed rest (12). Target exercise intensities for this protocol consisted of supine walking/running graded sequentially between 40 and 80% $VO_2$ peak (about 25% at high intensity and 75% at moderate intensity). Target speeds to achieve the different exercise intensities were based upon a linear relationship between treadmill speed and $VO_2$ determined during the ambulatory phase with a graded exercise test performed in upright posture. During the course of the 60-day BR, 29 exercise sessions were performed for each subject. Of the exercise session performed, the mean exercise time was 50±2min on each training day. Across all exercise sessions completed, the average LBNP was 52±3mmHg which corresponded to a mean loading of 1.0±0.1 BW sufficient to produce an adequate weight-bearing muscle load and cardiovascular support for supine treadmill exercise. A detailed description of the LBNP device and exercise protocol during the WISE study has been published elsewhere (13).

## 3.4. Energy intake

Throughout the experiment, the subjects were provided a diet with a macronutrients composition of 30% fat, 15% proteins and 55% carbohydrates. Food intake was calculated to match requirements during bed-rest with the objective to provide the subjects just the amount required to maintain EB. Importantly, the subjects were not required to finish their meal if not hungry. Subjects could also request larger portions when hungry. Snacks were offered to cover any leftovers, but the decision to eat them was left to the subjects. Additional snacks were also offered to the exercise group to allow them to compensate for the energy cost of the exercise sessions. Thus, EI was allowed to fluctuate around theoretical energy requirements in order to measure spontaneous changes in EI and ingestive behaviors associated with the physical inactivity induced by the bed rest.

During the control period, energy requirements for the base diet were calculated as RMR times a physical activity level of 1.4 (PAL). RMR was estimated from the Schoefield equation (14) and then confirmed on day five of the control period by indirect calorimetry. Measured and calculated RMR were within 3% of agreement on average. A PAL of 1.4 was selected during the control period for subjects with a low level of physical activity, based on the latest version of the DRI (15), to account for the confinement at the Institute. A PAL of 1.2 was selected for the bed rest based on previous studies (16). RMR was measured twice in each period, fat mass (FM) by Dual Energy X-RayAbsorptiometry (DXA) was assessed every 15 days and subjects were weighted daily. Those parameters were used to adjust the base energy prescription through out the study to account for by changes in body composition.

Diet was supplied by the hospital kitchen and controlled by three dieticians under the supervision of the investigators. Diet followed a seven-day rotating menu, with two daily choices, which was based on the subject's avoidances and preferences specified during the selection process. This was Western diet and meals were provided at breakfast (0800am-0900am), at lunch (1200am-1300pm) and dinner (0700pm-0800pm). Snacks were offered in the afternoon around 0400pm. A cook from the hospital kitchen was devoted to the study and was asked to strictly follow recipes so that precise ingredients were entered in the Geni Software (Frana, Nancy, France). All food and leftovers were weighted individually e.g. meat, vegetables, sauce, fruits, etc. The overall objective was to maximize the accuracy of the EI measurement which was recorded for each meal and averaged per day.

## 3.5. Perceived hunger profile

Visual analogue scales (VAS) 100-mm in length with words anchored at each end, expressing the most positive and the most negative ratings, were used to assess perceived hunger, satiety, fullness, desired (prospective) food consumption, and desire to eat something fatty or sweet. The questionnaires were made as small booklets showing one question at a time. Subjects did not discuss or compare their ratings with each other and could not refer to their previous ratings when marking the VAS. VAS were administered every week. 8 VAS distributed the meals were completed during these test-day. VAS were administered 15 minutes before and 15 minutes after each meal. Two other VAS were administered between at 1000am and 1600pm. Cumulated VAS (area under the curve (AUC) interpolated from the first measurement in the morning until the latest measurement in the evening was used in the analysis.

## 3.6. Total energy expenditure

TEE was determined at the end of the bed rest period (day 46 to 56) and one year after the bed rest by the doubly labeled water (DLW) methodology described by Schoeller et al. (17).

After providing baseline urine samples, the subjects ingested a premixed 2g/kg estimated total body water (TBW) dose of DLW. The dose was composed of 0.2 g/kg estimated TBW of 10% $H_2{}^{18}O$ (Cambridge Isotope Laboratory, USA) and 0.15 g/kg estimated TBW of 99% $^2H_2O$ (Cambridge Isotope Laboratory, USA). Urine samples were collected 3 and 4h post dose, after equilibration of the isotopes with body fluids. During the bed rest period, two urine voids were collected at one hour of interval on day 10 after DLW ingestion for end points

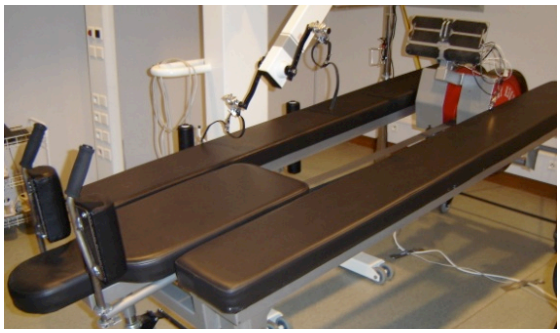
measurements. During the free-living conditions, the measurement period was extended to 14 days and two additional samples were collected on day 6 and 8 post-dose. For this free-living measurement, the volunteers were dosed at the Institute of Space Medicine but the sample collection on days 6, 8 and 14 were performed by the volunteers themselves at home, after having been thoroughly briefed on all the crucial details of the method. Urines were stored on sealed cryotubes and send by postal mail.

Urine were treated by black carbon and filtered on regenerated cellulose filters as previously described in details (18). 0.1µL of the treated samples was injected in an elemental analyzer (Flash HT, ThermoFisher) connected to a continuous flow isotope ratio mass spectrometer (Delta V, ThermoFisher). Samples was thermo-converted to $H_2$ and CO at 1400°C on glassy carbon. $H_2$ and CO were further separated at 104°C on a GC column before sequential analysis of deuterium and 18-oxygen isotopic abundances. The results were expressed relative to a standard mean ocean water scale using two laboratory standards. Analyses were performed in quadruplicate and repeated if the SD exceeded 2 ‰ for deuterium and 0.5 ‰ for 18-oxygen.

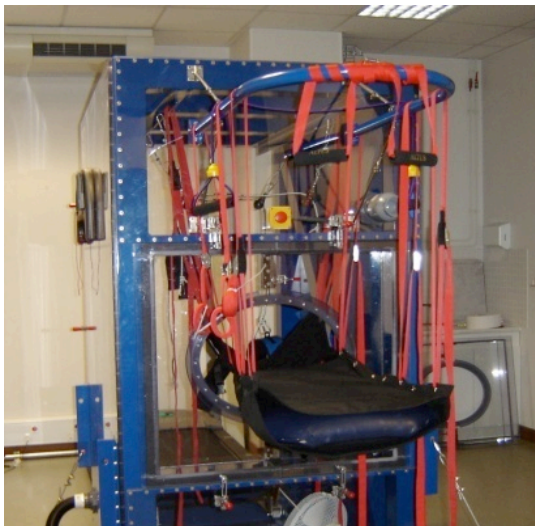
The TBW was calculated from the average of the dilution spaces of deuterium and oxygen-18 after correction for isotope exchange by 1.041 and 1.007, respectively (11). The average space dilution ratio was 1.021±0.009 in free-living conditions and 1.020± 0.003 during the bed rest. The $CO_2$ production was estimated according to Racette et al. (19), and the TEE was derived using the classical indirect calorimetry equations and assuming a respiratory quotient of 0.86.

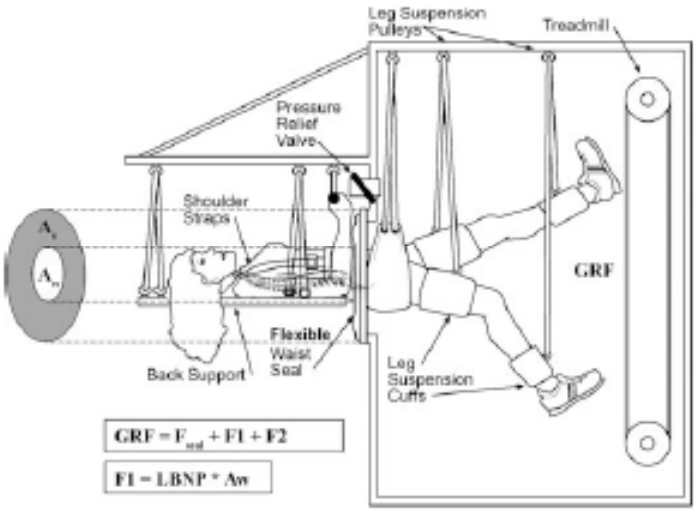
## 3.7. Resting metabolic rate, diet-induced thermogenesis, and activity-related energy expenditure

In ambulatory control period and at 32 days of bed rest, each subject was awakened at 0630am. The resting metabolic rate (RMR) was measured by indirect calorimetry (Deltatrac II, GE, USA) for 1 hour under the supervision of the investigators. The first 5-min of data were excluded as were 2-min of data following any movement or loss of wakefulness. Then, a breakfast representing 50% of the RMR in energy (44% carbohydrate, 15% protein and 41% fat) was offered to the participants. Diet-induced thermogenesis (DIT) was calculated as the increment of RMR during the 4-hour post breakfast and expressed in % of the energy intake. TEE minus RMR minus DIT was computed as an estimate of energy expended for physical activity (AEE). One year after the end of the bed rest, RMR, but not DIT, was measured for 1 hour in fasting state. Consequently, we used the DIT value measured in ambulatory control period to estimate free-living AEE. The physical activity level (PAL) was calculated as the ratio of TEE to RMR.



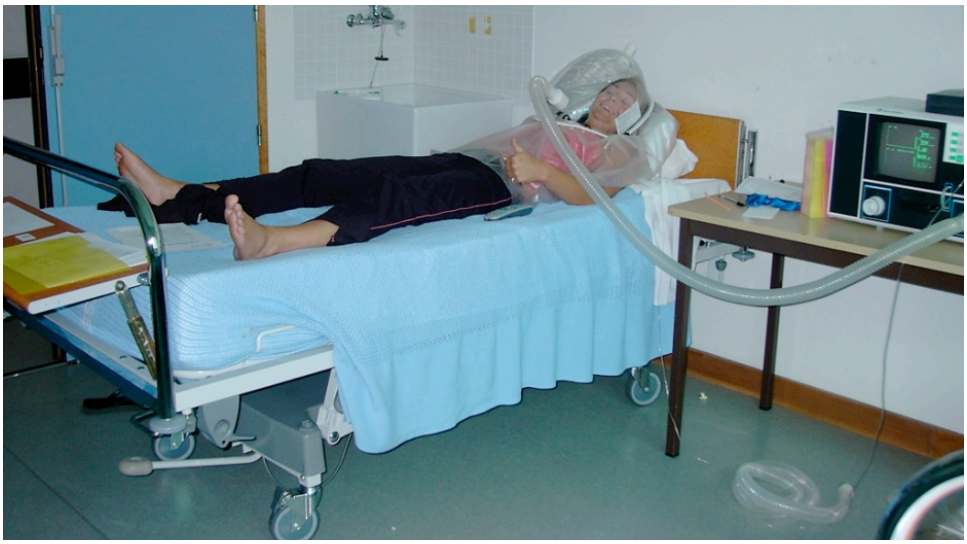
**Flywheel (Resistive exercise)**






**Low body negative pressure-treadmill (aerobic exercise)**

**Typical meal**



**Indirect calorimetry (DATEX)**

## 3.8. Body mass and composition

Body mass was measured daily on a special supine weighting device. FM and fat free mass (FFM) were measured twice during the ambulatory period, every fifteen days during the BR period and one year after the end of the experiment by Dual Energy X-RayAbsorptiometry (DXA) on QDR 4500 W scanner using the version software 11.2 (Hologic France). During bed rest, FFM measured by $^{2}H$ and $^{18}O$ dilution agreed within 2.5±2.6% with FFM measured by DXA ($y=1.025x$, $R^2=0.95$, $p<0.0001$ with a non significant intercept of -0.3kg being removed from the equation).

## 3.9. Hormone measurements

Fasting blood samples were collected at baseline, and at 30 and 60 days of bed rest. Fasting glucagon like-peptide 1 (GLP1), leptin and ghrelin were measured in duplicate using the Bioplex Diabetes® (Biorad, France). The overall intra and inter-assay precisions were ≤20 and ≤30%, respectively, the accuracy (%recovery) ranges between 70-130% and the cross-reactivity was <0.5%. Total PYY was measured in duplicate by the Millipore single Plex (Millipore France) having an intra- and inter-assay variation <11 and <19%, respectively and a recovery of 107%. Analyses were done at the core facility of the Saint Antoine Hospital in Paris, dedicated to micro-assays (IRSSA, Inserm IFR65).

## 3.10. Data and stastitical analysis

Prior analysis, most data were average to a 15-day time frame to increase the readability of the results and facilitate comparison with changes in body composition. Before the statistical analysis, the normality of the data was ascertained by using the Kolmogorov-Smirnov test. All variables were analyzed by a multiple analysis of variance (MANOVA) with time as the repeated measure (ambulatory versus bed rest) and group (control versus exercise) as main effect. Post hoc were performed using the Tuckey test. Punctual comparison between the control and exercise groups as well as between the ambulatory/free-living and bed rest periods were performed by unpaired and paired t-test, respectively. Simple regressions were performed to interpret the data. All statistics were performed using Statistica version 7.1.515.0 (Statsoft, Paris, France). Reported values are means±SD (unless otherwise stated), with $p<0.05$ statistically significant.

# 4. RESULTS

## 4.1. Body composition changes during the bed rest

Body mass and composition changes are shown in **Figure 1**. After 2 months of inactivity, body mass decreased similarly in both groups (-3.3±0.3 kg; $p<0.0001$). The loss in body mass was essentially due to a reduction of -2.9±0.1 kg in FFM ($p<0.0001$) in the control group while it was mainly explained by a decrease in FM (-1.9±0.3 kg; $p=0.001$) in the exercise group. The exercise training partially

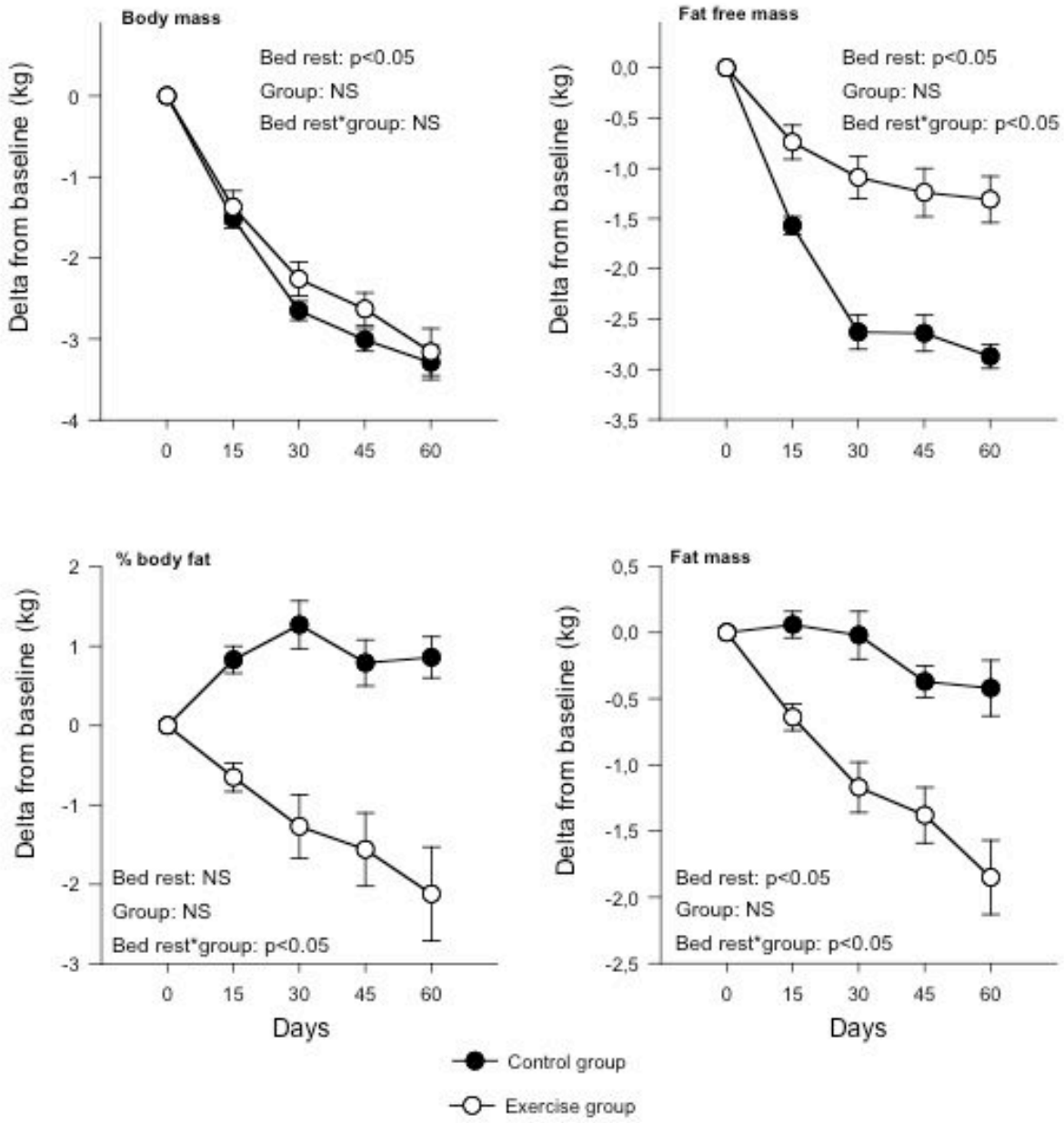



**Figure 1:** Body mass and composition during the bed rest expressed as changes (kg) from ambulatory baseline period in the control (n = 8) and exercise (n = 8) groups. Bed rest and group effects and bed rest-by-group interactions (Bed rest*group) are noted on each figure.

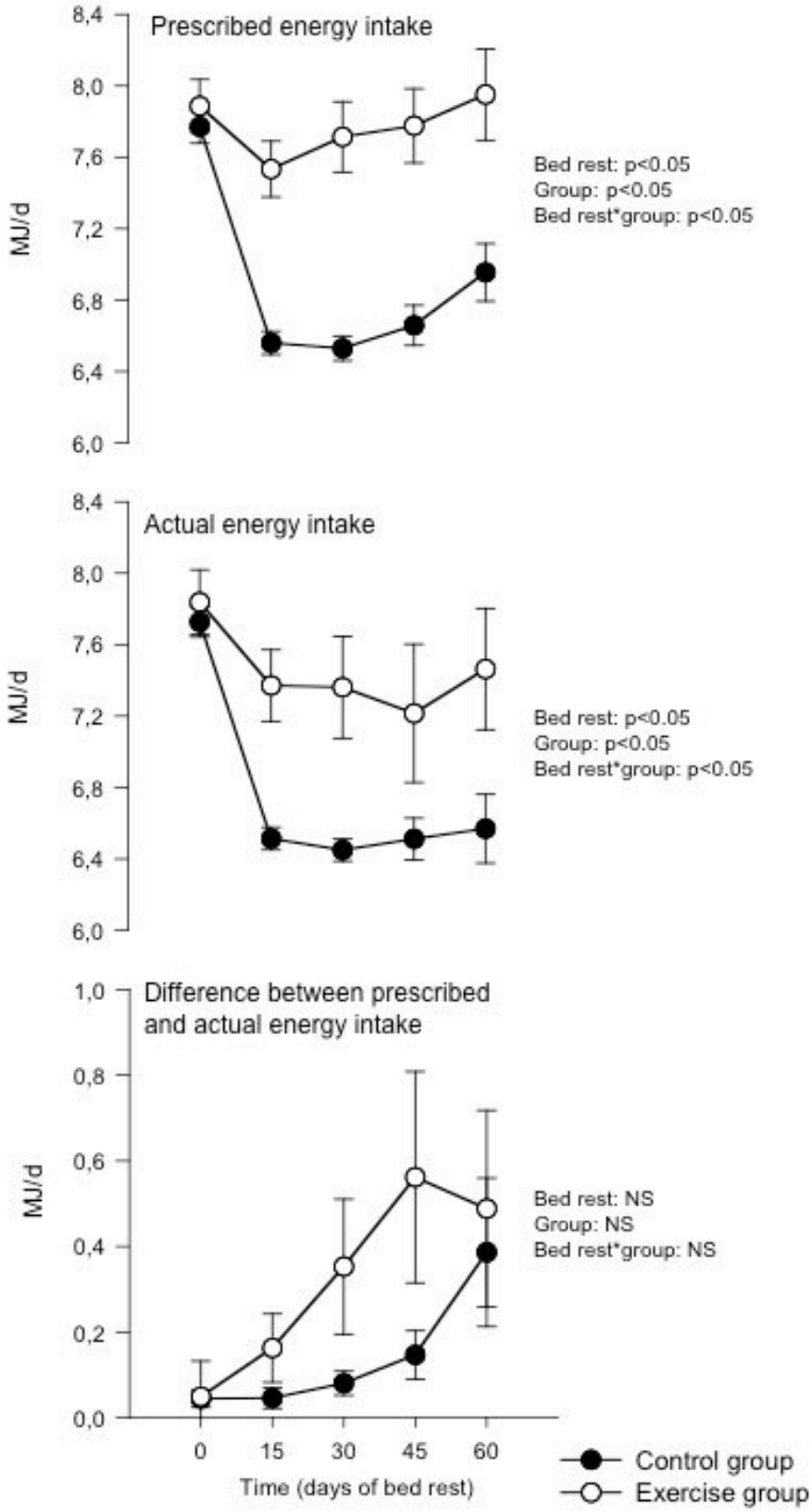



**Figure 2:** Time course of prescribed and actual energy intake and the differences between both of them during the bed rest (in MJ/d) in the control (n = 8) and exercise (n = 8) groups. Bed rest and group effects and bed rest-by-group interactions (Bed rest*group) are noted on the left side of each figure.

counteracted the physical inactivity-induced muscle atrophy (bed rest-by-group interaction: $p$=0.0006) and FM was maintained in the control group (bed rest-by-group interaction: $p$=0.005). These changes resulted in a significant drop in the percentage of body fat in the exercise group between the ambulatory period (24.6±4.9%) and the end of the bed rest (22.6±6.2%). No change was noted in the control group (26.2±5.6 vs 27.1±5.5%, bed rest-by-roup interaction: p=0.004).

## 4.2. Prescribed and spontaneous energy intake during the bed rest

Prescription of a maintenance energy intake is difficult during bed-rest. It is not possible to use body weight as an indicator of imbalance and thus adjust the prescription because bed-rest related muscle atrophy can lead to loss of fat-free that can mask fat mass gains and hence energy imbalance. Moreover, our study design called for provision of sufficient energy to maintain balance with *ad libitum* intake around that provision without weight clamping. To test the accuracy of our prescription, we compared it with TEE measured during the final phase of bed-rest.

The prescribed EI and diet composition provided to the control and exercise groups during the ambulatory control and bed rest periods of the study are compared to the spontaneous EI of the volunteers during the experiment in **Table 2**. The differences in the prescribed EI during the bed rest period between the two groups correspond to the estimated cost of the exercise training protocol. During the bed rest, the volunteers spontaneously reduced their EI below the prescription, which is represented by the increase in the difference between the prescribed and the actual EI (**Figure 2**). The volunteers in the control group consumed almost the total quantity of provided meals during all the period of the bed rest (99% and 97% during the ambulatory control period and the first 45 days of bed rest, respectively), but they ate 95% of the prescribed EI during the last 15 days of the bed rest (p=0.07 compared to the ambulatory period). The exercise group already consumed 93% of the prescribed EI between the 30$^{th}$ and the 45$^{th}$ day of bed rest and then 94% between the 45$^{th}$ and the last day of the bed rest.

## 4.3. Energy expenditure during the bed rest

The exercise group had a significant higher TEE than the control group during the BR (7.12±0.78 vs 8.85±1.14 MJ/d, p=0.004, **Figure 3A**). RMR did not differ between the control (4.98±0.45 MJ/d) and the exercise (5.30±0.40 MJ/d) groups. No between-group difference was also noted in DIT (control: 0.43±0.13 MJ/d; exercise: 0.52±0.17 MJ/d). Consequently, the difference in TEE between the two groups was mainly accounted for by a greater AEE of the exercise group than in the control group (3.02±1.09 vs 1.71±1.09 MJ/d, respectively, p=0.031) likely due to the exercise training.

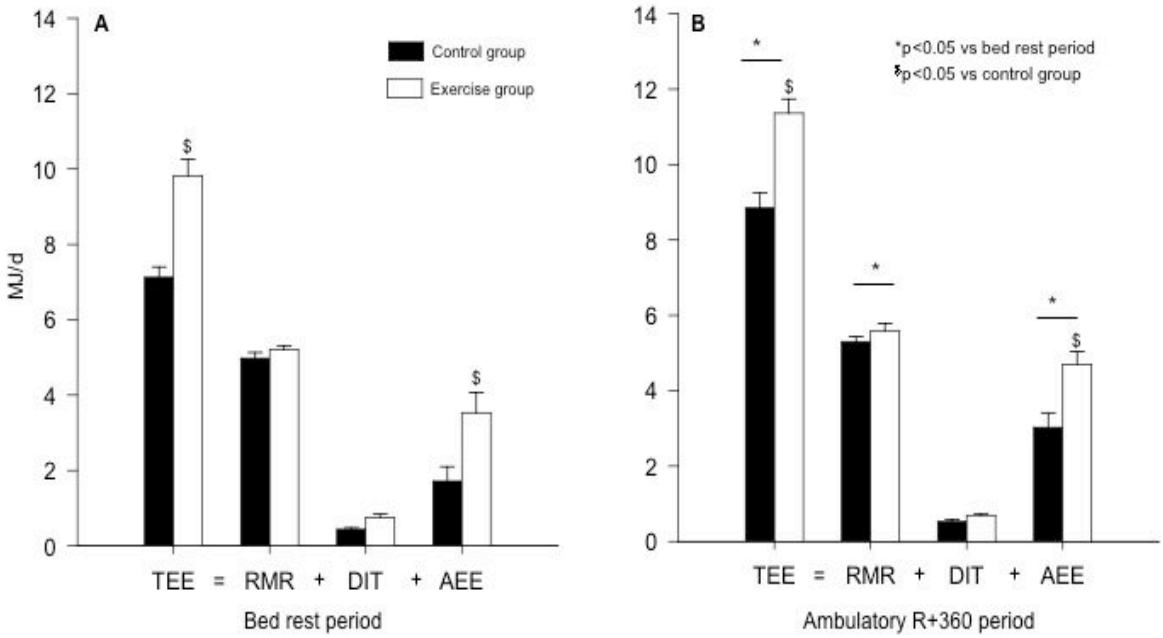



**Figure 3:** Total energy expenditure (TEE) and its components, i.e. resting metabolic rate (RMR), diet-induced thermogenesis (DIT), activity-related energy expenditure (AEE) expressed in MJ/d in bed rest period (Figure A; n=8 in both control and exercise groups) and ambulatory period one year after the end of the experiment (R+360; Figure B; n=6 in control group and n =8 in exercise group). Differences between the groups are noted on the figure.

## 4.4. Energy balance after 60 days of bed rest

EB relative to DLW TEE were negative in both the control (-0.6±1.0 MJ/d, p=0.13 vs zero) and exercise (-1.4±0.8 MJ/d, p=0.001 vs zero) groups (**Figure 4**). This is in good agreement with the EB calculated from the changes in body composition over the 60 days of the bed rest showing an EB of -0.5±0.4 MJ/d in the control group and -1.5±0.5 MJ/d in the exercise group. The difference between TEE and the prescribed EI during the DLW period was -0.2±1.0 MJ/d in the control group and of -0.9±0.8 MJ/d in the exercise group. This gap was only significantly different from zero (p=0.01) in the exercise group. During this same period, the difference between the prescribed and the actual EI was similar between the two groups (-0.4±0.2 MJ/d and -0.4±0.2 MJ/d, in control and exercise groups, respectively) and not different from zero in both groups. The difference between the prescribed EI and TEE explained 70.8% of the variability in EB ($R^2$=0.78; y=0.80 x – 0.52; p<0.0001). EB also tended to negatively correlate with the difference between the prescribed and actual EI ($R^2$=0.22; y=0.84 x – 0.55; p=0.066).

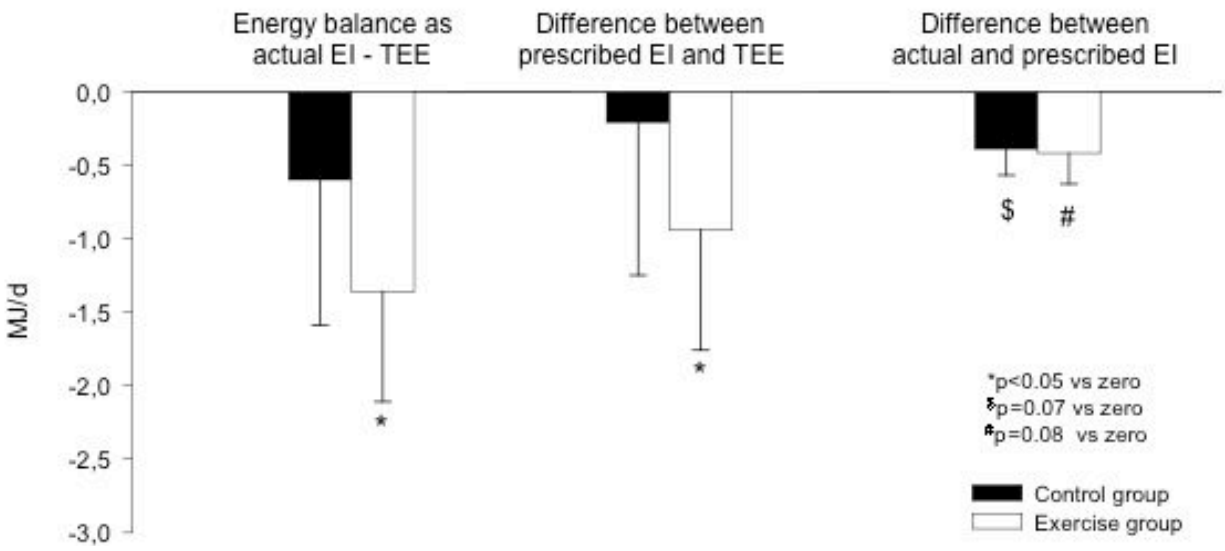



**Figure 4:** Energy balance calculated as energy intake (EI) minus total energy expenditure (TEE), difference between prescribed EI and TEE and difference between actual and prescribed EI in MJ/d measured between the 46th and 56th day of bed rest in the control (n=8) and exercise (n=8) groups. The differences with zero are noted on the figure.

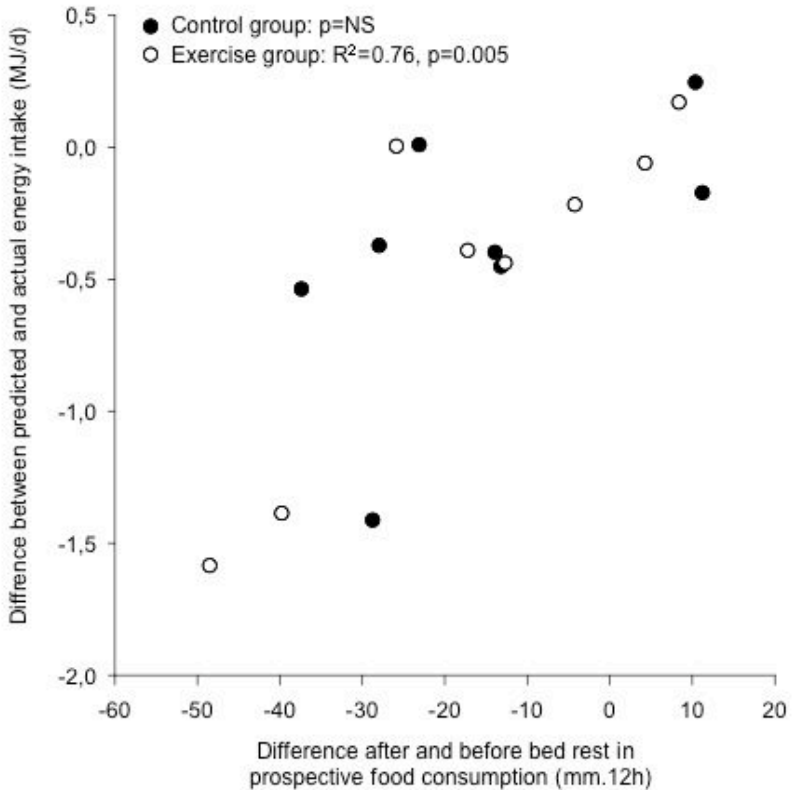



**Figure 5:** Regression analysis between the bed rest-induced change in 12h-cumulated desired food consumption (mm.12h) and the difference between actual and predicted energy intake (MJ/d) at the end of the bed rest in both control and exercise groups (n=8 in each group). As indicated on the figure, whereas a positive correlation was observed in the exercise group, no relationship was noted in the control group.

**TABLE 2 .** Average dietary intake in ambulatory control and bed rest periods

| | | Control group | | Exercise group | | MANOVA (p-value) | | |
|---|---|---|---|---|---|---|---|---|
| | **Units** | **Ambulatory period** | **Bed-rest period** | **Ambulatory period** | **Bed-rest period** | **Bed rest effect** | **Group effect** | **Bed rest-by-group interaction** |
| **n** | | 8 | 8 | 8 | 8 | | | |
| **Prescribed energy and macronutrients intake** | | | | | | | | |
| **Energy intake** | MJ/day | 7.7 ± 0.3 | 6.7 ± 0.3 | 7.9 ± 0.7 | 7.7 ± 1.0 | 0,0001 | 0,005 | 0,0002 |
| **Glucides** | g/day | 269 ± 8 | 225 ± 8 | 271 ± 12 | 267 ± 21 | 0,0001 | 0,001 | 0,0001 |
| | % | 58 ± 0 | 57 ± 1 | 58 ± 1 | 58 ± 1 | | | |
| **Lipids** | g/day | 62 ± 2 | 53 ± 2 | 63 ± 3 | 62 ± 4 | 0,0001 | 0,004 | 0,0001 |
| | % | 30 ± 0 | 30 ± 1 | 30 ± 0 | 30 ± 0 | | | |
| **Proteins** | g/day | 56 ± 4 | 55 ± 4 | 59 ± 7 | 57 ± 6 | 0,0001 | NS | NS |
| | % | 12 ± 0 | 13 ± 1 | 12 ± 1 | 12 ± 1 | | | |
| **Spontaneous energy and macronutrients intake** | | | | | | | | |
| **Energy intake** | MJ/day | 7.5 ± 0.2 | 6.5 ± 0.2 | 7.6 ± 0.5 | 7.4 ± 0.8 | 0.0001 | 0.039 | 0.004 |
| **Glucides** | g/day | 259 ± 7 | 219 ± 9 | 261 ± 16 | 252 ± 27 | 0.0001 | 0.030 | 0.002 |
| | % | 58 ± 1 | 56 ± 1 | 58 ± 1 | 57 ± 1 | | | |
| **Lipids** | g/day | 59 ± 2 | 52 ± 2 | 59 ± 4 | 59 ± 6 | 0.001 | 0.027 | 0.0018 |
| | % | 29 ± 0 | 30 ± 0 | 30 ± 0 | 30 ± 0 | | | |
| **Proteins** | g/day | 56 ± 4 | 54 ± 4 | 59 ± 6 | 56 ± 6 | 0.01 | NS | NS |
| | % | 12 ± 1 | 14 ± 1 | 13 ± 1 | 13 ± 1 | | | |

%, Percentage of daily energy intake.

Values are mean ± SD

No between-group differences were noted (unpaired t-test) in ambulatory period. The effect of bed rest was determined by MANOVA.

The between-group differences in the energy intake corresponds to the estimated cost of the exercise training protocol

**TABLE 3.** Mean daily subjective ratings of motivation to eat and food preferences during the bed rest

| | Ambulatory control period | | After 15 days of bed rest | | After 30 days of bed rest | | After 45 days of bed rest | | After 60 days of bed rest | | MANOVA (p-value) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Control | Exercise | Control | Exercise | Control | Exercise | Control | Exercise | Control | Exercise | Bed rest effect | Group effect | Bed rest-by-group |
| **Hunger** | 52 ± 10 | 52 ± 12 | 49 ± 14 | 42 ± 18 | 41 ± 23 | 38 ± 22 | 37 ± 22 | 40 ± 23 | 35 ± 21 | 43 ± 22 | NS | NS | NS |
| **Fullness** | 73 ± 11 | 69 ± 15 | 70 ± 10 | 76 ± 14 | 74 ± 15 | 78 ± 14 | 79 ± 17 | 78 ± 16 | 80 ± 16 | 76 ± 18 | NS | NS | NS |
| **Prospective consumption** | 53 ± 26 | 59 ± 28 | 52 ± 19 | 49 ± 18 | 48 ± 29 | 41 ± 28 | 40 ± 26 | 42 ± 25 | 38 ± 22 | 46 ± 21 | NS | 0.025 | NS |
| **Prospective fatty food** | 109 ± 18 | 101 ± 18 | 110 ± 12 | 105 ± 11 | 113 ± 9 | 110 ± 8 | 112 ± 11 | 111 ± 10 | 113 ± 8 | 110 ± 7 | NS | NS | NS |
| **Prospective sweet food** | 73 ± 19 | 76 ± 23 | 78 ± 18 | 82 ± 20 | 84 ± 26 | 83 ± 26 | 89 ± 25 | 81 ± 23 | 89 26 | 79 ± 24 | NS | NS | NS |

Values are mean ± SD

n = 8 in each group. The values are cumulated visual analog scales (VAS on 100mm) and expressed in mm.12h. Cumulated VAS are area under the curve interpolated from the first measurement in the morning until the latest in the evening.

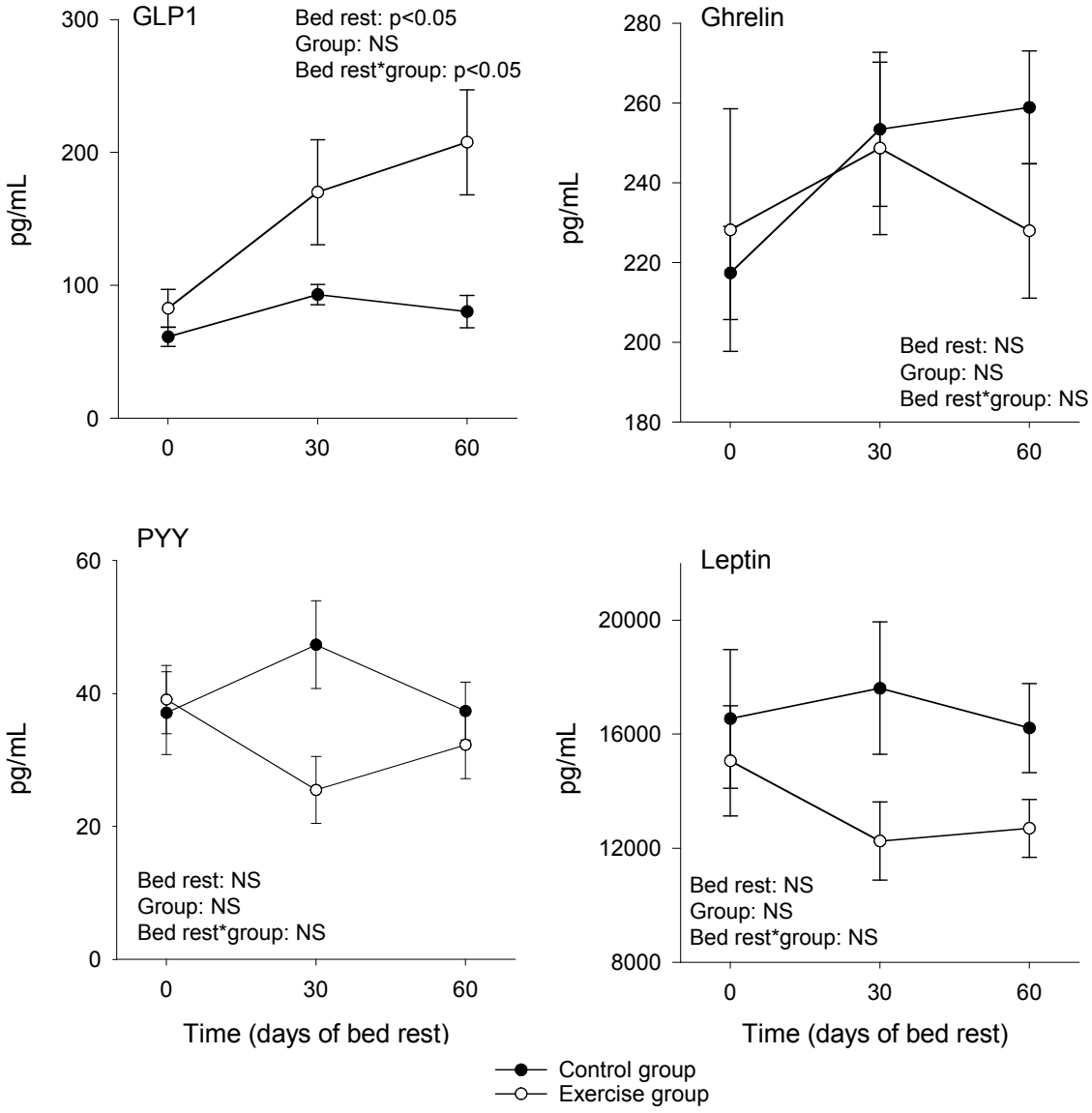



**Figure 6:** Time course of the fasting plasma glucagon like-peptide 1 (GLP1), leptin, ghrelin and total PYY concentration during the bed rest in control (n<8) and exercise (n<8) groups. Bed rest and group effects and bed rest-by-group interactions (Bed rest*group) are noted on each figure.

## 4.5. Subjective hunger changes during the bed rest

The average values for the subjective reports of hunger, fullness and desired (prospective) food consumption are shown in **Table 3**. There were no significant group effects for any of the subjective measures. The 12h-AUC for the desired food consumption decreased significantly by 25% gradually during the bed rest in both groups ($p=0.025$). The 12h-AUC for hunger and fullness did not significantly vary during the bed rest. The preferences towards fatty or sweet food were also not affected by the bed rest in both groups.

The variation in 12h-AUC for the prospective food consumption between the end of the bed rest and the ambulatory period positively correlated with the differences between the prescribed and actual EI averaged during the last 15 days of bed rest in the exercise group ($R^2 =0.76$; $y=0.028x - 0.014$; $p=0.005$; **Figure 5**). No such relationship was noted in the control group ($R^2=0.33$, $p=0.13$).

## 4.6. Hormonal pattern changes during the bed rest

With the Multiplex® and Bioplex® technologies, we did not obtained results from 1-2 subjects per hormone. In those cases the software reported that the values were out of the range of the standard curves. Bed rest-induced changes in fasting plasma hormone are represented in **Figure 6**. Fasting plasma ghrelin, PYY, and leptin did not vary during the bed rest period in both groups. There was no significant difference between the two groups for all these hormones.

At all time points, leptin concentrations were strongly associated with FM (at baseline: $R^2=0.89$, $p<0.0001$, **Figure 7**). Given the between group difference in FM changes during the bed rest, the lack of significant change in leptin was unexpected. Interestingly, the fasting concentrations of leptin adjusted for FM negatively correlated with the spontaneous EI ($R^2= 0.49$, $p=0.006$, Figure 7).

Fasting plasma GLP-1 was significantly higher in the exercise group than in the control group ($p=0.014$; Figure 6). GLP-1 concentration increased in the two groups ($p=0.0076$) but with a different pattern (bed rest-by-group interaction: $p=0.048$). A post-hoc Tukey test showed that in the control group GLP-1 rose from 61±20 pg/ml in the ambulatory period to 93±22 pg/ml after one month of bed rest ($p=0.025$) and then decreased to 80 ± 34 pg/ml after two months of bed rest. In the exercise group, GLP-1 continuously increased throughout the bed rest period to reach 208±112 pg/ml (vs. 83±40 pg/ml in the ambulatory period; $p=0.015$) at 60 days of bed rest. We observed a positive relationship between GLP-1 during the bed rest period and the 12h-AUC for desired food consumption ($R^2=0.29$; $p=0.04$, Figure 7).

## 4.7. Characteristics of the volunteers after one year of follow-up

All the volunteers recovered their initial body mass and composition one year after the end of the intervention (Table 1). In free-living conditions, the exercise group had a significant higher TEE than the control group (9.8±1.2 vs. 11.4±0.9 MJ/d, respectively; $p=0.02$, **Figure 3B**). The difference in TEE between the two groups was mainly accounted for by a greater AEE in the daily life of the exercise group (4.70±0.82 MJ/d; $p=0.04$) compared to the control group (3.53±1.52 MJ/d). AEE normalized for body mass was not different between the two groups in either period.

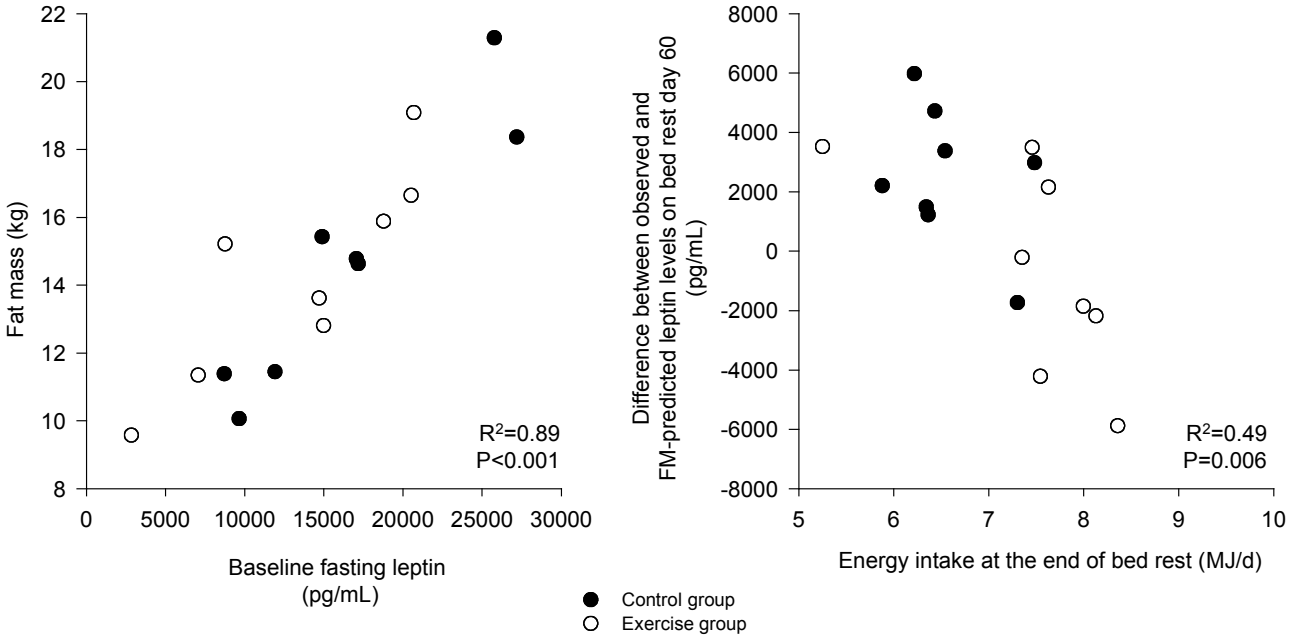



**Figure 7:** Regression analysis between fat mass (FM; kg) and fasting plasma leptin (pg/ml) at baseline (at left) and between the spontaneous energy intake at the end the bed rest and differences between observed and FM-predicted leptin levels on bed rest day 60 (at right) in the control (n=8) and exercise (n=8) groups. The relationships are noted on the figure.

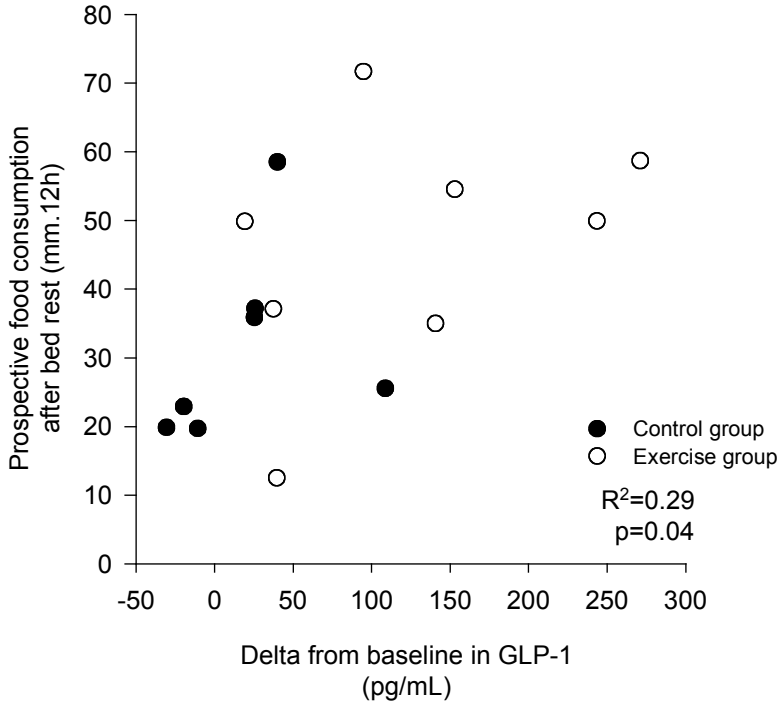



**Figure 8:** Regression analysis between the bed rest-induced change in glucagons-like peptide-1 (GLP1) in pg/ml and 12h-cumulated prospective food consumption at the end of bed rest in mm.12h in the control (n=8) and exercise (n=8) groups. The relationship is noted on the figure.

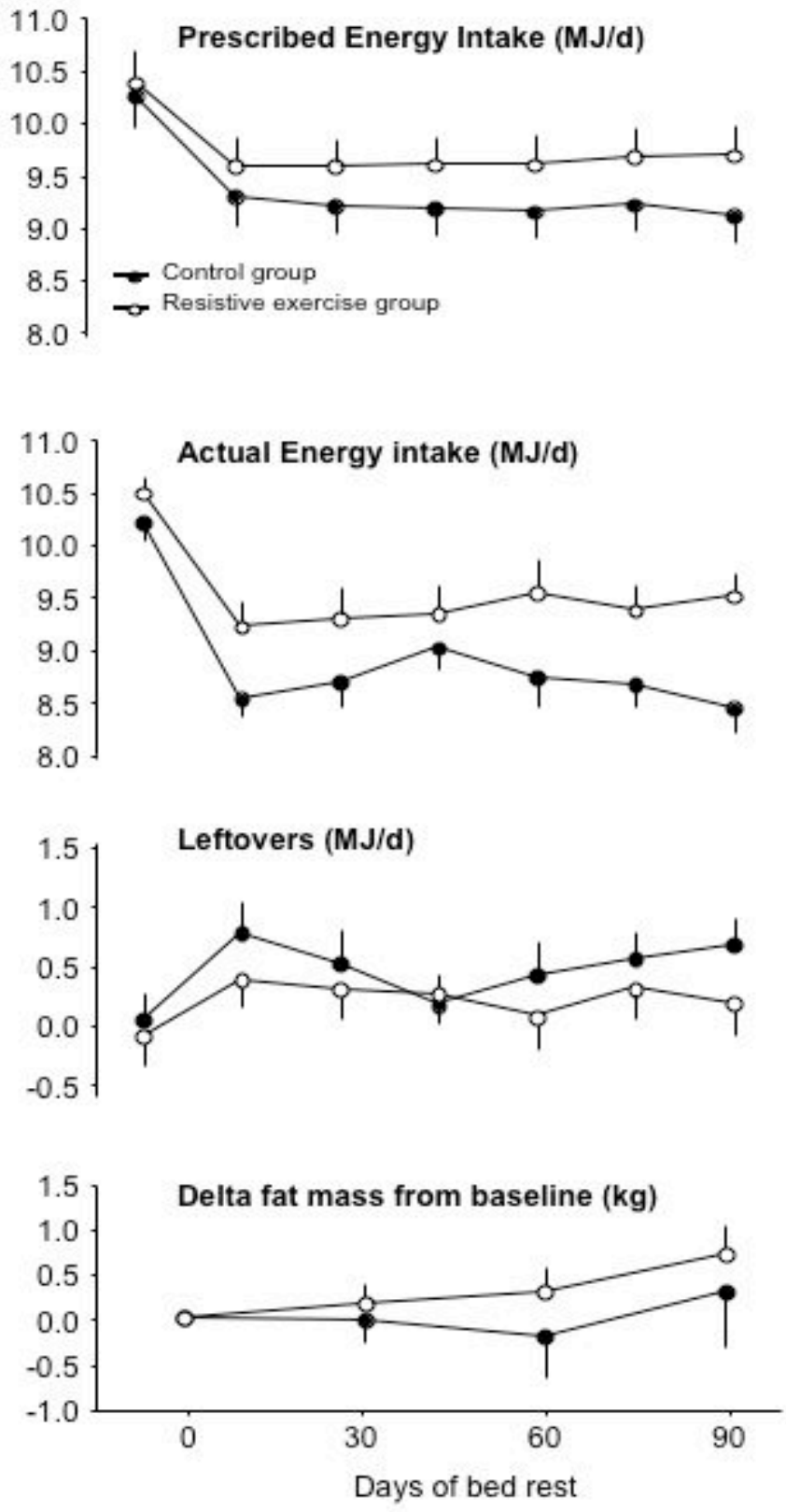



**Figure 9:** Results of prescribed and actual energy intake, leftovers and changes in fat observed during a 90-day bed rest conducted in men with (n=7) and without (n=8) resistive exercise performed for 35 min every three days at maximal power contraction. Adapted from reference (27).

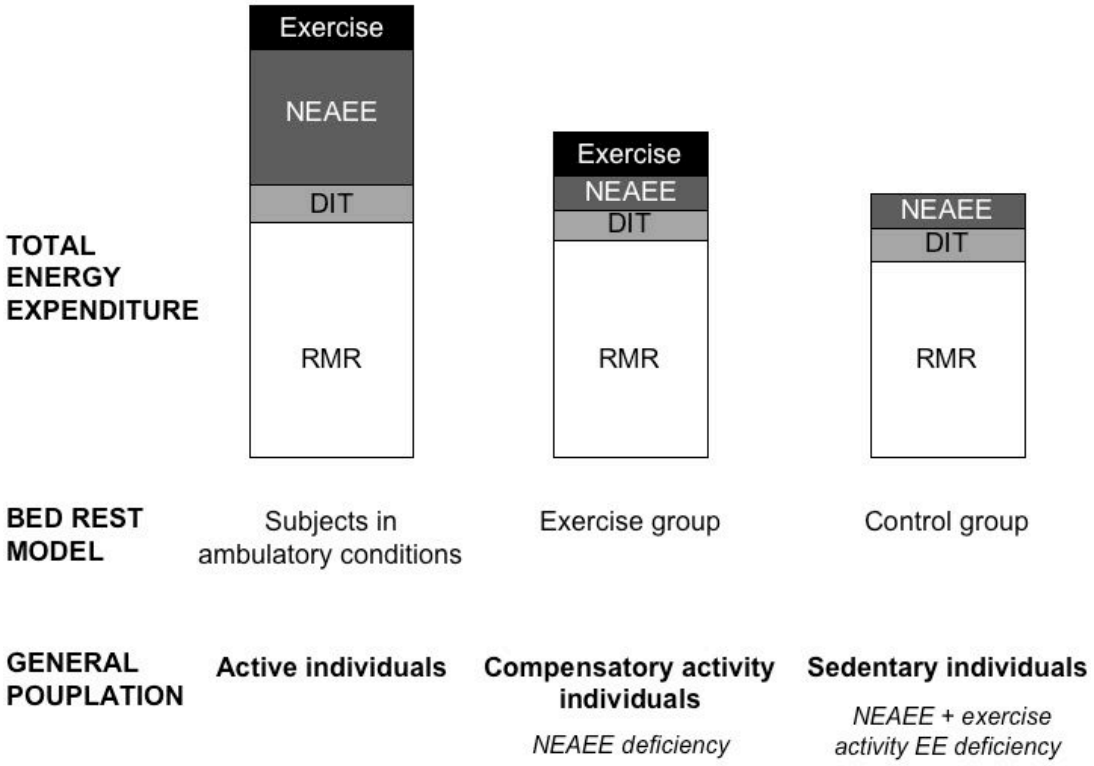



**Figure 10:** Schematic representation of the components of total energy expenditure during bed rest, conducted with or without exercise training. Exercise: exercise energy expenditure, NEAEE: non-exercise activity energy expenditure, DIT: diet-induced energy thermogenesis, RMR: resting metabolic rate.

# 5. DISCUSSION

The present study aimed to test whether or not a long-term decrease in TEE due to an extreme sedentary behavior will affect EB regulation. Two patterns of responses emerged from the study depending on whether or not exercise was added to bed rest. Those who were in the control group decreased their EI sufficiently to maintain EB, while those in the exercise group decreased their EI by a comparable amount, but due to their higher TEE, were in negative EB.

The female volunteers in the control group had a PAL of 1.45 during the 2-month bed rest due to a 51% decrease in AEE. A 42-day bed rest in male also reported a PAL of 1.47 (9). These PAL values are lower than the average ambulatory PAL of 1.80, but not as low as the generally accepted minimal PAL of 1.3 that is the requirement for total inactivity (20). A difference in our study is that we did not provide food at the ambulatory levels as have many other studies. This may have influenced our results, however, the volunteers were free to ask for extra-meal servings any time and were offered snacks. In addition they were not required to eat all the meals or snacks. Because of this, they had enough latitude to adjust their EI as part of their adaptation to the study conditions. Interestingly, rather than responding with positive EB as have been reported in short-term studies (3-5), they spontaneously decreased their EI by 16% compared to the ambulatory period and EB was not significantly different from zero. One may say that EB was mild negative (-0.6±1.0 MJ/d, p=0.13) at the end of the bed rest, even when more food was offered to them. However, we can note that the volunteers' EI reached significance relative to the prescribed EI only during the last 15 days of bed rest, which was when we measured TEE. It is therefore probable that those changes were dependent of the increase in the research activities due to the various outcome measures performed at the end of this particular bed rest.

Similar results were observed in a previous 42d-bed rest during which healthy men were provided excess amounts at meals in a more traditional *ad libitum* manner. In that study, the male subjects also decreased their dietary intake by 16% compared to ambulatory (21) and were in EB during bed-rest (9). To complete these observations and thus to have further evidences regarding the adaptive response of EB to physical inactivity, we reanalyzed data from the 90-day Toulouse long-term bed rest conducted in 2001/2002 in men and published elsewhere (REF). Energy prescription was based on a 35 kcal/kg/d food allotment reduced arbitrarily by 200 kcal/d at the beginning of the bed rest to avoid the well-described initial passive overeating period. As during WISE2005, the volunteers were free to ask bigger size portions or to not finish their meals. As illustrated in **Figure 9**, EI and leftovers remained quite stable over the 90-day study. A more detailed analysis of the actual EI revealed a small trend for overeating during the first 45 days of bed rest that was fully compensated during the last part of the bed rest, overall resulting in a global stable fat mass (change after 90 days of bed rest: 0.3±0.6kg). Taken together, these results suggest that the volunteers subjected to long-term physical inactivity induced by bed rest adjusted their EI to EE and were in EB during most of the studies.

The present results might appear contradictory to what has been observed repeatedly in the few short-term studies by Murgatroyd (3), Shepard (4) and Stubbs (5). Murgatroyd et al. (3) reported a positive energy balance of 2.6MJ/d in normal-weight men confined in a calorimeter chamber for 1d and fed *ad libitum*. Their volunteers were extremely sedentary with a PAL of 1.25. A PAL of 1.25 in subjects confined in a small area is however surprising compared to the physical activity of

1.45 x RMR that we measured in our control subjects. Nonetheless, a lack of compensation of EI for the decrease in EE was also reported in individuals with a PAL of 1.4 for 7days (5). Stubbs et al. observed no tendency for EI to begin to decrease in response to a consistent reduction in TEE. These contrasting findings, however, may indeed be complementary in that the regulatory process may require about one week to be fully effective. In our previous 42-day bed rest (9), we observed that adaptation of EI to TEE took place within the first week of bed rest. Although our results showed that EB can be achieved after long-term physical inactivity due to relatively regulated feeding behavior, we strongly think that the short-term studies are of particular importance in the context of obesity. In the general population, physical inactivity can be viewed as episodes of different duration, but of increasing frequency the past few decades. The short-term episodes are non-compensated for by changes in EI, and thus could lead to an ever cumulative positive EB, weight gain and ultimately obesity.

The secondary aim of the present study was to investigate the effect of a combined aerobic and resistance exercise training performed concomitantly to the bed rest on the adjustment of EI to TEE. Although TEE was significantly reduced by 22% due to a decrease in AEE by 36% compared to the EE measured in their daily life, the volunteers in the exercise group had a PAL of 1.68. This represents an active status for the general population (22, 23). Interestingly, the exercise group were in negative EB (-1.36 ± 0.75 MJ/d). This negative EB was due to an excessive decrease in EI compared to the drop in TEE during the bed rest. This reduced EI may be an influence of our experimental design coupled with an underestimation of the energy requirements related to the energy cost of the exercise. Importantly, the subjects were allowed to request more food, yet instead of asking for extra food, the volunteers spontaneously like the control group ate less than the prescribed EI. This voluntary reduction in EI is well represented by the relationship between the subjective desired food consumption and the differences between the prescribed and actual EI.

These results are interesting when considering the ongoing research on the non-exercise activity energy expenditure, also called non-exercise activity thermogenesis, and can be seen as highly complementary to the studies performed by Stubbs et al. in both men and women in intervention studies of 7d (24, 25) and 16d (26). In one of these studies (24), conducted in men over 7 days, a graded increase in TEE due to medium (about 1.6 MJ/d) or high exercise regimens (about 3.2 MJ/d) markedly elevated TEE and was not compensated by any increase in EI, which in turn precipitated a negative EB. Rather TEE decreased in both treatments because of the gradual drop in non-exercise activity energy expenditure. Whereas Stubbs et al. (26) assumed that accurate adjustments of EI to acute increases in TEE are likely to take weeks rather than days, our results suggest that this lack of compensatory effect on EI is independent of the duration of the experiment. In fact, the authors reported a partial compensation (about 30%) for the exercise induced-energy deficit (26) on a longer time-scale through a decrease in TEE in both treatments because of a gradual drop in NEAEE. Interestingly, during bed rest the volunteers can be considered as deficient in non-exercise activity expenditure (**Figure 10**). Consequently, an exercise-training program that significantly impacts on TEE is expected to induce a negative EB because no regulation can be achieved through non-exercise activity expenditure modulation. The direct corollary is that exercise program with small impact on TEE will have a small effect on EB regulation. The results of the present study strongly support those hypotheses. The combined resistive and aerobic exercises increased TEE by about 19% and results in a significant negative EB (-1.36±0.75 MJ/d) and a loss in fat mass. On the other hand, during the 90-day bed rest in men (27), the impact of the resistive exercise program performed every 3 days for 35 min had only an

estimated impact on TEE of 2% and, as seen in Figure 9, the fat mass remained stable with a light tendency to increase. Based on the body composition changes, the estimated EB was calculated at only 395kJ/d over the 90-day period.

In the present study, there was clearly no tendency for restoring EB through EI. Whereas Stubbs et al. (26) assumed that accurate adjustments of EI to acute increases in EE are likely to take weeks rather than days, our results suggest that this lack of compensatory effect on EI is independent of the duration of the experiment. Although our subjects cannot be compared to athletes such observation was already made (28, 29). Even a 20% increase in TEE for 40 weeks due to marathon training induced no increase in EI (28). It is therefore remarkable the extent to which the subjects did not compensated and so tolerated a marked negative EB over periods amounting to 7d (24, 25), 16d (26) and even 60d.

These results may appear to contrast to many long-term, outpatient studies (up to 12 weeks) that investigated the effect of added exercise on changes in body composition and that indicate that compensation for the exercise is improved as evidenced by a small loss of FM (no larger than 1kg on average) (30). In fact, we think these studies are complementary as compensation was shown to occur either by increases in EI or by decreases in non-activity exercise (32,33,34). The decrease in non-activity exercise is an interesting hypothesis to explain the difference between our study (in non-exercise deficient subjects in who compensation can only occur through modulation of EI) and the long-term outpatients studies (in subjects with large modulation possible through non-exercise activity).

The measurement of several satiety/satiation signals provided complementary data to understand EB regulation during physical inactivity with and without exercise. There are a variety of gut hormones and other peripheral signals that influence the hypothalamus and brainstem that control EB (31). These include leptin, ghrelin, PYY and GLP1 among others. While leptin is both a short-term and a long-term signal, the others are involved in short-term mechanisms. Whereas ghrelin is the only endogenous peripheral hormone that has been shown to induce hunger and increase food intake, leptin, PYY and GLP1 are satiety hormones, which inhibit EI (32).

Although the subjects desired eating less during the bed rest and actually decreased their EI, long-term physical inactivity did not significantly affect the fasting plasma concentrations of the gut hormones. The lack of changes in leptin in the context of a long-term negative energy balance is quite interesting. Although no variation in fasting leptin concentration was noted, fasting leptin adjusted for FM were higher during the bed rest in the control group, as it was previously observed in a 7d-bed rest (33). Except a putative relationship with the increase in circulatory inflammatory markers observed during the bed rest (data not shown), we do not know how to explain a higher FM-adjusted fasting leptin levels in this context. It is interesting to note that FM-adjusted fasting leptin levels were strongly related to EI at the end of the bed rest for both groups. This suggests that overt short-term period leptin, adjusted for FM represents a potentially good biomarker of EI. Similar relation was observed with the rhythm of daily body temperature (ref). Further studies are clearly needed to support that observation. Since Stubbs et al. (5) reported no change in the subjective hunger after 7d of inactivity, we can assume that the physical inactivity impact on the satiety signaling system is a long-term process. Other additional satiety hormones, such as neuropeptide Y, cholecystokinin, pancreatic peptide and glucose-dependent insulinotropic peptide may also be involved in the regulation of the feeding behaviour under physical inactive conditions.

Interestingly, in addition to these previous changes in hormones, the exercise training significantly increased the fasting concentration of GLP1. Moreover, the changes in GLP1 concentration were weakly related, but negatively, to the subjective desired food consumption in the exercise group. A rise in GLP1, but also in PYY, was previously reported during an acute exercise at 65% of the maximal heart rate and associated with a relative decrease EI compared to exercise-induced EE in both normal-weight males and females (34). However, contrary to the PYY concentration, the elevated GLP1 concentration was sustained in post-exercise period (34). This difference in the time delay of the GLP1 and PYY response to the exercise may explain why we detected changes in GLP1 but not in PYY. Taken these results together, the weak coupling between EI and EE at high levels of EE induced by acute or chronic exercise may be mediated, at least in part, through the action of GLP1. However, whereas the phenomenon of 'exercise-induced anorexia' which produces a negative EB was thought to be a short-term process, it appears to be sustained on at least several weeks under the conditions of our study.

In this study, we investigated for the first time the impact of both long-term physical inactivity with and without exercise on feeding behaviour, EI and EB in healthy adults. While the EB is well regulated in the long-term, no such adjustment in response to exercise-induced EE was observed. The role of the deficiency in non-activity exercise induced by bed rest warrants further investigations but appears as attracting data to complement the numerous publications in which added exercise does not increase weight loss beyond 1 kg of FM in fully efficient non-exercise activity outpatients. The hormonal mechanisms involved in the regulation of the appetite sensation appear to be different in response to enforced inactivity or exercise training in which GLP1, but not leptin, ghrelin, or PYY, may be involved. Rather, leptin and fat mass-adjusted leptin appear good markers for both EB and short term EI. Further studies are warranted to better underline the mechanisms controlling appetite in response to EE changes. Others gut hormones known to act on the satiety should be investigated and in particular in post-prandial conditions rather than in fasting state.

Lastly, it is important to point out that these present findings may be not applicable to other populations than healthy women such as men, obese, children or elders. Similarly, our results may be affected to some extent by the important muscular atrophy, GI transit time, and the hypovolemia induced by the model. However, we think that bed rest provide a good model to investigate the physiology of physical inactivity and to understand the role of non-exercise and exercise physical activity.

# 6. ACKNOWLEDGEMENTS

AB collected and analysed the data, conducted the statistical analysis and drafted the manuscript. IM participated to the writing of the manuscript and gave relevant advices in the interpretation of the results. SB designed the study, helped with the collection, the statistical analysis and the interpretation of the data, corrected the first draft of the manuscript and get funding. CS assisted with the statistical analysis and data interpretation. DAS helped with the interpretation of the results with special reference to the energetics and corrected the English of the manuscript. SN strongly helped with the study organisation and the collection of the data. AZ performed all the isotopic analysis to determine the total energy expenditure. BL analysed the gut hormones. ARH provided us the data concerning the energy cost of the exercise. None of the authors had any personal or financial conflicts of interest with regard to the study.

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# CHAPITRE 5

# Effect of physical inactivity on the oxidation of satured and monounsatured dietary fatty acids: results of a randomized trial

Audrey Bergouignan, Dale A. Schoeller, Sylvie Normand, Guillemette Gauquelin-Koch, Martine Laville, Timothy Shriver, Michel Desage, Yvon Le Maho, Hiroshi Ohshima, Claude Gharib & Stéphane Blanc

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# Résumé

## Introduction

L'obésité est caractérisée par un excès de masse grasse, une hypertriglycéridémie, une insulino-résistance et une incapacité à utiliser les lipides en tant que substrats. Comme cette dernière n'est pas améliorée après une perte de poids, il a été suggéré que cette oxydation lipidique réduite serait une cause plutôt qu'une réponse adaptative à l'obésité. Si la génétique détermine une susceptibilité individuelle à l'obésité, il est bien établi que des facteurs environnementaux, tels qu'une alimentation riche en graisses et un mode de vie sédentaire, s'associent pour favoriser la prise de poids et expliquent l'augmentation de l'obésité dans nos sociétés. Néanmoins, les données sur les effets délétères de l'inactivité physique restent indirectes et reposent principalement sur des études épidémiologiques ou sur les effets bénéfiques de l'exercice physique. Dans ce contexte la capacité réduite à oxyder les lipides, considérée comme causale dans le développement de l'obésité, pourrait être secondaire à la généralisation d'un mode de vie sédentaire.

## Objectifs

Dans cette étude, nous nous proposons de tester les hypothèses suivantes :

- L'inactivité physique, indépendamment des changements de la balance énergétique, entraîne une diminution de l'oxydation de lipides exogènes représentatifs de l'alimentation humaine : oléate (mono-insaturé) et palmitate (saturé).
- Un entraînement physique de type maximal résistif, qui permet de maintenir la masse maigre sans augmenter significativement la dépense énergétique totale, réduit les effets délétères induits par l'inactivité physique.

## Matériel et Méthodes

18 hommes sains et minces (moyenne ± sd ; 32,6±4,0 ans et IMC de 23,6±0,7 kg/m$^2$) ont participé en 2000-2001 à une étude d'alitement prolongé organisée par les agences spatiales internationales à la Clinique de l'Espace (Toulouse, France). Après 15 jours de période contrôle, les volontaires étaient aléatoirement répartis dans deux groupes : un groupe contrôle (n=9) qui restait strictement allongé pendant 3 mois, et un groupe exercice (n=9) qui était soumis, simultanément à l'alitement, à un entraînement d'exercice d'intensité maximale tous les 3 jours. Avant et après deux mois d'alitement, nous avons mesuré l'oxydation totale des macronutriments par calorimétrie indirecte et l'oxydation lipidique exogène de l'oléate et du palmitate, respectivement les principaux acides gras monoinsaturés et saturés de l'alimentation occidentale, à l'aide d'un double marquage aux isotopes stables. Nous avons aussi mesuré les métabolites et hormones plasmatiques par des dosages RIA et enzymologiques et la composition corporelle par DEXA (Dual Energy X-ray).

## Résultats

Au cours de l'alitement, la masse grasse est restée constante en moyenne (0.3±1. 4 kg, p=NS) ce qui indique une balance énergétique stable des volontaires. Par conséquent, indépendamment des changements de la balance énergétique, l'alitement prolongé a entraîné une élévation des concentrations plasmatiques à jeun d'acides gras libres (17%, p=0.03), de triglycerides (52%, p=0.02) et du

rapport insuline sur glucose (132%, $p<0.0001$). L'oxydation glucidique à jeun et post-prandiale a augmenté (17%, and 8%, $p=0.01$, respectivement) tandis que l'oxydation lipidique totale à jeun et après un repas a chuté (27%, $p=0.01$ and 36%, $p=0.005$, respectivement). De manière non attendue, l'inactivité physique a significativement réduit l'oxydation cumulée du palmitate de 8.2±1.6 % et de 11.1±2.2% 7h et 36h après la dose, respectivement ($p<0.0001$) mais n'a pas affecté l'oxydation cumulée sur 7h après l'ingestion des traceurs de l'oléate.

Malgré un effet protecteur de la masse maigre (-0.8±0.6 vs. -2.4±1.8 dans le groupe contrôle kg, $p<0.05$) et du métabolisme de repos (-0.05±0.29 vs. -0.53±0.54 MJ/d dans le groupe contrôle, $p=0.02$), l'exercice physique, sans augmentation de la dépense énergétique totale, ne fut pas suffisamment efficace pour contrer les altérations induites par l'inactivité physique.

## Discussion

L'inactivité physique, indépendamment des changements de la balance énergétique, induit chez des individus sains des caractéristiques physiologiques (hypertriglycéridémia, insulino-résistance et oxidation lipidique réduite) proches de celles que l'on observe chez des sujets obèses. De plus, l'inactivité physique diminue l'oxydation des acides gras alimentaires saturés mais pas celle des acides gras monoinsaturés. Ces altérations métaboliques ne sont pas atténuées par l'entraînement physique de type résistif et des études complémentaires sont nécessaires afin de tester d'autres protocoles d'exercice. Enfin, cette étude encourage le régime de type méditerranéen, riche en acides gras monoinsaturés, pour les personnes sédentaires ou obèses. De plus amples études sont requises en vue de mieux comprendre les mécanismes sous-tendant l'oxydation différentielle des acides gras saturés et monoinsaturés en conditions d'inactivité physique.

PLoS CLINICAL TRIALS

# Effect of Physical Inactivity on the Oxidation of Saturated and Monounsaturated Dietary Fatty Acids: Results of a Randomized Trial

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**Abbreviations:** CI, confidence interval; DIT, diet-induced thermogenesis; FFM, fat-free mass; FM, fat mass; LPL, lipoprotein lipase; MEDES, Institut de Médecine et Physiologie Spatiale (Institute for Space Physiology and Medicine); NPRQ, nonprotein respiratory quotient, RMR, resting metabolic rate; SD, standard deviation; TG, triglyceride

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## ABSTRACT

**Objectives:** Changes in the way dietary fat is metabolized can be considered causative in obesity. The role of sedentary behavior in this defect has not been determined. We hypothesized that physical inactivity partitions dietary fats toward storage and that a resistance exercise training program mitigates storage.

**Design:** We used bed rest, with randomization to resistance training, as a model of physical inactivity.

**Setting:** The trial took place at the Space Clinic (Toulouse, France).

**Participants:** A total of 18 healthy male volunteers, of mean age ± standard deviation 32.6 ± 4.0 y and body mass index 23.6 ± 0.7 kg/m$^2$, were enrolled.

**Interventions:** An initial 15 d of baseline data collection were followed by 3 mo of strict bed-rest alone (control group, $n = 9$) or with the addition of supine resistance exercise training every 3 d (exercise group, $n = 9$).

**Outcome measures:** Oxidation of labeled [$d_{31}$]palmitate (the main saturated fatty acid of human diet) and [1-$^{13}$C]oleate (the main monounsaturated fatty acid), body composition, net substrate use, and plasma hormones and metabolites were measured.

**Results:** Between-group comparisons showed that exercise training did not affect oxidation of both oleate (mean difference 5.6%; 95% confidence interval [95% CI], −3.3% to 14.5%; $p = 0.20$) and palmitate (mean difference −0.2%; 95% CI, −4.1% to 3.6%; $p = 0.89$). Within-group comparisons, however, showed that inactivity changed oxidation of palmitate in the control group by −11.0% (95% CI, −19.0% to −2.9%; $p = 0.01$) and in the exercise group by −11.3% (95% CI, −18.4% to −4.2%; $p = 0.008$). In contrast, bed rest did not significantly affect oleate oxidation within groups. In the control group, the mean difference in oleate oxidation was 3.2% (95% CI, −4.2% to 10.5%; $p = 0.34$) and 6.8% (95% CI, −1.2% to 14.7%; $p = 0.08$) in the exercise group.

**Conclusions:** Independent of changes in energy balance (intake and/or output), physical inactivity decreased the oxidation of saturated but not monounsaturated dietary fat. The effect is apparently not compensated by resistance exercise training. These results suggest that Mediterranean diets should be recommended in sedentary subjects and recumbent patients.

### Editorial Commentary

**Background:** Obesity is an important contributor to the burden of chronic diseases, particularly type II diabetes, cardiovascular disease, hypertension, and stroke. Being inactive is a risk factor for all of these conditions. However, the physiological effects of inactivity are not well understood. In this trial, supported by the European Space Agency, a group of researchers aimed to further understand the effects of physical inactivity on the way that fat from the diet is metabolized (i.e., broken down to generate energy). 18 healthy male volunteers were randomized into two groups, both of whom underwent 90 days of bed rest, aiming to mimic sedentary behavior. One group also received an exercise training program during the 90 days' bed rest. The researchers examined to what extent two different types of fatty acids common in the diet were metabolized over the duration of the trial: oleate (monounsaturated fat) and palmitate (saturated fat). As secondary objectives of the study, body weight, water, fat, and energy expenditure were also examined in the participants.

**What this trial shows:** The researchers did not see any statistically significant changes between the groups—that is, participants receiving bed rest, and those receiving bed rest plus exercise training—for any of the primary or secondary outcomes, except for resting metabolic rate, which was higher in the exercise group. However, they did see physiologically relevant changes in fat metabolism of one of the fatty acids, palmitate, over the course of the trial within both groups studied. Although metabolism of oleate (monounsaturated fat) did not show significant changes over the course of the trial, metabolism of palmitate (saturated fat) dropped by nearly 10% in both groups (bed rest, and bed rest plus exercise).

**Strengths and limitations:** The study design was appropriate to the questions being posed, and the techniques for examining fat metabolism were relevant. Although the number of participants was very small, this problem is true of many such studies due to the cost and complexity of the interventions. The model for inactivity used in this trial—90 days' bed rest—is very extreme. Very few studies of this type have been performed, with most of the evidence relating to activity and fat handling coming from training studies in otherwise sedentary people.

**Contribution to the evidence:** It is already known that physical activity has numerous health benefits, including the prevention of obesity. This trial provides data showing that inactivity lowers the ability to metabolize fat, specifically saturated fat, from the diet, which would therefore be more likely to be stored in the body.

*The Editorial Commentary is written by PLoS staff, based on the reports of the academic editors and peer reviewers.*

## INTRODUCTION

Obesity is reaching pandemic proportions, affecting all sexes, races, and ages. Currently more than 1 billion adults are overweight and at least 300 million of them are clinically obese. Overweight, and ultimately obesity, is a major contributor to the global burden of chronic diseases and disabilities such as type 2 diabetes, cardiovascular diseases, hypertension, and stroke, and certain forms of cancer [1]. As a consequence, obesity accounts for 2%–6% of total health care costs in several developed countries.

Obesity is a fat storage disease. In humans, stored lipids originate largely from the diet. Obesity thus represents a failure in dietary fat balance. Using [1,1,1,-$^{13}$C]triolein, a long-chain triacylglycerol, Binnert et al. [2] showed that dietary fat oxidation is decreased by 50% in obese women as compared to lean counterparts. Furthermore, it has been reported that there is no improvement in such preferential dietary fat channeling away from oxidation amongst post-obese weight-stable women [3], lending support to the hypothesis that the partitioning of dietary fat to storage is a causal factor in obesity rather than an adaptive response to obesity. Studies in obese Zucker rats has shown that this impaired partitioning between oxidation and storage is likely due to a preferential trafficking of meal-derived fatty acids towards adipose tissue for storage [4]. Understanding the factors which regulate dietary fat oxidation is therefore crucial to deciphering the causes of obesity.

We hypothesize that the generalized sedentary behaviors of our modern societies, which have been increasing since the beginning of the last century [5], play a key role in the reduced capacity to use dietary fat as fuel. It is already well accepted that physical inactivity on its own represents a risk factor for numerous chronic diseases including obesity and, as such, has been classified as the second cause of death in the US [6]. Nevertheless, it is also important to note that our knowledge of the detrimental health effects of inactivity is somewhat indirect and based on the positive effects of exercise training on sedentary populations [7]. Because there are very few longitudinal studies of increasing sedentarianism, our knowledge of the development of the "sedentary death syndrome" as proposed by Lees and Booth remains weak [8].

We investigated the effects of enforced physical inactivity induced by three months of strict bed rest on dietary fat oxidation and tested the efficacy of a low-volume, high-intensity resistance training program to mitigate the effects of this extreme sedentary behavior. This paper reports the primary outcomes investigating nutrition. Data on other physiological functions in this inactivity regimen are already published [9–16] or are to appear elsewhere.

## METHODS

### Participants

A call for candidates was made via the Internet on the MEDES (Institut de Médecine et Physiologie Spatiale, Toulouse, France) and ESA (European Space Agency) Web sites and by press announcements. The selection was carried out in two phases.

**Preselection: First screening.** Preselection was based on the volunteers' application files, comprising first a general questionnaire on the participant's way of life, education, and professional experience; and second, a medical questionnaire on personal and family medical history, by phone. The purpose was to select volunteers who met the following requirement criteria: healthy, male, European Community citizen, aged 25 to 45, nonsmoker, no alcohol, no drug dependence, no medical treatment, height 165 to 185 cm, no overweight nor excessive thinness (body mass index as the ratio of weight [kg] to height [m$^2$] between 20 and 27), no personal nor family history of chronic or acute disease (e.g., hypertension, diabetes) that could affect the physiological data and/or create a risk for the participant during the experiment. In addition, any participant covered by a Social Security system was to be free of any engagement during four consecutive months.

Specific exclusion criteria were: having given blood (more than 300 ml) in a period of three months or less before the start of the experiment, participant already participating in a

clinical research experiment, poor tolerance to blood sampling, past record of orthostatic intolerance, cardiac rhythm disorders, allergies, intensive sport training, fractures or tendon laceration since less than one year, chronic back pain, history of thrombophlebitis, presence of metallic implants, special dietary requirements, sleep disorders, or photosensitive epilepsy. Out of the 730 applications received, 124 participants were preselected.

**Selection: Second screening.** The 124 preselected participants were invited to MEDES to undergo medical and psychological examinations to select the participants most apt to participate in the long-duration bed-rest intervention. Tests lasted 48 hours and were carried out by physicians and psychologists from MEDES who were not involved in the individual scientific protocols. The purpose of the first step was to select 17 participants (14 participants, three replacements) for the first experimentation phase in 2001. Similarly, the second selection process started at the end of the first period. The medical checkup included: clinical examination and questionnaire to verify that no exclusion criteria were present; 12-lead electrocardiogram and measurement of arterial blood pressure and heart rate; testing for orthostatic hypotension; a questionnaire to evaluate the quality of participants' sleep; ophthalmologic examination (visual acuity and fundus examination); and biochemistry, hematology, serology, and toxicology analyses. In addition, in specialized departments (Rangueil Hospital, Toulouse, France) the following tests were conducted: echo-Doppler measurements of the lower limbs (to eliminate participants with venous insufficiency), front and side chest radiography, a dental panoramic radiography, abdomen radiography (to ensure the absence of lithiasis), abdomen echography, DEXA (dual-energy-X-ray absorptiometry) measurement of bone density (which should be no more than 1 standard deviation [SD] above or below the age/sex-matched mean), and a measurement of maximal oxygen consumption. The preselected participants underwent psychiatric and psychological evaluations to ensure that participants would tolerate the conditions of the experiment. The selection protocol included psychological tests applied to the candidates in a group, a projective test, and an interview.

**Participant aptitude.** Candidates were declared able to participate in the experiment if the two following conditions were fulfilled: first, that the informed consent form had been carefully read and that candidates had the opportunity to ask any additional questions concerning the research project. After having given satisfactory answers to these questions, participants signed an Information and Consent Form that was approved by the Institutional Review Board of Midi-Pyrénées I (France). Second, after the results of the numerous medical and psychological tests were checked, a last verification was made to ensure that all inclusion criteria were present and that no exclusion criteria existed. Out of the 124 preselected participants, 15 declined to participate and 85 were excluded for medical and psychological reasons. Thus, 24 participants were included.

## Interventions

The intervention on activity lasted for 4 mo and was divided into three periods: a 15-d ambulatory control period, 90 d of bed rest in a head-down tilt position (–6°), and a 15-d recovery period. During the control and recovery periods, the participants were confined to MEDES. During the bed rest, all activities of daily living (i.e., showering, restroom needs, eating, etc.) were performed in supine position. Standing or seating positions were forbidden. Cameras were used at night to ensure compliance of the participants to these conditions. During bed rest, the subjects were divided into three groups: a control group that remained in bed ($n = 9$), an exercise group subjected to a supine resistance exercise training protocol concomitantly with the bed rest ($n = 9$), and a group receiving a single intravenous infusion of bisphosphonate (Pamidronate) to prevent bone demineralization ($n = 6$). Interventions on this latter group will not be further described as it was not part of the present protocol. Details can be found elsewhere [15].

**Training program.** The resistance training program was performed on the flywheel ergometer [17]. This device allows participants to perform maximal concentric and eccentric actions in the supine squat and calf press. The training sessions were programmed every 3 d during the bed rest and lasted 35 min. Progressive warm-ups preceded four sets of seven maximal concentric and eccentric repetitions in the squat, followed by four sets of 14 repetitions in the calf press. Periods of rest lasting 2 min were allowed between sets and 5 min between exercises. The energy expenditure of a session was on average 640 kJ. Such training prevents lean body mass loss and mitigates muscle strength loss while not significantly impacting total daily energy expenditure during the entire bed-rest period (theoretical impact of 0.03 on the physical activity level defined as the ratio of total energy expenditure to resting metabolic rate [RMR]).

## Diet

In order to dissociate the effects of physical inactivity from those of a positive energy balance induced by physical inactivity, we attempted to provide a eucaloric energy intake and thus maintain energy balance during ambulatory and bed-rest periods. However, diet monitoring represents a difficult challenge during bed rest, because body composition changes due to muscle atrophy and, as a consequence, changes in body mass do not reflect energy balance. Energy requirements were calculated as RMR times a physical activity factor of 1.4 and 1.2 during the control and bed-rest periods, respectively [18,19]. RMR was measured twice in each period to adjust intake for changes in fat-free mass (FFM). Water intake was provided at 3 l/d. Snacks and extra water were provided to the exercise group on the days of training to cover the energy cost of the session. The macronutrient composition of the diet was set at 30% fat, 15% protein, and 55% carbohydrate. Diet was supplied by the hospital kitchen and controlled by a dietician under the supervision of the investigators. The participants were asked to finish all food given. Leftovers, if any, were weighed and snacks were provided later during the day to cover the energy deficit. Macronutrients and energy intake were calculated based on the hospital recipes and Geni software (Geni, Micro 6, Nancy, France).

## Objectives

We hypothesized that physical inactivity, independent of its effects on energy balance, reduces the oxidation of dietary fat. To test this hypothesis we used a unique space physiology-derived model of strict bed rest to induce three months of

physical inactivity in healthy participants. An additional objective was to test the hypothesis that a high-intensity, low-volume resistance exercise training protocol would mitigate the effect of inactivity on dietary fat oxidation.

## Outcomes

**Primary outcome measures.** Dietary fat oxidation was measured before (control period day 11) and at the end of the bed rest (bed rest day 78), 36 h after the last bout of exercise, through a combination of indirect calorimetry and a recently developed method by which fatty acids labeled by stable isotopes are mixed into a standard breakfast. Oleic (18:1) and palmitic (16:0) acids were selected for the study. These fatty acids represent the main monounsaturated and saturated fatty acids of the human diet (38% and 20% of total intake, respectively). Baseline breath and urine samples and fasting blood samples were collected. Then a breakfast representing 50% of the RMR (expressed as MJ/24 h) in energy (55% carbohydrates, 15% protein, and 30% fat) was offered to the participants. Part of this breakfast was composed of a liquid replacement meal (Boost High protein, Mead Johnson, United States) in which 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) were homogenized at 65 °C or above (melting point of palmitate). Then, hourly breath and fresh urine samples were collected for 7 h. Breath $^{13}CO_2/^{12}CO_2$ and urinary $^{2}H/^{1}H$ ratios were measured on an isotope ratio mass spectrometer (GV Instruments, France). Recovery of [1-$^{13}$C]oleic acid was calculated as the instantaneous recovery of $^{13}$C in expired $CO_2$ hourly sampled over the 7 h of the test and expressed as a percentage of the dose. Recovery of [$d_{31}$]palmitate was calculated as the cumulated recovery of $^{2}$H in total body water hourly sampled through urine voids.

**Secondary outcome measures.** Body weight was measured daily on a special supine weighing device. Total body water was measured by hydrometry based on the isotope dilution of $H_2{}^{18}O$ in body water. Fat mass (FM) and FFM were measured by DEXA on QDR 4500 W (Hologic, Massy, France) scanner using the QDR System Software for version 11.2. Energy expenditure; total fat, carbohydrate, and protein oxidation rates; and the nonprotein respiratory quotient (NPRQ) and lipogenesis were calculated from hourly indirect calorimetry data. Fasting hormones and metabolites were measured on the day of the oral lipid load. Details on the methods for primary and secondary outcomes assessment can be found in Protocol S1.

## Sample Size

Eleven projects from international laboratories investigating each physiological function were selected by peer review for participation in this long-term bed rest experiment. Consequently, the selection of one particular primary outcome for sample size calculation is impossible for bed rest, and no preliminary data exist for such a long-term study. One of the most conservative changes induced by bed rest is muscle atrophy. Based on results obtained during a 42-d bed rest study [18], we observed a loss in muscle mass of 3.2 ± 2.0 kg (mean ± SD) on seven healthy men with a power of 94%. Nine subjects per group were required to detect the same changes at a power of 99%.

## Randomization

For the whole study, the participants were housed two per room. The pairing of subjects was based on thorough psychological examinations by psychologists expert in confinement situations, who made their decisions independently of the principal investigators. This procedure was considered essential to avoid conflicts between two participants to be confined together in a 25 $m^2$ room for three months. Pairs of subjects were then written on a small piece of paper, folded in an envelope, and mixed in a dark bag. Each intervention was also written on a small piece of paper, folded in an envelope, and mixed in a dark bag. An envelope containing a participant pair and an envelope containing an intervention group were randomly and sequentially selected for group allocation in the control, exercise, and bisphosphonate groups.

## Blinding

No blinding was performed in this study. Blinding was not possible given (1) the permanent inpatient design of this longitudinal study and (2) the type of countermeasures applied and the heavy schedules associated with the complicated planning of experiments in supine positions.

## Statistical Methods

Variables were analyzed by a two-way multiple ANOVA with time as the repeated measure (ambulatory versus bed rest) and group (control versus exercise) as main effect. RMR was analyzed by a repeated measures (ambulatory versus bed rest) analysis of covariance with FFM as the covariate and group (control versus exercise) as main effect. When necessary, within group comparisons were further evaluated by a paired t-test. Due to the low sample size inherent in such a study, all statistical results were confirmed by the Wilcoxon sign-rank. The Pearson product-moment correlation coefficient was used to investigate the relationships between relevant variables. All statistics were performed using JMP version 5.1.1 (SAS Institute, North Carolina, United States), and reported values are means ± SD (unless otherwise stated), with $p < 0.05$ considered statistically significant.

# RESULTS

## Participant Flow

The participant flow is represented in Figure 1.

## Recruitment

Recruitment of half the participants started in January 2001, and the experiment lasted from August 2001 to December 2001. The recruitment of the other half of subjects started in June 2001, and the experiment lasted from March 2002 to July 2002.

## Baseline Data

Table 1 shows the baseline characteristics of the participant groups. No major differences in these characteristics were noted at baseline.

## Numbers Analyzed

Nine participants in the control group and nine in the exercise group underwent the intervention. One subject of the exercise group was excluded from any procedures requiring ingestion or injection of products because of

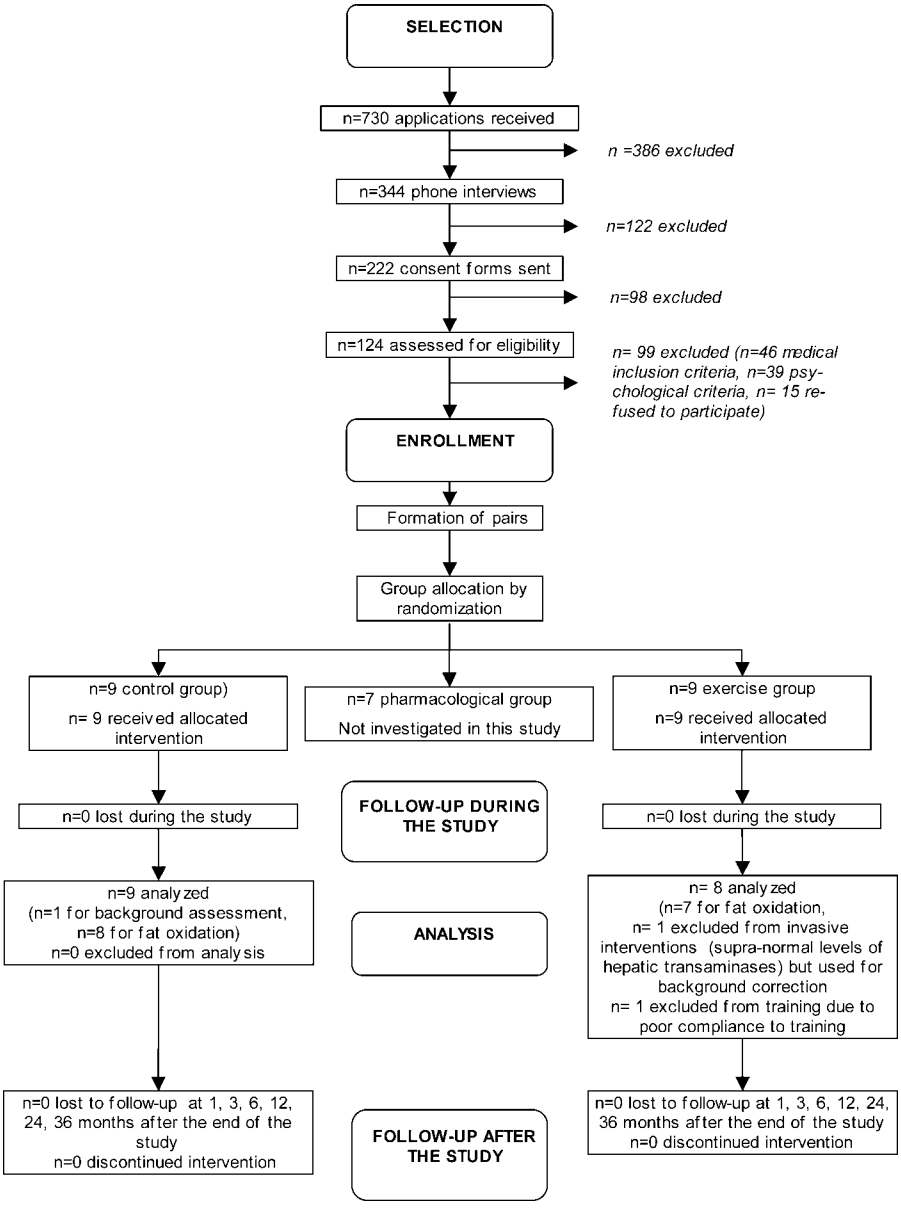


**Figure 1.** Flow Chart Showing the Progress of Participants in the Study

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marginally high hepatic transaminase levels in the middle of the bed-rest period. This participant was used as the background subject given the small expected changes in background isotope enrichment over the time course of the study. One participant from the exercise group failed to comply with the training protocol due to a knee injury not reported during the selection process. He was also excluded from the analyses.

## Outcomes and Estimation

**Primary outcomes.** We did not observe any differences between the randomized groups for the primary outcomes. At the end of the bed rest, the mean difference between groups was 5.6% (95% CI, −3.3% to 14.5%; $p = 0.20$) for oleate cumulative recovery and −0.2% (95% CI, −4.1% to 3.6%; $p = 0.89$) for palmitate cumulative recovery at 7 h postdose. Within-subject changes were, however, noted.

The 7 h postdose cumulative recovery of palmitate (Figure 2) decreased after bed rest. In the control group, palmitate recovery was 22.7% ± 5.1% during the ambulatory period and 15.1% ± 3.9% during the bed-rest period (mean

**Table 1.** Characteristics of the Participants

| Variable | Unit | Control Group | Exercise Group |
|---|---|---|---|
| *n* | | 9 | 9 |
| Age | years | 32 (4) | 33 (5) |
| Height | m | 1.73 (0.03) | 1.75 (0.05) |
| Body mass | kg | 70.9 (6.1) | 70.6 (5.8) |
| Fat mass | % | 18.4 (4.7) | 15.6 (3.6) |
| Body mass index | kg/m$^2$ | 23.6 (1.9) | 23.0 (2.6) |

Values in parentheses indicate SD.

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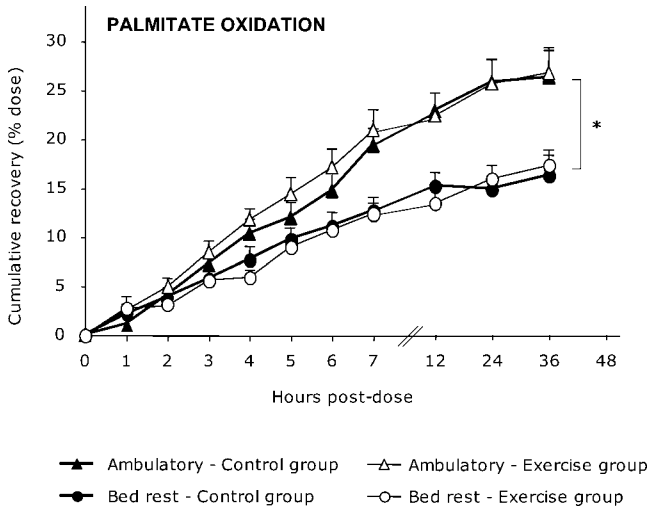


**Figure 2.** Hourly Cumulative Percent Recovery of [$d_{31}$]Palmitate (16:0) Before and After Bed Rest

This experiment included control ($n = 8$) and exercise ($n = 7$) groups. No between-group difference was noted after bed rest. *$p < 0.05$ compared to ambulatory period.
DOI: 10.1371/journal.pctr.0010027.g002

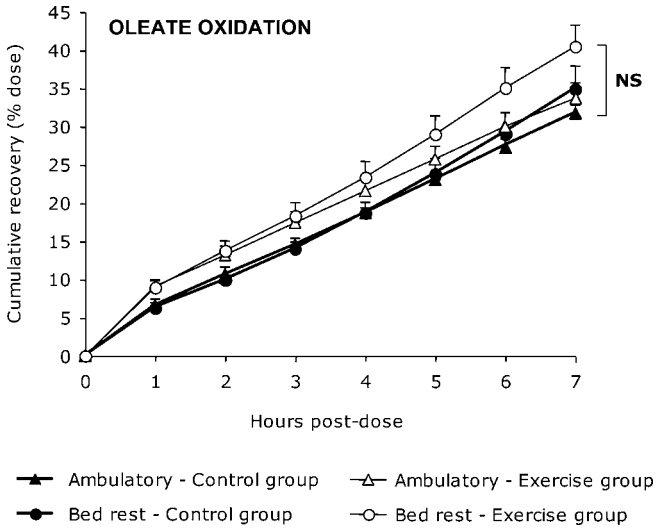


**Figure 3.** Hourly Cumulative Percent Recovery of [1-$^{13}$C]Oleate (18:1) Before and After Bed Rest

This experiment included control ($n = 8$) and exercise ($n = 7$) groups. Values are corrected by the acetate correction factor. No effects of exercise training or bed rest were noted (NS, not significant).
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difference −6.7%; 95% CI, −10.5% to −3.0%; $p = 0.004$). In the exercise group, palmitate recovery was 22.3% ± 6.2% and 13.4% ± 4.7% during the ambulatory and bed-rest periods, respectively (mean difference −8.5%; 95% CI, −14.1% to −3.0%; $p = 0.01$). These changes were maintained for 36 h postdose. Cumulative recovery of palmitate of the control group at 36 h postdose was 28.5% ± 7.6% during the ambulatory period and 17.5% ± 5.6% during bed rest (mean difference −11.0%; 95% CI, −19.0% to −2.9%; $p = 0.01$). In the exercise group, oxidation dropped from 28.5% ± 7.0% to 17.2% ± 3.3% after bed rest (mean difference −11.3%; 95% CI, −18.4% to −4.2%; $p = 0.008$). Taken together, bed rest resulted in a −8.2% decrease in palmitate oxidation at 7 h postdose (95% CI, −10.4% to −4.7; $p < 0.0001$) and −11.1% at 36 h postdose (95% CI, −15.8% to −6.5%; $p < 0.0001$).

In contrast, no bed rest effects were noted on the 7 h postdose cumulative recovery of oleate (Figure 3). In the control group, oleate recovery was 31.7% ± 3.2% during the ambulatory period and 34.9% ± 8.6% during the bed rest (mean difference 3.2%; 95% CI, −4.2% to 10.5%; $p = 0.34$). In the exercise group, oleate recovery was 35.4% ± 6.8% and 39.4% ± 7.4% during the ambulatory and bed-rest periods, respectively (mean difference 6.8%; 95% CI, −1.2% to 14.7; $p = 0.08$).

All results were confirmed by the nonparametric Wilcoxon sign-rank test ($p$-value not detailed).

**Secondary outcomes.** Energy intake via diet was 10.2 ± 0.7 MJ/d during the control period for both groups. By design, energy intake was decreased during the bed-rest period in the control (8.7 ± 0.7 MJ/d) and exercise (9.4 ± 0.6 MJ/d) groups to match calculated requirements. The between-group difference represents the estimated energy cost of exercise. For both groups, the macronutrient composition of the diet was 54% ± 1% carbohydrate, 29% ± 1% fat, and 16% ± 1% protein during the control period. During the bed-rest period the diet composition was 51% ± 2% carbohydrate, 31± 2% fat, and 17% ± 1% protein.

We observed a significant bed-rest effect on body mass and FFM, but the presence of a bed rest × group interaction indicated that the effect of bed rest differed between the control and exercise groups (Table 2). After three months of inactivity, body mass of the control group changed by −2.9 kg (95% CI, −5.1 to −0.6 kg), whereas no changes were noted in the exercise group (0.02 kg; 95% CI, −1.2 to 1.2 kg). The loss in the control group was essentially accounted for by the −2.4 kg change in FFM (95% CI, −3.9 to −0.9 kg) and no significant change in fat mass (−0.4 kg; 95% CI, −2.0 to 1.1 kg). The loss in FFM was confirmed by a Wilcoxon rank-sign test ($p = 0.008$). In the exercise group, the changes were not significant for FFM (−0.5 kg; 95% CI, −1.5 to 0.5 kg) and FM (0.5 kg; 95% CI, −0.5 to 1.5 kg). Of note, although FM showed no significant change over the time course of the bed rest and indicated that the control group averaged a 200 J/d negative energy balance and the exercise group a 300 J/d positive energy balance, between-participant variability in fat balance was large for both groups, ranging from −3.4 to +2.1 kg. These changes correlated with both energy (Pearson $r = 0.74$, $p < 0.01$) and fat intake (Pearson $r = 0.63$, $p < 0.05$) during the bed-rest period.

The fasting hormone and metabolite response to bed rest were not different in the control and exercise groups (Table 3). Except for glycerol and glucose, which remained unchanged, bed rest induced an increase in fasting β-hydroxybutyrate (30%), leptin (49%), insulin (132%), free fatty acids (17%), triglycerides (TGs) (52%), and the insulin to glucose ratio (132%). Leptin correlated strongly with FM during both the ambulatory and the bed-rest periods (Pearson $r = 0.68$; $p < 0.01$, and $r = 0.83$; $p < 0.0001$, respectively).

As expected, FFM was a determinant of RMR in both groups during both the ambulatory and bed-rest periods (Pearson $r = 0.65$; $p < 0.01$, and $r = 0.59$; $p < 0.01$, respectively). We observed a significant bed rest × group interaction for RMR (Table 4). Indeed, although RMR decreased by 8% after bed

**Table 2.** Body Mass and Composition

| Variable | Unit | Control Group | | Exercise Group | | ANOVA | | |
|---|---|---|---|---|---|---|---|---|
| | | Ambulatory Period | Bed-Rest Period | Ambulatory Period | Bed-Rest Period | Bed Rest | Group | Bed Rest × Group |
| *n* | | 9 | 9 | 8 | 8 | | | |
| Body mass | kg | 70.9 (6.1) | 68.0 (4.6) | 69.0 (3.4) | 69.0 (4.2) | 0.03 | 0.84 | 0.02 |
| Fat mass | kg | 13.2 (4.1) | 12.8 (3.0) | 10.5 (2.8) | 11.0 (3.4) | 0.94 | 0.18 | 0.28 |
| Fat-free mass | kg | 57.6 (4.2) | 55.2 (2.8) | 58.5 (2.3) | 58.0 (1.6) | 0.003 | 0.21 | 0.03 |

Values are means (SD).

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rest in the control group, no change was noted in the exercise group. These results were similar after adjustment for FFM. The average change for the control group was −630 kJ (95% CI, −929 to −332 kJ; Wilcoxon sign-rank test, $p = 0.008$) and −4 kJ (95% CI, −242 to 234 kJ, Wilcoxon test $p = 0.94$) for the exercise group. This suggests that the bed rest-induced decrease in RMR was independent of the active metabolic mass changes.

A similar shift in fasting substrate use was noted in both groups, either expressed in g/h or mg/kg FFM/h. The results per group are indicated in Table 4, but for clarity both groups have been combined in the following section. In mg/kg, FFM/h fasting glucose oxidation increased by 17% (23.1 mg/kg FFM/h; 95% CI, 11.7 to 34.5 mg/kg FFM/h; Wilcoxon sign-rank test, $p = 0.002$), and lipid oxidation decreased by 27% (–8.9 mg/kg FFM/h; 95% CI, −14.8 to −2.9 mg/kg FFM/h; Wilcoxon sign-rank test, $p = 0.01$). As a result, the NPRQ significantly increased by 4% (0.033; 95% CI, 0.054 to 0.013). Fasting lipid oxidation in g/min correlates with FM during both the ambulatory and bed-rest periods (Pearson $r = 0.68$; $p < 0.01$, and $r = 0.63$; $p < 0.05$, respectively). The 7 h postmeal cumulative substrate use expressed in g or mg/kg FFM showed the same pattern of response (Table 4). No group effect emerged, suggesting that resistance exercise training had no effect on the partitioning of postprandial substrate use. Expressed in mg/kg FFM and cumulative for both groups, postprandial glucose oxidation increased by 124 mg/kg FFM (95% CI, 28 to 220 mg/kg FFM; Wilcoxon sign-rank test $p = 0.005$) and lipid oxidation decreased by 36% (–53 mg/kg FFM; 95% CI, −86 to −21 mg/kg FFM; Wilcoxon sign-rank test $p = 0.003$). Lipogenesis, as measured by indirect calorimetry, increased by 129% (0.9 g; 95% CI, 0.2 to 1.6 g) but remained low in absolute numbers. Fasting and postprandial protein oxidation remained unaffected by bed rest, as did diet-induced thermogenesis (DIT).

### Ancillary Analyses

No associations were noted between the 7 h cumulative oxidation of oleate and various metabolic variables during the ambulatory or bed-rest periods (Table 5). Although the ambulatory 7 h cumulative palmitate oxidation showed the same results, strong associations were noted during bed rest. Palmitate oxidation correlates positively with fasting lipid oxidation and thus negatively with NPRQ. A positive relationship was also noted between palmitate oxidation and total fat oxidation over the 7 h postprandial phase (Table 5).

### Adverse Events

No adverse effects from the intervention were noted, and the participants tolerated all the experiments well.

## DISCUSSION

### Interpretation

Although the resistance exercise training program mitigated the physical inactivity-induced muscle atrophy (0.5 ± 1.2 kg lost in the exercise group versus 2.4 ± 1.9 kg lost in the control group), the substrate oxidation rates, the hyperinsulinemia, and the hyperlipidemia induced by bed rest were not restored by exercise to their basal values. Three points

**Table 3.** Fasting Hormones and Metabolites

| Variable | Unit | Control Group | | Exercise Group | | ANOVA | | |
|---|---|---|---|---|---|---|---|---|
| | | Ambulatory Period | Bed-Rest Period | Ambulatory Period | Bed-Rest Period | Bed Rest | Group | Bed Rest × Group |
| *n* | | 8 | 8 | 7 | 7 | | | |
| Leptin | ng/ml | 1.7 (1.3) | 2.5 (1.4) | 2.0 (1.0) | 3.0 (2.1) | 0.04 | 0.53 | 0.80 |
| Insulin | mU/l | 25.8 (10.8) | 55.8 (23.2) | 20.6 (6.9) | 51.9 (10.2) | <0.0001 | 0.37 | 0.80 |
| Glucose | mmol/l | 4.9 (0.5) | 4.7 (0.2) | 4.6 (0.3) | 4.9 (0.3) | 0.61 | 0.78 | 0.06 |
| Insulin/glucose | | 5.3 (2.1) | 11.9 (4.7) | 4.5 (1.5) | 10.8 (2.7) | <0.0001 | 0.36 | 0.97 |
| β-hydroxybutyrate | μmol/l | 46.5 (17.3) | 58.0 (30.1) | 42.5 (8.9) | 57.6 (22.7) | 0.04 | 0.72 | 0.64 |
| Glycerol | μmol/l | 36.6 (13.8) | 39.6 (13.6) | 34.1 (12.7) | 39.9 (15.6) | 0.40 | 0.92 | 0.86 |
| Free fatty acids | μmol/l | 232.5 (138.1) | 275.5 (112.5) | 270.1 (56.3) | 311.3 (80.0) | 0.03 | 0.53 | 0.86 |
| Triglycerides | μmol/l | 897.0 (463.4) | 1341.5 (740.1) | 835.8 (390.6) | 1294.0 (966.7) | 0.02 | 0.89 | 0.97 |

Values are means (SD).

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**Table 4.** RMR and Fasting and Postprandial Substrate Oxidation

| Variable | Variable Subcategory | Units | Control Group | | Exercise Group | | MANOVA | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Ambulatory Period | Bed-Rest Period | Ambulatory Period | Bed-Rest Period | Bed Rest | Group | Bed Rest × Group |
| *n* | | | 8 | 8 | 7 | 7 | | | |
| RMR | | MJ/d | 6.4 (0.4) | 5.9 (0.3) | 6.4 (0.3) | 6.4 (0.5) | 0.06 | 0.10 | 0.02 |
| $RMR_{FFM}$ | | MJ/d | 6.4 (0.3) | 5.8 (0.3) | 6.3 (0.3) | 6.3 (0.3) | 0.002 | 0.20 | 0.002 |
| Fasting substrate oxidation | NPRQ | | 0.890 (0.063) | 0.926 (0.058) | 0.890 (0.030) | 0.920 (0.040) | 0.01 | 0,91 | 0.79 |
| | Glucose | g/h | 7.5 (2.0) | 9.1 (1.9) | 8.0 (1.6) | 9.1 (1.4) | 0.003 | 0.79 | 0.49 |
| | | mg/kg FFM/hr | 134.2 (40.5) | 160.4 (37.4) | 134.2 (20.2) | 153.8 (20.7) | 0.01 | 0.74 | 0.49 |
| | Lipids | g/h | 1.9 (1.1) | 1.2 (0.9) | 1.9 (0.4) | 1.5 (0.7) | 0.001 | 0.84 | 0.55 |
| | | mg/kg FFM/hr | 32.6 (19.0) | 21.7 (16.0) | 31.8 (7.8) | 25.2 (10.4) | 0.01 | 0.85 | 0.46 |
| | Protein | g/h | 3.6 (0.6) | 3.6 (0.0) | 4.0 (0.6) | 3.9 (0.8) | 0.93 | 0.23 | 0.86 |
| | | mg/kg FFM/hr | 63.2 (10.0) | 64.1 (11.8) | 66.9 (11.5) | 66.6 (15.7) | 0.94 | 0.57 | 0.86 |
| 7 h postmeal cumulative substrate oxidation | DIT | % | 9.2 (2.1) | 10.6 (2.3) | 9.2 (2.0) | 9.9 (1.8) | 0.27 | 0.55 | 0.76 |
| | Glucose | g | 78.4 (11.8) | 82.9 (13.5) | 80.3 (12.8) | 89.7 (9.7) | 0.02 | 0.46 | 0.36 |
| | | mg/kg FFM | 1395 (249) | 1477 (230) | 1384 (187) | 1520 (171) | 0.01 | 0.99 | 0.33 |
| | Lipids | g | 7.5 (6.6) | 4.5 (5.0) | 10.0 (3.4) | 6.8 (3.4) | 0.004 | 0.34 | 0.93 |
| | | mg/kg FFM | 132 (117) | 79 (89) | 168 (59) | 114 (56) | 0.005 | 0.40 | 0.97 |
| | Protein | g | 25.3 (3.0) | 28.7 (5.7) | 27.7 (5.5) | 25.6 3.8) | 0.65 | 0.85 | 0.09 |
| | | mg/kg FFM | 452 (56) | 519 (131) | 464 (81) | 434 (56) | 0.51 | 0.33 | 0.10 |
| | Lipogenesis | g | 1.1 (1.6) | 1.9 (1.8) | 0.3 (0.4) | 1.3 (1.6) | 0.02 | 0.31 | 0.77 |

Values are mean (SD).

$RMR_{FFM}$, resting metabolic rate adjusted for fat-free mass.

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can explain this lack of effect. First, resistance exercise training was shown to induce a decrease in baseline TG concentrations by 19% and an increase in resting fat oxidation by 21% when exercise was performed within 16 h postexercise [20]. We attempted to determine the effects of training, not the acute effects of the exercise. Our tests took place 36 h after the last session of training, and the delay may have been too long for the effect of training to pertain. Second, the impairment in oxidative capacity and the increase in glycolytic capacity observed in both groups may be partly explained by the pattern of changes in muscle fibers. Although the resistance exercise program was effective for maintaining whole muscle size, physical inactivity was shown to induce a shift from slow oxidative (type I) to fast glycolytic (types IIa and IIa/IIb) fibers and an apparition of hybrid fibers (type I/IIa/IIb), for which oxidative patterns are still unknown [21]. A higher proportion of type IIb fibers in skeletal muscle tissue was observed in obese and diabetic participants [22].

**Table 5.** Pearson Correlation Coefficients for Dietary Fat Oxidation

| Variable | Unit | Oleate 7 h Postdose | | Palmitate 7 h Postdose | |
|---|---|---|---|---|---|
| | | Ambulatory | Bed Rest | Ambulatory | Bed Rest |
| Body mass | kg | 0.35 | −0.28 | −0.19 | 0.31 |
| FFM | kg | 0.19 | −0.11 | 0.08 | 0.17 |
| FM | kg | 0.31 | −0.26 | −0.42 | 0.30 |
| RMR | MJ/d | 0.18 | −0.05 | 0.06 | 0.10 |
| Fasting NPQR | | −0.02 | 0.41 | 0.29 | −0.74** |
| Fasting lipid oxidation | g/mn | 0.10 | −0.39 | −0.29 | 0.77*** |
| Fasting glucose oxidation | g/mn | 0.17 | 0.17 | 0.16 | −0.61* |
| Breakfast energy content | kcal | −0.27 | 0.10 | −0.15 | 0.11 |
| Breakfast lipid content | kcal | −0.04 | −0.16 | 0.22 | 0.44 |
| Breakfast glucose content | kcal | −0.33 | 0.20 | −0.06 | −0.04 |
| 7 h postmeal lipid oxidation | g | 0.24 | −0.15 | 0.15 | 0.74** |
| 7 h postmeal glucose oxidation | g | −0.23 | −0.08 | −0.15 | −0.38 |
| DIT | % | 0.36 | 0.14 | 0.03 | −0.41 |
| Insulin | mU/l | −0.03 | 0.05 | −0.15 | −0.07 |
| Glucose | mmol/l | −0.07 | 0.17 | −0.15 | −0.23 |
| Free fatty acids | μmol/l | 0.35 | −0.06 | 0.32 | 0.50 |
| Triglycerides | μmol/l | 0.01 | 0.36 | −0.47 | −0.37 |

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.0001$

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Finally, the modest energy expenditure induced by our resistance exercise training program (about 640 kJ per session) may not have been sufficient to fully protect the muscle fiber pattern and the related oxidative capacity. In support of that, an early bed-rest study suggested a relationship between the amount of energy expenditure and the degree of insulin resistance [23]. Overall, physical inactivity may induce a muscle fat oxidative capacity impairment that leads to a drop in fat oxidation rate and to insulin resistance. This study provides evidence that physical inactivity itself triggers physiopathological characteristics observed in obese and diabetic individuals.

The second finding of our investigation indicated that physical inactivity per se induces a preferential partitioning of dietary fat from oxidation toward storage. Studies on exercise training and detraining [24–26] may provide insights into putative mechanisms of this process, as the effects of exercise training on postprandial lipidemia have been well documented. The rise in dietary TG clearance after prior exercise is likely to be mediated in part by an up-regulation of lipoprotein lipase (LPL) activity. Exercise for 5–13 consecutive days increases LPL mRNA in skeletal muscle with no obvious changes in adipose tissue LPL mRNA [27]. Conversely, a two-week period of detraining in runners decreased LPL activity in skeletal muscle but increased LPL activity in adipose tissue [28]. Further investigations are therefore needed to better understand the fat oxidation regulation mechanisms according to physical activity level.

A notable finding of this study is that the longitudinal effects of physical inactivity on dietary fat oxidation depend on the nature of the fatty acid. Physical inactivity decreased saturated, but not monounsaturated, fatty acid oxidation. We observed that oleate recovery as a percentage was higher than that for palmitate, regardless of the testing period. This result is in agreement with previous studies showing that polyunsaturated and monounsaturated fatty acids are more oxidized in inactive persons than are saturated fatty acids [29]. Recent investigations in men have shown that dietary fat type substitution may affect energy metabolism and ultimately body mass. Piers and colleagues [30] have reported that the postprandial fat oxidation rate is higher, and carbohydrate oxidation rate is lower, after a monounsaturated fatty acid, rather than after saturated fatty acid meal. A further four-week dietary intervention on the same participants in which saturated fatty acids were replaced by monounsaturated fatty acids induced a significant loss of body weight and fat mass without any change in total energy or fat intake [31].

Recently, Kien et al. [32] fed healthy young adults either with a high palmitic acid diet or with a high oleic acid diet and showed a reduction in postprandial lipid oxidation and daily energy expenditure following the high-palmitate diet and no changes after the high-oleate one. In our study, the environmental intervention did not involve energy or fat balance, but rather involved physical activity level. Previous studies clearly showed that dietary fat oxidation is unrelated to global fat oxidation, and we confirmed this result in the control period for both oleate and palmitate. Under bed-rest conditions, however, although oleate oxidation seemed to remain independent of global substrate use, palmitate oxidation was strongly correlated with fasting NPRQ as well as with postprandial lipid oxidation. This decrease in palmitate oxidation induced by physical inactivity may be, at least in part, explained by the shift from red to white muscles. Palmitate transport into giant sarcolemmal vesicles and hence, palmitate oxidation, are significantly greater in red oxidative muscles than in white glycolytic muscles [33]. Additionally, the muscle fiber type muscle proportion may also explain the inefficiency of resistance exercise training on palmitate oxidation. The reason for the unchanging oleate oxidation are unknown. A similar differential fat oxidation pattern was noted in type 2 diabetes, a disease that shares numerous features with obesity, including a decreased capacity to use fat as fuel. A study in cultured human myotubes from diabetic participants reported a higher oxidation of oleate compared to palmitate. Whereas oleate preferentially appeared into the intramyocellular free-fatty acid pool, palmitate was incorporated into intramuscular TG [34]. The pathways at play require further investigation.

## Overall Evidence and Generalizability

Although epidemiological studies have demonstrated relationships between sedentary behaviors and morbidity [6], most of our knowledge on mechanism comes from studies on the beneficial effects of adding exercise training. This is also the case for our understanding of the regulation of fat oxidation [20,35–37]. One study showed that occasional inactivity lowered fat tolerance to high-fat diet, leading potentially to weight gain in the long term [38]. Although impaired fat oxidation is considered causative in obesity, our knowledge on the chronic effects of sedentary behaviors on oxidative balance, independently of energy balance disturbances, is still weak. The present study is one of the first longitudinal studies to show that physical inactivity per se impairs dietary fat oxidation. We used a unique model of physical inactivity mainly utilized by space agencies for microgravity simulation: long-term bed rest, which decreases physical activity level from 1.7 to 1.2 [18]. Although we acknowledge that the physical inactivity induced by strict bed rest is severe and might not represent the level achieved in the general population, such a study design clearly helps to clarify mechanisms and possible levers of action on which countermeasures may be tested.

With the understanding that inactivity is no longer applicable at a certain volume of exercise, the key is to find the minimal amount of exercise that will restore dietary fat oxidation. This notion of minimal activity level is currently a matter of great debate in the literature. We failed to show that high-intensity low-volume resistance exercise training could mitigate the changes induced by bed rest on fat oxidation. The low level of energy expenditure attained during resistance exercise might explain those results. Another trial has been completed that will provide evidence on the effect of training at higher expenditure (SB, personal communication).

## Study Limitations

Several limitations have to be considered in our study. First, we acknowledge the fact that the sample size is low. Long-term bed rest of such duration represent a clear challenge to implement, and large sample sizes are not realistic from both practical and economic points of view. The involvement of different teams testing different study hypotheses on the

same group of participants limits the a priori calculation of power.

Second, a modest but significant 2 g per meal increase in fat intake occurred during the bed-rest period despite a strict monitoring of nutritional conditions. Such a change in percentage fat intake is, however, unlikely to be responsible for the reduction in postprandial fat oxidation, as a major change in fat intake (20 to 50 g) has only modest effects on dietary fat oxidation [39]. We also failed to accurately stabilize fat mass and interindividual variations were large and ranged from −3.4 to +2.1 kg. Nevertheless, energy imbalance is unlikely to explain the results because average fat mass remained stable (0.3 ± 1.4 kg).

Last, we observed a trend toward higher oleate oxidation in the exercise group after bed rest ($p = 0.08$). We can not exclude the possibility that time points over the 7 h postdose might result in a significant effect of training on oleate, as observed by Votruba et al. [40]. This was not possible due to experiment constraints in this bed-rest study, but is currently being tested in women submitted to two months of bed rest.

### Conclusion

The present study shows that physical inactivity plays a key role in the portioning of saturated fat to oxidation, independently of its effects on energy balance, and results in a metabolic state comparable to that observed in obesity. Further investigations are necessary to better understand the underlying mechanisms and the parameters of an adequate exercise training program (in terms of frequency, intensity, duration, and type of exercise). Our study suggests that the Mediterranean diet (that is, one low in saturated fats, high in monounsaturated fats) would be helpful if promoted in sedentary populations and in recumbent patients, two groups at risk for weight gain.

## SUPPORTING INFORMATION

**CONSORT Checklist**
Found at DOI: 10.1371/journal.pctr.0010027.sd001 (54 KB DOC).

**Trial Protocol Part A**
Found at DOI: 10.1371/journal.pctr.0010027.sd002 (467 KB DOC).

**Trial Protocol Part B**
Found at DOI: 10.1371/journal.pctr.0010027.sd003 (1.1 MB DOC).

**Protocol S1.** Detailed Methods
Found at DOI: 10.1371/journal.pctr.0010027.sd004 (67 KB DOC).

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### Author Contributions

GGK, HO, CG, and SB designed the study. SN, ML, TS, and SB analyzed the data. DAS, SN, GGK, MD, and HO collected data or did experiments. AB wrote the first draft of the paper. AB, DAS, SN, ML, YLM, CG, and SB contributed to the writing of the paper.

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# CHAPITRE 6

## Extreme physical inactivity differentially alters dietary oleate and palmitate trafficking

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***Diabetes, sous presse***

# Résumé

## Introduction

Dans l'étude précédente, nous avons vu que l'inactivité physique, indépendamment des changements de la balance énergétique, diminue l'oxydation lipidique à jeun et en situation post-prandiale. De manière non attendue, elle réduit l'oxydation des acides gras exogènes saturés, mais n'affecte pas celle des acides gras monoinsaturés. L'inactivité physique semble par conséquent altérer les mécanismes de régulation impliqués dans le métabolisme des lipides totaux et exogènes, tels que l'absorption et la clairance plasmatique, l'acheminement vers le tissu adipeux et le muscle, et/ou la capacité oxydative. Malgré un maintien de la masse métaboliquement active, le protocole d'entraînement physique de type résistif précédemment testé s'est révélé inefficace pour contrer les effets délétères induits par l'inactivité physique.

## Objectifs

Dans cette étude, nous nous proposons de :

- Caractériser une partie des mécanismes responsables du métabolisme des lipides alimentaires en conditions d'inactivité physique extrême induite par un alitement prolongé de deux mois.
- Déterminer l'efficacité d'un protocole d'entraînement physique combinant un exercice de type résistif et aérobic.

## Matériel et Méthodes

16 femmes minces et en bonne santé (moyenne±sd, âge : 33,5±3,4 ans ; IMC : 21,5±1,3kg/m$^2$) ont participé en 2005 à l'étude WISE (Women Space Simulation for Exploration study) organisée par des agences spatiales internationales à la Clinique de l'Espace (Toulouse, France). Après une période ambulatoire de 20 jours, un groupe contrôle (n=8) est resté allongé pendant deux mois tandis qu'un groupe exercice (n=8) a effectué, simultanément à l'alitement, un protocole d'exercice physique de type résistif et aérobic. Avant et après un mois d'alitement, l'acheminement et l'oxydation des acides oléiques et palmitiques exogènes ont été suivis par double marquage isotopique. L'expression génique des principales protéines impliquées dans le métabolisme lipidique au niveau musculaire a été mesurée par qPCR et le contenu lipidique dans les muscles a été quantifié par IRM. Enfin, les concentrations plasmatiques des métabolites et des hormones ont été dosées par les méthodes RIA et enzymologiques et la composition corporelle a été déterminée par DEXA.

## Résultats

Comme nous l'avons observé chez les hommes, l'inactivité physique entraîne chez les femmes une élévation de la lipémie à jeun (37% ; $p$=0.025) et à la suite d'un repas (27% ; $p$<0,01), un shift dans l'oxydation des substrats au profit des glucides et une hyperinsulinémie en situation post-absorptive ($p$<0,0001 ; $p$=0,01, respectivement) et post-prandiale ($p$<0,0001 ; $p$=0,02, respectivement). Cependant, aucun changement de la concentration des acides gras libres n'a été noté. Les concentrations cumulées sur les 10 heures de test de [1-$^{13}$C]oléate et [$d_{31}$]palmitate

dans les triglycerides (+51% and +25% ; $p$=0,01 et p=0,02, respectivement) et les acides gras libres (+47% and +48% ; $p$=0,004 et $p$=0,002, respectivement) augmentent. Le rapport acides gras libres sur triglycerides cumulés sur 10h demeure inchangé aussi bien pour le [1-$^{13}$C]oléate que pour le [$d_{31}$]palmitate, suggérant qu'une quantité similaire de traceurs est captée par les tissus périphériques en période contrôle et au cours de l'alitement. Pourtant comme précédemment, l'inactivité physique réduit l'oxydation du [$d_{31}$]palmitate cumulée sur 12h de 8,2±4,9% ($p$<0,0001) et n'affecte pas celle du [1-$^{13}$C]oléate. Au niveau musculaire, l'inactivité physique diminue l'expression génique de la lipoprotéine lipase ($p$=0,043) et des transporteurs FAT/CD36 ($p$=0,043) et CPTI ($p$=0.043), et élève la quantité de lipides stockés ($p$=0,05). Une corrélation négative entre l'accumulation de lipides musculaires et la réduction de l'oxydation du palmitate a été notée dans les deux groupes ($y = -0.11 x + 18.13$ ; $r^2 = 0.48$; $p = 0.003$).

Excepté une maintenance partielle de la masse maigre (-1,3±0,2kg vs. -2,9±0,1kg dans le groupe contrôle; $p$<0,001), de la capacité oxydative musculaire (expression génique de la citrate synthase inchangée), de la sensibilité à l'insuline après un repas ($p$=0,050) et de l'oxydation lipidique totale en situations post-absorptives (augmentation de 4% du quotient respiratoire non protéique vs. 9% ; $p$<0,05) et post-prandiale ($p$<0,001), l'entraînement physique de type résistif et aérobic a été inefficace pour contrer les effets délétères de l'inactivité physique.

## Discussion

L'inactivité physique induite par deux mois d'alitement prolongé entraîne le développement d'une insulino-résistance et un shift dans l'utilisation des substrats au profit de l'oxydation des glucides totaux en situation post-absorptive et post-prandiale. Cette diminution de l'oxydation lipidique totale est probablement due, en partie, à la diminution du transport des acides gras dans le myocyte et la mitochondrie. Elle induit aussi une augmentation de l'absorption intestinale et/ou une diminution de la clairance plasmatique des acides gras exogènes résultant en une hypertriglycéridémie et un "spill-over" accru des acides gras libres provenant de l'hydrolyse des lipoprotéines par la lipoprotéine lipase. Bien que les acides oléique et palmitique alimentaires semblent avoir une absorption, un acheminement et une captation par les tissus périphériques similaires, l'inactivité physique diminue seulement l'oxydation du palmitate. Ainsi l'inactivité physique altèrerait la répartition des acides gras saturés au profit d'un stockage au niveau musculaire. Cette hypothèse semble étayée par la relation négative obtenue entre la réduction d'oxydation du palmitate et la quantité de lipides au niveau musculaire. Ce résultat est d'autant plus intéressant lorsque l'on considère la relation établie entre l'insulino-résistance et les triglycérides intra-musculaires.

Grâce au maintien de la masse musculaire et des capacités oxydatives musculaires, l'entraînement physique aérobic et résistif a permis de freiner l'altération de la réponse à l'insuline et la diminution de l'oxydation lipidique totale à jeun et après un repas. Toutefois, cet entraînement physique n'a pas été suffisant pour éviter les changements induits par l'inactivité physique dans l'oxydation des acides gras saturés exogènes et l'accumulation des lipides dans les muscles. De plus amples études sont donc nécessaires afin de mieux comprendre les mécanismes impliqués dans le métabolisme différentiel des acides gras saturés et monoinsaturés ainsi que les relations entre activité physique, oxydation lipidique totale et exogène.

# 1. ABSTRACT

**Objective:** Obesity and diabetes are characterized by the incapacity to use fat as fuel. We hypothesized that this reduced fat oxidation is secondary to a sedentary lifestyle.

**Research Design and Methods:** We investigated the effect of a 2-month bed rest (BR) on the dietary oleate and palmitate trafficking in lean women (control group, n=8) and the effect of concomitant resistance/aerobic exercise training as a countermeasure (exercise group, n=8). Trafficking of stable isotope-labelled dietary fats was combined with muscle gene expression and MRI-derived muscle fat content analyses.

**Results:** In the control group, BR increased the cumulative [1-$^{13}$C]oleate and [$d_{31}$]palmitate appearance in TG (37%, $p$=0.009 and 34%, $p$=0.016, respectively) and NEFA (37%, $p$=0.038 and 38%, $p$=0.002), and decreased muscle LPL ($p$=0.043) and FAT/CD36 ($p$=0.043) mRNA expressions. Plasma NEFA/TG ratios for [1-$^{13}$C]oleate and [$d_{31}$]palmitate remained unchanged, suggesting that the same proportion of tracers enters the peripheral tissues after BR. BR unaffected [1-$^{13}$C]oleate oxidation but decreased [$d_{31}$]palmitate oxidation by -8.2±4.9% ($p$<0.0001). Despite a decreased spontaneous energy intake and a reduction of 1.9±0.3kg ($p$=0.001) in fat mass, exercise training did not mitigate these alterations, but partially maintained fat-free mass, insulin sensitivity and total lipid oxidation in fasting and fed states. In both groups, muscle fat content increased by 2.7% after BR and negatively correlated with the reduction in [$d_{31}$]palmitate oxidation ($r^2$=0.48; $p$=0.003).

**Conclusion:** While saturated and monounsaturated fats have similar plasma trafficking and clearance, physical inactivity impacts the partitioning of saturated fats towards storage likely leading to an accumulation of palmitate in muscle fat.

# 2. INTRODUCTION

In our search of the environmental factors that fuelled the pandemic of obesity, we face a paradox. Although sedentary lifestyle has been highlighted for decades as one of the main factors triggering weight gain, the physiology of physical inactivity has received little attention (1). Clearly, the causal relationships between sedentary behaviours and obesity are essentially based on epidemiological studies or on the indirect beneficial effects of exercise training (2). None of these studies provide evidence to support a cause-and-effect relationship.

Obesity is a fat storage disease characterized by insulin resistance and a decreased capacity to oxidize lipid (3) in fasting (4) and post-prandial (5) conditions. Because weight reduction was not associated with improvement in fat utilization (6), it was suggested as a primary impairment in the etiology of obesity, rather than an adaptive response. Consequently, the delineation of the causes responsible for this reduced capacity to oxidize fat appears a fundamental pre-requisite to develop efficient strategies against obesity.

We previously extended the early Mayer hypothesis (7) and hypothesized that the decreased fat oxidation observed in obese and post-obese subjects is due to the generalized adoption of sedentary behaviors (8). Using strict bed-rest (BR) as a model, we showed that physical inactivity *per se*, i.e. independent of the known physical inactivity-induced energy balance changes, lowers fasting and post-prandial fat oxidation (9). Unexpectedly, whereas monounsaturated dietary fat (oleate) oxidation remained unaffected by BR, saturated fat (palmitate) oxidation decreased by 11% (9). These results are interesting when considering the north/south gradient in obesity prevalence in France that was not associated with the overall energy intake but in the greater amount of saturated fat in the diet (10).

The main objective of our present study was to investigate the mechanisms involved in metabolism of dietary fat during 2-months of BR in women. The key for obesity prevention is to determine the minimal volume of exercise that will restore fat oxidation. Since aerobic exercise training protocols in sedentary lean (11) or obese individuals (12, 13) result in increased fat oxidation during exercise, we tested, as a second objective, the efficacy of combined aerobic and resistance exercise training on fat oxidation.

# 3. RESEARCH DESIGN & METHODS

Sixteen women volunteered in a 60-day BR study. For logistical reasons, the study was divided into two sessions separated by 2 months; each involving half of the volunteers. The subjects were selected if they engaged on at least 30 minutes of moderate activity per day; this being achieved either with structured exercise or with activities of daily living. Athletes and extremely fit individuals were excluded. The subjects were non-smokers, were free of clinical or biomedical diseases and were asked to stop birth control pills 3 months before the study. The study was approved by the local IRB (Midi-Pyrénées I, France).

The BR in head-down tilt position (-6°) was preceded and followed by two 20-day periods of ambulatory control and recovery, respectively. This duration was necessary for the different teams involved in the study to complete baseline data collection. During the control period, subjects were asked, under professional supervision, to exercise in order to help offset detraining possibly occurring while living confined to the Institute. After the 20-day control period, the subjects were randomly divided into a control group that remained in bed and an exercise group that was subjected to combined supine resistance and aerobic exercise training for 60 days (n=8, each). Subjects were continuously in bed

24h/day and standing or seating positions were forbidden. A general overview of the protocols, indicating the specific days of the tests, is illustrated Figure 1.

For the exercise group, resistance training was performed at maximal effort on a flywheel ergometer (14) to train the thigh muscle groups using supine squat exercises. 19 training sessions of 35 minutes were programmed every three days, as previously described (9, 15). Aerobic training was performed three to four times per week (29 sessions of 50 ± 2 min total) on a vertical treadmill in a lower body negative pressure chamber, with intensities varying from 40 to 80% pre-BR maximal oxygen uptake, as previously described (15-17).

Throughout the experiment, we aimed to maintain energy balance. Energy requirements during control and recovery periods were calculated as RMR times a physical activity level (PAL) of 1.4 selected for individuals with a low level of activity (18). During BR a PAL of 1.2 was selected based on a previous BR experiment (19). RMR was measured twice in each period to adjust intake for changes in FFM. Water intake was provided at 3L/day. The macronutrient composition of the diet was set at 30% fat, 15% proteins and 55% carbohydrates. The subjects were asked to finish all food given. Snacks were provided to the subjects to cover leftovers, if any, to cover the cost of the exercise sessions in the exercise group, and more generally when the subjects felt hungry.

FM and FFM were measured twice during the ambulatory period and every fifteen days during the BR period by DXA on QDR 4500 W scanner using the version software 11.2 (Hologic France).

Vastus lateralis biopsies were obtained prior to and after 59 days of BR; 4 hours after lunch and at least 24 hours from the last exercise session, as previously described (20). Due to tissue sharing, we obtained biologic material for only five subjects in both groups. After total RNA extraction, the relative expression levels of ACADL, CD36, CPT1B, COX4I1, GPD1, and LPL were analyzed on the ABI 7900HT Sequence Detection System, as previously described (21), and RT-PCR was performed by using a random primer from a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems) to reverse the RNA. TaqMan Universal PCR Master Mix and Assays-on-Demand Gene Expression probes (Applied Biosystems) were used for the PCR step, and by using the comparative amplification detection threshold of target gene expression ($C_T$) method for analysis. Helicase with zinc finger (HELZ) was taken as an internal standard. Thus, mRNA levels were measured by determining the cycle number at which $C_T$ was reached. In each sample, $C_T$ was normalized to HELZ expression, performed in parallel ($\Delta C_T$). Normalized $\Delta C_T$ values from each time point, in samples 'ambulatory control period', were then subtracted from each time point in samples 'BR' ($\Delta\Delta C_T$) to determine the relative abundance values ($2^{-\Delta\Delta CT}$). Results were expressed in percentage changes from the ambulatory period.

The gastrocnemius/soleus muscle fat content was estimated 14 days prior to and after 29 and 56 days of BR from axial T1-spin echo weighted images obtained from the superior aspect of the calcaneus to the proximal tibia of both legs (PHILIPS INTERA 1.5 T MRI), as previously described (22). We used a relaxation time of 425 ms, an excitation time of 16 ms based on one excitation. The field of view was 400mm x 400mm, matrix size of 192x384 with a slice thickness of 7 mm and spacing of 1 mm.

Dietary fat oxidation and trafficking were measured 15 days prior to and after 32 days of BR. Upon waking (0600am), an intravenous catheter was inserted into the forearm vein for arterialized blood sampling. Baseline breath and urine samples and fasting blood sample were collected. The subjects then ingested 0.4 g/kg of $H_2{}^{18}O$ (10% enriched, CIL Andover MA, USA) to measure TBW, and, subsequently, RMR was measured for 1 hour using indirect calorimetry (Deltatrac II, GE, USA). Then, the participants ingested a fixed moderately high fat breakfast (41%) representing approximately 50% of each subject's RMR in energy (i.e. 3.1MJ in the ambulatory period for both groups and 2.7MJ and 3.1MJ during BR for control and exercise groups, respectively). The breakfast included a liquid replacement meal in which 15mg/kg of [$d_{31}$]palmitic acid (>98% enriched, CIL) and 10mg/kg of [1-$^{13}$C]oleic acid (>99% enriched, CIL) were homogenized. Following ingestion of the meal, hourly breath and urine samples were collected for 12 hours. Equilibration time for $H_2{}^{18}O$ was taken at 3 and 4-hr post-dose. During the 10 hours of the test, total substrate use and NPRQ were measured hourly by continuous indirect calorimetry and nitrogen excretion. Blood samples were collected every hour for 10 hours

to assess the post-prandial response of metabolites and hormones. At 1300, subjects were given a moderately high carbohydrate (58%) lunch (2.7MJ in the ambulatory period for both groups and 2.7MJ and 2.4MJ during BR for control and exercise groups, respectively.

Breath sample $^{13}CO_2/^{12}CO_2$ ratios were measured on a continuous flow inlet system connected to an isoprime IRMS (GV Instruments). The oxidation rate of monounsaturated fat was inferred from the recovery of [1-$^{13}$C]oleate calculated as the instantaneous recovery of $^{13}$C in expired $CO_2$ expressed as a percentage of the dose and corrected for isotope sequestration by assuming an acetate correction factor of 51% (23). $^{2}H/^{1}H$ from urine samples were analyzed as previously described (24). The oxidation rate of saturated fat was inferred from the cumulative recovery of $^{2}$H in TBW. The method was validated at rest and during exercise by comparison with classical $^{13}$C-labelling corrected for isotopic sequestration (25, 26). $^{18}$O enrichments in TBW urine samples were decolorized by black carbon and reduced to CO by carbon reduction at 1400°C in an elemental analyzer (Flash HT, ThermoFisher) coupled to a Delta V IRMS. TBW was determined using the $^{18}$O isotope dilution method. The detailed calculations of the percentage recoveries are described in detail elsewhere (25).

To determine dietary [$d_{31}$]palmitate and [1-$^{13}$C]oleate trafficking, total lipids were extracted from plasma. NEFA and TG fractions were separated by solid-phase extraction and derivatized to methyl esters (27). 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 GC/MS analysis (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 MPE by the concentration of its corresponding unlabeled compound.

Insulin was measured by RIA (DSL), and glucose (Biomérieux), NEFA (Wako) and TG (Biomérieux) were measured by enzymatic methods. Total fat, carbohydrate and protein oxidation rates as well as the NPRQ, were calculated from indirect calorimetry data and urinary nitrogen (28).

All variables were analyzed by a multiple analysis of variance with time as the repeated measure (ambulatory versus BR), group (control versus exercise) as main effect and sessions as covariate. The changes in gene expressions were analyzed using a Wilcoxon rank-sign test to determine the BR effect (ambulatory versus BR) and a Mann-Whitney test to analyze the between-group differences because normality was not respected. Statistics were performed using Statistica version 7.1.515.0 (Statsoft) and reported values are means±SD, unless otherwise stated.

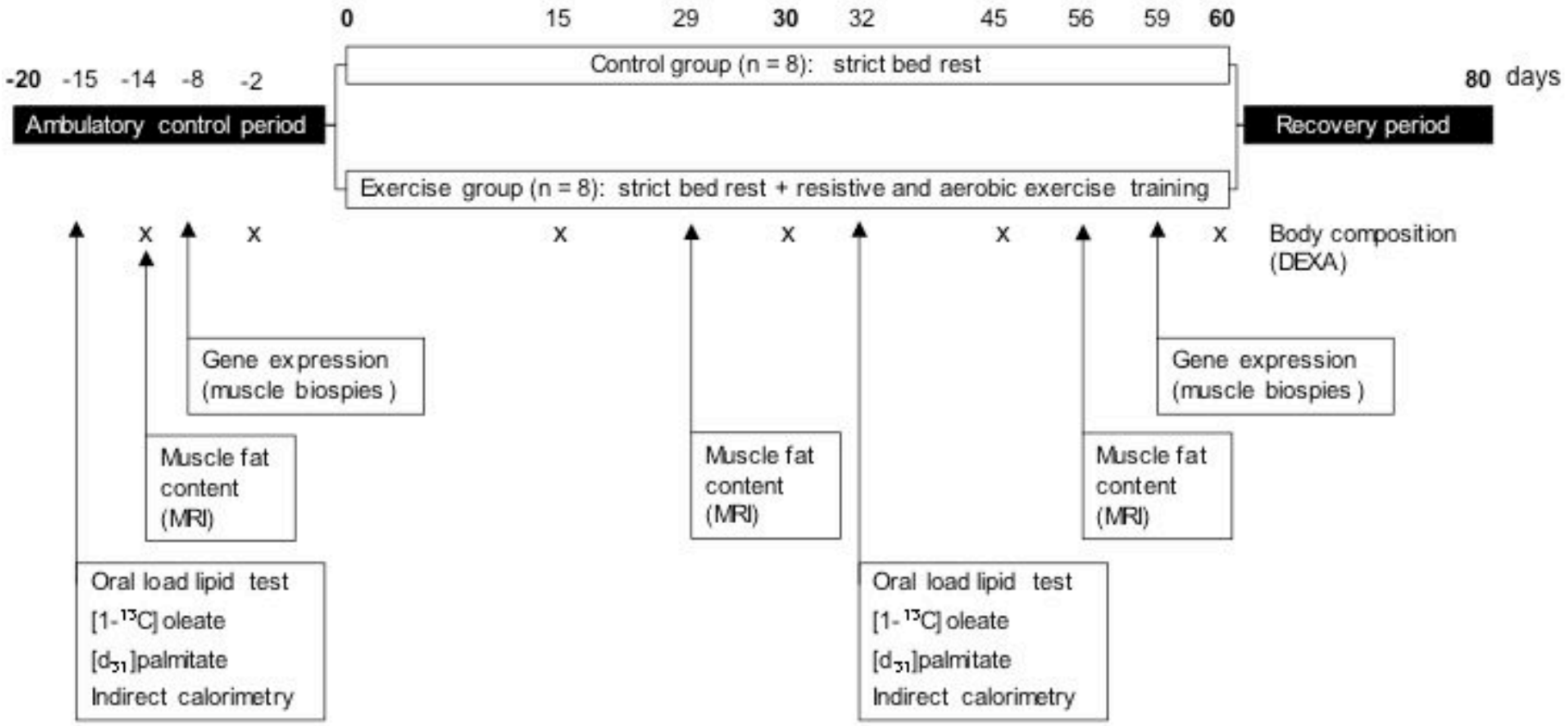


**Figure 1:** Overview of the protocols conducted during the study.

**TABLE 1.** Characteristics of the participants in ambulatory period and body mass and composition and dietary intake evolution during the bed rest

| | | | Ambulatory period | | 30 days of bed rest | | 60 days of bed rest | | MANOVA (p-value) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Units | Control | Exercise | Control | Exercise | Control | Exercise | Bed rest effect | Group effect | Bed rest-by-group interaction |
| CHARACTERISTICS | **n** | | 8 | 8 | 8 | 8 | 8 | 8 | | | |
| | **Age** | years | 34 ± 4 | 33 ± 4 | | | | | | | |
| | **Height** | m | 1.63 ± 0.06 | 1.65 ± 0.07 | | | | | | | |
| | **BMI** | kg/m² | 21.3 ± 1.4 | 21.7 ± 1.4 | | | | | | | |
| | **$VO_2$peak** | l/min | 1.9 ± 0.4 | 2.1 ± 0.4 | | | | | | | |
| | | ml/kg/min | 34.5 ± 7.7 | 35.1 ± 4.4 | | | | | | | |
| BODY MASS AND COMPOSITION | **Body mass** | kg | 55.6 ± 3.8 | 58.4 ± 6.5 | 52.9 ± 4.1 | 55.8 ± 6.0 | 52.3 ± 3.8 | 54.9 ± 6.1 | 0.0001 | NS | NS |
| | **Fat free mass** | kg | 40.8 ± 3.1 | 43.8 ± 5.8 | 38.3 ± 3.2 | 42.7 ± 5.5 | 38.0 ± 3.0 | 42.5 ± 5.7 | 0.0001 | NS | 0.0006 |
| | **Fat mass** | kg | 14.8 ± 3.7 | 14.5 ± 3.2 | 14.7 ± 3.8 | 13.1 ± 3.5 | 14.3 ± 3.5 | 12.4 ± 3.6 | 0.001 | NS | 0.005 |
| | | % | 26.4 ± 5.5 | 25.0 ± 5.0 | 27.5 ± 5.8 | 23.5 ± 5.8 | 27.1 ± 5.5 | 22.6 ± 6.2 | 0.06 | NS | 0.006 |
| DIETARY INTAKE | **Energy intake** | MJ/day | 7.5 ± 0.2 | 7.6 ± 0.5 | 6.5 ± 0.2 | 7.4 ± 0.8 | 6.5 ± 0.2 | 7.4 ± 0.8 | 0.0001 | 0.039 | 0.004 |
| | **Carbohydrates** | g/day | 259 ± 7 | 261 ± 16 | 219 ± 9 | 252 ± 27 | 219 ± 9 | 252 ± 27 | 0.0001 | 0.030 | 0.002 |
| | | % | 58 ± 1 | 58 ± 1 | 56 ± 1 | 57 ± 1 | 56 ± 1 | 57 ± 1 | 0.0001 | NS | 0.002 |
| | **Lipids** | g/day | 59 ± 2 | 59 ± 4 | 52 ± 2 | 59 ± 6 | 52 ± 2 | 59 ± 6 | 0.001 | 0.027 | 0.0018 |
| | | % | 29 ± 0 | 30 ± 0 | 30 ± 0 | 30 ± 0 | 30 ± 0 | 30 ± 0 | 0.0001 | NS | NS |
| | **Proteins** | g/day | 56 ± 4 | 59 ± 6 | 54 ± 4 | 56 ± 6 | 54 ± 4 | 56 ± 6 | 0.01 | NS | NS |
| | | % | 13 ± 1 | 12 ± 1 | 14 ± 1 | 13 ± 1 | 14 ± 1 | 13 ± 1 | 0.0002 | NS | 0.0001 |

Values are mean ± SD

BMI: body mass index; VO2peak, peak O2 consumption.

No difference were noted between groups (t-test) in ambulatory period. The effect of bed rest was determined by a multiple analysis of variance (MANOVA).

The between-group differences in the energy intake corresponds to the estimated cost of the exercise training protocol

# 4. RESULTS

The baseline characteristics of the volunteers, the energy intake and the diet composition provided to the control and exercise groups during the study are summarized in Table 1. No between-group differences were observed at baseline.

## 4.1. Body composition changes

After BR, BW decreased by 4.6% in both groups (Table 1). The loss in BW was essentially due to a 6.2% reduction of FFM in the control group but to a 9.9% decrease in FM in the exercise group. Exercise training partially counteracted the loss in FFM compared to the control group (-2.5% vs. -6.2%).

## 4.2. Insulin resistance and shift in substrate utilization

After one month of inactivity we observed an increase in fasting TG (37%; $p$=0.025), insulin (22%; $p$=0.014) and HOMA (38%; $p$=0.01) in both the control and exercise groups (Figure 2), whereas fasting glucose and NEFA remained unchanged. NPRQ increased from 0.791±0.033 to 0.859±0.030 during BR in the control group ($p$<0.0001). This shift in fasting substrate use was mitigated by exercise training (4% increase in NPRQ vs. 9% in control group; BR-by-group interaction: $p$=0.037) and was not explained by a shift in macronutrient composition of the diet, as the food quotient remained close to 0.88 in all subjects throughout the study.

After meal ingestion, the cumulative glucose and NEFA responses did not vary and TG concentration increased significantly by 27% in both groups during BR (Figure 2). BR induced a 36% increase in post-prandial insulin concentration in the control group that was not seen following exercise training, as evidenced by a significant BR-by-group interaction (Figure 2). After BR the ratio of cumulative carbohydrate/fat oxidation increased significantly by 2.5 fold in the control group and by 1.6 fold in the exercise group. The significant BR-by-group interaction suggests, however, that exercise training partially attenuated the effect of sedentariness (Figure 3).

## 4.3. Proportion of dietary fatty acid uptake

The concentrations of [1-$^{13}$C]oleate and [$d_{31}$]palmitate in plasma TG and NEFA were not different between the control and exercise groups (Figure 4). Cumulative dietary labeled oleate and palmitate in TG increased significantly by 51% and 25%, respectively, during BR in both groups. Similarly, a 1.5 fold increase in both cumulative oleate and palmitate labeled NEFA concentrations were noted during BR (Figure 4).

In the exercise and control groups the NEFA/TG ratio remained unchanged during BR for both [1-$^{13}$C]oleate (14% vs. 13% during ambulatory conditions) and [$d_{31}$]palmitate (10% vs. 11%).

## 4.4. Palmitate and oleate oxidation

An overall group effect was noted for both oleate and palmitate oxidation. The higher oxidation values observed in the exercise group were essentially accounted for by differences in initial FFM (Figure 5). In both the exercise and control groups, BR decreased palmitate oxidation by -8.2 ± 4.9% and -6.4 ± 4.8% of the dose, respectively (Figure 5).

Conversely, BR did not affect the 12h post-dose cumulative recovery of [1-$^{13}$C]oleate in either group. The differential oxidation rates between oleate and palmitate was not partly attributable to differences in the kinetics of oxidation, as suggested from the 12hr instantaneous percentage recovery curves (Figure 5). Indeed, breath and urine samples collected 24hr post-dose confirmed that in both groups oleate remained unaffected by physical inactivity (-0.70±8.6%, BR effect: $p$=0.8), whereas palmitate oxidation decreased by -6.0±8.1% (BR effect: $p$=0.01).

## 4.5. Muscle expression of key lipid metabolism proteins

Muscle mRNA LPL expression decreased after BR in the control group ($p$=0.043; Figure 6) but not in the exercise group. FAT/CD 36 ($p$=0.043, for each group) and CPTI ($p$=0.043, for each group) mRNA expressions decreased in both groups whereas mRNA expressions of ACADL and GPD1 remained unchanged. COX4 mRNA expression was decreased during BR in the control group ($p$=0.043) but not in the exercise group. No significant group differences were noted using a Mann-Whitney test.

## 4.6. Muscle fat content

BR induced a significant 2.7% increase in muscle fat content in both groups (Figure 7). The T1 signal values measured after one month of BR and adjusted for baseline values negatively correlated with the reduction in palmitate oxidation (Figure 7). Both groups were combined for this analysis since no BR-by-group interaction was observed for both palmitate oxidation ($p$=0.87, Figure 5) and T1 signal ($p$=0.54, Figure 7). This demonstrates no between-group differences in the slopes of the relationships. No relation was noted between the T1 signal adjusted for baseline and the changes in oleate oxidation after BR ($r^2$=0.02; $p$=0.68). An outlier test failed to reveal extreme values in both these relationships.

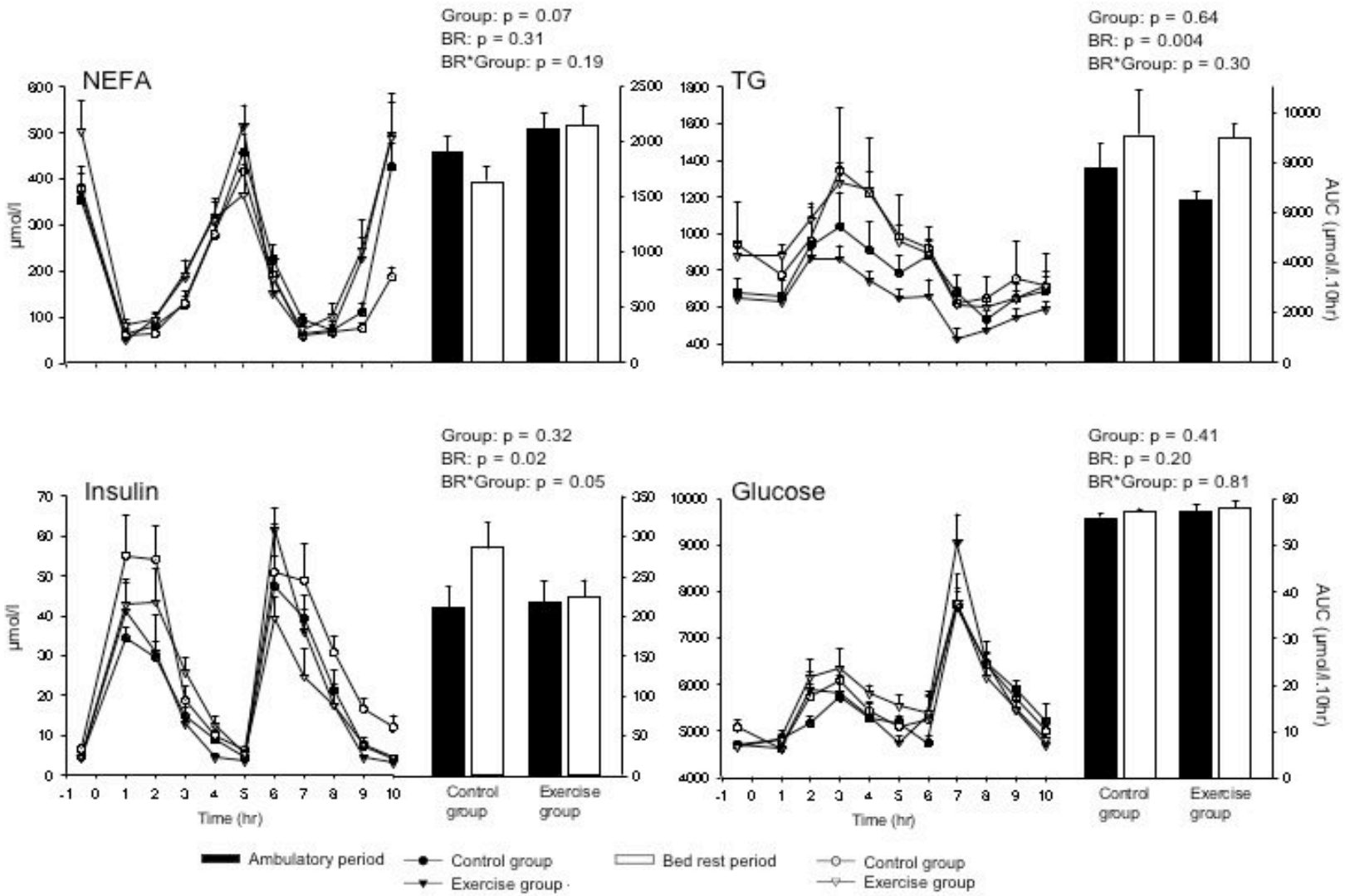


**Figure 2:** Time course of NEFA, TG, insulin and glucose concentrations (µmol/l) in the control (n = 8) and exercise (n = 8) groups 15 days before bed rest and after 32 days of bed rest (BR). Time 0 corresponds to the standard breakfast ingestion. The post-prandial cumulative responses of these parameters were calculatedby the area under the curve (AUC) over the 10hr postdose. Data are mean ± sem.

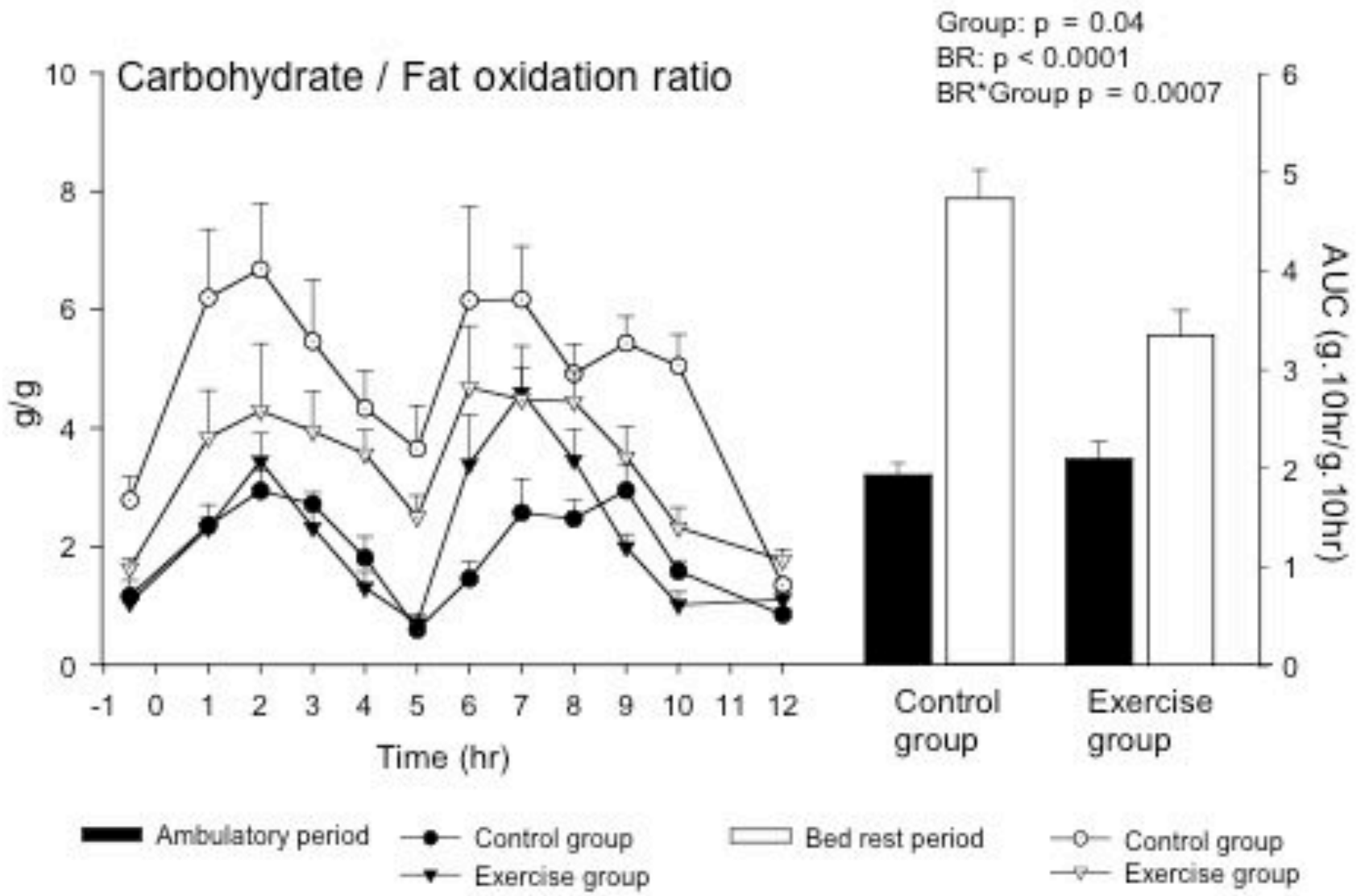


**Figure 3:** Time course of the ratio between carbohydrate and fat oxidations (g/g) in the control (n = 8) and exercise (n = 8) groups 15 days before bed rest (BR) and after 32 days of BR. Time 0 corresponds to the standard breakfast ingestion. The ratio of the post-prandial cumulative carbohydrate to cumulative fat oxidation were calculated by the area under the curve (AUC) over the 10hr postdose. Data are mean ± sem.

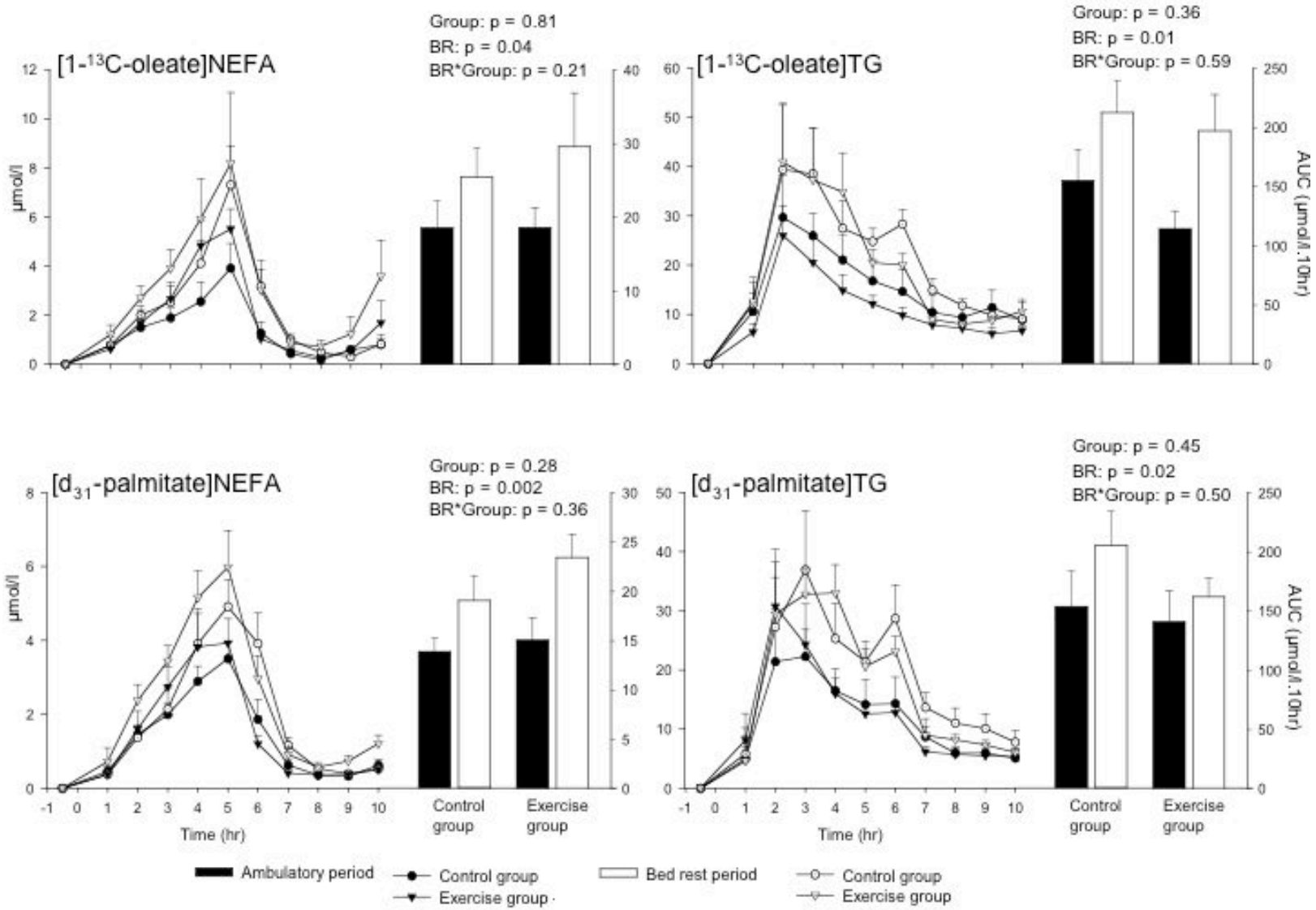


**Figure 4:** Time course of labeled dietary [1-$^{13}$C]oleate and [$d_{31}$]palmitate in TG and NEFA in the control (n = 8) and exercise (n = 8) groups 15 days before bed rest (BR) and after 32 days of BR. Time 0 corresponds to the standard breakfast ingestion. The cumulative responses of these parameters were calculated by the area under the curve (AUC) over 10hr postdose. Data are mean ± sem.

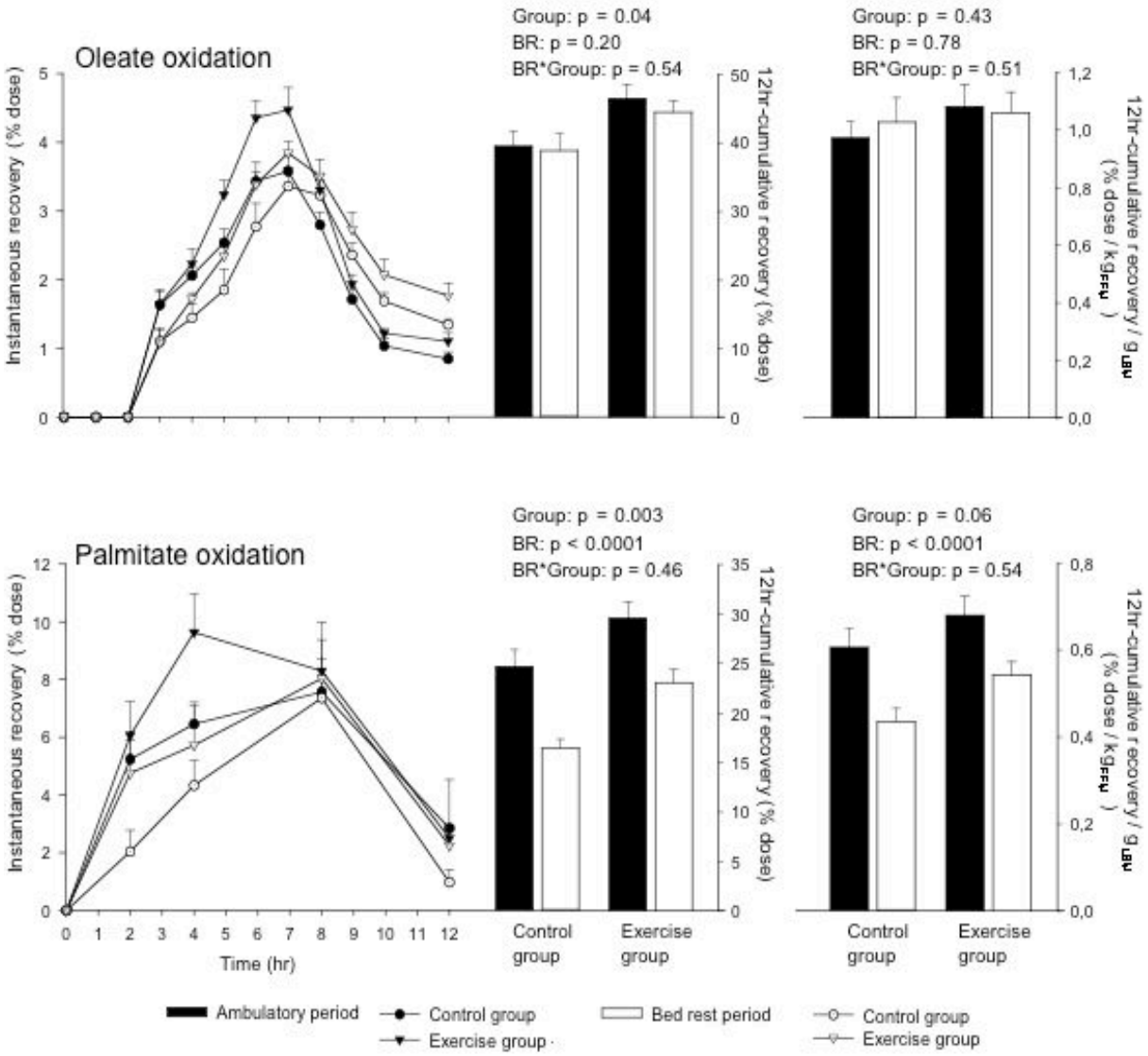


**Figure 5:** Hourly instantaneous percent recovery of [1-$^{13}$C]oleate and [$d_{31}$]palmitate in the control (n = 8) and exercise (n = 8) groups 15 days before bed rest (BR) and after 32 days of bed rest. Time 0 corresponds to the standard breakfast ingestion. Recoveries of [1-$^{13}$C]oleic and [$d_{31}$]palmitic acids were calculated as the instantaneous recovery of $^{13}$C in expired $CO_2$ hourly sampled over the 12 hours of the test and as the cumulative recovery of $^{2}$H in total body water hourly sampled through urine, respectively. Both of these recoveries are expressed as a percentage of the dose. The cumulative percent recoveries of [1-$^{13}$C]-oleate and [$d_{31}$]-palmitate were calculated by the area under the curve (AUC) over the 12hr postdose and then normalized by kg of fat free mass (FFM). Data are mean ± sem.

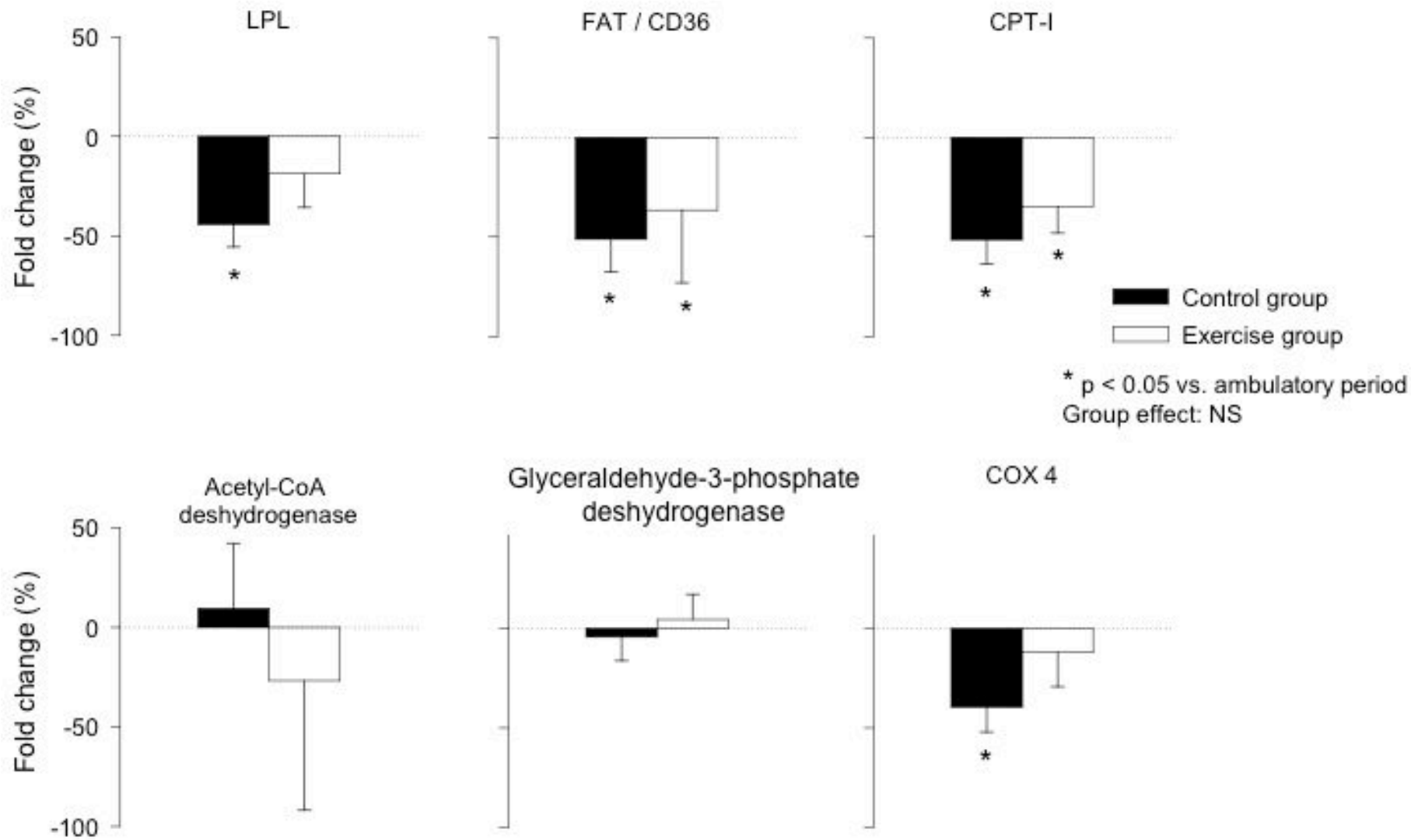


**Figure 6:** Bed rest-induced changes in expression of skeletal muscle lipoprotein lipase (LPL), fatty acid transporter CD36 (FAT/CD36), carnitine palmitoyl transferase I (CPTI), AcetylCoA deshydrogenase, glyceraldehydes 3 phosphate deshydrogenase (GPD1) and citrate synthase (COX4) mRNAs measured between 8 days before bed rest and after 59 days of bed rest and expressed in percentage of fold change in the control (n = 5) and exercise (n = 5) groups. Data are mean ± sem.

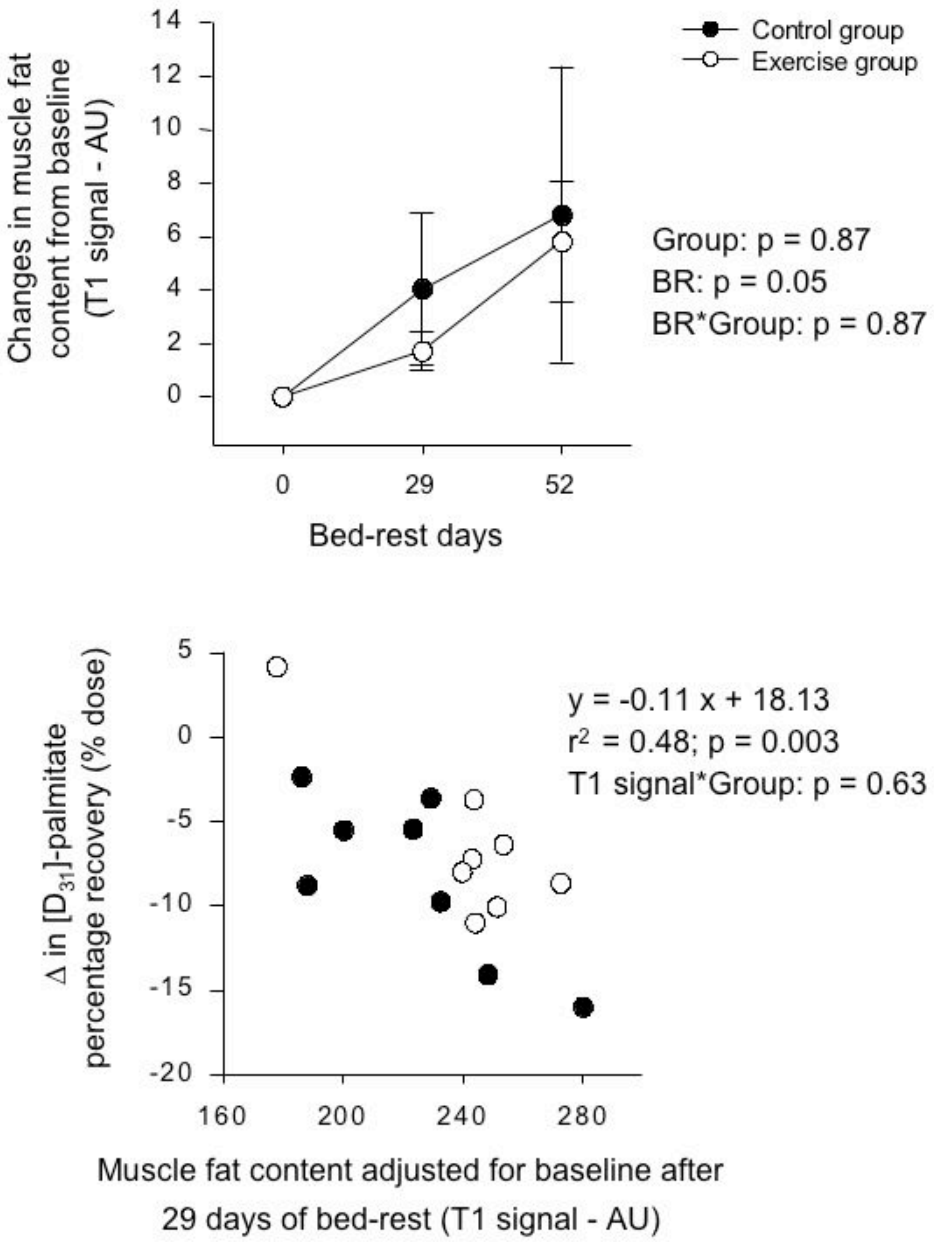


**Figure 7:** Changes in the gastrosoleus muscular fat content during bed rest (BR) from ambulatory period (top). Regression analysis between the BR-induced changes in cumulative [$d_{31}$]palmitate (middle) and [1-$^{13}$C]oleate (bottom) percentage recoveries (% dose), and the gastrosoleus muscle fat content after one month of BR. Muscle fat content was measured before bed rest and after one and two months of BR by Magnetic Resonance T1 signal intensity (arbitrary unit, AU) in the control (n = 8) and exercise (n = 8) groups. BR induced a significant increase in muscle fat content in both groups ($p$ = 0.051). After one month of BR, T1 signal adjusted for baseline values negatively correlated with the reduction in palmitate (y = -0.11 x + 18.13 ($r^2$ = 0.48; $p$ = 0.003). Data are mean ± sem.

# 5. DISCUSSION

The present study extends our previous results obtained during a 90d-BR in men (9) to women. Extreme physical inactivity, independent of changes in energy balance and macronutrient intake, induces a hypertriglyceridemia, a decrease in insulin sensitivity and a decrease in total lipid oxidation in favor of an increase in total carbohydrate oxidation in both fasted and fed states. As in men, inactivity decreases saturated but not monounsaturated fatty acid oxidation. The present study suggests some mechanisms involved in these metabolic alterations.

BR induces an increase in plasma TG concentration, which may be due to a greater lipid absorption in enterocytes and/or a lower clearance of TG. The head-down position used to simulate microgravity may induce a larger blood volume in the visceral areas (29) leading to greater macronutrient absorption. Our data suggest that hydrolysis of TG-rich lipoproteins by LPL may be reduced by BR, which is caused by both muscle atrophy and the reduction in muscle LPL gene expression. Using the hindlimb tail rat model, Bey et al. (30) already demonstrated that acute or chronic periods of inactivity decrease muscle LPL activity. The chylomicron-released NEFA can either enter the plasma pool or the peripheral cells (adipocyte or myocyte). During physical inactivity, we observed a greater spillover of the dietary NEFA, indicating a lower uptake by the peripheral tissues. At the muscle level, FAT/CD36 gene expression dropped during BR, which reduces muscle uptake of NEFA, as was previously reported in inactive muscle of rats (31). Despite the greater dietary NEFA spillover, we did not observe greater plasma NEFA concentrations. Both the higher insulin concentration in the post-prandial period acting on the adipose tissue and the VLDL metabolism may be involved. The NEFA pool constitutes the major source for VLDL synthesis in the liver (32). Because our volunteers developed insulin resistance during BR, it is likely that the lower NEFA concentration and the higher TG concentration are interrelated and involve VLDL synthesis. In support of that, Hodson et al. (33) showed that the higher TG concentrations observed in the insulin resistant males is attributed to a higher post-prandial VLDL concentration. Further investigations are required to delineate the chylomicrons and VLDL metabolism during physical inactivity.

Extreme physical inactivity induces a shift in substrate use with a decrease in lipid oxidation in favour of carbohydrate oxidation in both fasting and fed states. This shift was observed during all BR studies and seems unrelated to energy balance as it was observed in conditions of positive, stable or negative energy balance (34). This phenomenon is partially due to the muscle atrophy associated with a shift in the muscle fiber types characterized by a decrease in the oxidative twitch fibers (MHCI and MHCIIa) and an increase in the glycolytic twitch fibers (MHCIIx) (35, 36). Such a fiber type pattern with a higher proportion of glycolytic fibers in skeletal muscle was observed in obese and diabetic subjects (37). Moreover, the decrease in CPTI mRNA expression could likely explain the blunted fat oxidation. Using the paradigm of suspension in rat and affimetrix® technology, Stein et al. (38) showed an increase in glycolytic capacity and a drop in oxidative capacity in atrophied slow type soleus muscle. In our study, we failed to show such changes in GPD1 and ACADL gene expressions in the mixed muscle type vastus lateralis. Further investigations are required to better understand the physical inactivity-induced impairments of the mechanisms regulating the metabolism of carbohydrates and lipids.

Physical inactivity does not affect dietary oleate oxidation but decreases palmitate oxidation by -8%, which is in accordance with the 11% decrease observed in the 3-month BR in men (9). In the present study, labeled oleate and palmitate incorporation into the NEFA and TG fractions in the ambulatory and BR periods were similar, suggesting that oleate and palmitate present a similar absorption rate, plasma trafficking, and clearance as already reported by Evans et al. (39) under normal conditions. The labeled NEFA/TG ratio can be considered an index of the proportion of NEFA from LPL-mediated hydrolysis of TG-rich lipoprotein entering the NEFA pool. In our study, the ratios indicated a similarity of the two dietary fatty acids with regard to uptake by the peripheral tissues in both ambulatory and BR conditions.

To our knowledge, no differences between oleate and palmitate binding affinities were reported in the human FABPs studied so far, including liver, muscle (40) and adipose tissue

(41). Furthermore, studies which have measured fasting fractional uptake of fatty acids by heart (42), liver or forearm (43) have failed to demonstrate any difference between palmitate and oleate. Taken together, these results suggest that the differential metabolism between the dietary saturated and monounsaturated fats is more likely due to differences in the handling between storage and oxidation within the myocytes than to a differential plasma trafficking.

Interestingly, we found a significant correlation between the decrease in palmitate oxidation and the gastrosoleus fat accumulation induced by physical inactivity, suggesting a preferential channelling of palmitate towards muscle fat. Gaster et al. (44) have previously reported similar results in cultured myotubes from diabetic subjects expressed as a reduction in palmitate oxidation and no change in oleate oxidation associated with a differential handling by myotubes: palmitate accumulates as diglycerides and TG, whereas oleate accumulates as intracellular free fatty acids. The key is to understand the mechanisms of regulation, which are negatively impacted by the physical inactivity, or by T2D, that may explain this differential oxidation and partitioning in myocyte. It was found that rat liver CPTI have a lower affinity for oleyl-CoA than palmitoyl-CoA (45) and that enzyme activity towards oleyl-CoA was more sensitive to inhibition by malonyl-CoA than was the activity towards palmitoyl-CoA (46). Both of these findings would favour oxidation of palmitate. Recently, it has been shown that a mitochondrial isoform of glycerol-3-phosphate acyltransferase (mtGPAT) may direct the flux of fatty acids toward glycerolipid synthesis and away from beta-oxidation (47). Interestingly, mtGPAT also presents a higher affinity for saturated than for monounsaturated fatty acids (47). Because both mtGPAT and CPTI are located on the outer mitochondrial membrane, they can compete for acyl coenzymes A. In fact, AMP-activated kinase (AMPK) reciprocally regulates TG synthesis and fat oxidation in liver and muscle via an inverse regulation of CPTI and mtGPAT (48). During exercise, AMPK is activated (49) and decreases TG synthesis and up-regulates beta-oxidation. Since muscle unloading down-regulates AMPK (50), an opposite process under physical inactivity would represent a good hypothesis for explaining the fat accumulation that we measured in muscles and the negative correlation between muscle fat content and reduced saturated fat oxidation. Despite some changes in the kinetics of oleate oxidation, the daily oleate oxidation was unchanged by physical inactivity, likely due to a preferential accumulation of oleate as free fatty acids (44). Because a clear relationship is observed between IMTG and their derivated products such as diglycerides or ceramides and the development of insulin resistance in diabetic subjects (51, 52), further investigations are clearly required to better understand the relationship between IMTG and dietary fatty acids according to their nature.

In this present study, we tested the efficacy of combined resistance and aerobic exercise training, performed concomitantly with BR, to mitigate the deleterious effects of physical inactivity. Similarly to what we observed during the 90d-BR performed in men (9), the BR-induced hypertriglyceridemia was not counteracted by exercise training. Such a lack of effect might be partially attributed to the absence of recent exercise (36-hr) prior to the test (53). Nevertheless, the combined aerobic/resistive exercise training partially maintained the muscle mass, as did the resistance training alone, but also partially protected the muscle fiber type pattern (15, 35, 36), the amount of mitochondria and the oxidative capacity, as expressed by the maintenance of COX4 gene expression. Interestingly, contrary to the resistance exercise training performed during the previous BR (9), we observed that this exercise training protocol partially counteracted the shift in substrate utilization in the fasting and fed states and the higher post-prandial insulin concentration observed in the control group. Consequently, the comparison of the results from these two BR may highlight the major role of the aerobic type exercise and its effect on energy balance in the protection of the muscle metabolic pattern. It also suggests that rather than the muscle mass, the anatomic and metabolic characteristics of the muscle may be the key factors determining macronutrient oxidation. However, only about 50% of the capacity to use fat as fuel was maintained by exercise training, suggesting at least some metabolic alterations in the exercise group. Indeed, the exercise training only tended to mitigate the LPL gene expression changes and had no effect on the muscle FAT/CD36 or CPTI expression alterations. Contrary to the total lipid oxidation, no beneficial effect of the exercise training was detected on the exogenous palmitate oxidation. However, the dietary palmitate oxidation normalized for FFM decreased by 20% in the exercise group compared to 29% in the control group, suggesting a slight protective effect of training. Yet, the question remains regarding how much physical activity is required to maintain both total

and exogenous lipid oxidation. Lastly, the relationship between muscle fat accumulation and BR-induced reduced dietary palmitate oxidation was also observed in the exercise group. Because high IMTG content were observed in both sedentary obese subjects and athletes (54), it is likely that this relationship does not have the same impact in health outcomes such as insulin resistance. However, further studies are needed to ascertain this relationship as well as to better understand the interactions between physical activity, total and exogenous lipid oxidation.

Several limitations of our study have to be considered. First, because multiple research teams participated in this study, there were limits on tissue availability and assays (ie. no VLDL separation from chylomicrons possible). Second, the strict BR paradigm used in this study created a physical inactivity at the extreme level of what is typically seen in our society. Although this model is relevant to highlight mechanisms of regulation underlying the lipid metabolism, further studies are required on the general population.

Despite the tight monitoring of the diet, the volunteers of the exercise group did not ingest the entire energy intake that they required to maintain their initial FM and consequently, were in negative energy balance, which does not allow us to clearly dissociate the effect of negative energy balance on substrate use from those solely due to exercise. Indeed, based on the changes in body composition, we estimated an average energy deficit of 1.48±0.54 MJ/d in the exercise group compared to 0.54±0.38 MJ/d in the control group. These estimations match to 88.5% and 98.7 % the energy balance measured from intake and doubly labeled-water derived-total energy expenditure in the control and exercise groups, respectively. Interestingly the negative energy balance of the exercise group was due to increased leftovers, not false energy intake prescription, suggesting strong effect of the BR/countermeasure on satiety (data not shown). Nevertheless, it is important to note that the previous BR studies reported that the substrate shift was observed in subjects being either in positive, negative or in energy balance (34). This suggests that the effect of physical inactivity might be, to some extent, independent of major changes in energy balance.

The present study clearly shows that extreme physical inactivity, independent of its effects on energy balance, impacts the partitioning of saturated fatty acids towards storage versus oxidation likely via a preferential accumulation of palmitate in muscle fat. Additionally, saturated and monounsaturated fatty acids present a similar absorption rate, trafficking and uptake by peripheral tissues independent of the physical activity level. Thus, our study provides interesting areas for future research in insulin resistance since it strongly correlated with IMTG. Further investigations are indeed necessary to better understand the mechanisms involved in the trafficking of the dietary fatty acids towards the different peripheral tissues (muscle or adipose tissue) according to their nature and in the lipid metabolism at the muscle level. Although this study also highlights the key role of the aerobic type exercise training in the regulation of the lipid oxidation, investigations on the relationship between the type of exercise and the exogenous lipid oxidation are also required.

# 6. ACKNOWLEDGEMENTS

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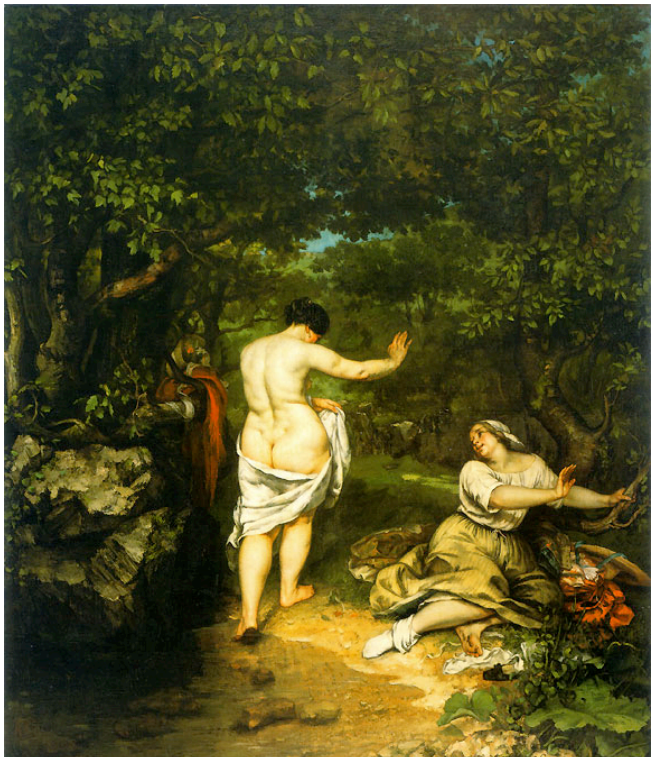
# RESULTATS

## PARTIE METHODOLOGIQUE



*Les Baigneuses de Courbet (1853)*

# CHAPITRE 7

# 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

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***European Journal of Applied Physiology, 95(4), p.344-50, 2005***

# Résumé

## Introduction

Le principal but des deux études précédentes était d'étudier l'effet direct de l'inactivité physique sur le métabolisme lipidique, indépendamment des effets confondants d'une éventuelle balance énergétique positive. Par conséquent, une des conditions fondamentales mais délicate de ce type d'étude est le maintien d'une balance énergétique stable. Pour cela, les investigateurs doivent ajuster l'apport calorique à la dépense énergétique totale et ce, de manière individuelle. *A priori*, le suivi de l'évolution de la masse corporelle pourrait représenter un indice fiable. Toutefois, à cause d'une sollicitation très limitée des muscles, l'alitement prolongé provoque une atrophie musculaire et ainsi altère la composition corporelle. La masse corporelle n'est donc pas représentative de l'état de la balance énergétique et le calcul des besoins énergétiques doit plutôt être basé sur les changements de masse grasse (MG). Parmi les différentes méthodes utilisées pour mesurer la masse grasse, la méthode des plis cutanés (PC) est la moins coûteuse, la plus facile et la plus rapide. Cependant, elle est largement critiquée à cause de son manque de précision et des biais importants qu'elle peut engendrer.

## Objectif

Nous avons cherché dans cette étude à tester la précision de la méthode du pli cutané, par comparaison avec la méthode de dilution isotopique (DIL), pour tester son utilisation dans la quantification des besoins énergétiques des volontaires lors d'alitements prolongés.

## Matériel et Méthodes

Nous avons compilé les données de trois campagnes d'alitement prolongé tête déclive à -6° : une chez la femme (n=8) effectuée en Novembre 1998 et deux chez l'homme (n=8) réalisée en Décembre 1997 (sans contremesure) et en Janvier 1998 (avec un garrot autour de la cuisse comme contremesure). La composition corporelle était mesurée après 6 jours d'alitement. Le pourcentage de masse grasse était dérivé de la mesure du pli cutané au niveau des biceps, des triceps, de la zone sous-scapulaire et de la zone iliaque basée sur la méthode de Durnin et Wormersly (1974). Le même jour, la masse maigre était déterminée par dilution isotopique en assumant un facteur d'hydratation de la masse maigre à 0,73. La masse grasse était calculée par soustraction avec la masse corporelle.

## Résultats

D'après des tests de précision, la méthode du pli cutanée peut mesurer avec précision des changements de masse grasse de seulement 1,1% pour une seule mesure. Aussi bien les interceptes ($F_{4,30}$=0,89 ; $p$=0,45) que les pentes ($F_{4,30}$=0,74 ; $p$=0,57) des relations pli cutanés vs. dilution isotopique n'étaient pas affectés par les périodes (Décembre vs. janvier), les conditions expérimentales (contrôle vs. alitement vs. alitement contremesure) ou le sexe. Basé sur ces observations, il a été possible de dériver l'équation suivante : $\%MG_{PC} = 0,94 * \%MG_{DIL}$ ($F_{1,47}$=97,9 ; $p$<0,0001), avec un biais entre les deux méthodes de -1,7±2,0%MG. Une mauvaise préparation de l'expérimentateur se traduit, cependant par des erreurs majeures dans l'estimation de la masse grasse.

## Discussion

La méthode des plis cutanés permet donc d'obtenir des mesures fiables de la masse grasse et par conséquent du statut énergétique à moindre coût et de manière facilitée. Ainsi, les besoins énergétiques des volontaires au cours des études d'alitement peuvent être rapidement ajustés permettant le maintien d'une balance énergétique stable et l'étude des effets de l'inactivité physique sur le métabolisme lipidique indépendamment des changements du statut énergétique des sujets. Toutefois, compte tenu du facteur de précision lié à l'expérience de l'expérimentateur, il est conseillé d'associer d'autres estimations de la composition corporelle lorsque cela est possible.

**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**

Alexandre ZAHARIEV, Audrey BERGOUIGNAN, Michel CALOIN, Sylvie NORMAND, Guillemette GAUQUELIN-KOCH, Claude GHARIB and Stéphane BLANC

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# CHAPITRE 8

## The acetate recovery factor to correct tracer derived dietary fat oxidation in humans

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# Résumé

## Introduction

Dans le cadre des études nutritionnelles, les isotopes stables sont classiquement utilisés pour mesurer l'oxydation des lipides. Comme nous l'avons fait dans nos propres études, 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. Néanmoins, il a été montré en conditions de perfusion que ce facteur, bien que reproductible, présentait une importante variation interindividuelle (12%). Il faut donc le déterminer pour chaque sujet. L'usage des isotopes radioactifs étant fortement limité dans les pays européens, ces résultats imposent d'effectuer les expériences en double dans des conditions similaires à quelques jours d'intervalle afin de déterminer l'oxydation lipidique et le facteur de correction acétate pour chaque sujet. Une telle procédure est envisageable lorsque les traceurs sont perfusés puisqu'un état stationnaire est atteint au bout de 2h environ. En revanche, cela devient beaucoup plus contraignant pour les études sur l'oxydation des lipides alimentaires 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. L'obligation de respecter ces contraintes expérimentales lourdes et coûteuses n'a pourtant jamais été vérifiée dans le cas d'ingestion des traceurs.

## Objectifs

En combinant les données provenant de 6 études distinctes (n=69 sujets) sur l'oxydation des acides gras exogènes, nous nous proposons dans cette étude de :

- Déterminer la variabilité inter-individuelle du facteur de correction acétate exogène,
- Déterminer les facteurs pouvant expliquer cette variabilité,
- Déterminer l'effet du niveau et des modifications de l'activité physique sur le facteur de correction acétate exogène,
- Déterminer la reproductibilité de ce facteur.

## Résultats

Chez des sujets sains et minces, le facteur acétate exogène est égal à 50,6 ± 5,4% de la dose (n=56) avec un coefficient de variabilité inter-individuelle de 10,6% au repos et de 9,2% après des modifications de l'activité physique. Comme les interventions sur le niveau d'activité physique n'affectent pas ce facteur de correction, nous avons pu calculer le coefficient de variation intra-individuelle qui est de 4,6%. Nous n'avons pas mis en évidence de déterminants anthropologiques ou physiologiques. Le fait d'appliquer un facteur de correction acétate individuel ou moyen ne modifie pas la moyenne et la variabilité des valeurs d'oxydation exogène aussi bien au repos qu'après modifications de l'activité physique. Il est important de noter qu'utiliser un facteur moyen fait varier de moins de 10% l'oxydation lipidique individuelle, contre 30% lors de perfusion. Par ailleurs, l'utilisation d'un facteur moyen ou individuel ne change pas la relation observée

entre le quotient respiratoire à jeun et l'oxydation lipidique exogène.

## Discussion

Cette étude valide l'utilisation d'un facteur de correction acétate moyen de 51% chez des sujets sains aussi bien pour des études déterministiques que comparatives mesurant l'oxydation des lipides alimentaires. 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. Des études similaires sont cependant nécessaires chez des sujets atteints de physiopathologies telles que l'obésité et le diabète.

**The acetate recovery factor to correct tracer-derived dietary fat oxidation in humans**

Audrey BERGOUIGNAN, Dale A. SCHOELLER, Susanne VOTRUBA, Chantal SIMON, and Stéphane BLANC

**American journal of physiology. Endocrinology and metabolism, 2008, vol. 294, E645–E653**

# CHAPITRE 9

## Bio-distribution of an oral load of 14(R,S)-[$^{18}$F]fluoro-6-thia-heptadecanoic acid in pigs

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***Applied Radiation & Isotopes, soumis***

## Résumé

### Introduction

L'obésité est le résultat d'une altération de la répartition des acides gras alimentaires entre stockage et oxydation, avec un acheminement préférentiel vers le tissu adipeux en défaveur du muscle. Dans ce contexte, l'étude du devenir des lipides représente un pré-requis fondamental à la mise en place de stratégies préventives et thérapeutiques de l'obésité. Cependant, les protocoles classiquement utilisés pour l'étude du métabolisme des lipides alimentaires sont lourds et invasifs. En effet, ils sont principalement basés sur des biopsies tissulaires, des prises de sang fréquentes, l'utilisation de composés marqués aux isotopes stables, les mesures des échanges gazeux par calorimétrie indirecte ou dans une chambre calorimétrique confinant les volontaires pendant plusieurs heures. La tomographie à émission de positrons (TEP) pourrait être une méthode non invasive alternative à l'étude du métabolisme des lipides alimentaires, et qui pourrait éviter ces protocoles lourds. La TEP repose sur la perfusion de molécules marquées.

### Objectif

Évaluer dans une étude préliminaire si l'acide 14(R,S)-[$^{18}$F]fluoro-6-thia-heptadecanoique [$^{18}$F]FTHA, considéré comme un bon analogue des acides gras en conditions post-absorptives, peut représenter un traceur potentiel des acides gras exogènes.

### Matériel et Méthodes

Nous avons étudié la distribution du [$^{18}$F]FTHA dans l'organisme de 4 cochons anesthésiés 30, 210 ou 270 min après l'ingestion d'un repas standard contenant le traceur. Chez un cochon, nous avons aussi comparé la distribution du [$^{18}$F]FTHA dans l'organisme avec celle du [$^{3}$H]palmitate exogène, traceur des acides gras classiquement utilisé dans les études sur le métabolisme.

### Résultats

La méthode semble être reproductible puisque l'accumulation de la radioactivité du $^{18}$F dans les différents compartiments du corps est similaire entre les deux cochons anesthésiés 30 min après la charge orale. Le pourcentage de $^{18}$F par rapport à la dose 300 min après l'ingestion est 1,05%, 1,40% et 3,84% dans le sang, 0,94%, 5,59% et 7,43% dans les urines et 1,72%, 6,86% et 8,89% dans le foie chez les cochons anesthésiés 30, 210 et 270 min après l'ingestion du traceur, respectivement. Cette augmentation du pourcentage de $^{18}$F dans le sang, les urines et les tissus, associée au délai entre l'anesthésie et l'ingestion du traceur, suggère un effet inhibiteur de l'anesthésie sur les processus de digestion. Le $^{18}$F s'accumule préférentiellement dans le foie et les urines. Au contraire, les résultats de la radioactivité du $^{3}$H montrent des taux d'apparition dans le sang et de captation du [$^{3}$H]palmitate dans les tissus plus élevés et une radioactivité plus faible dans les urines comparés au $^{18}$F.

### Discussion

Le [$^{18}$F]FTHA semble être directement transporté par la veine porte au foie où il est métabolisé et excrété dans les urines. Ce résultat suggère que le [$^{18}$F]FTHA ne

reflète pas proprement le métabolisme des acides gras alimentaires. Des études sont donc nécessaires pour établir un nouveau candidat qui pourrait tracer le devenir des acides gras exogènes pour les études de tomographie à émission de positrons.

# 1. ABSTRACT

**Background:** A non-invasive measurement of dietary fat trafficking by positron emission tomography scan would avoid classic cumbersome clinical protocols and provide crucial data in the etiology of metabolic diseases.

**Aim:** The objective of this study was to evaluate 14(R,S)-[$^{18}$F]fluoro-6-thia-heptadecanoic acid [$^{18}$F]FTHA considered as a good fatty acid analog in the fasting state as a potential dietary fatty acid tracers.

**Methodology:** We study the biodistribution of [$^{18}$F]FTHA in 4 pigs anesthetized 30, 210 or 270 min after a standard meal containing the tracer. In one pig, we also compared the [$^{18}$F]FTHA biodistribution with that of dietary [$^{3}$H]palmitate, a fatty acid tracer widely used in metabolic studies.

**Results:** The method seems to be repeatable since $^{18}$F radioactivity accumulation in the body compartments are similar in the two pigs anesthetised 30 min after the oral load. The 300min post-ingestion $^{18}$F dose percentage is 1.05%, 1.40% and 3.84% in blood, 0.94%, 5.59% and 7.43% in urine and 1.72%, 6.86% and 8.89% in liver when pigs are anesthetized 30, 210 and 240 min after the tracer ingestion, respectively. This increase in 18F dose percentage in blood, urine and tissues with the delay between anaesthesia and the tracer ingestion suggests an inhibitory effect of anaesthesia on digestion process. $^{18}$F is preferentially accumulated in liver and in urine. The comparison with $^{3}$H results shows higher rates of appearance in blood and uptakes in tissues of [$^{3}$H]palmitate and lower $^{3}$H radioactivity in urine than $^{18}$F.

**Conclusions:** [$^{18}$F]FTHA likely bypasses the lymphatics pathway and is directly transported by the vein portal to the liver to be metabolised and excreted in urine, which suggests that [$^{18}$F]FTHA does not reflect the dietary lipid metabolism. Further studies are required to establish a new tracer candidate to mimic dietary fatty acid fate for PET studies.

# 2. INTRODUCTION

Obesity, and its associated co-morbidities, is increasing worldwide [1]. By definition obesity is a fat storage disease. Since lipogenesis in humans is negligible except during heavy carbohydrate overfeeding [2], it essentially represents the consequence of an altered partitioning of dietary fat between oxidation and storage, which is likely due to a preferential channelling of dietary fat towards adipose tissue and away from muscle [3].

Consequently, any interventions that may act on dietary fat portioning may prove successful for the prevention and treatment of obesity. However, the protocols usually applied to test such intervention are clearly cumbersome. The studies in humans rely on tissue biopsies, frequent blood samples, stable isotopic labelled compounds, gas exchange measurements and confinement in a GCRC for several hours. In animals the studies are generally terminal limiting longitudinal studies.

Positron emission tomography (PET) may, within certain objectives, avoid these limitations. Indeed, PET provides quantitative images reflecting the accumulation of a radiolabelled substrate in each region of the body. The method has been largely validated and used, in animals or humans, to study the lipid metabolism in the heart [4-7], the skeletal muscle [6-8] or the liver [9,10] in pathological conditions of cancer [11], myocardium dysfunction [12] and diabetes [8,13].

In all the PET studies conducted so far, however, the radiolabelled substrate is infused which, in case of lipids, is best adapted to study plasma fatty acid metabolism. The problem is more complicated for dietary fat. From a physiological point of view, the study of exogenous fat metabolism requires packaging of the resynthesized triglycerides into lipoproteins from which determines tissular distribution. From a technical point of view, as the maximal rate of fat oxidation appears around 6 hours after the meal in humans, the dose of the tracer has to be calculated in respect of a trade-off between the digestion time, the half-time life of the tracer, the threshold of radioactivity detectable by PET and the radioactivity allowed for humans.

In this study, we tested the feasibility of a non-invasive measurement of dietary fat trafficking between organs by PET scan in pigs. Feasibility was tested by studying the biodistribution in pigs of an oral load of 1,4(R,S)-[$^{18}$F]fluoro-6-thia-heptadecanoic acid ([$^{18}$F]FTHA) mixed in a standard meal along with gamma camera imaging. [$^{18}$F]FTHA, which has been proven to reflect the uptake rate of plasma fatty acids in skeletal muscle [7] and liver [9] and the oxidation rate in heart [4,5,7], is particularly suited for the purpose of the study. It has a highly first-pass uptake in the main organs implied in lipid metabolism, a long retention time into the cells, and a physical decay half-time life of 110 min.

# 3. MATERIEL & METHODS

## 3.1. Radiopharmaceutical synthesis

[$^{18}$F]FTHA was synthesized according to the method of De Grado [14] using a precursor benzyl-14(R,S)-tosyloxy-6-thia-heptadecanoate. Fluorine-18-fluoride was produced by bombardment of $^{18}$O target with protons in the Department of Medical Physics RDS Cyclotron (Madison), as previously described by Nickles et al. [15].

## 3.2. Study design

Four pigs (weight 35-38kg) were studied. The animal preparation protocol was reviewed and approved by the University of Wisconsin-Madison Research Animal Resource Committee. The study protocol is represented on Figure 1. The study started with a Boost™ (Med. Jonhson, USA) meal habituation for 5 days after an overnight fasting. On the experiment day, the pigs had fasted 24h before starting the protocol with an ingestion of an oral [$^{18}$F]FTHA load mixed in 250 ml of Boost. The ingested $^{18}$F dose varied between the animals from 7.5 to 44.3 mCi. Because the results obtained in the first three pigs raised questions about the use of [$^{18}$F]FTHA as a dietary fatty acid analog, pig 4 also received a 10 mCi dose of [$^{3}$H]palmitate, a tracer widely used in lipid metabolic studies, in order to compare the metabolism of the two tracers.

Pigs 1 & 4, 2 and 3 were respectively anesthetized 30 min, 210 min and 270 min after the oral load. Anesthetic state was induced with a mixture of telazol and atropine intramuscularly. The initial anesthetic state was achieved with sodium thiopenthal intravenously. A tracheotomy was performed in supine position, and the pigs were ventilated using 100% oxygen. The bladder and the femoral artery were catheterized for urine and blood samplings collected every 30 min to assess the rate of appearance of the tracers.

300 min after the ingestion of the tracers, all the animal were euthanized with 100mg/kg sodium pentobarbital. After euthanization, organs implied in cardio-respiratory, excretion, reproduction and digestion functions as well as pancreas, thyroid, muscles and adipose tissue and gastrointestinal tract contents were removed and weighed.

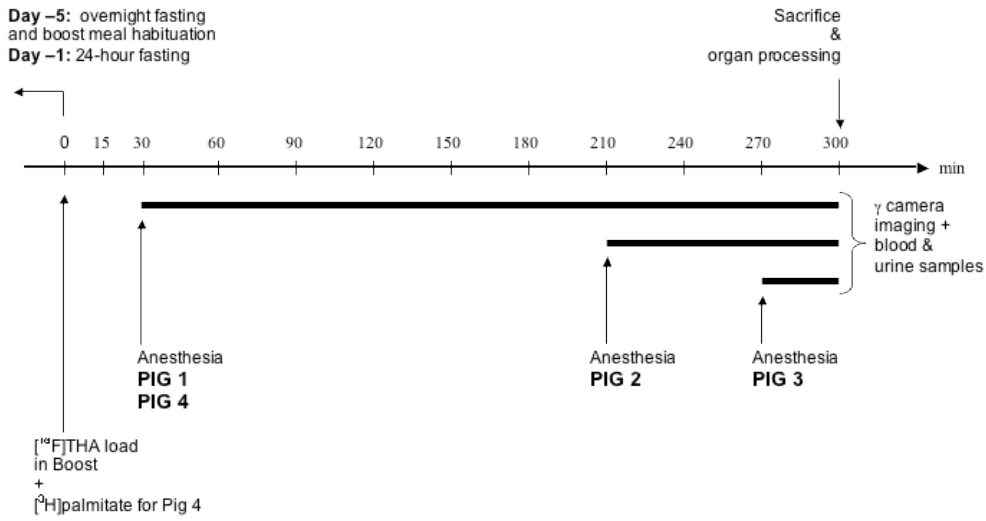


**Figure 1:** Study protocol

## 3.3. Biochemical analysis

Five samples (1g) of each organ/tissue and blood/urine were placed in a tube and for $^{18}F$ assayed by gamma counter (Packard, Cobra, Canberra Company, USA).

Only for the pig 4, $^{3}H$ radioactivity was also determined by scintillation counting. For this, total lipid fraction was extracted from plasma, urine and organ/tissue samples by the Folch procedure. Two aliquots of 1ml from blood or urine sample and five aliquots of 1g of tissue were assayed in duplicate. Organic solvent extraction of the samples was performed with serial addition of chloroform/methanol (1:2), chloroform and 2M potassium chloride/hydrogen chloride. After centrifugation the aqueous layer was removed and then treated with a chloroform, methanol and potassium chloride/hydrogen chloride solution (1:1:0.9). The aqueous layer was counted for $^{3}H_2O$ in a Packard liquid scintillation counter with results reported as the average of the four values.

The $^{18}F$ and $^{3}H$ radioactivity values in blood were expressed as the percentage of the tracer dose times the total estimated blood volume (6.5% of the weight of the animal) or in urine times the urine volume. For every time and organ, the radioactivity was corrected for the decay based on the activity at the time of sacrifice and was expressed as the percentage of the dose per organs but also as the standard uptake values (SUV) in % dose per kg organ/g aliquot. We used the %dose to compare the results with others studies.

**TABLE 1.**

| | [$^{18}$F]fluoro-6-thia-heptadecanoic acid | | | | | | | | [$^{3}$H]palmitate | |
|---|---|---|---|---|---|---|---|---|---|---|
| | PIG 1 | | PIG 2 | | PIG 3 | | PIG 4 | | PIG 4 | |
| | % dose | SUV | % dose | SUV | % dose | SUV | % dose | SUV | % dose | SUV |
| ***hemato - cardio-respiratory*** | | | | | | | | | | |
| **heart** | 0,09 | 0,03 | 0,82 | 0,03 | 0,89 | 0,20 | 0,07 | 0,02 | 0,92 | 0,26 |
| **lung** | 0,11 | 0,01 | 0,47 | 0,00 | 0,68 | 0,05 | 0,17 | 0,01 | 1,78 | 0,13 |
| **spleen** | 0,04 | 0,02 | 0,08 | 0,00 | 0,16 | 0,08 | 0,04 | 0,01 | 0,45 | 0,08 |
| **blood** | 1,05 | 0,02 | 1,40 | 0,02 | 3,84 | 0,06 | 0,92 | 0,01 | 2,78 | 0,04 |
| ***metabolization/excretion*** | | | | | | | | | | |
| **liver** | 1,72 | 0,07 | 6,86 | 0,03 | 8,89 | 0,32 | 1,94 | 0,08 | 17,18 | 0,69 |
| **kidneys** | 0,18 | 0,03 | 0,74 | 0,02 | 0,40 | 0,13 | 0,10 | 0,04 | 0,58 | 0,23 |
| **bladder** | 0,05 | 0,09 | 0,17 | 0,04 | 0,03 | 0,05 | 0,02 | 0,02 | 0,04 | 0,05 |
| **urine** | 0,94 | 1,10 | 5,59 | 9,12 | 7,43 | 6,59 | 2,06 | 1,89 | 0,26 | 0,24 |
| ***endocrine/exocrine*** | | | | | | | | | | |
| **pancreas** | 0,04 | 0,02 | 0,08 | 0,00 | 0,13 | 0,07 | 0,04 | 0,02 | 1,00 | 0,47 |
| **thyroid** | 0,00 | 0,01 | 0,00 | 0,00 | 0,00 | 0,03 | 0,00 | 0,01 | 0,03 | 0,14 |
| ***reproduction*** | | | | | | | | | | |
| **ovary** | 0,00 | 0,01 | 0,00 | 0,02 | 0,00 | 0,03 | 0,00 | 0,01 | 0,00 | 0,00 |
| ***muscles/fat*** | | | | | | | | | | |
| **gastrocnemius** | 0,05 | 0,01 | 0,09 | 0,01 | 0,06 | 0,02 | 0,03 | 0,01 | 0,27 | 0,06 |
| **soleus** | 0,04 | 0,00 | 0,01 | 0,01 | 0,06 | 0,02 | 0,04 | 0,01 | 0,66 | 0,15 |
| **SC fat** | 0,77 | 0,03 | 0,58 | 0,00 | 1,21 | 0,04 | 0,23 | 0,01 | 0,02 | 0,56 |
| **IA fat** | 1,72 | 0,06 | 1,83 | 0,01 | 2,76 | 0,10 | 1,47 | 0,06 | 0,31 | 1,18 |
| ***gastrointestinal tract*** | | | | | | | | | | |
| **stomach** | 0,56 | 0,07 | 0,56 | 0,05 | 0,59 | 0,07 | 1,60 | 0,20 | 5,18 | 0,64 |
| **duodenum** | 0,33 | 0,13 | 6,19 | 0,39 | 0,39 | 0,17 | 1,59 | 1,28 | 1,78 | 1,95 |
| **jejunum** | 0,91 | 0,16 | 5,51 | 0,19 | 0,03 | 0,04 | 1,87 | 0,08 | 5,41 | 0,24 |
| **ileum** | 0,77 | 0,04 | 0,26 | 0,38 | 2,05 | 0,07 | 0,01 | 0,00 | 0,10 | 0,19 |
| **caecum** | 0,02 | 0,01 | 0,04 | 0,02 | 0,06 | 0,05 | 0,06 | 0,03 | 0,60 | 0,29 |
| **asc. /desc. colon** | 0,10 | 0,05 | 0,07 | 0,02 | 0,13 | 0,04 | 0,17 | 0,03 | 1,60 | 0,32 |
| **trans. /rectum** | 0,09 | 0,03 | 0,65 | 0,07 | 0,26 | 0,07 | 0,03 | 0,02 | 1,37 | 0,92 |
| ***gastrointestinal tract contents*** | | | | | | | | | | |
| **stomach** | 39,46 | | 1,89 | | 1,58 | | 23,24 | | 34,39 | |
| **duodenum** | 0,81 | | 0,06 | | # | | 0,19 | | 0,61 | |
| **jejunum** | 0,23 | | 3,19 | | 0,03 | | 4,99 | | 8,33 | |
| **ileum** | 4,18 | | # | | 0,98 | | 0,00 | | 0,02 | |
| **caecum** | * | | 0,01 | | 0,12 | | 0,00 | | 0,09 | |
| **asc. /desc. colon** | * | | 0,02 | | 0,03 | | 0,04 | | 1,96 | |
| **trans. /rectum** | * | | 0,06 | | 0,01 | | 0,01 | | 0,75 | |

% dose are expressed per organ except for fat that is expressed

SUV: standard uptake values expressed in % dose kg/g.

SC fat: subcutaneous fat, IA: intraabdominal fat, asc. /desc. Colon: ascendent and descendent colon, Trans: transverse colon.

Blood and urine data represent samples taken at time of sacrifice.

* samples lost because of a gamma counter problem.

# no content found.

# 4. RESULTS

## 4.1. Repeatability of the method (Pigs 1 & 4)

The ingested [$^{18}$F]FTHA dose and the study schedule were similar for the pigs 1 and 4. The comparison of the $^{18}$F radioactivity in the body compartments (Table 1) shows differences only in the gastrointestinal tract contents. Whereas pig 1 accumulated 0.23% of the ingested [$^{18}$F]FTHA in jejunum and 4.18% in ileum, the percentages for pig 4 were 4.99% and 0.00%, respectively. A differential rate of digestion may explain these differences in radioactivity values. Otherwise, the [$^{18}$F]FTHA apparition rates in both blood and urine (Figure 2) as well as the [$^{18}$F]FTHA uptake rates in all the organs presented a good agreement between the two pigs.

## 4.2. $^{18}$F appearance rate in blood and urine (Pigs 1, 2 & 3)

The $^{18}$F radioactivity concentration in blood and urine are represented in Figure 2. The longer the delay between the oral load and the anesthesia is, the greater is the rate of $^{18}$F appearance in blood and urine. Indeed, the 300min post-ingestion $^{18}$F dose percentage is 1.05%, 1.40% and 3.84% in blood, and 0.94%, 5.59% and 7.43% in urine for pigs 1, 2 and 3, respectively. These results likely represent the inhibitory effect of the anesthesia on the digestion process. Nevertheless, a high clearance in blood and urine is illustrated by the decrease in 18F radioactivity from 2.2% at 210min to 1.4% at 300min post-ingestion in blood and from 7.6% at 270min to 5.6% at 300min post-ingestion in urine. This represents the metabolic fate of the molecule.

## 4.3. $^{18}$F uptake in tissues (Pigs 1, 2 & 3)

The uptake of [$^{18}$F]FTHA in tissues, expressed in percentage of the ingested dose per organ, was measured 300min after the tracer ingestion in euthanized animals and is shown in Table 1. As previously, the $^{18}$F dose percentage in each organ increases with the delay between the oral load and the anesthesia. The radioactivity can be considered as negligible in all the organs except in the stomach content of the pig 1 with a dose percentage of 39.46%, likely explained by the anesthesia 30 min after the meal. The highest radioactivity values was observed in the liver of all the animals with 1.72%, 6.86% and 8.89% in the pigs 1, 2 and 3 respectively anesthetized 30, 210 and 240 min after the oral load. These results combined with the high rates of $^{18}$F appearance in urine suggest that the tracer [$^{18}$F]FTHA is mainly metabolized in liver and then excreted in urine. No radioactivity was found in the bones (data not shown).

## 4.4. $^{18}$F radioactivity versus $^{3}$H radioactivity (Pig 4)

In pig 4, the [$^{18}$F]FTHA uptake as a percent of dose was compared with that of the tritiated palmitate, a classic fatty acid analog widely used in metabolic studies. Firstly, the rate of appearance in blood of [$^{3}$H]palmitate was by 302% greater than that of [$^{18}$F]FTHA, which suggests that the packaging in lipoproteins of [$^{18}$F]FTHA–triglycerides was impaired. Consequently, the [$^{3}$H]palmitate uptake in the organs is higher than the one of [$^{18}$F]FTHA. For example, there is 66 times more $^{3}$H in soleus muscle than $^{18}$F. As [$^{18}$F]FTHA, [3H]palmitate is greatly uptake by the liver with a $^{3}$H dose percentage of 17.18% in this organ. But only 0.26% of the dose ingested is present in the urines 300min after the oral load, which suggests that [$^{3}$H]palmitate is stored in liver likely in the form of triglycerides but it is not metabolised and excreted in the urines as [$^{18}$F]FTHA.

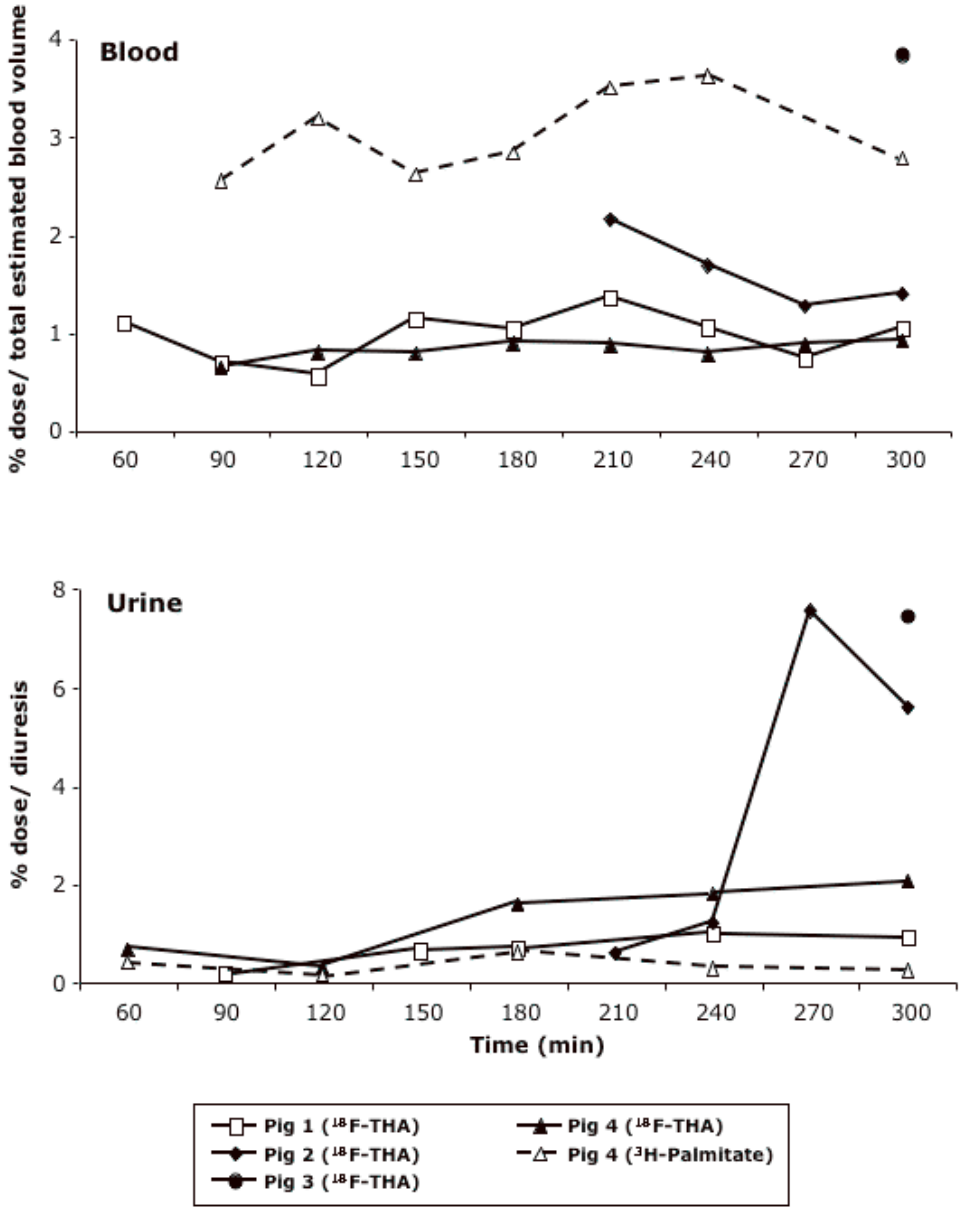


**Figure 2:** Percentages of tracer dose ingested by total estimated blood (6,5% of pig weight) in blood (upper panel) and by diuresis in the urine (lower panel) every 30 min from 30 min after the anaesthesia to the euthanasia of the animals.

# 5. DISCUSSION

$^{18}$F is considered as an excellent candidate for PET studies, thanks to low positron energy, which not only limits the dose rate to the patients, but also provides high-resolution images. Furthermore, the 110 min physical half-life permits an off-site production of radiotracer and dynamic studies for assessing metabolic processes. Firstly used for assessing carbohydrate metabolism with the $^{18}$FDG tracer [16], 18F has then been used, through [18F]FTHA, to study lipid metabolism in heart [4,5,7], liver [9] or skeletal muscle [7].

[$^{18}$F]FTHA is a false long-chain fatty acid substrate and inhibitor of fatty acid metabolism [4]. After transport into mitochondria, it undergoes initial steps of beta-oxidation and is thereafter trapped in the cell. The CPT-I inhibitor POCA decreased mouse cardiac [$^{18}$F]FTHA uptake by 85% [4]. The rate of radioactivity accumulation in the myocardium would, therefore, directly reflect the beta-oxidation rate of long-chain fatty acids [4,5]. Most of the studies investigating human myocardial free fatty acid uptake have been performed in fasting state [12,17,18] but also under hyper-insulemic conditions showing a suppression of the myocardial free fatty acid uptake rates in response to insulin [6]. Results on [$^{18}$F]FTHA uptake in skeletal muscle are more controversial. In humans, the fraction directed to beta-oxidation has been found to be 34-58% in forearm or leg muscles [19-21]. Takala et al. [7] compared the myocardial and skeletal muscle [$^{18}$F]FTHA metabolism and showed that in the heart 89% of [$^{18}$F]FTHA accumulation enters myocardial mitochondria whereas only 36% of [$^{18}$F]FTHA accumulation in skeletal muscle appears to directly enter into mitochondria. The majority of [$^{18}$F]FTHA is taken up by other cell fractions. Therefore, [$^{18}$F]FTHA traces mainly free fatty acid beta-oxidation in the heart but only free fatty acid uptake in the skeletal muscle. In liver, [$^{18}$F]FTHA is considered as a valuable tool for the investigation of hepatic free fatty acid turnover in humans and shows a liver free fatty acid uptake expressed by unit mass 50 times higher than that reported in skeletal muscles [9]. Despite some controversial results in these previous studies, [$^{18}$F]FTHA appeared to be a good candidate to trace lipid trafficking in the fasting state and consequently, may be used for dietary fatty acid trafficking studies.

Nevertheless, our pilot descriptive study indicated that [$^{18}$F]FTHA does not mimic the metabolism of dietary fatty acids. Indeed, although the method seems to be repeatable, both the rates of appearance in blood and urine as well as the uptake in all the organs are significantly different between [$^{18}$F]FTHA and the classic tracer used in lipid metabolic assessment, [$^{3}$H]palmitate. The low rates of [$^{18}$F]FTHA appearance in blood suggest that the transport of [$^{18}$F]FTHA through the enterocyte barriers and the packaging into chylomicrons are impaired. It is well known that when lipids are given in the form of free fatty acids, more fatty acids are transported via portal system rather than by lymphatics and particularly, when triglycerides quantity is very low. Moreover, the portal transport of lipids has certain selectivity according to the chain-length and the level of unsaturation of fatty acids [22]. Monounsaturated fatty acids and fatty acids with an atypical structure impinging the re-esterification in triglycerides are mainly transported in the blood stream whereas lymph is the major agent of transport of absorbed long-chain saturated fatty acids [23,24]. Because $^{18}$F introduces changes in the biochemical structure and properties of the molecule, and is also subject to defluorination reactions, which would be evident by $^{18}$F activity accumulating in the bone tissue, it is likely that [$^{18}$F]FTHA mainly bypass the lymphatic pathway,

and is directly transported to liver and metabolised, which is contrary to fate of [$^{3}$H]palmitate,. This process is clearly supported by both the high $^{18}$F radioactivity accumulations in liver and the appearance kinetics in urine. Consequently, despite the excellent characteristics of [$^{18}$F]FTHA for PET studies, [$^{18}$F]FTHA does not appear to be an adequate dietary fatty acid tracer.

Taken together, these results suggest that the dietary lipid metabolism assessment requires a tracer that does not change the biological and chemical properties of the molecules. $^{15}$O may be an adequate radioisotope as oxygen is a natural atom of lipid molecules but with a very short half-life of 2 min, it cannot be used for metabolic studies performed by PET scan. [1-$^{11}$C]palmitate has been investigated as a natural tracer of fatty acid metabolism, in particular for myocardium investigations in fasting state [25-27]. Both the release of the oxidative catabolic, $^{11}CO_2$, and backdiffusion of [1-$^{11}$C]palmitate contribute to the early myocardial clearance pattern. Since the diffusion rates of $^{11}CO_2$ and unoxidised [1-$^{11}$C]palmitate are both rapid, the fractional contribution of the two clearance processes cannot be determined from kinetic analysis of regional time-activity curves. As a result of this ambiguity, the interpretation of [1-$^{11}$C]palmitate kinetics in heart has remained strictly qualitative and subject to uncertainty [25-27]. Moreover because of the short half-life of $^{11}$C (20 min), this radiotracer is not optimal for many PET studies in humans. Nevertheless, this tracer may be used for metabolic studies in small animals with short digestion process.

This pilot study was a preliminary approach in order to validate the tracer [$^{18}$F]FTHA, classically used for endogenous lipid metabolism assessment, as a dietary fatty acid analog. We show that despite the repeatability of the method, [$^{18}$F]FTHA was not assimilated in a similar way than the long-chain fatty acid. Indeed, [$^{18}$F]FTHA likely bypasses the lymphatic pathway in favour to a transport in portal vein directly to the liver to be metabolised and excreted in the urines. Further studies are required to establish a new tracer candidate to assess the dietary fatty acids fate by in vivo non-invasive PET technique.

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# DISCUSSION

*Les Granges Baigneuses (Renoir, 1918)*

Over the last century, our modern western societies have adopted a generalized sedentary lifestyle that has been considered conducive to weight gain and its associated risks factors, including cardiovascular and coronary diseases, stroke, cancer and type 2 diabetes. The causal role of sedentary behaviours is, however, essentially based on observational epidemiological studies or on the indirect beneficial effects of exercise training. So far, very few studies provided mechanistic or interventional evidence to support a stronger case for the cause-and-effect relationship and our knowledge of the metabolic consequences of physical inactivity remains sparse.

The main objective of the present work was to longitudinally investigate in healthy adults the direct impact of physical inactivity on the two main regulators of body weight: the energy and oxidative balances and to delineate some of the involved mechanisms. To investigate if physical inactivity impacts body weight regulation we tested two main hypotheses: 1) in the long-term, physical inactivity reduces total energy expenditure (TEE) that is not compensated by proportional changes in satiety (and thus energy intake (EI)), conducing to a positive energy balance (EB), 2) 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, physical inactivity induces, independent of energy balance, a decrease in total and dietary lipid oxidation. Fatty acids represent a large heterogeneous family; we further hypothesized that the effect of physical inactivity on dietary fat metabolism will be dependent on their chemical natures.

In this last section, we aimed to integrate our results within long-term objectives and to propose some guidelines for future research. These objectives, that represent the frame of this discussion, are:

- to better understand the physiology of physical inactivity and, in a second step, to appreciate the role of the sedentary behaviours adopted by the western societies in the aetiology of obesity and its related metabolic disorders,
- to characterize the mechanisms underlying the deregulation of lipid metabolism during physical inactivity, hoping that it may help to open new areas to develop efficient strategies of prevention and treatment of both obesity and diabetes,
- to better determine the impact of physical inactivity on the oxidation of fatty acids of different chemical nature,
- to better understand the beneficial effects of physical activity and exercise on body weight and lipid metabolism regulation. In this line of investigation, we propose to discuss the differences between non-exercise activity energy expenditure (NEAEE) and exercise and in relation to the current physical activity recommendations.
- Finally, because we used the model of the head-down bed rest (HDBR) usually employed in space medicine, the last objective was to integrate our results in the context of space medicine with the long-term objective to better develop efficient countermeasures for the success of space missions.

# 1. INFERENCES TO PHYSIOPATHOLOGIES

In this work, we showed that physical inactivity *per se* induced by two or three months of bed rest leads to numerous metabolic alterations in both healthy men and women. We summarised our findings and the associated conclusions and perspectives in **Figure 1**.

## 1.1. Physical inactivity and energy balance regulation

### 1.1.1. Physical inactivity and feeding behaviour

In the short term, physical inactivity reduces TEE but does not impact satiety feelings (Stubbs *et al.*, 2004). No compensatory decrease in EI in line with a consistent drop in TEE is noted (Murgatroyd *et al.*, 1999; Shepard, 2001; Stubbs *et al.*, 2004). Thus, sedentary lifestyle appears to have considerable scope to conduce a positive EB, and hence body weight gain. However, these studies were conducted only in the short- and medium-term and the long-term effect of physical inactivity on EB and feeding behaviour remains to be established.

During 60 days of strict bed rest (**Chapter 4**), we confirmed that physical inactivity decreases TEE due to a decrease in all its components: resting metabolic rate (RMR), diet-induced thermogenesis (DIT) and activity energy expenditure (AEE). The reduced TEE is, however, mainly the consequence of a 51% drop in AEE. Interestingly and contrary to our hypothesis, the volunteers maintained an adjusted EI to the reduced TEE suggesting that EB can be regulated after long periods of inactivity. Interestingly this compensatory decrease in EI was partly voluntary as indicated by the rate of subjective appetite reported by the volunteers. Stubbs et al (Stubbs *et al.*, 2004) noted no effects of change in TEE on hunger and appetite during short/medium term studies and suggested that appetite control may take two to four weeks. Our results support this hypothesis.

### 1.1.2. Physical inactivity and satiety signals

Appetite is regulated by a complex system of central and peripheral interacting signals, which includes gut hormones and peripheral adiposity factors (Valassi *et al.*, 2008). Gut hormones include ghrelin which stimulates appetite, and glucagon like peptide-1 (GLP1), oxyntomodulin, peptide YY (PYY), cholecystokinin and pancreatic polypeptide which inhibit appetite. Leptin is a peripheral satiety signal secreted by adipocytes. All of them influence the hypothalamus and brainstem that control EB (Neary *et al.*, 2004). In our study, we examined for the first time the effect of physical inactivity on fasting plasma PYY, ghrelin, GLP1 and leptin. No change in these hormone concentrations was noted during bed rest. The lack of changes in leptin concentrations was, however, not expected based on the changes in fat mass observed throughout the bed rest. In fact, the observed versus FM-predicted leptin concentrations correlated with EI of the volunteers. Therefore under physical inactivity conditions the decrease in EI seems to be

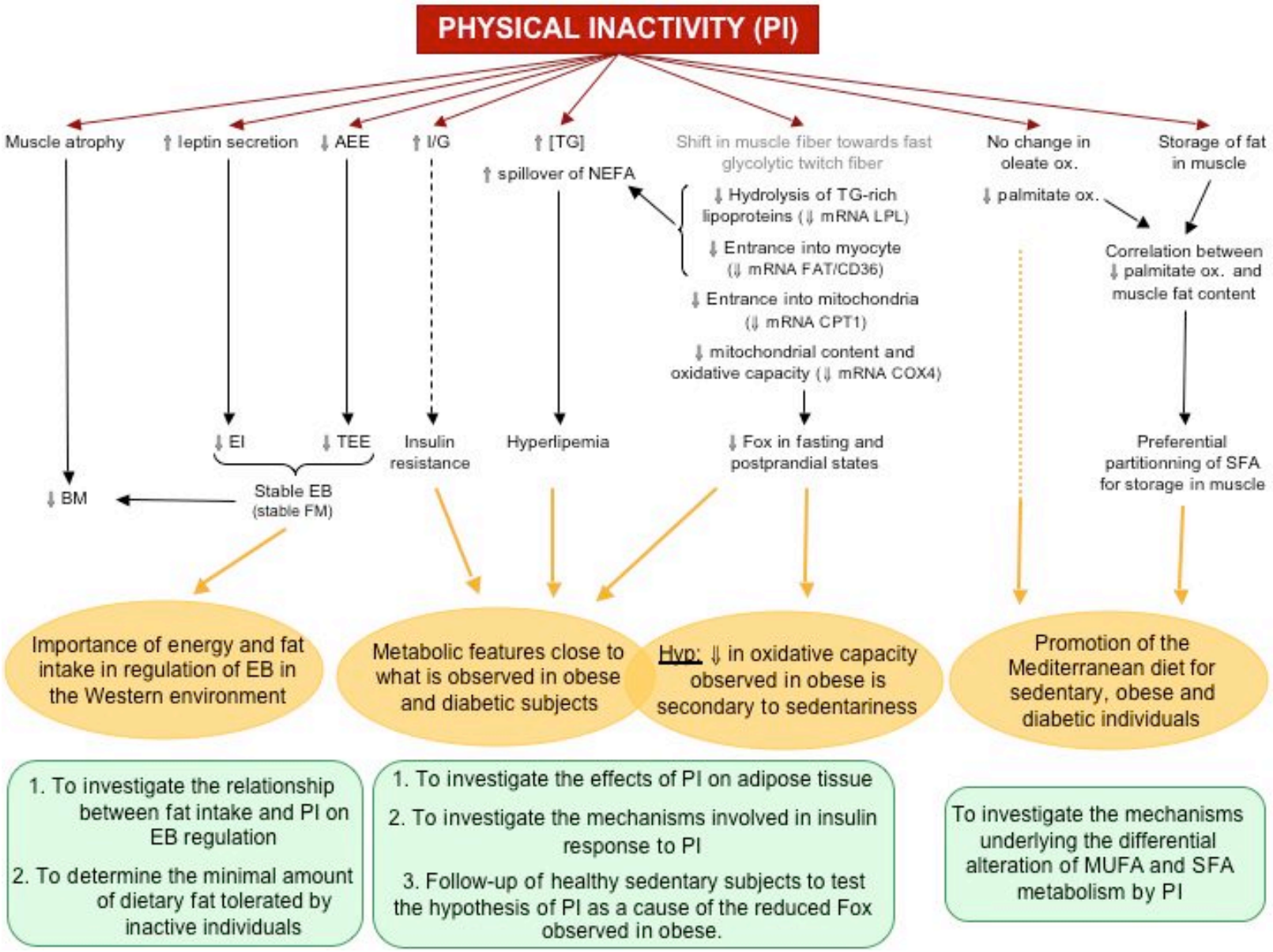



**Figure 1:** Summary of the physical inactivity-induced metabolic alterations observed in our studies and of the associated conclusions and perspectives.
Grey text indicates the results obtained by another team involved in the same bed rest study, conclusions are in yellow circles and perspectives in green rectangulars.
AEE, activity-related energy expenditure; I/G, ratio between fasting insulin and glucose concentrations (index of insulin resistance); TG, triglycerides, NEFA, non esterified fatty oxidation; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; EI, energy intake; TEE, total energy expenditure; BM, body mass; FM, fat mass; EB, energy balance; Fox, fat transporter CD 36; CPT1, carnitine palmitoyl transferase 1; COX4, citrate synthase.

at least in part, under the control of leptin secretion although the likely role of other hormones needs to be investigated in the future. Interestingly, such an elevation in the secretion of leptin independently of fat mass was previously observed in a 7d bed rest study (Blanc, 2000b). Moreover, two studies recently observed an inverse relationship between plasma leptin levels and physical activity energy expenditure independently of body composition (Franks *et al.*, 2003; Franks *et al.*, 2007). These authors suggested that it is unlikely that leptin causes a decline in physical activity level but more likely that physical activity modifies a number of mechanisms underlying leptin action.

Further studies are therefore required to characterize the physical inactivity-induced alterations on the regulation of leptin secretion which will allow a better understanding of the mechanisms underlying the compensatory decrease in response to a reduced TEE on the long-term.

### 1.1.3. Relevance to the current modern environment

Compared to the short- and medium-term studies (Murgatroyd *et al.*, 1999; Shepard, 2001; Stubbs *et al.*, 2004), our study showed that EB is regulated during long periods of physical inactivity. Therefore, from a fundamental point of view, physical inactivity is associated with a coupling between TEE and EI and may not generate a positive EB and body weight gain. However, these results were obtained in an artificial environment which does not reflect daily life. Indeed, the general population is inactive during frequent but short periods of time rather than during periods including months. Because short-term episodes will not trigger appetite control by the satiety signal system, weight gain can be seen as the cumulative consequences of these imbalances. Lastly, the free-living diet is more variable and the percentage of fat in the diet is closer to 40% than to the 30% in our study.

Proteins, fats and carbohydrates generate different sets of physiological responses that produce different effects on the intensity and duration of satiety (Blundell & King, 1996). The nutrient composition of food and the overall energy density influence control of meal size and post-ingestive inhibition. Particular sensory and nutrient combinations in foods can facilitate passive overconsumption. The organism compensates to some extent the overconsumption induced by high-fat diet and energy-dense food but it is commonly accepted that the satiety signals are too weak or triggered too late to avoid the ingestion of fatty meals. Intermittent dietary fat intake is therefore particularly susceptible to override physiological satiety signals leading to a positive EB and weight gain on the long-term (Blundell & King, 1996). By investigating the feeding behaviour of subjects fed *ad libitum* with high or low-fat diets during long-term physical inactivity, we may provide interesting evidence regarding the relationship between diet and physical inactivity on the regulation of EB. However, the resultant EB may be highly dependent on the lipid balance and also on the capacity to oxidise fat as fuel.

## 1.2. Are the developments of obesity and diabetes secondary to physical inactivity?

### 1.2.1. Physical inactivity induces a hypertriglyceridemia, a shift in substrate use and an insulin resistance

Physical inactivity affects the lipid metabolism independently of EB. Indeed, we showed (**Chapters 5 and 6**) that physical inactivity induced in both fasting and fed

states a hypertriglyceridemia, a shift in substrate use with an increase in carbohydrate oxidation and a decrease in lipid oxidation and a development of a resistance to the effects of insulin. These results were previously observed in bed rest of short and medium-term but only in fasting state. We extended these observations to the post-prandial state in both healthy men (**Chapter 5**) and women (**Chapter 6**). Based on these results, we can conclude that physical inactivity triggers the development of metabolic features close to what it is observed in both obese and diabetic individuals.

The incapacity to use fat as fuel observed in obese individuals has been reported in fasting (Colberg, 1995) and post-prandial (Binnert *et al.*, 1996) states and during exercise (Ezell *et al.*, 1999). Interestingly, fat oxidation is not improved after weight loss but with the performance of exercise (Berggren *et al.*, 2008). Therefore, we may hypothesize that the reduced fat oxidation observed in fasting and post-prandial states in obesity and diabetes is primary caused by physical inactivity. The direct corollary is that the development of these physiopathologies is, at least partly, secondary to a generalized sedentary lifestyle. Clearly further studies are needed to support our hypothesis. It is nevertheless of importance that the major changes in lipid metabolism observed in healthy lean inactive subjects are comparable to those observed in obese and diabetic individuals.

### 1.2.2. Comparison of the altered mechanisms involved in lipid metabolism between inactive, obese and diabetic persons

During the 2-month bed rest study (**Chapter 6**), we showed that the high triglyceridemia is likely due to alterations in the mechanisms involved in the regulation of the lipid trafficking. Indeed, physical inactivity impairs the clearance of the triacylglycerides (TAG)-rich lipoproteins from the circulation; probably due to a decrease in the gene expression of the lipoprotein lipase (LPL) and of the fatty acid transporter FAT/CD36. A decrease in LPL activity associated with a lower TAG uptake by red skeletal muscles was previously observed in inactive rats (Bey & Hamilton, 2003). We also showed that this process is associated with a greater spill-over of non-esterified fatty acids (NEFA) released by the hydrolysis of TAG-rich lipoprotein by LPL, which may be explained by the diminished fatty acid transport into the muscle. However, NEFA concentration does not change in post-prandial conditions in women. This phenomenon may be explained by the increase in insulin concentration induced by physical inactivity, which may favour the lipid storage and reduce the adipose tissue lipolysis, respectively. To better understand the causes of the hyperlipemia observed in inactive conditions, investigations on the metabolism of the lipoprotein are clearly required. LPL is poorly characterised in diabetic and obese individuals, so it is therefore difficult to draw clear comparisons between the mechanistic defects leading to the hyperlipemia between our inactive subjects and persons with obesity and T2D.

We showed (**Chapter 6**) that the muscle atrophy, the altered transport of fatty acids into both the myocyte and the mitochondria, the lower oxidative capacity of muscles associated with a shift in the muscle fibres pattern towards the fast glycolytic twitch fibres (reported by another team participating in the same bed rest study (Trappe *et al.*, 2007a; Trappe *et al.*, 2007b)) are likely involved in the decrease in fat oxidation observed under inactive conditions. Interestingly, an alteration of the muscle fiber pattern also seems to be associated with obesity and T2D. Indeed, some authors (Wade *et al.*, 1990; Helge *et al.*, 1999) reported an inverse relationship between the percentage of fat mass and the percentage of fast muscle fibres. More recently, it has been observed that patients with T2D have 16% less slow oxidative twitch fibres and 49% more fast glycolytic fibres than healthy subjects after adjustment for age and BMI (Oberbach *et al.*, 2006).

However, others studies did not confirm those results (Simoneau *et al.*, 1995; Kempen *et al.*, 1998). Moreover, several studies showed a reduced mitochondrial oxidative capacity, which depends on the type of muscle fiber (Simoneau *et al.*, 1995; Kempen *et al.*, 1998; He *et al.*, 2001) as well as a diminished beta-oxidation (Astrup *et al.*, 1996; Blaak *et al.*, 2000) in the muscle from obese or insulin resistant subjects. In general, the metabolic pattern of the enzymes and proteins involved in the lipid metabolism seems to be driven towards an esterification rather than a lipid oxidation (Simoneau *et al.*, 1999). As under physical inactivity conditions, the transport into mitochondria is impaired in obese and diabetic individuals because of a lower CPT1 activity in the muscles (Simoneau *et al.*, 1999). Contrary to our result obtained during enforced inactivity conditions, no reduced expression of FAT/CD36 in muscle from obese individuals has been reported (Bonen *et al.*, 2004). However, a preferential trafficking of fatty acids towards adipose tissue for storage rather than towards muscle for oxidation is observed in both obese and diabetic people. This is partly explained by the greater content of FAT/CD36 in adipocytes compared to myocytes in those patients (Bonen *et al.*, 2006).

Thus, our study highlights similar patterns in the key proteins involved in lipid metabolism in healthy inactive subjects and obese or diabetic people (**Table 1**). These observations are in support of our hypothesis that the defects in lipid metabolism considered conducive to weight gain might be secondary to the adoption of generalized sedentary behaviours in the general population. However, it appears necessary to better investigate the effects of physical inactivity at adipose tissue level in order to complete the comparison between the metabolic defects related to physical inactivity and the physiological characteristics met in obesity and diabetes. Although we did not investigate the mechanisms underlying the development of the resistance to the effects of insulin in this work, such a study would also be interesting in regard to the relationship observed between diabetes and sedentary behaviours. Our results and recently published data obtained in response of overnutrition can be used to infer interesting hypotheses.

**Table 1 Comparison of the altered lipid metabolism observed in our healthy lean inactive subjects and in obese and diabetic individuals**

| Parameters | Healthy inactive subjects | Obese and/or diabetic subjects |
|---|---|---|
| Body mass | ⇓ | ⇑ |
| Fat mass | Stable | ⇑ |
| Fat-free mass | ⇓ | ⇑ |
| Lipemia | ⇑ in [TG] with a greater spillover of NEFA and putative associated increase in VLDL | ⇑ in [TG], [NEFA] and [VLDL] in insulin resistant individuals |
| Insulinemia | ⇑ | ⇑ |
| Total fat oxidation | ⇓ | ⇓ |
| **Muscle** | | |
| Muscle fiber pattern | ⇑ of fast glycolitic twich fiber and of ⇓ low oxidative twitch fiber | ⇑ of fast glycolitic twich fiber and of ⇓ low oxidative twitch fiber |
| LPL | ⇓ in gene expression | ? |
| FAT/CD36 | ⇓ in gene expression | no change reported |
| CPT1 | ⇓ in gene expression | ⇓ in gene expression and activity |
| mitochondrial content | ⇓ | ⇓ |
| mitochondrial oxidative capacity | ⇓ | ⇓ |
| **Adipose tissue** | | |
| LPL | ? | ? |
| FAT/CD36 | ? | ⇑ in content |

TG, triglyceride; NEFA, non-esterified fatty acid; VLDL, very-low density lipoprotein; LPL, lipoprotein lipase; FAT/CD36, fatty acid transporter CD36.

## 1.3. Physical inactivity, intra-muscular triglycerides turnover and insulin resistance

In response to high-fat diet, an increased lipemia is observed. The overload dietary nutrients first accumulate in the adipose tissue. But when the lipid-derived metabolites overridden the fat oxidation, they begin to accumulate outside of the adipose depots, including muscle, liver and heart essentially. This results in the redirection of long-chain acyl CoAs (LC -CoAs) into the endoplasmic reticulum and cytosolic lipid species, such as diacylglycerols (DAG), ceramides and TAG. In muscle, levels of lipid signalling molecules, such as LC-CoA, DAG and ceramides, positively correlate with TAG content and negatively correlate with insulin sensitivity (Shulman, 2000; Hulver *et al.*, 2003). Although a role for these cytosolic lipids in the development of hepatic insulin resistance is well supported, the link with skeletal muscle is still mainly based on circumstantial evidences. However, ceramide and DAG are thought to engage stress-activated serine kinases that interfere with insulin signal transduction (Yu *et al.*, 2002; Holland *et al.*, 2007). DAG is a potent allosteric activator of both conventional and novel PKC isoforms. In skeletal muscle cells, PKCΘ is the most abundant PKC isoform (Griffin *et al.*, 1999; Cortright *et al.*, 2000; Itani *et al.*, 2000). PKCΘ phosphorylates IRS1 (Li *et al.*, 2004), the main mediator of insulin response in muscle (Kido *et al.*, 2000), and decreases IRS1 associated PI3 kinase activity (Yu *et al.*, 2002), leading to impaired insulin signalling. In addition, PKCΘ has the unique ability among the PKC isoforms to activate pro-inflammatory nuclear factor NFkB (Griffin *et al.*, 1999), which has been linked to fatty acid-induced impairment of insulin action in skeletal muscle in rodents (Kim *et al.*, 2001; Yuan *et al.*, 2001). Ceramides may mediate an antagonistic effect on insulin signalling via inhibition of the phosphorylation of Akt (Schmitz-Peiffer *et al.*, 1999; Summers, 2006) independently of changes in the ability of insulin to stimulate IRS1 phosphorylation or PI3 kinase activation. Increased ceramides are also associated with processes such as oxidative stress, inflammation and apoptosis (Summers, 2006). However, as it is well explained in Summers' review (Summers, 2006), a precise understanding of the molecular mechanism by which ceramides regulate insulin action and impact cell viability is still elusive.

Interestingly, we showed that physical inactivity *per se*, i.e. independent of overfeeding, induces a hyperlipemia, a decrease in fat oxidation (**Chapters 5 and 6**) and an accumulation of fat in muscle in association with the development of an insulin resistance (**Chapter 6**). Therefore, we can speculate that the low energy turnover due to reduced physical activity induces a low intra-muscular triglycerides (IMTG) turnover, which in turn conduces to the accumulation of complex bioactive lipids and hence, to insulin resistance. Consequently, future research on IMTG turnover, DAG and ceramide contents associated with insulin response is highly warranted to gain a better understanding of relationship between T2D, insulin resistance and sedentary behaviours.

Lastly, Koves et al. (Koves *et al.*, 2008) recently provided new results concerning additional factors that could be involved in muscle insulin resistance. In the context of a high-fat feeding, they showed that, while beta-oxidation was not altered, the TCA cycle was impaired, which results in an incomplete beta-oxidation and consequently, in the accumulation of acyl-carnitines in mitochondria and myocytes. Acylcarnitines are thought, via their role in the protein acetylation/acylation processes, to activate serine kinases that act on IRS1 in association with the reactive oxidation species. In the light of these recent findings, it would be also interesting to verify if the TCA cycle rather than beta-oxidation is

impaired in physical inactivity conditions and to measure the acyl-carnitines in mitochondria and myocytes from inactive subjects.

### 1.4. Interaction between energy and lipid balances under physical inactivity conditions

Whereas the performance of regular moderate intensity exercise allows one to achieve fat balance on a diet with 40% of energy, the same volunteers in sedentary conditions will have positive energy and lipid balances and consequently a high risk of weight gain (Stubbs *et al.*, 1995). However, when faced with a diet comprising 20% of energy as fat, they attained energy and fat balances. These results suggest that a fundamental interaction between the physical activity level and the dietary fat proportion determines EB. Based on our own results, we can hypothesize that the lower tolerance of inactive individuals faced with a high-fat diet may likely be due to the physical inactivity-induced incapacity to oxidise fat. Therefore, despite the achieved EB during long periods of physical inactivity, high content of fat in the diet may lead to accumulation of fat mass and probably to weight gain. The precise quantity of dietary fat that overrides the oxidative capacity of the organism according to the physical activity level is still unknown. However, in our study, female volunteers fed with a moderate fat diet (30% of total energy intake) were in stable EB and fat mass. Therefore, although further studies are clearly required to better determine this threshold in dietary fat content, we can propose that a diet with 30% of energy as fat may be adequate for sedentary subjects and populations with a PAL around 1.45.

## 2. DIFFERENTIAL METABOLIC FATE BETWEEN MUFA AND SFA

### 2.1. Importance of the nature of fatty acids

The key role of dietary fat in the development of obesity has been highlighted in 1998 by Bray et al. (Bray & Popkin, 1998) based on a positive relationship between the percentage of fat in the diet and the percentage of obesity in adults from surveys conducted in twenty countries. This was supported by numerous studies (Golay & Bobbioni, 1997; Hill *et al.*, 2000; Bray *et al.*, 2004), but the relevance of this association had been criticized based on the observation that the percentage of fat in the western diet has recently been reduced while obesity rates continued to rise (Heini & Weinsier, 1997). It should be noted, however, that even if the percentage of fat in the diet has decreased, the total EI continued to increase and, consequently, the absolute fat intake probably also increased. In addition to the amount of fat, further evidence shows that the quality of fat is an important factor to consider in the development of obesity (Storlien *et al.*, 1996). Some cross-sectional studies identified positive associations between the consumption of saturated fatty acids (SFA) and the risk of obesity and its related disorders, and negative associations for that of monounsaturated fatty acids (MUFA) (Doucet *et al.*, 1998; Gonzalez *et al.*, 2000; Williams *et al.*, 2000; Brunner *et al.*, 2001). In the review in **Annex 1**, we compiled recent data regarding how the metabolic fates of MUFA and SFA differ. We briefly reviewed the epidemiological data and frame

this within the concept of fat balance. Then, we compiled available data on the different steps of dietary fat metabolism from digestion to cellular mechanisms in muscle and adipose tissues. We additionally tried to propose some hypothesis on recently identified proteins involved in the partitioning of fatty acids. Finally in light of our results and other recent data, we demonstrated the critical interaction with physical activity and how this might alter the metabolic fate of SFA and MUFA. It is this final part that we report here.

## 2.2. Physical inactivity differentially alters the oxidation of monounsaturated and saturated fatty acids

The decrease in fat oxidation that we observed under physical inactivity was dependent on the biochemical properties of the FA. Indeed, physical inactivity differentially alters the metabolism of oleate (18:1) and palmitate, (16:0) the main MUFA and SFA of our western diet, respectively. We showed that whereas physical inactivity does not affect dietary oleate oxidation, it decreases dietary palmitate oxidation by 11% and 8% in both men (**Chapter 5**) and women (**Chapter 6**), respectively. This represents the main and new finding of our work. Interestingly, Votruba *et al.* (Votruba, 2003) reported, using the same stable isotope double labelling method, that prior light, moderate or heavy exercise significantly increases the dietary [1-$^{13}$C]oleate oxidation but not dietary [$d_{31}$]palmitate oxidation. Taken together, our and their results suggest that the mechanisms involved in the metabolism of MUFA and SFA are triggered or altered by physical activity and inactivity. Moreover, we reported that the reduced dietary palmitate oxidation correlated with the physical inactivity-induced increase in muscle fat content as measured by MRI (**Chapter 6**). As we did not observe an alteration of MUFA and SFA trafficking and uptake under physical inactivity conditions, these results indicate that physical inactivity-induced reduction in palmitate oxidation would be explained, at least in part, by a preferential channelling of palmitate towards muscle fat. Clearly, the physical inactivity-induced altered partitioning of SFA in muscle may be due to a reduced oxidative capacity of SFA at mitochondria level and/or an enhanced incorporation of SFA into complex lipids for storage in myocyte. In support of that, Stettler *et al.* (Stettler, 2005) reported that 60h of bed rest combined with a high-saturated fat diet (45% fat, of which approximately 60% was saturated) increases intramyocellular triglycerides. However, we did not find any literature studies that investigated the effect of physical inactivity on the key proteins involved in the regulation of the partitioning of MUFA or SFA in muscle. Further studies are required to better understand the mechanisms involved in this differential metabolism of MUFA and SFA. Based on current knowledge on fatty acids (FA) metabolism, however, we can propose a mechanistic hypothesis to explain the complex fate of FA within the physical activity continuum.

## 2.3. Potential mechanisms that might alter the fate of SFA and MUFA under different conditions of energy turnover

The first committed step of glycerolipid biosynthesis is the acylation of glycerol-3-phosphate catalyzed by glycerol-3-phosphate acyltransferase (GPAT). Two isoforms have been described: a microsomal and a mitochondrial isoform (mtGPAT) (Coleman & Lee, 2004). In tissues other than liver, mtGPAT activity is approximately 10 % of the total GPAT activity (Coleman *et al.*, 2000). Because both mtGPAT and CPT1 are located on the outer mitochondrial membrane, they

can compete for acyl-CoA. Thus, they can regulate the partitioning of FA between degradative and biosynthetic fates (Muoio *et al.*, 1999). The coordinated regulation of CPT1 and mtGPAT is mediated by AMP-activated kinase (AMPK) which reciprocally regulates TAG synthesis and fat oxidation in liver and muscle (Muoio *et al.*, 1999). AMPK is a regulatory sensor of the energy stores, which protects cells against the consequences of ATP depletion by inhibiting biosynthetic pathways and stimulating energy-generating pathways (Hardie & Carling, 1997). In response to increase in the cellular AMP/ATP ratio, AMPK inactivates acetyl-CoA carboxylase by phosphorylation (Carling & Hardie, 1989) resulting in a decrease in its product, malonyl-CoA, an intermediate in *de novo* synthesis of FA and an allosteric inhibitor of CPT1 (McGarry *et al.*, 1977). By decreasing malonyl-CoA, AMPK relieves the inhibition on CPT1 and thereby increases fat oxidation (Merrill *et al.*, 1997; Velasco *et al.*, 1997). In both muscle and liver, activated AMPK also inhibits FA esterification into TAG by AMPK dependent inactivation of mtGPAT, but not diacylglycerol transferase, the enzyme responsible for the synthesis of TAG from diacylglycerol (DAG), or microsomal GPAT (Muoio *et al.*, 1999). AMPK might also participate in the regulation of ceramide synthesis. The rates of ceramide synthesis depend largely on the availability of long-chain saturated fat, which together with serine are the rate limiting substrates in the *de novo* ceramide synthesis by serine palmitoyltransferase (SPT) (Merrill, 2002). SPT is specific for SFA. Interestingly, 5-aminoimidazole-4-carboxamide (AICA) riboside, which enters cells and is converted to AICA ribotide, an ATP analog, has been shown to inhibit palmitate-induced SPT activity and *de novo* ceramide synthesis in rat astrocytes (Blazquez *et al.*, 2001) and bovine retinal pericytes (Ruderman *et al.*, 2003). AMPK was also reported to inhibit the palmitate-induced increase in NFkB pathway (Cacicedo *et al.*, 2004).

Interestingly, the AMPK is stimulated in human skeletal muscle during exercise, and the degree of activation is dependent on the exercise intensity (Chen *et al.*, 2000; Fujii *et al.*, 2000; Wojtaszewski *et al.*, 2000; Cacicedo *et al.*, 2004). Consequently, exercise training decreases the concentration of malonyl-CoA and increases the expression and activity of malonyl-CoA decarboxylase in human muscle (Kuhl *et al.*, 2006) enhancing fat oxidation and inhibiting lipid biosynthesis. Furthermore, four weeks of endurance training in male Sprague-Dawley rats (Lessard *et al.*, 2007) and eight weeks in obese humans (Bruce *et al.*, 2006), decreased DAG and ceramide contents and increased insulin sensitivity but did not affect muscle TAG content. Conversely, muscle unloading down-regulates AMPK (Han *et al.*, 2007), which may induce the opposite metabolic cascade. Interestingly, Kump *et al.* (Kump *et al.*, 2006) showed that cessation of physical activity after 21 days of wheel running in rats increases epididymal fat mass in association with a concomitant increase in mtGPAT activity and protein levels. Clearly, a similar study on the effect of physical inactivity on muscle lipid content and mtGPAT activity is warranted.

Nevertheless, mtGPAT has a 3- to 10-fold higher activity with palmitoyl-CoA than oleyl-CoA (Coleman & Lee, 2004). Therefore, the coordinated regulation of CPT1 and mtGPAT mediated by AMPK may be a good candidate pathway to explain the relationship between the physical activity level and the differential oxidation and storage in muscle fat of dietary oleate and palmitate, i.e. the physical inactivity-induced reduced palmitate oxidation and the physical activity-induced increased oleate oxidation observed in our studies (**Chapters 5 and 6**) and in Votruba *et al.* (Votruba, 2003), respectively. However, further studies are required to better determine the mechanism of regulation involved in the relationship between the physical activity level and the SFA oxidation.

By its inhibitory effect on SPT, mtGPAT and the palmitate-induced NFkB pathway, AMPK also reduces the accumulation of ceramides, DAG and TAG and

thus prevents the development of insulin resistance. Therefore, AMPK may also represent a good candidate to explain the relationship between insulin sensitivity and the physical activity level observed in epidemiological studies (**Figure 2**). However, further studies are required to clearly demonstrate this association between energy demand, i.e. physical activity or inactivity, AMPK levels, lipid intermediaries content and insulin responsiveness.

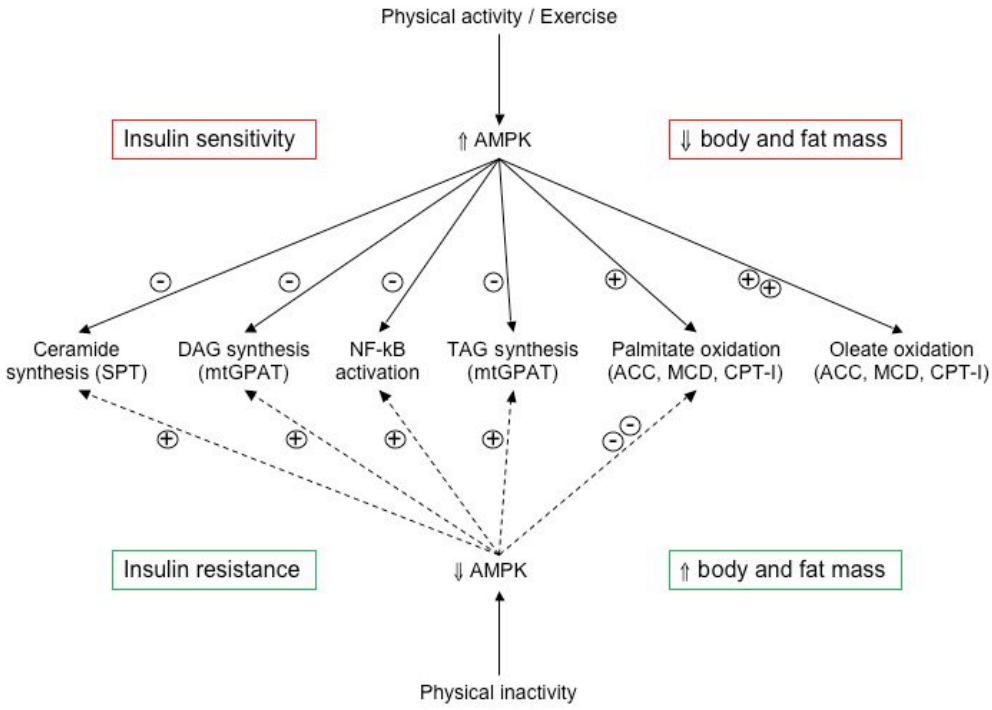



**Figure 2:** Effect of the physical activity level on lipid biosynthesis and oxidation in muscle cell via the regulation of AMPK concentration, and the ultimate consequences on insulin responsiveness and body weight regulation.

SPT, serine palmitoyl transferase; DAG, diacylglycerol; mtGPAT, mitochondrial glycerol-3-phosphate transferase; TAG, triacylglycerol; ACC, acetylCoA carboxylase; MCD, malonylCoA deshydrogenase; CPT1, carnitine palmitoyl transferase 1

## 2.4. Perspectives

In this line of investigation, a more detailed protocol to study the effect of physical inactivity/activity on dietary MUFA and SFA metabolism and more specifically on the plasma trafficking and muscle partitioning is warranted. Based on the recent data, we summarised the plasma trafficking in MUFA and SFA in normal weight individuals in **Figure 3**. We propose to longitudinally study the direct effect of physical inactivity during a bed rest on the plasma trafficking of MUFA and SFA in the different class of lipids and in lipoproteins. In adipose tissue, some mechanisms underlying fat metabolism are still unclear. In regard of our current knowledge, we propose to analyse the gene expression or the activity of the key proteins involved in lipid metabolism such as LPL, FAT/CD36, acylation stimulating protein and hormone sensitive lipase. Such a study will also provide further evidence on adipose tissue lipid metabolism to complete the comparison between healthy lean inactive subjects and obese people as we previously proposed. Regarding the differential partitioning of MUFA and SFA in myocyte, our current knowledge is summarised in **Figure 4** and further details are provided in the review (**Annex 1**). The effect of physical inactivity on MUFA and SFA oxidation rates and storage in complex lipids such as LC-CoA, TAG, DAG and ceramides should be examined. Moreover, the gene expression or activity of CPT1, SPT, microsomal and mtGPAT, steraoyl desaturase, diacylglycerol transferase and hormone sensitive lipase would have to be assessed. Interestingly, such a study may also help us to gain a better understanding of the mechanisms underlying the relationship between physical inactivity and insulin resistance. Moreover, as we previously explained, acylcarnitines resulting from an incomplete beta-oxidation have been recently proposed to alter the insulin response. To complete the studies that we previously propose, it could be interesting to measure the acylcarnitines into mitochondria and myocyte but taking into account their origin, i.e. dietary palmitate or oleate. All of these investigations may provide a better understanding of the mechanisms underlying the impaired insulin response induced by physical inactivity and the respective role of SFA and MUFA in these alterations.

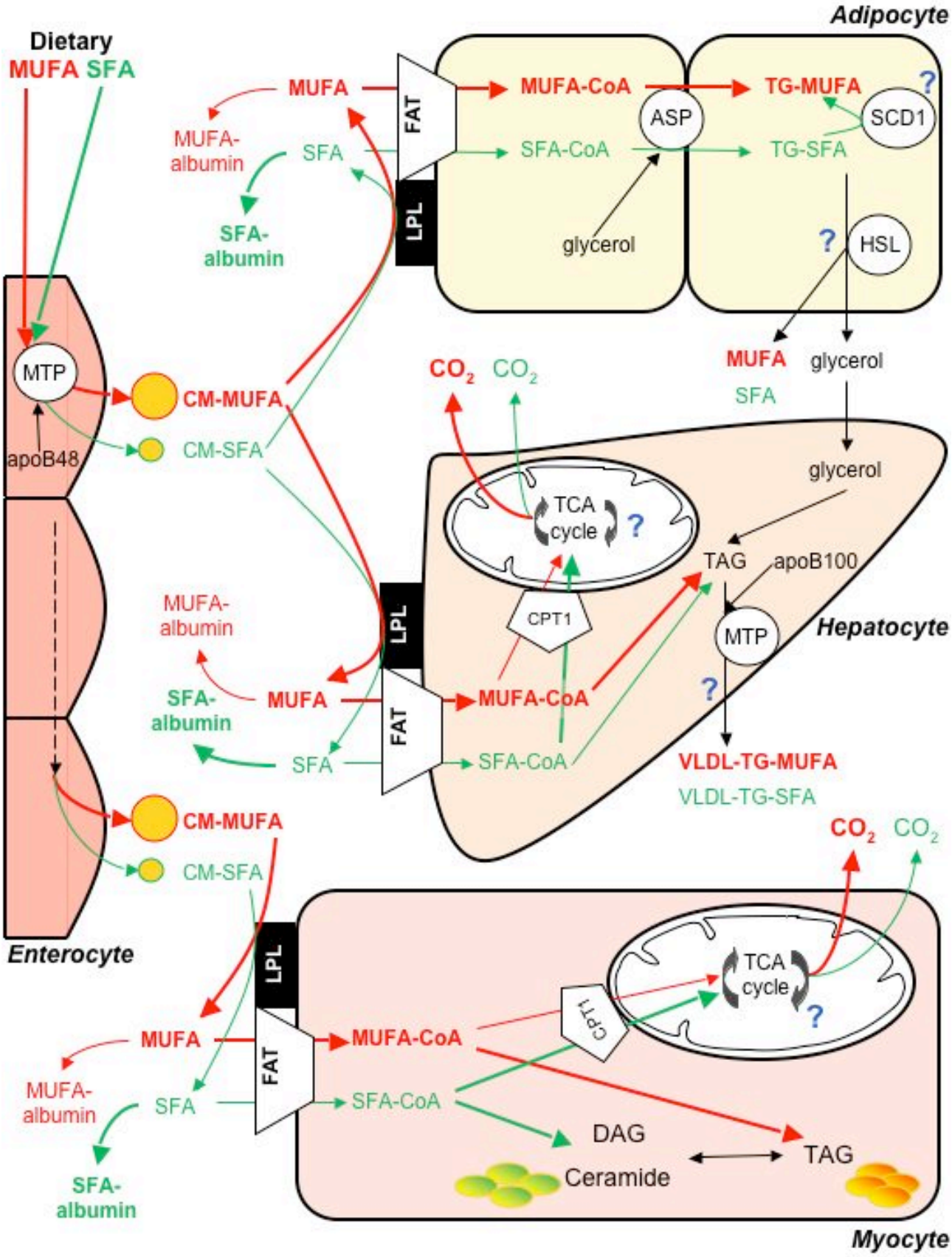
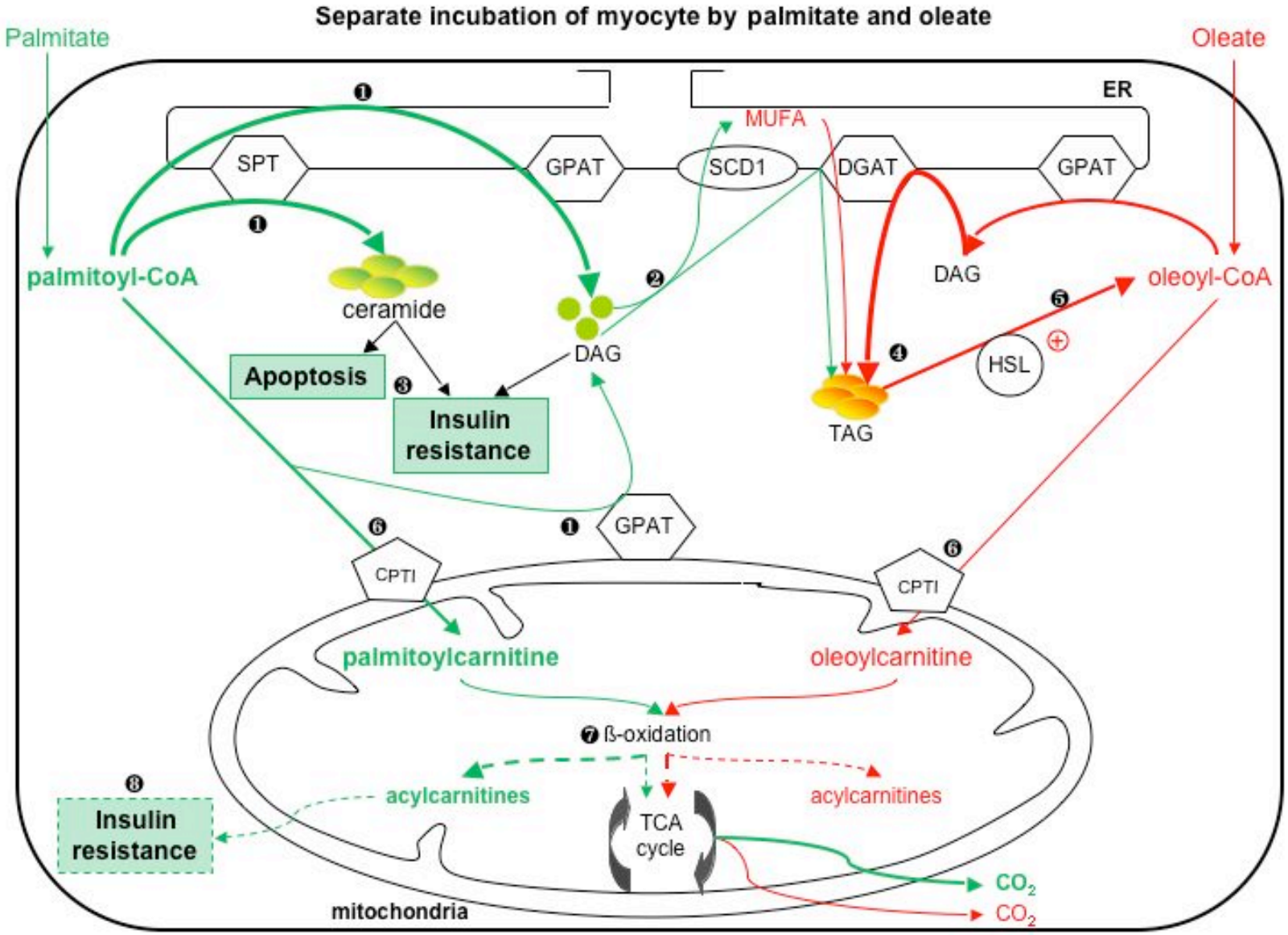

Adipocyte
Dietary
MUFA SFA
MUFA
FAT
MUFA-CoA
TG-MUFA
ASP
SCD1
MUFA-
albumin
SFA
SFA-CoA
TG-SFA
LPL
glycerol
HSL
SFA-
albumin
MTP
CM-MUFA
CM-SFA
apoB48
CO2
CO2
MUFA
glycerol
SFA
TCA
cycle
TAG
apoB100
CPT1
MTP
Hepatocyte
VLDL-TG-MUFA
VLDL-TG-SFA
Enterocyte
Myocyte
DAG
Ceramide

**Figure 3:** Dietary saturated (SFA) and mono-unsaturated (MUFA) fatty acids trafficking based on *in vivo* studies. The green and red arrows correspond to the MUFA and SFA channelling, respectively. After a similar digestion and assimilation, MUFA are predominantly packaged in larger chylomicrons (CM) than SFA which is likely due to a differential affinity of the intestinal microsomal triacylglyceride transfer protein (MTP) towards fatty acids varying in the degree of saturation. The larger CM are better hydrolyzed by lipoprotein lipase (LPL) than the smaller CM. MUFA is preferentially taken up by the peripheral tissues via a greater affinity of the fatty acid transporters (FAT) for MUFA than SFA and in turn, SFA are spilled over in NEFA pool in higher proportions. Although MUFA may be released in priority, MUFA is the main fatty acid component of the adipose tissue either because of its greater entrance in adipocytes or because of a putative desaturation process of SFA by the steraoyl desaturase (SCD1). In the hepatocyte and myocyte, the carnitine palmitoyl transferase 1 (CPT1) presents a greater affinity for SFA than MUFA but MUFA are greater oxidised than SFA. This may be explained by a mass effect of MUFA on CPT1 and/or by a greater incorporation of SFA in complex lipids (ceramides and DAG) than SFA. This results in a higher retention of SFA in liver and in a possible greater synthesis of MUFA-rich very low density lipoproteins (VLDL) due to a better affinity of the liver MTP for MUFA than SFA. In the myocyte, MUFA are preferentially incorporated in triacylglycerol (TAG).

**Figure 4:** Differential oxidation and incorporation in complex lipids in myocytes incubated separately with either excess palmitate or oleate. The green and red arrows correspond to the oleate and palmitate channelling, respectively. The solid arrows represent results from relevant *in vitro* studies whereas dotted arrows are only hypothetical interpretations. ❶ Palmitate is preferentially incorporated in ceramide and diacyl glycerol (DAG) because of the high affinity of the serine palmitoyl transferase (SPT) and the mitochondrial glycerol-3-phosphate acytransferase (GPAT) and the lower affinity of the diacylglycerol transferase (DGAT) for saturated fatty acids. ❷ A small proportion of palmitate is incorporated in triacylglycerol (TAG) pool as mono-unsaturated fatty acyl-CoA (MUFA) after a previous desaturation process via the steraoyl desaturase (SCD1), which is close to the DGAT on the endoplasmic reticulum (ER) membrane. ❸ However, palmitate is mostly incorporated in ceramide and DAG, which triggers the development of an insulin resistance and apoptosis. ❹ On the other hand, oleate preferentially accumulates as triacylglycerol (TAG) due to the high affinity of DGAT for unsaturated fatty acids. However, in spite of the increased TAG content, ❺ TAG turnover may be high since oleate activates the gene expression of the hormone sensitive lipase (HSL). Nevertheless, ❻ because of the higher affinity of the carnitine palmitoyl transferase 1 (CPT1), the transport of palmitate into mitochondria is greater than of oleate, which results in a greater oxidation of palmitate than oleate in *in vitro* conditions. However, we assume that a greater proportion of palmitate is incompletely oxidised ❼ inducing accumulation of acylcarnitines, which have been recently proposed to alter the insulin signals ❽


Separate incubation of myocyte by palmitate and oleate
Palmitate
Oleate
ER
MUFA
SPT
GPAT
SCD1
DGAT
GPAT
palmitoyl-CoA
ceramide
DAG
DAG
oleoyl-CoA
HSL
Apoptosis
Insulin resistance
TAG
GPAT
CPTI
CPTI
palmitoylcarnitine
oleoylcarnitine
ß-oxidation
acylcarnitines
acylcarnitines
Insulin resistance
TCA cycle
$CO_2$
$CO_2$
mitochondria

# 3. NON-EXERCISE ACTIVITY OR STRUCTURED EXERCISE TO PREVENT AN UNHEALTHY BODY WEIGHT?

Physical activity may play a role in weight maintenance. It has also been reported to improve the insulin sensitivity (Dolkas & Greenleaf, 1977; Venables & Jeukendrup, 2008) and the postprandial lipemia (Tsetsonis & Hardman, 1996b; Tsetsonis & Hardman, 1996a; Malkova *et al.*, 1999; Herd, 2000). Fat oxidation, however, 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 (Smith, 2000) and women (Hansen *et al.*, 2007). A large body of data effectively reported that exercise promotes a preferential channelling of lipids towards oxidation associated with a decrease in lipid storage (Calles-Escandon *et al.*, 1996; Friedlander, 1998). Therefore, the secondary objective of this present work was to test the efficiency of two different types of exercise training to protect the energy balance and the lipid metabolism against the deleterious effects of the physical inactivity.

In this context, central and crucial questions are: (1) What are the beneficial effects of physical activity on energy and fat balances? and (2) How much physical activity is enough to prevent unhealthy weight gain and metabolic disorders? Finally, the overall challenge in the battle against the rising levels of obesity is to translate this knowledge into practical actions for a general public health benefit.

## 3.1. Physical activity and energy balance regulation

We have previously seen that EB can be regulated during long periods of physical inactivity. However, the daily environment is characterized by appetizing fatty food which have a weak effect on the satiety signal system and by physical inactivity periods probably too short to modulate appetite control and adjust EB. Increasing physical activity, mostly through exercising, was demonstrated as an efficient strategy to minimize positive EB due to eucaloric high fat diet (Department of Health, 1995).

### 3.1.1. Effect of short- and medium-term exercise training on energy balance

To examine the issue of acute compensatory changes in hunger and food intake short-term intervention studies have been conducted that deliberately impose acute bouts of exercise with subsequent measure of food intake. Contrary to a widespread belief, there is no short-term compensatory increase in hunger and food intake (King & Blundell, 1995; Westerterp-Plantenga *et al.*, 1997). On the contrary, there is a loose coupling between exercise-induced EE and EI over these time frames. Moreover, there is a suppression of hunger during and for a short-term after an intense exercise, which was named 'exercise-induced anorexia' (King & Blundell, 1995; Westerterp-Plantenga *et al.*, 1997).

On the medium-term, Stubbs *et al.* (Stubbs *et al.*, 2002a; Stubbs *et al.*, 2002b; Whybrow *et al.*, 2008) conducted an interesting series of studies in which

they used similar methodologies. In an initial study, they examined the effect of graded increases in exercise-induced EE on appetite, EI, TEE and body weight over a period of 7d in 6 healthy women (Stubbs *et al.*, 2002b) and then in 6 healthy men (Stubbs *et al.*, 2002a) living in their normal environment. Each subject was enrolled in three protocols corresponding to no exercise (0 MJ/d), medium exercise level (approximately 1.3MJ/d) and high exercise level (approximately 2.6 MJ/d). They showed that the treatments did not affect hunger, appetite or body weight. They observed no compensatory increase in EI in response to the exercise-induced increased TEE, which in turn precipitated a negative EB that was roughly proportional to the energy cost of the exercise. Interestingly, they reported in both groups a tendency to decline TEE, suggesting that the restoration of EB occurs by a reduction of NEAEE rather than by an increase in EI. Recently, they extended these studies with the same protocols to a period of 16d in 6 men and 6 women (Whybrow *et al.*, 2008). They observed that subjects compensated for 30% of the exercise-induced energy expenditure in average. However, a considerable inter-individual variation in the extent of the compensation was noted. These studies appear to capture the first stages of a change in EI in order to match a markedly elevated EE. Thus, concentrating only on short- and medium-term effects without considering the longer-term timescale may fail to reveal the way in which food intake and EB are actually controlled, since it likely takes a number of weeks for EI and EE to achieve a new balance.

### 3.1.2. Effects of long-term exercise training

Based on these previous observations, we proposed to characterise during the WISE study the effect of long-term exercise training performed concomitantly to the bed rest, on appetite, changes in EI around a pre-determined amount of dietary intake estimated to cover energy needs and on EB regulation (**Chapter 4**). Eight healthy women were subjected to a combined resistive and aerobic exercise training (moderate and high-intensity exercise) during the 60 days of bed rest. After an intervention on TEE for 60 days, we observed no compensatory EI to cope with the exercise-induced TEE. Although this lower EI compared to TEE may be partly forced by the wrong estimation of the energy cost of the exercise by the investigators, the volunteers did not increase spontaneously their dietary intake. Consequently, the volunteers were in negative EB (-1.36 ± 0.75 MJ/d), which conduces to a drop in fat mass of -1.9kg on average. Interestingly, the energy deficiency was roughly equivalent to the energy expended during exercise (1.31 MJ/d), as previously observed by Stubbs *et al.* (Stubbs *et al.*, 2002a; Stubbs *et al.*, 2002b) in medium-term studies.

### 3.1.3. Exercise training and satiety signal system

In this study (**Chapter 4**), we additionally examined for the first time to our knowledge the effects of long-term exercise training on the main hormones involved in the satiety signal system. As in the control group, the concentration of fasting total PYY and ghrelin concentration did not vary with the intervention whereas leptin secretion was related to changes in EI. We reported in the exercise group an additional increase in GLP1, a satiety gut hormone, which negatively correlated with the prospective food consumption of the volunteers. A similar increase in GLP1 concentration was recently observed during and after an acute intense exercise of one hour (Martins *et al.*, 2007). Thus, whereas the phenomenon of 'exercise-induced anorexia' which produces a negative EB was thought to be a short-term process, it appears to be sustained for at least several weeks. This study provides only preliminary results regarding the relationship between increased TEE, satiety signal system regulation and feeding behaviour and other satiety

hormones such as neuropeptide Y, cholecystokinin, pancreatic peptide and glucose-dependent insulinotropic peptide involved in the regulation of dietary intake would have to be studied. Moreover, as most of these hormones are satietogenic, assessment of the post-prandial concentrations are warranted.

### 3.1.4. Exercise intervention and body weight regulation

Therefore, contrary to the assumption of Stubbs *et al.* (Whybrow *et al.*, 2008), a compensatory EI in response to high exercise-induced EE does not occur even in the long-term. Taken together, these results provide evidences that exercise performed on short-, medium- or long-term induces a negative EB, which conduces, but only on the long-term, to a loss of fat mass and hence on body mass. Although our subjects cannot be compared to athletes such observation was already made (Westerterp & Saris, 1991; Westerterp *et al.*, 1992). Even a 20% increase in TEE for 40 weeks due to marathon training induced no increase in EI (Westerterp *et al.*, 1992). These results suggest that exercise might prove an efficient strategy to prevent or treat weight gain or regain. Schoeller *et al.* (Schoeller *et al.*, 1997) and Weinsier *et al.* (Weinsier, 2002) using DLW, determined the minimal physical activity level (PAL) for former-obese women to avoid weight regain. In their studies, women who are successful in maintaining a normal body weight over one year have a PAL of 1.7-1.75, which corresponds approximately to 80 min of moderate activity or 35 min of vigorous activity per day added to a sedentary lifestyle. To prevent the transition of overweight to obesity, current physical activity guidelines promote 45 to 60 minutes of moderate intensity activity over everyday day (Saris, 2003).

Although cross-sectional population-based studies reported negative relationship between physical activity and body weight or fat mass, exercise alone is generally less efficacious compared with dietary restriction in attaining weight loss (Miller *et al.*, 1997). The lack of results of exercise intervention is likely due to a non adherence to such heavy exercise prescriptions in overweight individuals but also probably to a concomitant decrease in the daily AEE as observed by Stubbs' team (Whybrow *et al.*, 2008). Therefore, instead of promoting heavy exercise prescriptions which are not motivating for sedentary or overweight persons and difficult to follow, further studies may investigate the role of NEAEE in the prevention and treatment of weight gain. However, exercise seems required since it affects the satiety signal system and thus may help in the adherence to dietary restriction intervention.

Although exercise appears to play a weak role in the regulation of EB, it modulates the lipid balance.

## 3.2. Exercise and fat balance

### 3.2.1. Intensity, duration and type of exercise

The intensity of exercise is typically described by the percentage of the peak volume of oxygen consumed by an individual ($VO_2$peak). The effect of the intensity on the metabolic response has been extensively described. Whereas low intensity exercise primarily relies on fat substrate, high-intensity exercise elicits the oxidation of carbohydrate. The maximal fat oxidation is reached with exercise at approximately 50-60% $VO_2$peak (Brooks & Mercier, 1994; Achten *et al.*, 2002). Lipid substrates are found in circulation as fatty acids released from adipose tissue or from diet as FA, TAG or lipoproteins but also in muscle fat stores (van Loon *et al.*, 2003). It is important to keep in mind that post-exercise phases also influence

lipid oxidation probably due to the replenishment of glycogen stores (Borsheim & Bahr, 2003). Studies have reported an increased fat oxidation and an attenuated postprandial lipemia after both endurance and endurance exercises (Horton *et al.*, 1998; Petitt, 2002; Gill & Hardman, 2003). Resistance exercise oxidised more fat than an isocaloric aerobic exercise. However, resistance exercise usually increases TEE less than endurance exercise. TEE defined by the intensity and the duration of the exercise seems to be the primary factor influencing fat oxidation (Tsetsonis & Hardman, 1996a; Tsetsonis & Hardman, 1996b). For example, isocaloric exercises at 66% $VO_2$peak for 45 minutes and 33% $VO_2$peak for 90 minutes resulted in similar 6 hour recovery non protein respiratory quotient and fat oxidation (Thompson *et al.*, 1998). Nevertheless, despite the post-exercise period, the acute effects of exercise have to be clearly distinguished from chronic adaptation to training.

### 3.2.2. Resistive versus aerobic exercise training effects on fat oxidation

Most of the longitudinal and cross-sectional studies investigating aerobic or resistance training effects on fat metabolism reported a significant decrease in respiratory exchange ratio ($VCO_2/VO_2$) during (Friedlander, 1998; Bergman, 1999; Friedlander *et al.*, 1999; Tunstall *et al.*, 2002) or after exercise (Pratley *et al.*, 1994; Treuth *et al.*, 1995), supporting the notion that training increases the reliance on fat as an energy source. However to our knowledge, only few studies investigated the effect of exercise training on fat oxidation at rest (Friedlander *et al.*, 1999; Schrauwen *et al.*, 2002) and no study examined post-prandial fat oxidation. Moreover, most of the studies focused on total and plasma fatty acid oxidation (Friedlander *et al.*, 1999; Schrauwen *et al.*, 2002). Except Votruba *et al.* (Votruba, 2003) who investigated the effect of prior acute exercise on exogenous oleate and palmitate oxidation, no studies addressed the question of the effect of exercise training on dietary fat oxidation.

During the two long-term bed rest studies, we investigated the effect of a resistive exercise training performed every 3 days for 35 minutes by male volunteers (**Chapter 5**) and of a combined resistive (every 3 days for 35 minutes) and aerobic (3-4 times per week, graded intensity ranged between 40% and 80% of $VO_2$peak, 50 minutes in average) exercise training performed by female volunteers (**Chapter 6**) on the total fat oxidation at rest and after a meal and on the exogenous palmitate and oleate oxidation. As the volunteers were concomitantly in bed rest, we examined the efficiency of these two training regimes in preventing the deleterious alterations induced by physical inactivity. Despite the maintenance of fat-free mass, the resistive exercise training did not mitigate the decrease in total fat oxidation in fasting and fed states as well as the reduction in exogenous palmitate oxidation induced by physical inactivity. On the contrary, the combined aerobic and resistive exercise training partially counteracted the drop in fat oxidation in both fasting and post-prandial conditions, which is likely due to the partial maintenance of both muscle mass and muscle oxidative capacity. However, only 50% of the fat oxidation was maintained may be because the training only tended to protect the decrease in LPL gene expression and did not impact the gene expression of the fatty acid transporters at mitochondria and myocyte levels (CPT1 and FAT/CD36, respectively).

Based on these results, we can draw several assumptions. First, muscle fibre type pattern in association with the muscle oxidative capacity may be the primary factors determining fat oxidation rather than muscle mass. The comparison of the effects of the two trainings highlights the greater impact of aerobic than resistive exercise on fat metabolism. However, the sex differences may influence fat

metabolism. Indeed evidence suggests that women oxidise proportionally more fat compared with males (Froberg & Pedersen, 1984; Horton *et al.*, 1998; Carter *et al.*, 2001) but conclusions are still not clear. Moreover, although some authors reported a greater fat oxidation during luteal compared to follicular phase (Zderic *et al.*, 2001; Ruby *et al.*, 2002), likely due to differences in circulating estradiol concentration, other authors did not report influence of the menstrual cycle phase on lipid metabolism (Melanson *et al.*, 1996). For our part, based on data provided by Dr Wade who investigated steroidgenesis during the same bed rest study, we did not observe a statistical effect of the menstrual cycle on our results (data not shown). As these bed rests were longitudinal studies during which each subject was its own control, the differences in the efficiency to counteract physical inactivity between the resistive and the combined resistive and aerobic exercise may be essentially accounted for by the differences in the training-induced TEE (approximately 275kJ/d versus 1.3MJ/d for resistive exercise alone and resistive and aerobic exercise together, respectively). In support of that, resistive exercise has been reported to increase fat oxidation only when it induces significant energy expenditure (Poehlman & Melby, 1998). Nevertheless, the combined resistive and aerobic exercise training did not mitigate the reduction in dietary palmitate oxidation, suggesting an independency between total and exogenous FA oxidation and thus that this exercise-induced TEE was not enough to maintain dietary palmitate oxidation at its baseline value. Because stored lipids mainly originate from the diet, future studies addressing the question of how much physical activity is enough to maintain both total and exogenous fat oxidation will be necessary.

### 3.2.3. Resistive versus aerobic exercise and post-prandial lipemia and hyperinsulinemia

Post-prandial lipemia and insulin responsiveness are related to fat oxidation. However, it is interesting to observe that these parameters respond differently to the exercise training. No beneficial effect of the resistive exercise alone was noted on both lipemia and insulinemia. However, the combined resistive and aerobic exercise partially counteracted the physical-inactivity induced hyperinsulemia in both fasting and fed states, which suggests once again the key role of TEE on lipid metabolism. In an early study, Dolkas *et al.* (Dolkas & Greenleaf, 1977) estimated that at least 4200kJ/day must be provided by supplemental exercise to restore the hyperinsulinemia to control levels during bed rest. We showed however that an additional energy expenditure induced by exercise of 1.3MJ/d already significantly improved insulin response. Intervention studies have reported that a period of endurance training can reduce postprandial concentrations of TAG and TAG-rich lipoproteins and in some instances increase TAG clearance (Gill, 2003). Although the intensity (more than 40-60% $VO_2$max) (Zhang *et al.*, 2006) and the duration (Zhang *et al.*, 2007), i.e. more than 45minutes, of our aerobic exercise were similar to acute exercise that effectively attenuates post-prandial hypertriglyceridemia, the combined aerobic and resistive exercise training did not mitigate the hypertriglyceridemia induced by physical inactivity. As our tests took place at least 36 hr after the last session of training to study the effect of the training, and not the acute effects of the exercise, the delay may have been too long to highlight the effects of the training. Indeed, the favourable conditions of exercise are rapidly reversed in the absence of recent exercise, i.e. less than 16hr after the last bout of exercise (Petitt, 2002; Gill, 2003). As studies reported beneficial effects of walking on post-prandial lipemia (Tsetsonis & Hardman, 1996b; Tsetsonis & Hardman, 1996a), we can questioned whether NEAEE likely because of the more continuous increase in TEE rather than the intermittent practice of exercise, may be more efficient in the prevention of postprandial hyperlipemia.

Based on our results, we can assume that the beneficial effects of training on lipid metabolism essentially seem to be dependent on exercise-induced energy expenditure but also on the frequency of the exercise sessions during the training. Additionally, the inefficiency of the combined resistive and aerobic exercise training protocol to mitigate the decrease in exogenous fat oxidation and the postprandial hyperlipemia may be due to a not sufficient exercise-induced energy expenditure or to the lack of NEAEE.

## 3.3. The key role of non-exercise activity energy expenditure

Classically, TEE can be separated into three components: RMR, DIT and AEE. The high variations in TEE observed between individuals are mainly due to variations in AEE. But AEE can also be differentiated between NEAEE, defined as 'any bodily movement' and exercise, defined as 'a subset of physical activity that is characterized by planned and purposeful training' (Caspersen *et al.*, 1985). 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 100kcal/d, NEAEE has been reported to vary up to 2000kcal/d (Levine, 2007). NEAEE has also been reported to be a predictor factor in weight gain (Levine *et al.*, 1999). Indeed, Levine et al. observed that the individuals who increased the most their NEAEE during overfeeding were those who gain the least fat, whereas those who did not increase their NEAEE gain the most fat. As lipogenesis is negligible in humans, these results suggest that the subjects who gain least fat were those who oxidised the highest proportion of dietary fat.

During the bed rest studies, the exercise group performed a heavy exercise training protocol but concomitantly to bed rest. Therefore, we can consider these subjects as exercisers with a NEAEE deficiency (**Figure 6**). Interestingly, 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, we can suppose that this lack of impact on exogenous fat oxidation is rather caused by the NEAEE deficiency rather than by an insufficient exercise-induced energy expenditure. We can extrapolate this idea and assume that whereas total lipid oxidation may be function of exercise-induced energy expenditure, dietary fat oxidation would be a function of NEAEE. In this line, the higher capacity to adapt fat oxidation to a high-fat diet reported by Smith *et al.* (Smith, 2000) and Hansen *et al.* (Hansen *et al.*, 2007) in active men and women respectively, but also the higher tolerance to high fat diet observed by Stubbs *et al.* (Stubbs *et al.*, 1995) in active adults compared to inactive adults may be related to differences in daily NEAEE (**Figure 5**). Clearly, these hypotheses may be considered as an extreme simplification of the lipid metabolism, but it could be worthy to guide future research in this direction.

In other words, because NEAEE is highly variable, it has a large potential to impact EB and body weight. In this line, some authors assumed that dysregulation of NEAEE may have a considerable influence on body weight regulation and obesity. For our part, based on the Levine's study (Levine *et al.*, 1999) and our results, we propose that NEAEE may also modulate the other key regulator of body weight : lipid balance. Specifically, we think that although exercise clearly affects total fat oxidation and in some extent body fat stores, NEAEE may rather impact the partitioning of exogenous fat oxidation. Thus, the double compartment model of Flatt that we presented in the Introduction (**Figure**

**6, Chapter 1**) of this manuscript may be updated one day by adding the NEAEE to the parameters regulating the outflow from the large turbine (body fat reserves).

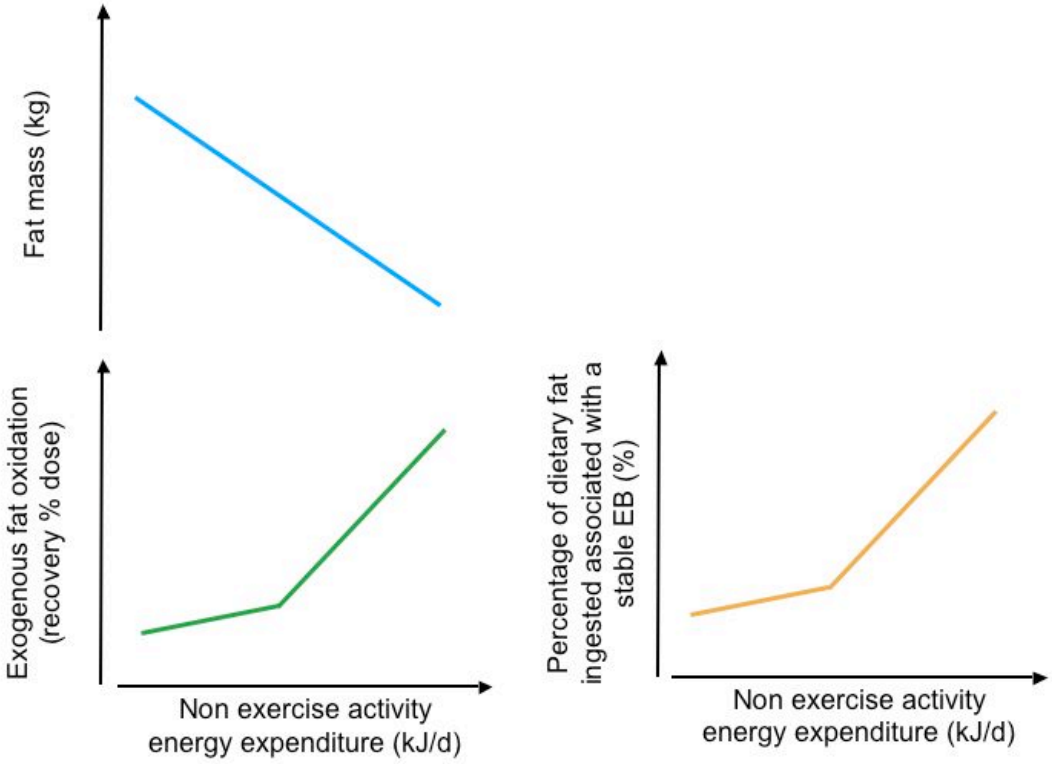



**Figure 5:** Hypothetical relationships between non exercise activity energy expenditure and fat mass, exogenous fat oxidation and the tolerance to dietary fat expressed as the percentage of dietary fat associated with a stable energy balance (EB). (Adapted from (Stubbs *et al.*, 1995) and (Levine *et al.*, 1999)

Moreover, it is interesting to remind that while mitochondrial content and oxidative capacity were partially maintained by the exercise training performed by our volunteers **(Chapter 6**), other key proteins involved in dietary lipid metabolism, such as FAT/CD36, CPT1 or LPL were not (fully) protected by the training. Therefore, in addition of the differences highlighted by Hamilton (Hamilton *et al.*, 2007) between the exercise physiology and the physical inactivity physiology, we propose that another scale has to be considered the NEAEE physiology.

## 3.4. The relevance of the bed rest model for future research

In the context of research investigating the relationships between NEAEE, exercise energy expenditure, physical inactivity and body weight regulation through the examination of their respective impact on energy and lipid balances, the bed rest model is highly relevant (**Figure 6**). Firstly, contrary to most of the studies in this area, the referential control group, different from the control inactive group during bed rest, is composed of active subjects who are placed in physical inactivity conditions instead of athletes who are detrained or sedentary individuals who are trained. Thus, this logic allows the direct study of the physical inactivity physiology instead of applying the exercise physiology to the physical inactivity physiology, as both likely appear to be delineated by different mechanisms (Hamilton *et al.*, 2007). Moreover, this approach better reflects the reality of the decrease in physical activity observed during recent decades than the experimental designs classically used in the others studies. Indeed, the recent emergence of global obesity occurred in parallel with the emergence of industrialization and technological progress, which discouraged physical activity. The physical inactivity is therefore the abnormal physiological state and the crucial point to investigate.

More specifically, TV viewing, computer, the Internet, cars, dishwashing machines and all the others devices accompanying our current life are elements decreasing the energy cost of living. In this line, the current NEAEE has been estimated to be approximately 1500kcal/d lower than that of our Palaeolithic ancestors (Hayes *et al.*, 2005). Therefore, the modern societies seems to primary suffer from NEAEE deficiency. Despite some practice of exercise to compensate NEAEE deficiency, these individuals can be considered physically inactive. This NEAEE deficient population can be compared to the exercise group of the bed rest studies. Thus, this exercise group seems adequate for intervention studies seeking to examine the direct effect of NEAEE deficiency on the organism. Moreover, the exercise prescription can be modulated and adapted according to the physiological system or the parameter investigated.

However, as Hamilton recently rose it (Hamilton *et al.*, 2007), the current crucial question is: 'Will more sitting worsen the average person's risk' of developing chronic diseases? This is likely plausible because of the constant technological progress, the increasing sedentary employment associated with growing urbanisation but also because the percentage of obese is continuously increasing and obese persons have been reported to sit more than lean persons (Tryon *et al.*, 1992). Therefore sedentary behaviours can be defined as a deficiency of both exercise and NEAEE. Interestingly, the control group in bed rest studies had a PAL of 1.45, which is similar to that estimate in sedentary populations and thus reflects this state of sedentariness. Consequently, it allows to directly answering to the question: Does a sedentary lifestyle 'cause' the onset of some chronic diseases including obesity?

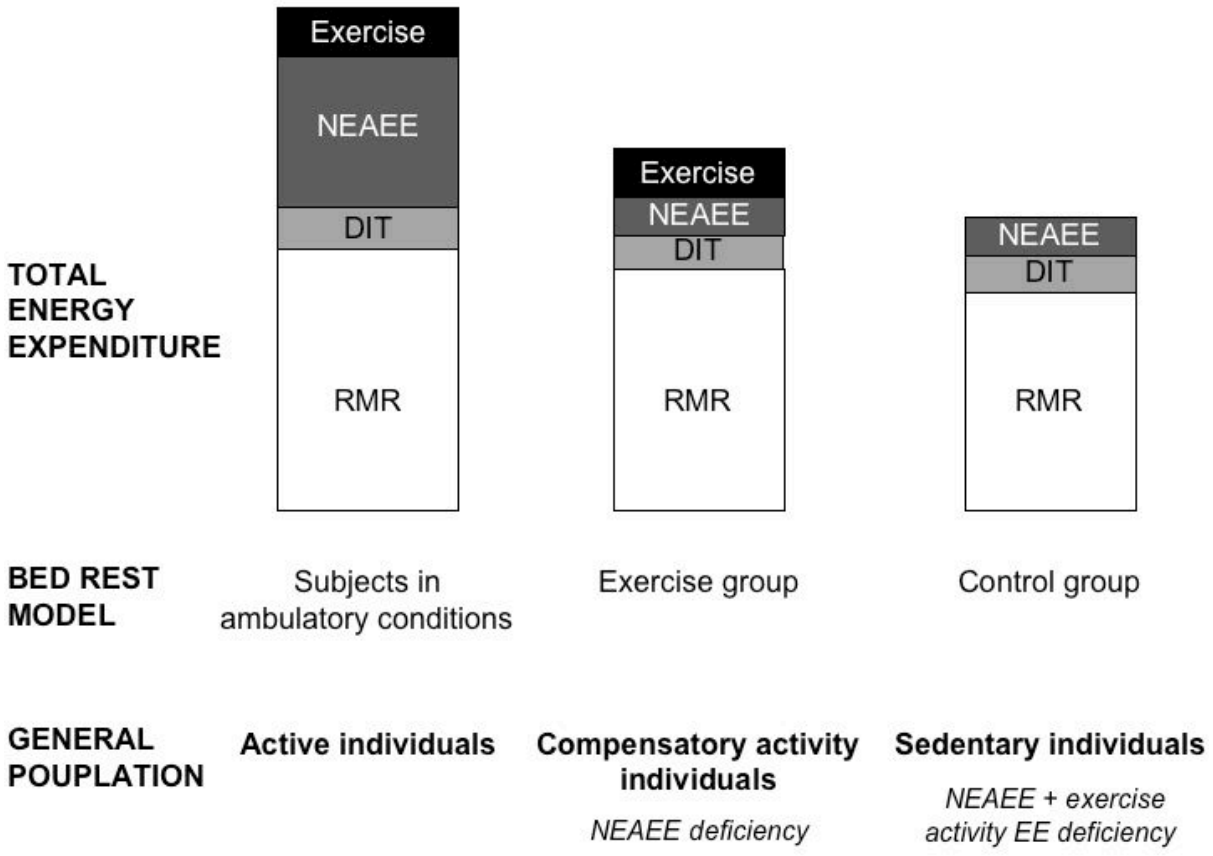



**Figure 6:** Comparison of the total energy expenditure of subjects from control and exercise groups in ambulatory conditions and during the bed rest with that of the general population.

EE, energy expenditure; NEAEE, non exercise activity energy expenditure, DIT, diet-induced thermogenesis; RMR, resting metabolic rate

In summary, the bed rest model seems to be appropriate to investigate the effects of the recent environmental changes that occurred in our modern societies, i.e. the decrease in NEAEE but also probably in exercise practice and the adoption of a generalised sedentary behaviours. Moreover, bed rest studies present the advantage to take place in a highly controlled environment, which allows accurately monitoring food and permits mechanistic approach as we did.

Given all the advantages of the bed rest model, we suggest that further bed rest studies will have to be conducted to further investigate the respective impact of TEE, exercise and NEAEE on the body weight regulation. The current environment is also associated with high-fat energy dense diets which contribute to the epidemic of obesity through an overriding of the satiety signal system and of the oxidative capacity of the organism resulting in an increase in EI and in storage of fat mass. Therefore, bed rest studies combined with high-fat diet may help to clarify the interaction between the two components of physical activity and diet in the regulation of energy and lipid balances. Such studies may provide a better understanding of the causes responsible for the epidemic of obesity but may also help to find some prevention strategies. For example, by varying the fat content in the diet during a bed rest, it may be possible to appreciate the minimal dietary fat quantity tolerated by an individual according to its physical activity. The next step of these investigations will be to translate this knowledge in practical recommendations for the general population.

## 3.5. Transition from the theory to practical physical activity recommendations

After the IASO 1st Stock Conference in Bangkok in 2002 (Saris, 2003) held to discuss the question: "How much physical activity is enough to prevent unhealthy weight gain?", experts concluded: "The current physical activity guideline for adults of 30 minutes of moderate intensity activity daily, preferably all days of week, is of importance for limiting health risks for a number of chronic diseases including coronary heart diseases and diabetes. However for preventing weight gain or regain this guideline is likely to be insufficient for many individuals in the current environment. There is compelling evidence that prevention of weight regain in formerly obese individuals requires 60-90 minutes of moderate intensity activity or lesser amounts of vigorous intensity activity. 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".

Clearly, exercise has very significant and independent health benefits such as lowered mortality, decreased risk for cardiovascular disease and some cancers, enhanced insulin sensitivity and subsequent reduced risk of developing T2D, reduced risk of osteoporosis and favoured body composition alterations, thus lowered rates of obesity and associated comorbidities (Surgeon General's Report on Physical Activity and Health). However, it is striking to note that the role of NEAEE in body weight regulation was not discussed during this conference and that none of these physical activity guidelines refer to it. However, it appears that as long as the increase in energy expenditure is sufficient, low-intensity endurance exercise is likely to generate beneficial metabolic effects that would be essentially similar to those produced by high-intensity exercise. Walking has been proved to be successful in the improvements of metabolic risk profile and should be more promoted. Moreover, it seems easier for sedentary or obese persons to adhere to public health interventions promoting walking than exercising. In this line, Hill et

al. (Rodearmel *et al.*, 2006) suggest to add 2000 steps per day to the daily activity to prevent unhealthy weight gain. However, the NEAEE deficit in obesity is closer to 2-3h of walking throughout the day corresponding to 2000-2500 kcal/week (Levine *et al.*, 2005).

To integrate an additional 2-3h of walking throughout the day in the daily life is clearly a challenge in regard of the time spent by people in their occupation and the modern environment discouraging such an activity. Political action is therefore imperative to effect physical and social environment changes to enable and encourage physical activity. Settings in which these environmental changes can be implemented include the urban and transportation infrastructure, schools and workplaces. In this line, Simon et al. (Simon, personal communication) conducted a pilot study (ICAPS) in four French comprehensive schools (954 pupils). They promoted, with the assistance of professional of sports, the practice of physical activity at school thanks to adapted schedules for exercise and leisure-time activities proposed between and after lessons. The participation to the activities was free and no competition was organised. After 6 months of intervention, they recorded a high participation in the activities, a significant decrease in the proportion of pupils who do not practice activity after school associated with a reduction in the percentage of children spending more than 3h/d in sedentary activities. After four years of follow-up, the normal-weight children participating in this intervention significantly reduced their TV viewing, and more importantly had a lower increase in BMI than control students. This population-based intervention study provided the first strong evidence of the beneficial impact of physical activity promotion at school on the prevention of unhealthy weight gain. On his side, Levine et al. (Levine & Miller, 2007) proposed to re-engineer the environment with, for example, computer treadmill for office workers which allows to spend 100kcal/h or to convert sedentary screen time to active screen time through activity-promoting video games which doubles energy expenditure in children (Lanningham-Foster *et al.*, 2006). Clearly, such interventions or innovations will have to be promoted and governments have to play a major role in these improvements of the environment.

# 4. APPLICATIONS TO SPACE SCIENCES

Although we used the bed rest as a unique model to study the direct effects of the physical inactivity on the human metabolism, it is important to note that this scientific project initially took place in the context of space science. It seems necessary to briefly present the space environment-induced physiological alterations, to explain the initial aims of the long-term bed rest studies involved in this work and to discuss the energy and oxidative balances response to space environment in the light of our results in order to fully and properly understand the global impact of this present thesis.

## 4.1. Adaptations to the microgravity environment

The full utilisation of the International Space Station will require flights of many astronauts for missions lasting up to six months duration. Ultimately the future planned exploration-missions to Mars will expose crew to reduced gravity environments for up to three years.

The space environment induces physiological changes which can affect health and performances of astronauts. Living in space environment and specially in weightlessness induces modifications on all the physiological systems, mainly on the cardio-vascular and sensori-motor systems, on the muscles and bones (Vernikos, 1996). Indeed, microgravity induces physical inactivity which mainly leads to muscle atrophy, bone demineralisation, alterations of fluids, electrolytes and hormones and modifications of metabolism. On the other hand, microgravity causes a loss of hydrostatic pressure, which induces a fluid shift from the lower to the upper part of the body. This fluid shift results in a transient increase of plasma volume as more fluid moves into the vascular compartment from the lower body than is filtered out of capillaries into the upper body (Gharib & Hughson, 1992; Maillet *et al.*, 1994). This thoraco-cephalic fluid shift together with the plasma volume expansion stimulate central volume carotid, aortic and cardiac receptors inducing an increase in diuresis and natriuresis and a decrease in plasma volume. This represents part of the cardiovascular deconditioning characterised by orthostatic intolerance and reduced exercise capacity.

In regard of all these physiological alterations, it is therefore necessary to improve our knowledge on physiological adaptations to space environment in order to develop preventive medicine including nutrition, therapy and rehabilitation, collectively referred to as countermeasures.

## 4.2. The head-down long-term bed rest (-6°) studies

Considering the limited number of flight opportunities, the difficulties related to the performance of in-flight experiments (operational constraints for astronauts, limited capabilities or in-flight biomedical devices and limited number of subjects), ground based experiments have been developed. The long-term bed rest simulates the physical inactivity and the head down tilt (-6°) position mimics the loss of hydrostatic pressure. Such experimental models are used to better understand the mechanisms of physiological adaptations, and to design and validate different countermeasures.

These were the aims of the two long-term HDBR studies on which our project was based on. The first one, organised by European, French, and Japanese Space Agencies in 2000-2001 in men, investigated the simulated weightlessness-induced deleterious alterations in men. In this study, a resistance exercise training protocol was tested as an exercise countermeasure to mainly counteract cardiovascular deconditioning and muscle atrophy and a group received a single intravenous infusion of biphosphanate (Pamidronate) to prevent bone demineralisation. Considering the expected future increase in the number of female astronauts (currently women represent 20% of the astronaut corps in the USA), it was also essential to improve knowledge on gender-specific differences. Because of the relatively small size of the female astronaut population compared with male astronaut population and because most of the ground-based studies were conducted in male volunteers, so far it was impossible to draw valid conclusions about gender differences. The Women International Space Simulation for

Exploration (WISE) study was organised by the European, French, American and Canadian Space Agencies in order to study weightlessness-induced physiological modifications and responses in female population and to compare the data to males. The ultimate goal was to delineate optimised countermeasure-protocols adapted for both genders. A combined resistance and aerobic exercise training protocol was tested and a group (nutrition group) received a dietary protein supplementation (0.6 g/kg body weight/day increase in protein intake) to prevent muscle atrophy. Both of these two HDBR studies took place at the Institute of Space Medicine in Toulouse (France).

These two long-term HDBR studies were unique by their integrative approach of the physiological systems. Effectively, several international teams were involved and simultaneously conducted experimental protocols to study the cardiovascular, muscle, bone, neuro-vestibulatory, immunological, hormonal, and metabolic systems. A psychological approach of the general behaviour and adaptation of the volunteers in such an isolated and confined environment was also conducted.

## 4.3. The tangle relationship between nutrition and adaptations to space environment

This paragraph 4.3. is essentially based on a small review that we published in the context of the COSPAR congress: Bergouignan A and Blanc S. Energy and oxidative balances in response to the space environment. COSPAR, 36th Scientific Assembly, p.102. 16-23, July 2006.

### 4.3.1. Importance of nutritional aspects for space missions

Nutrition has never been a high priority, given the relative short duration of flights. With the construction of the International Space Station and the related foreseen long-term missions, the interest in nutrition has dramatically increased (Lane *et al.*, 1998). Indeed, an increasing body of data has shown a direct relationship between nutrition and the deleterious adaptation to space in regards of both EB and the regulation of the energy substrate oxidation (Stein *et al.*, 1999a; Blanc *et al.*, 2000).

In support of that, Muslim army pilots undergoing a month of Ramadan partial fast have a decreased EI and lose body and fat mass. Both maximal exercise capacity and orthostatic tolerance are impaired in same way to what is observed in astronauts after a space flight. This suggests that nutrition may play a role in the cardiovascular deconditioning syndrome. These observations have been extended to muscle atrophy (Biolo *et al.*, 2007), but need to be tested for immuno suppression, bone demineralisation and overall well-being.

### 4.3.2. Energy deficit during space flight

Among the four studies published so far that investigated EB, three demonstrated a severe ongoing negative EB during space flight (Rambaut *et al.*, 1977; Stein *et al.*, 1996; Lane *et al.*, 1997; Stein *et al.*, 1999b). After a 16-day space flight, Stein *et al.* (Stein *et al.*, 1999b) reported an energy deficit as large as 5.7±0.3 MJ/d (n=4), which would cause a weight loss >5kg/month if sustained. If such a deficit is physiologically tolerable for short-term missions because of the high energy density of fat mass, it becomes a significant detrimental issue jeopardizing health and performance for long-term missions. Giving this chronic under-nutrition, the

energy and macronutrients requirements as well as the study of the mechanisms of regulation need to be precisely evaluated for long-term missions as foreseen of Mars and Moon.

### 4.3.3. The metabolic alterations induced by weightlessness

Endocrine and metabolic profiles during both space flight and HDBR simulations suggest that the organism perceived the weightlessness environment as a stress and responds accordingly. On the $SLS_1$ and $SLS_2$ missions, the whole protein turnover (synthesis and degradation), acute phase hepatic proteins, cortisol secretion, IL-1 and IL-10 increased during the first day of flight (Stein *et al.*, 1996). Data from Skylab, however, show a variation in the duration of the metabolic stress suggesting a mission dependency response. Short-term HDBR (3 days) (Acheson *et al.*, 1995) supports this notion of stress early in a mission by reporting an increase in resting metabolic rate. For longer duration of HDBR (42 days), Blanc *et al.* (Blanc, 1998) observed in men an increased urinary excretion of cortisol, urea, creatinine and GH to last around 4-5 weeks. Moreover, an insulin-resistance has been reported as soon as 3 days of HDBR and then confirmed in the 42d-HDBR, with in addition a reported shift from lipid toward carbohydrate oxidation. During the 2- and 3-month HDBR in women (**Chapter 6**) and men (**Chapter 5**) respectively, we confirmed the insulin-resistance and this decrease in reliance on fat in both fasting and post-prandial states.

### 4.3.4. What are the underlying mechanisms?

We additionally investigated the mechanisms underlying such a shift in substrates oxidation. This shift may be partly accounted for by a shift in muscle fibre types from slow oxidative to fast glycolytic twitch fibre concomitantly to the loss of muscle. The decrease in beta-oxidation is also caused by a drop in the FA availability due to a decrease in muscle LPL gene expression and fatty acid transporters, and by a decrease in mitochondria capacity to oxidise fat (**Chapter 6**). Based on the hind-limb tail suspended rat model, Stein *et al.* (Stein *et al.*, 2002) studied, at the gene expression level, the activity of the enzymes involved in metabolic pathways and showed a decrease in oxidative capacity and an increase in glycolytic capacity in atrophied muscle. They additionally observed a parallel increase in glucogenic capacity of the liver and suggested that the increase in reliance on glucose metabolism at muscle level may be rather caused by an increase in glucose, which may lead to a rise in malonyl-CoA and a subsequent inhibition of CPT1 decreasing the FA availability (Stein *et al.*, 2005). Although we effectively observed a decrease in the gene expression of CPT1 during the HDBR in women, so far, no elevation in the neoglucogenesis was reported during HDBR (Blanc, 2000a). However, the reduced CPT1 activity may decrease the entrance of long-chain FA into mitochondria, which in turn may accumulate as lipid droplets in cytosol. Interestingly, we reported a fat accumulation in muscle during the HDBR in women (**Chapter 6**), which may be partly responsible for the insulin resistance.

### 4.3.5. Functional consequences on muscular system

These considerations have functional consequences since the fatigability of muscles is increased under weightlessness, which is known to participate in the reduced exercise capacity. Indeed, the mechanical efficiency of walking on a treadmill was estimated to be as low as 11% in space compared with 20% on Earth (Fitts *et al.*, 2001). The regulation of substrate mobilisation and oxidation at cell level plays an

important role in exercise capacity and performances (Ferretti *et al.*, 1997). As long-term missions are planned and as increased plasma lipids are risk factors for cardiovascular disease, studies are needed to determine countermeasures will counteract or mitigate these deleterious effects induced by microgravity environment.

## 4.4. Countermeasures

### 4.4.1. Exercise countermeasures

With the understanding that physical inactivity is no longer applicable at a certain volume of exercise, the key is to find a sufficient amount of muscular activity, applicable during space flight because of mission requirements and constraints, which will maintain fitness. During the 2000/2001 HDBR (**Chapter 5**), resistive exercise training program without any significant increase in TEE was applied. However, apart of a prevention of the HDBR-induced muscle atrophy neither the lipemia and insulinemia levels nor the FA oxidation were improved. On the contrary, probably through the partial maintenance of the muscle mass and oxidative capacity, the combined resistive and aerobic exercise training performed by the women (**Chapter 6**) mitigated the simulated weightlessness-induced alterations of the insulin response and of lipid oxidation in both fasting and post-prandial states. The comparison of these two types of exercise training highlights the importance of the greater efficiency of the aerobic exercise-induced increase in TEE to counteract the metabolic alterations induced by HDBR. However, the female volunteers did not adjust their EI to this high TEE and were in negative EB. Consequently, it is difficult to conclude whether the mitigated decrease in fat oxidation was the result of the exercise or that of the negative EB. This confounding effect between exercise and EB represents the main limit of our results obtained during the HDBR in women.

### 4.4.2. Exercise induces an energy deficit

This negative EB related to the performance of intense exercise training is of particular importance regarding the energy deficit observed during space flights. Interestingly, astronauts who exercise during space flights are more subject to energy deficiency than those who do not train. On LMS mission which had a large exercise requirement, the EI failed to meet energy needs, whereas in SLS missions 1 and 2, the astronauts did not exercise, ate more and were in approximate EB (Stein *et al.*, 1996). In Shuttle/MIR missions, during which exercise was also required, after more than 3 months in space, astronauts have still not adapt their EI to the TEE and also had an energy deficit. Our results obtained on the ground after 2 months of HDBR are in accordance with the energy deficiency associated with exercise observed during space flight (**Chapter 4**). Indeed, the control group who did not exercise was in approximate EB, whereas the exercise group submitted to a high volume of exercise consumed less energy than required and was in negative EB. Taken altogether, these results indicate that although exercise is partially effective to counteract muscle atrophy and metabolic alterations, it induces a significant energy deficit which is critical for the health of astronauts.

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### 4.4.3. Consequences of an energy deficit on health

The long-term consequences of a chronic energy deficit are serious. On the ground a chronic energy deficiency results in decreased physical performance together with

increased fatigability (Edgerton *et al.*, 1995; Riley *et al.*, 1995), and susceptibility to infection progressively increases (Keusch & Farthing, 1986). Wound healing is also compromised with chronic under-nutrition, which may be a problem if injury ever occurs during space flight (Kirkpatrick *et al.*, 1997). During space flight, a decreased immunocompetence has been reported (Levine & Greenleaf, 1998) (Taylor *et al.*, 1997). Moreover, some authors (Stein, 2000) reported that the decrease in protein synthesis and an estimate of the inflight energy deficit shows a good correlation. As muscle atrophy is essentially accounted for by a decrease in protein synthesis rather than by an increase in protein catabolism, exercise performance may be counter-efficient for the maintenance of fat-free mass at a certain volume. In addition to under-nutrition, astronauts have to face to malnutrition due to altered nutrient bioavailability, an inadequate food system and physiologic changes. Indeed, non adequate intake of mineral salts (calcium, sodium, potassium) and oligo elements (iron) as well as reduced vitamin D stores have been reported during ground-based and/or space flight studies (Smith *et al.*, 2001). This can exacerbate the physiological alterations induced by microgravity environment such as bone and muscle losses or oxidative stress (Smith *et al.*, 2001). Therefore, nutrition through intake of both macronutrients and micronutrients plays a crucial role for the long-term health and safety of astronauts.

### 4.4.4. Reasons for the negative energy balance

The decrease in EI observed during space flights can firstly be explained by space motion sickness, preoccupation with mission objectives and stress, to which are added the high EE induced by exercise associated with the adverse effects of exercise on EI. Stein (Stein, 2000) suggested that the energy imbalance induced by exercise is due to problems in disposing of the metabolic by-products during exercise. According to him, these defects would be mainly the results of less efficient thermo-regulatory mechanisms in microgravity (or bed rest) than on the ground. This essentially includes impaired heat dissipation partly due to a low air flow at the surface of the skin in space shuttle, reduced blood flow at the extremities of the body and decreased sweat losses. He also proposed that during exercise a large part of blood volume is diverted towards muscle and away from the gut. This means that the gut is not ready to process the food, which in turn triggers certain gut hormones and reduces the appetite accordingly.

Interestingly, we showed in the 2-month HDBR in women (**Chapter 4**) that the no compensatory EI of the volunteers face to the exercise-induced energy expenditure was associated with an increase in GLP1 concentration, a satiety gut hormones and with no change in ghrelin concentration, the only known orexigenic hormone. The loss of appetite was probably related to the well known 'exercise-induced anorexia'. Although the lack of adjustment to energy needs was a spontaneous response of the volunteers, it is important to note that the negative EB observed in the exercise group was partly related to a wrong estimation of the energy cost of the exercise by the investigators. Indeed, the energy imbalance was roughly proportional to the energy cost of the exercise. Such results raise once again the importance to accurately assess the energy requirements of the astronauts during space missions.

### 4.4.5. Assessment of the energy requirements

The issues of the EB is only important during long-term space missions since the large reserves of body fat can buffer the negative EB for short periods. In a previous 42d HDBR (Blanc, 1998), the energy requirements for men was evaluated to 1.47 x RMR + energy cost of exercise. We showed that the energy requirements

for women are very close, i.e. 1.45 x RMR + energy cost of exercise (**Chapter 4**). Interestingly, these equations are similar to that derived during space flight by Stein et al. (Stein *et al.*, 1999b) based on DLW derived TEE on 4 male astronauts: 1.4 x RMR + cost of physical exercise. Therefore, energy needs seem to be similar on grounds and in space.

As previously observed, we confirmed that the body mass does not reflect the EB because of the muscle atrophy. Indeed, the female volunteers in the control group were in approximate EB despite the decrease in body mass (**Chapter 4**). Thus, clamping fat mass during HDBR studies instead of body mass as American investigators used to do (Krebs *et al.*, 1990), avoid conducing to positive EB and fat gain. Therefore, the body fat is a greater index of the EB in microgravity. It is important to be able to follow the day-to-day changes in EB during space flight in order to rapidly adjust the dietary intake. In this line, the skinfold thickness method that we validated in the **Chapter 7** by comparison with the dilution method can be helpful to evaluate the nutritional state of the astronauts and rapidly adjust their energy requirements. However, we showed that the high energy needs of the aerobic exercise are not compensated by an adjusted EI due to the 'exercise-induced anorexia'. Therefore, we can wonder what would be the impact of an increase in the prescribed dietary intake in individuals who loose appetite. Higher fat diet may be provided to the astronauts since fat has a weak effect on the satiety signal system and enhances passive overconsumption (Blundell *et al.*, 1993; Blundell & King, 1996). However, we can wonder what would be the effect of a diet with high proportion in fat on the lipid balance, which is already characterized by an impaired fat oxidation, and in turn on the insulin response. If EI cannot be easily increased, we may be should pay attention to the other side of the EB: TEE. Indeed, we feel that the energy deficiency issue has to be though differently and others types of countermeasures have to be considered.

### 4.4.6. Future research guidelines: Find a compromise between the different countermeasures

The resistive and aerobic exercises are crucial to counteract the weightlessness-induced muscle atrophy and cardiovascular deconditioning as well as insulin resistance, hyperlipemia and the decrease in fat oxidation (**Chapter 6**). However the high energy needs of the aerobic exercising is compatible neither with EB nor with nitrogen balance. Indeed, it creates a decrease in protein synthesis, which in turn leads to a loss in muscle mass. Thus, exercise, at a certain volume, is counter-efficient for both EB and maintenance of muscle mass. Interestingly, no muscle breakdown occurred in MIR mission whereas astronauts were free to select the amount of exercise they wanted (Stein *et al.*, 1999b). Their musculoskeletal muscle was able to adapt better to space flight than those of astronauts forced to do a pre-determined type and amount of exercise. As Stein proposed (Stein, 2000), a vigorous exercise program may be applied to rebuild the atrophied muscles 2 or 3 weeks before landing, since the negative EB on this short period would have little consequences on the organism.

Another alternative approach would be to combine nutrition and exercise countermeasures to mitigate the muscle atrophy, which may allow reducing the exercise prescription. Based on the observation that ingestion or infusion of essential amino acids provides a potent acute anabolic stimulus in healthy young and elderly subjects (Volpi *et al.*, 1998; Volpi *et al.*, 1999; Paddon-Jones *et al.*, 2003), dietary supplementation of essential amino acids has been tested to mitigate the muscle atrophy. In this line, a high-leucine high-protein diet was provided to a nutrition group during the WISE study. Biolo and colleagues (personal communication) reported that dietary protein supplementation decreases loss of

muscle mass compared to the control group, in association with an approximate nitrogen balance. Additionally, the volunteers in the nutrition group consumed more energy than those in the control and exercise groups, suggesting a positive effect of protein on appetite. However, Lejeune *et al.* (Lejeune *et al.*, 2006) reported that a high-protein diet has incidences on ghrelin and GLP1 concentrations and increases the 24h-satiety. Therefore, before applying a high-protein diet combined with exercise during space missions, it appears necessary to study the effect of protein on the feeding behaviour. Another side effect of the protein supplementation is a hypercalciuria and a bone resorption likely linked to changes in pH. Therefore, the aim for future research is to find the adequate dose between exercise and dietary protein supplementation to mitigate the muscle atrophy and the metabolic alterations without increasing the loss of appetite and hence, the energy imbalance as well as the bone demineralisation already induced by the microgravity environment.

For our part, we observed that the simulated microgravity induces a decrease in palmitate oxidation which is correlated to the muscle fat accumulation (**Chapter 6**). In the previous paragraph, we reported that a reduced free FA availability and a high muscle fat content may participate in the muscle dysfunction and fatigability (Brooks, 1997). Moreover, a relationship between IMTG and insulin resistance is established (Kelley *et al.*, 2002). Similarly, epidemiological studies have associated the diets rich in palmitic acid with an increased risk for insulin resistance and T2D (Vessby *et al.*, 2001). A previous bed rest reported an increase in IMTG within subjects fed with a high-saturated fat diet and this in association with a decrease in insulin sensitivity (Stettler, 2005). On the contrary, oleate oxidation is not affected by microgravity and improves insulin sensitivity through a protective effect against the palmitate-induced insulin resistance and cell viability. The cellular mechanisms involved in oleate and palmitate metabolism at muscle level as well as the associated insulin responsiveness are fully described in the review in **Annexe 1**. Taking into account these results, it is tempting to speculate that a diet rich in oleic acid for astronauts may partially mitigate the reduced fat oxidation, the alterations in insulin response and the muscle fat accumulation and consequently, the deleterious consequences on muscle functions induced by the microgravity environment. Clearly, the potential beneficial effects of this type of diet would have to be previously investigated during a long-term bed rest study.

Finally, pharmacological countermeasures may also be proposed to mitigate the muscle dysfunction due to muscle atrophy and the reduced fat oxidation. Resveratrol, a natural polyphenolic compound mainly found in the skin of grapes and in red wine is well known for its antioxidant properties (Baur & Sinclair, 2006) and for its beneficial action on lipid metabolism through the maintenance of a normal lipid flux (Lagouge *et al.*, 2006). It has also been shown to significantly increase SIRT1 activity (Howitz *et al.*, 2003), which leads to an activation of the gene expression of PGC1alpha (Rodgers *et al.*, 2005), protein known for its stimulating effect on mitochondrial biogenesis and thus lipid oxidation. Favouring the fat oxidation, resveratrol may also limit the development of insulin resistance. Preliminary results obtained at the DEPE showed that this molecule prevent the loss of muscle mass and strength in hind limb tail rats (Momken, personal communication). Future HDBR studies should test resveratrol as a pharmacological countermeasure to prevent muscle atrophy, alterations in lipid metabolism, insulin resistance but also oxidative damages.

# 5. CONCLUSIONS

This work shows that in spite of a stable energy balance, physical inactivity itself triggers a metabolic pattern close to what is observed in obesity and diabetes supporting the hypothesis that these physiopathologies may be, at least in part, secondary to the adoption of a generalized sedentary lifestyle over the last century. Therefore, we may assume that obesity, and particularly the metabolic patterns related to obesity are not only the result of a chronic positive energy balance. Concerning the exercise intervention strategies, although exercise training has clearly beneficial effects on health, it may be not suitable for sedentary and overweight persons in particular in our current modern environment. Therefore, the role of the non-exercise activity energy expenditure in the regulation of both energy and lipid balances seems to be a priority for future research. A better understanding of the interaction between dietary fat, with a particular attention on the nature of fatty acids, and physical activity will also have to be elucidated in order to better face the obesity epidemic in the future. Nevertheless, waiting for these future results, this present thesis already clearly promotes a simple recommendation for the general population to prevent obesity and its related comorbidities, which is: "Get moving!". Finally, in addition of the new insights for the understanding of the aetiology of obesity and its related metabolic disorders, this present work promoted the importance of nutrition for space flight missions and proposed new countermeasures.

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*(XXème siècle)*

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# ANNEXES

*Danseuse à la barre de Botero (2001)*

# ANNEXE 1

## Metabolic fate of saturated and mono-unsaturated dietary fats:

## The Mediterranean diet revisited from epidemiological evidences to cellular mechanisms with activity energy expenditure as a central actor

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# 1. ABSTRACT

Increasing evidence indicates favourable effects of the Mediterranean diet partly associated to its monounsaturated fatty acids (MUFA) content on both obesity and diabetes. However, neither the underlying mechanisms by which the Mediterranean diet exerts its protective effect, nor the interplay with other environmental factors (i.e. physical activity), are fully characterised. In this review, we examined recent data on how the metabolic fate of MUFA and saturated fatty acids (SFA) differs. Because of differential packaging into lipoproteins, hydrolysis of triacylglycerol-rich lipoproteins by lipoprotein lipase and transport into oxidative tissues, MUFA are oxidised more than SFA. This high MUFA oxidation favour lipid oxidation and according to the oxidative balance concept reduces the risk of obesity. It also improves the intra-muscular triacylglycerol turnover, which mitigates the SFA-induced accumulation of diacylglycerol and ceramides, and thus protects the insulin sensitivity and cell viability. Finally, physical activity through its action on the energy turnover differentially regulates the metabolism of SFA and MUFA. The putative combined role of AMPK-activated kinase and mitochondrial glycerol-3-phosphate transferase on the intra-muscular partitioning of MUFA and SFA provides a new area of research to better understand the beneficial effects of the Mediterranean diet and physical activity on obesity and diabetes.

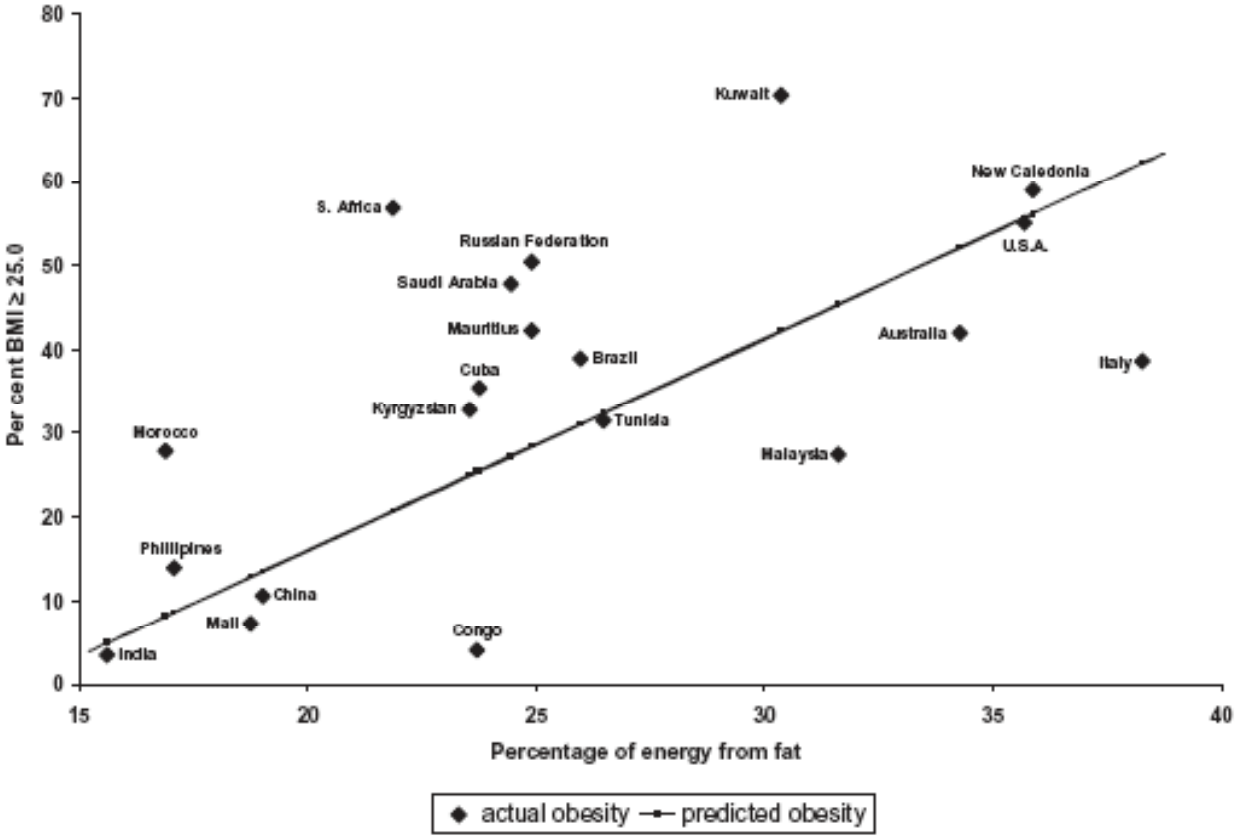


**Figure 1 :** The relationship between the percentage of the population who are obese and the proportion of energy intake from fat. From Bray et al. [3].

# 2. INTRODUCTION

Obesity is a worldwide health problem that affects both genders as well as different ages and race classes. Currently more than 300 million individuals are obese (body mass index (BMI) of 30 kg/m$^2$ or higher). Overweight, and ultimately obesity, is a serious risk factor for multiple chronic diseases, including cardiovascular disease, hypertension, diabetes, cancers, and arthritis, which cause almost 300,000 deaths and costs 117 billion dollars each year in the United States alone [1]. Although genetic predisposition is a risk factor, the rapidly increasing prevalence of obesity in both developed and developing countries [2] suggests environmental factors, such as changes in the dietary and physical activity patterns are key contributors in the etiology of obesity.

In 1998, Bray et al. [3] highlighted the key role of dietary fat in the development of obesity based on a positive relationship between the percentage of fat in the diet and the percentage of obesity in adults from surveys conducted in 20 countries (Figure 1). This was supported by numerous studies [4-6], but the relevance of this association had been criticized based on the observation that the percentage of fat in Western diets has recently been reduced while obesity rates continued to rise [7]. It should be noted, however, that even if the percentage of fat in the diet has decreased, the total energy intake continued to increase and, consequently, the absolute fat intake increased too. In addition to the amount of fat, further evidence showed that the quality of fat is an important factor to consider in the development of obesity [8]. Some cross-sectional studies identified positive associations between the consumption of saturated (SFA) fatty acids and the risk of obesity and its related disorders, and negative associations for that of monounsaturated fatty acids (MUFA) [9-12].

An additional line of conjecture comes from the study of paleonutrition. Growing evidence indicates that humans evolved from an animal protein diet with 35% of fat, including 7.5% of saturated fats of total energy intake [13] to a Western diet with 41% of fat, including 15.4% from SFA [14]. Moreover, fat from wild species were rich in PUFA with an appreciable quantity of n-3 (4%) [15], whereas the Western diet contains PUFAs rich in the n-6 series such as arachidonic (20:4) and linoleic (18:2) fatty acids [16].

The importance of fat quality on health outcomes have been well demonstrated by population-based, cross-cultural comparisons of diets with large differences in the type of fat ingested. Among those the Mediterranean diet has been the most studied. It was first described in the 1960s by Angel Keys, based on his observation of food habits of some populations in the Mediterranean region. The diet he observed was based on a large variety of foods, mostly vegetables, fresh fruits and fish, as well as a moderate consumption of alcohol. It was characterized by a high fat consumption in Greece (40% of daily energy intake) or by a moderate fat consumption in Spain (30% of daily energy intake) [17]. The Mediterranean diet, however, was low in SFA (7-8% of energy), because the fat source consisted primarily of olive oil [18, 19] resulting in a higher ratio of MUFA to SFA than in other places of the world, including Northern Europe and North America [20, 21].

The health outcomes associated with the Mediterranean diet have been extensively studied and numerous reviews have been written detailing how the adherence to a diet rich in MUFA was associated with decreases in mortality, cardiovascular diseases, prevalence in metabolic syndrome, various types of cancers

and ultimately obesity and diabetes [22]. Nevertheless, neither the underlying mechanisms through which the Mediterranean diet exerts its protective effects, nor the interplay with other environmental factors such as the level of physical activity have been well characterised.

This review was conducted to compile recent data, and to propose hypotheses, regarding how the metabolic fates of MUFA and SFA differ. Herein, we briefly review the epidemiological data and frame this within the concept of fat balance. In addition, we compile available data on the differential digestion, assimilation, trafficking and cellular metabolism of SFA and MUFA. Despite previous reviews, such a compilation is missing from literature, because most of the reviews for the last decade have focused on other aspects of the metabolism of SFA, MUFA and PUFA. Here, we took into account results on PUFA metabolism to explain central pathways only when no data on MUFA were available. Finally in light of recent data, we demonstrate the critical interaction with physical activity and how this might alter the metabolic fate of SFA and MUFA.

# 3. EPIDEMIOLOGICAL EVIDENCE

Epidemiological studies that looked for associations between the Mediterranean diet and obesity or diabetes were recently reviewed by Moussavi et al. [23].

## 3.1. Cross-sectional studies

Four cross-sectional studies are representative of what is generally observed in the literature. In a representative Mediterranean Spanish population consisting of 1547 men and 1615 women, Schröder et al. [24] observed that the adherence to the traditional Mediterranean dietary pattern, characterized by high intakes of vegetables, fruits, legumes, fish, cereals, and nuts, and low and moderate consumption of meat and wine, respectively, was negatively associated with body mass index (BMI). The obesity risk also decreased in both men and women with increasing adherence to the traditional Mediterranean dietary pattern. The negative association between Mediterranean diet and BMI was also later observed by Shubair et al. [25] in 759 adults from Ontario (USA) and by Panagiotakos et al. [26] in 1514 men and 1528 women from Greece. This last study also reported a negative relationship between the Mediterranean diet and the waist-to-hip ratio [26]. On the other hand, Trichopoulou et al. [27] did not observe an essential relationship between adherence to the Mediterranean diet and BMI in a large general Greek population sample from the Greek European Prospective Investigation into Cancer and Nutrition (EPIC) study (23,597 adults).

## 3.2. Longitudinal studies

Several cohort studies also demonstrated association between the Mediterranean diet and change in body weight. A longitudinal analysis of 6319 Spanish men and women showed that subjects with a high adherence to the Mediterranean diet had lower crude increments of weight (+0.45kg) after 2 years compared to subjects

with a low adherence to the Mediterranean diet (+0.73kg) [28]. In 17,238 women and 10,589 men from the Spanish cohort of the EPIC study who were not obese ($20 < BMI < 25$ kg/m$^2$) at baseline and followed for 3.3 years, a high adherence to a Mediterranean diet was associated with a significant lower risk of becoming obese after follow up in both men (odds ratio with 95% CI: 0.69) and women (odds ratio with 95% CI: 0.68) [29]. On the contrary, Bes-Rastrollo et al. [30] carried out a study in 7368 Spanish young males and females and showed that a high levels of olive oil consumption is not associated with lower weight gain or a significantly lower risk of developing overweight or obesity in the context of the Mediterranean food pattern.

### 3.3. Interventional studies

McManus et al. [31] randomized 101 overweight men and women to either a moderate fat, Mediterranean-style, energy restricted diet (35% energy from fat) or a low-fat, energy-restricted diet (20% energy from fat). After 18 months, the moderate-fat group had a 4.1 kg decrease in body weight, while the low-fat group had increases of 2.9 kg. In the KANWU study [32], 162 healthy men and women were randomized to a controlled isoenergetic diet for 3 months containing either a high proportion of SFA or MUFA. The high SFA diet significantly impaired the insulin sensitivity by 10% whereas there was no change with the isoenergetic high MUFA diet.

Taken together cross-sectional, longitudinal and interventional studies indicate that there is a favourable effect of Mediterranean-type diets on weight maintenance and glucoregulation. These include cross-sectional, longitudinal and interventions studies. The studies, by their nature, however, do not specify the mechanisms through that mediate these positive results.

## 4. WHOLE BODY DIFFERENTIALLY DIETARY FATTY ACID OXIDATION AND MAINTENANCE OF BODY WEIGHT

### 4.1. The central concept of energy and fat balances

Based on the laws of thermodynamics, body composition and usually body mass can remain stable over time only if the metabolizable energy ingested matches the energy being expended, i.e. if the individual remains in energy balance. Energy is expended for three main purposes: the resting metabolic rate which maintains basic physiologic functions, the digestion and storage processes associated with ingested food and physical activity which is the most variable component.

The stability of body mass also requires that the proportion of the substrates oxidised summed across all the cells of the body matches the macronutrient composition of the diet. This is acutely and tightly achieved for glucose and amino acids through appropriate changes in their oxidation after meals [33]. On the other

hand, adjustment of fat oxidation to fat intake was shown to take up to a week. Thus, in the short term, whereas any excess in glucose or amino acids is oxidized, excess in fat are stored. This low priority for fat oxidation in the hierarchy of substrate use explains that energy balance and fat balance are strongly correlated [34].

These two levels of complexity in the regulation of energy balance are further complicated by the observation that fat oxidation rate, and thus fat balance, depends on the chemical structure of individual fatty acids.

## 4.2. Differential oxidation of dietary fatty acids

Using fatty acids labelled with radioactive or stable isotopes, it was shown in both rats [35-39] and humans [40-42] that dietary fat oxidation vary according to the nature of the fatty acids acutely ingested (Table 1). The short- and medium-chain fatty acids and the unsaturated oleate (18:1) and linoleate (18:2) are more rapidly and greater oxidized than the long-chain fatty acids and the SFA palmitate (16:0) and stearate (18:0). For example, in humans, the cumulated oxidation over a 9h-test period was 41%, 18%, 16%, 13% for laurate (12:0), oleate, palmitate and stearate, respectively [41].

## 4.3. Effect of different dietary fatty acids on total lipid oxidation, energy expenditure and body weight

The above short-term studies indicate that are some differences in the fate of different fatty acids. In the long-term, diets enriched in specific fatty acids have been shown to influence fat balance, body mass, and possibly energy expenditure.

As reviewed elsewhere, animal studies have demonstrated different rates of weight gain in response to the consumption of different types of dietary fat [43]. In humans (Table 1), Kien et al. [44] fed during 28 days healthy young adults either with a high palmitic acid (16:0) or with a high oleic acid (18:1) diet and showed a reduction in postprandial lipid oxidation following the high-palmitate diet and no changes after the high-oleate one. Piers et al. [45] reported in men that the postprandial fat oxidation rate is higher and carbohydrate rate lower, after a MUFA (virgin olive oil) than after a SFA (cream) meal. A further four-week intervention on the same participants in which SFA were replaced by MUFA induced a significant loss of body weight and fat mass (-1.6 ± 1.1 kg and -1.1 ± 0.7 kg, respectively) without any change in total energy or fat intake [46].

In healthy humans [44] and in particular in men [47], 28 days of a high palmitic acid diet reduce the daily energy expenditure whereas a high-oleic acid diet did not affect it. In rats [48] a long-term SFA intake, compared with the intake of vegetable oils rich in MUFA, decreases diet-induced thermogenesis through a decline in sympathetic activity in brown adipose tissue, resulting in body fat accumulation. In humans [49], a diet with a low PUFA:SFA ratio reduced both the resting metabolic rate and the diet-induced thermogenesis together with a lower basal fat oxidation, compared to a diet with a high PUFA:SFA ratio. These results were confirmed after a prolonged period (14 days) of diets contrasted in their PUFA:SFA ratio [50]. However, Soares et al. [51] found a higher diet-induced thermogenesis (5.1 ± 2.0% vs. 2.5 ± 2.9%, respectively) after an acute high-fat meal including extra virgin olive oil (MUFA) compared to an isoenergetic diet including cream (SFA) in obese subjects but not in normal-weight subjects. In

**Table 1. Association between dietary fat composition with change in oxidation rate, energy expenditure, resting metabolic rate, diet-induced thermogenesis and body weigth and fat in rats and humans.**

| | | | Outcomes | | | | |
|---|---|---|---|---|---|---|---|
| **Authors** | **Subjects** | **Design of the study** | **Oxidation rate** | **Total energy expenditure** | **Resting metabolic rate** | **Diet-induced thermogenesis** | **Body weigth and fat changes** |
| Leyton et al. [35] | 56 Sprague Dawley female rats | Oral load of radiolabelled lauric, palmitic, stearic, oleic, linoleic, linolenic or arachidonic acids. 24h of test. | Linolenic > oleic > arachidonic > linoleic > lauric > palmitic > myristic > stearic acids | | | | |
| Jones et al. [40] | 6 lean men | Oral load of stable isotope labelled stearate, oleate and linoleate. 9h of test. | Oleate > Linoleate > Stearate | | | | |
| Delany et al. [41] | 4 healthy men | 40% of total fat. Laurate, palmitate, stearate, oleate, elaidate, linoleate, linolenate. 9h of test. | Laurate > others PUFA and MUFA > SFA | | | | |
| Schmidt et al. [42] | 10 healthy men | 40% of total fat. Emulisfication of stable isotope labelled oleate and palmitate. 7h of test. | Oleate > Palmitate | | | | |
| Kien et al [44] | 21 healthy women and 22 healthy men | 40% of total fat. High palmitate diet (16.8% palmitate and 16.4% oleate) or high oleate diet (1.7 % palmitiate and 31.4% oleate) for 28d. | ⇑ after high oleate diet | ⇓ after high palmitate diet | | | |
| Piers et al [45] | 14 lean to obese men | 43% of total fat. Acute breakfast rich in MUFA (olive oil, 25% MUFA, 7% SFA) or in SFA (cream, 13% MUFA, 24% SFA). 12h of test. | ⇑ and ⇓ in post-prandial fat and carbohydrates oxidation, respectively after MUFA-rich meal. | | | | |
| Piers et al. [46] | 8 overweight or obese men | 40% of total fat. High MUFA diet (MUFA 22%, SFA 11%) or high SFA diet (MUFA 13%, SFA 24%) for 4 weeks. | | No change | | | ⇓ in both body and fat mass after high MUFA diet. |
| Kien et al. [47] | 21 healthy women and 22 healthy men | 40% of total fat. High palmitate diet (16.8% palmitate and 16.4% oleate) or high oleate diet (1.7 % palmitiate and 31.4% oleate) for 28d. | ⇑ and ⇓ after high oleate and palmitate diet, respctively, in women | ⇑ and ⇓ after high oleate and palmitate diet, respectively, in men | | | |
| Takeuchi et al. [48] | 76 male rats | 39.4% of total fat. Isoenergetic diet with lard or high olive oil as fat for 12 weeks. | | | | ⇓ with diet rich in SFA | |
| Jones et al. [49] | 8 healthy men | Low PUFA:SFA (0.24) or a high PUFA:SFA (1.65) ratio for 1 week. | ⇓ fat oxidation with the low PUFA:SFA ratio | | ⇓ with the low PUFA:SFA ratio | ⇓ with the low PUFA:SFA ratio | |
| Van Marken Lichtenbelt et al. [50] | 6 healthy men | 46% of total fat. Low PUFA:SFA (0.19) or a high PUFA:SFA (1.67) ratio for 2 weeks. | | | ⇓ with the low PUFA:SFA ratio | ⇓ with the low PUFA:SFA ratio | |
| Soares et al. [51] | 12 lean to obese women | Acute test meal rich in SFA (cream, 40% of total fat, MUFA:SFA ratio = 3.0) or in MUFA (olive oil, 41% of total fat, MUFA:SFA ratio = 7.0) | | | | ⇑ after MUFA-rich meal in obese women. | |

Mono-unsaturated fatty acid (MUFA); saturated fatty acid (SFA); polyunsaturated fatty acid (PUFA).

contrast, we have recently completed a study investigating the interaction with physical activity energy expenditure and four days of feeding 50% of energy as fat with either high palmitic acid or high oleic acid content and find no effect of fatty acid composition on 24h energy expenditure (Schoeller, personal data).

Taken together these studies have demonstrated an acute differential oxidation of fatty acids that is function of their chemical structure in both human and animals: SFA being less oxidized than unsaturated fatty acids. Interventional studies further demonstrated that such differential oxidation affects body weight and composition.

# 5. DIGESTION, ASSIMILATION OF DIETARY MUFA AND SFA AND RESULTANT LIPEMIA

Several steps in the metabolic fate of fatty acids may be involved in the differential metabolism between the MUFA and SFA: absorption through the enterocytes, trafficking between the peripheral tissues (liver, adipose tissue or skeletal muscle) and/or handling into myocytes and mitochondria.

## 5.1. Digestion and assimilation processes

A typical meal contains lipids mainly in the form of triacylglycerides (TAG), with small amounts of free fatty acids (FFA), cholesterol and other sterols. Digestion of dietary lipids begins in the stomach with partial hydrolysis of TAG and the formation of large fat globules that also contains FFA, phospholipids and sterols. Gastric lipases hydrolyze the TAG containing short-chain fatty acids (FA) faster than those containing medium- or long –chain FA, but no differential hydrolysis rates of the TAG based on the degree of saturation of the FA that they contain was reported [52]. Nevertheless, diets high in olive oil were shown to promote gastrointestinal secretions and to stimulate stomach emptying [53], which would increase the rate of supply of monoacylglycerols and FA to the enterocyte. As these fat globules enter the intestinal lumen, they are mixed with bile salts and hydrolyzed by pancreatic lipases to monoacylglycerols and long-chain FFA, which then enter enterocytes by either a passive or facilitated process. The later is responsible for the greater portion of uptake and includes fatty acid transporter proteins such as the fatty acid binding protein (FABP) which are associated with the brush border membrane [54]. To our knowledge, only one study investigated the transport of FA through intestinal FABP and reported no discrimination among the different FA [55]. Finally a study of Jones et al. [56], investigated the malabsorption of different fatty acids given within an emulsion, and reported little difference. Relatively small amounts of [1-$^{13}$C]palmitic acid (1.2%) and [1-$^{13}$C]oleic acid (1.9%) were excreted in stool, indicating almost a complete absorption. Only [1-$^{13}$C]stearate was less absorbed (91% of absorption). Thus, current knowledge suggests that assimilation of dietary fat is not highly influenced by the dietary FA composition of meals. It is consequently not expected that lipemia differs when meals vary in dietary FA.

## 5.2. Effect of acute and chronic meals varying in dietary fatty acids on postprandial lipemia

Roche et al. [57] provided to healthy normolipidaemic men three test meals containing 12, 17 and 24% of energy as MUFA representing respectively current UK, current Mediterranean and traditional Mediterranean MUFA intakes (Table 2). The different proportions of MUFA in these acute test meals had no effect on the postprandial plasma TAG, indicating that MUFA and SFA have a same rate of digestion and assimilation. These results were confirmed in a second additional study conducted by the same team and using the same proportions of MUFA in the acute test meals [58]. These two studies suggest that varying meal MUFA composition across a range, which reflects worldwide dietary variation, has no effect on postprandial lipaemic response.

In contrast, the postprandial TAG concentrations have been shown to be altered in some studies when meals are highly enriched in specific dietary FA in healthy men. Thosltrup et al. [59] tested meal tests high (approximately 43% of energy) in stearic acid (18:0), palmitic acid (16:0), palmitic (16:0) + myristic (14:0) acid, oleic acid (18:1) and elaidic acid (trans 18:1), in 16 healthy young men. Intake of meals rich in the long-chain SFA stearic and palmitic acids resulted in a relative lower lipemic response than did intake of a meal rich in unsaturated fatty acids oleic and elaidic acids. Such result observed when meals are experimentally highly enriched in different individual FA, was also found when food components naturally enriched in different type of FA varied in the diet. For example, when 40g of fat was provided by olive oil MUFA source, the postprandial TAG concentration was higher than when the same amount of fat was provided by butter SFA source [60].

Gender seems also to influence postprandial TAG concentrations when meal fatty acid composition is altered. The plasma TAG concentration is higher after a MUFA (67% MUFA, 20% SFA of total energy) than after a SFA meal (33% MUFA; 53% SFA of total energy) in men while the patterns of TAG responses is identical after the MUFA and SFA meals in women [61].

These heterogenic results in lipaemic response to an acute meal varying in dietary FA types may be likely explained by differences in the percentage of MUFA and SFA in the acute test meals. The findings from long-term dietary intervention have been more consistent.

There are a number of different types of studies, which have evaluated the impact of altered background diet on postprandial lipemia. But most of them have addressed the question of whether a change in background dietary FA intake enriched in PUFA affected the postprandial lipemia [62-66]. Herein, we report the most relevant studies designed to compare the effect of long-term dietary intervention with meals enriched either in MUFA or in SFA. After a 8-week diet rich in olive oil, the postprandial plasma TAG concentration were significantly greater in the early postprandial period than after 8 weeks of a control SFA-enriched diet [67]. Such results were confirmed in diabetic subjects after a similar dietary intervention of 3 weeks [68]. A study showed different patterns of postprandial lipemia after standard fat-containing meals in northern (diet characterized by 13.6% SFA and 11.1% MUFA of total energy) and southern (diet characterized by 14.1% SFA and 18.2% MUFA of total energy) European males [69], suggesting that background dietary MUFA consumption may influence the nature and extent of postprandial lipemia. In the southern Europeans, plasma TAG concentrations were much greater during the early postprandial phase and returned to near-fasting concentrations much earlier than in the northern Europeans.

**Table 2. Effect of dietary fatty acid composition on post-prandial lipemia, chylomicrons secretion and size, NEFA spillover, very low density lipoprotein synthesis and lipoprotein lipase activity.**

| | | | Outcomes | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Authors** | **Subjects** | **Design/Intervention** | **Total TAG** | **Chylomicrons** | **Apolipoprotein B48** | **NEFA** | **VLDL** | **LPL activity** |
| Roche et al. [57] | 30 healthy men | 40g of fat. 3 acute test meals with 12, 17 and 24% of MUFA. 9h of test. | No differences | No differences | No differences | lower after high MUFA intake | | |
| Jackson et al. [58] | 15 healthy men | 40g of fat. 3 acute test meals with 12, 17 and 24% of MUFA. 9h of test. | No differences | No differences | No differences | lower after high MUFA meal | | |
| Tholstrup et al. [59] | 16 healthy men | Acute high fat test meals: 43% of stearic, palmitic, palmitic + myristic, oleic, elaidic acids. 8h of test. | unsaturated oleic, elaidic acids > SFA stearate and palmitate. Faster return to near-fasting concentration after unsaturated FA intake. | | | | | ⇑ in plasma after high unsaturated FA intake |
| Mekki et al. [60] | 10 healthy men | Test meals with 40g of fat: olive oil (MUFA) or butter fat (SFA). 7h of test. | MUFA > SFA | Concentration and diameter higher after MUFA intake | | | | |
| Koutsari et al. [61] | 9 healthy men and 10 healthy women. | Test meals rich in MUFA (67%) or in SFA (53%). 6h of test. | MUFA > SFA in men. No effect in women | | | No differences | | |
| Roche et al. [67] | 23 healthy men | 8 weeks of olive oil rich diet (18.3% MUFA, 12.0% SFA) vs. SFA-rich diet (13.8% MUFA, 17.1% SFA). 9h of test. | MUFA > SFA | | Higher in CM in the early post-prandial phase in MUFA rich diet | | | No differences in plasma |
| Rivellese et al. [68] | 11 T2D patients | 3 weeks of diet rich in MUFA (23%, SFA 8%) or in SFA (17%, MUFA 15%). 6h of test. | No differences | | | | | ⇑⇑ in adipose tissue LPL after MUFA diet. |
| Zampelas et al. [69] | 30 Northern and 30 Southern healthy male Europeans | Comparison of Northern (17% SFA, 12% MUFA) and Southern (5.4% SFA, 24.1% MUFA) diet. 9h of test. | Sourthern diet > Northern diet. Faster return to near-fasting concentration in Southern Europeans. | | | | | |
| Higashi et al. [72] | 8 healthy men | 40g of fat. Acute test meals with olive oil (70.7% oleic acid of total FFA), milk fat (69.3% of SFA). | Higher after olive oil intake | Higher after MUFA intake | | | | |
| Jackson et al. [74] | 10 healthy postmenopausal women | 40g of fat. Test meals with palm oil (SFA) or olive oil (MUFA). 8h of test. | No differences | | ⇑⇑ after olive oil rich meal | | No differences in Sf 60-400 and Sf 20-60 fractions | |
| Jackson et al. [75] | 9 healthy postmenopausal women | 40g of fat. 4 test meals with palm oil (SFA) or olive oil (MUFA). 8h of test. | No differences | | ⇑⇑ in Sf > 400 fraction after olive oil rich meal | | No differences in SF 60-400 and Sf 20-60 fractions | |
| Silva et al. [76] | 12 healthy men and 13 healthy women | 16 weeks of a moderate MUFA (13% SFA, 15% MUFA) or high MUFA (10% SFA, 18% MUFA) diet. 8h of test. | No differences | | Moderate and high ⇓⇓ in moderate and high MUFA diet, respectively. | | | |
| Bysted et al. [100] | 16 healthy men | 5 acutre test meals: 51% of fat: palmitate, stearate, trans-18:1 isomers, oleate or linoleate. 8h of test. | | Similar FA composition than the diet | | No differences | Different FA composition than the diet. | |
| Gill et al. [104] | 17 men and 18 women with a moderate hypercholesterolemia | 6 weeks of diet with low (7.8%), moderate (10.3%) or high (13.7%) MUFA content. 12h of test. | | | | | No differences in Sf 60-400 and Sf 20-60 fractions | |

Mono-unsaturated fatty acid (MUFA); saturated fatty acid (SFA); chylomicrons (CM), very low density lipoprotein (VLDL), lipoprotein lipase (LPL)

The difference in postprandial triglyceridemia observed between chronic diets enriched in MUFA and SFA may be due to different rates of either the synthesis and secretion of the chylomicrons (CM) from the enterocytes to the circulation or the clearance of TAG-rich lipoproteins from the circulation.

## 5.3. Dietary fatty acids and chylomicrons secretion and synthesis

In the enterocyte, dietary FFA are used as substrates for esterification of lysophosphatidic acid or monoacylglycerol, leading to formation of diacylglycerol (DAG) and TAG. Next, mediated by a specific lipid transfer complex, the microsomal TAG transfer protein (MTP) located in the endoplasmic reticulum lumen, TAG are packaged with apolipoprotein B 48 (apoB48) to form CM, which then enter the circulation via the lymph [70].

Early studies investigating intestinal lipoprotein secretion in animal models involved the use of duodenal infusions of FA, followed by collection of lymph lipoproteins. These studies that investigated only SFA and PUFA, showed that infusion of palmitic acid (16:0) stimulated the output of very low density lipoproteins (VLDL) and CM-sized lipoproteins, whereas linoleic acid (18:2) led to the formation of larger CM-sized particles [71]. In humans (Table 2), more recent studies that measured CM-specific markers in response to acute meals varying in SFA and MUFA compositions, provide strong evidence that different FA have direct effects on the formation of CM. In response to a meal with olive oil (MUFA) as source of fat, CM concentrations were higher than after a meal with butter (SFA) as the fat source [60]; however, only an olive oil rich meal produced an increase in apoB48 concentration. These data are consistent with earlier reports of higher postprandial lipemia and exaggerated retinyl esters and apoB48 responses in CM fraction in response to olive oil compared to SFA rich meals [72-75]. The increase in apoB48 in CM-TAG fraction after a MUFA rich meal has been reported in the early phase of postprandial curve [57]. However, the increase in CM-TAG fraction was lower than that of apoB48, which indicate the formation of a larger number of smaller CM when test meals rich in MUFA rather than in SFA are fed.

Chronic effects of background diets varying in FA were also reported on CM synthesis and secretion, as well as on the size and composition of the secreted particles. Conversely to acute MUFA rich meal, high MUFA content in the background habitual diet results in an attenuated apoB48 response, with no difference in CM-TAG response, indicating formation of fewer CM but larger particle size [76]. These data are in accordance with the study of Zampelas et al. [69] comparing post-prandial responses of southern and northern Europeans acclimated to diet rich in either MUFA or SFA, respectively. In Southern European subjects, a larger increase in TAG, but not in apoB48, concentrations was observed 2h after the ingestion of a standard test meal suggesting earlier entry of larger CM particles.

These data from human studies indicate that the mechanisms responsible for the altered CM size and composition in response to a single high MUFA meal differ from those resulting from chronic changes in diet. The differential underlying mechanisms are well reviewed by Williams et al. [77]. Most of the data come from in vitro studies using Caco-2 cells i.e. human colon carcinoma cells that spontaneously differentiate into polarised enterocytes on microporous membranes. In Caco-2 cells, as in single meal studies in humans, unsaturated fatty acids appear to produce a higher number of CM (oleic acid) than do SFA [78]. This is

consistent with the observation that oleic acid is preferentially esterified into TAG in the enterocyte [79] and the most potent stimulator for secretion of newly synthesized TAG-rich lipoproteins compared to others unsaturated or saturated FA [80]. Van Greevenbroek et al. [81, 82] later suggested that the stimulation of CM secretion by oleic acid compared to SFA may be explained by a preferential translocation of unsaturated TAG into lipoproteins by MTP and/or by a greater activation of MTP by oleic acid-rich TAG than by palmitic acid. Caco-2 cells cultured with oleic acid also produce lipoproteins containing greater amounts of TAG relative to apoB than those cultured with palmitic acid [82], indicating again the ability of MUFA to synthesize larger CM particles, containing higher TAG and lower phospholipids per particle than SFA [82].

As above reported, adherence to Mediterranean diet not only results in higher peak lipemia, but also a faster decrease in TAG concentration. Similarly, postprandial TAG concentrations return faster to near-fasting level after an acute high MUFA test meals (43% of total energy) than after high SFA test meals [59] or in healthy adults with a dietary background MUFA consumption rather than with SFA consumption [69], suggesting a greater clearance of unsaturated TAG from circulation. Consistent with the MUFA-induced larger-sized CM observation, Karpe et al. [83] showed that larger CM are cleared faster from the plasma compartment than small VLDL-sized intestinal lipoproteins. In this study, the postprandial dietary FA oxidation was measured through the recovery of $^{13}C$ label on breath as $CO_2$ after ingestion of $^{13}C$ labelled palmitic acid. The dietary fat oxidation was higher at 3h and 4h postdose on the MUFA than on the SFA diet and supports the possibility of faster rates of $^{13}C$ labelled palmitate oxidation from the hydrolyzed larger CM.

Altogether these results indicate that the greater postprandial TAG response during chronic intake of MUFA-rich diets, compared to SFA-rich diet, is due to the production of a greater number of large-sized CM, and these are cleared faster from plasma. These differential clearances may be due to differences in trafficking at the whole organism level.

# 6. DIFFERENTIAL TRAFFICKING OF DIETARY FATTY ACIDS

## 6.1. Dietary fatty acids trafficking at the whole organism level

Once digested, assimilated and incorporated into CM-TAG lipoproteins, dietary FA are channelled towards the peripheral tissues and partitioned between storage and oxidation. To our knowledge, only one study directly studied the trafficking of different dietary FA into tissues. Bessesen et al. [39] examined the effects of degree of saturation on the postprandial movement of dietary tracer stearic (18:0) and oleic (18:1) acids between tissues in rats. The postprandial oxidation of the saturated stearic acid was found to be considerably less than that of oleic acid due to a retention within the liver and skeletal pools. The retention of saturated fat in liver 24h after tracer administration compared with mono-unsaturated fats was

earlier observed by Leyton et al. [35]. On the other hand, they observed at 8- and 24-h after the ingestion of the tracers a greater adipose tissue tracer content in the oleate fed group. Such differential trafficking was, however, not observed in humans, at least at the subcutaneous adipose tissue and skeletal muscle levels. Using stable isotopic labelling in conjunction with arteriovenous difference measurements in 8 healthy subjects, Evans et al. [84] found that [1-$^{13}$C]palmitic acid and [1-$^{13}$C]oleic acid ingested in equal amounts present a similar rate of absorption, trafficking and uptake by adipose tissue and skeletal muscle. The trafficking of MUFA and SFA is summarised in Figure 2.

Thus animal and human studies do not allow drawing clear conclusions about a plasma trafficking that is different between SFA and MUFA. In the fed state, the lipoprotein lipase (LPL) activity represents the first step in controlling dietary FA trafficking. From this step, is indeed dependent, dietary NEFA spillover, VLDL production rate and tissue uptake.

## 6.2. Dietary fatty acids and lipoprotein lipase activity

As CM-TAG enter the circulation, they interact with LPL, which are located on the luminal surface of capillary endothelial cells, resulting in hydrolysis of TAG to FFA and monoacylglycerol. LPL is essentially up-regulated in the early phase of the postprandial period (2-4h) in response of the increased postprandial lipemia [59, 85, 86] and in particular in adipose tissue [87], indicating a fatty acid feedback control of LPL.

The higher clearance of CM after chronic MUFA ingestion may be explained by differences in LPL activity. It has been reported that the consumption of single meals high SFA stearic (18:0) and palmitic (16:0) acid induce a smaller postprandial increase in plasma heparin releasable LPL activity (analyses performed after suppression of hepatic LPL) than did the consumption of high oleic acid (18:1) meals [59] (Table 2). Rivellese et al. [68] also clearly showed in diabetic subjects an increase in both adipose tissue LPL mRNA and activity after 3 weeks of MUFA diet. Previous studies in animals [88] or in vitro [89] also reported that the degree of saturation and chain length of FA affects plasma heparin LPL activity with a greater hydrolysis of short-chain unsaturated monoacylglycerol and TAG.

Roche et al. [67], on their part, did not report changes in post-heparin LPL after 8 weeks of a Mediterranean-type diet (MUFA: 18% and SFA: 12% of total energy) or after a typical current diet from northern Europe (MUFA: 14% and SFA 17% of total energy) in 23 healthy men. These controversial results may be explained by the small differences in the dietary fatty acid proportions between these two diets. Nevertheless, Summers et al. [90] confirmed those results by showing that FA differences in the rate of extraction from CM-TAG disappeared when expressed in relation to their proportions in CM-TAG.

These results indicate that the total plasma TAG concentration [91] and the size of the CM particle resulting from the type of dietary FA ingested seems to be the primary factors affecting LPL activity, and consequently, the clearance of the TAG-rich lipoproteins. Indeed, although the unsaturated fatty acids TAG may stimulate the LPL activity, LPL does not seem to present preferential affinity towards unsaturated or saturated TAG. Nevertheless it is important to note that apart from a few *in vitro* studies of milk LPL, which showed a greater hydrolysis of unsaturated TAG [92] and no differences with PUFA-rich TAG [93], we did not

find studies focussing on tissue-specific activity of LPL (muscle, adipose tissue or liver) and MUFA. Clearly, such data are needed to better understand the trafficking of the dietary FA with different chemical structures towards storage or oxidation.

## 6.3. Post-prandial non-esterified fatty acid release

A significant proportion of the hydrolysed postprandial TAG-rich lipoprotein fatty acids are not taken up into peripheral tissues but "spilled over" to the circulation as non-esterified fatty acids (NEFA) bound to albumin [94]. Differences in affinities for oleate and palmitate have been reported in three binding sites of bovine serum albumin [95]. Despite low differences in the MUFA proportions in their three acute test meals (12, 17 and 24% of energy) only, Roche et al. [57] observed a lower incremental area under the curve of postprandial plasma NEFA concentrations following high MUFA meals (Table 2) suggesting either a lower spillover from the LPL hydrolyzed TAG-rich lipoproteins, a higher inhibition of hormone sensitive lipase (HSL), and/or a higher NEFA uptake by peripheral tissues. There is some circumstantial evidence that SFAs are more likely to be released into the plasma after the action of LPL [96, 97], although this was not confirmed *in vivo* in adipose tissue [98, 99]. Other studies investigating the effect of dietary FA composition in meal on post-prandial lipemia did not observe changes in plasma NEFA concentration after an acute high MUFA or SFA meal [61, 100] or after a long-term dietary intervention [68].

Although these results are controversial likely because of the differences in the experimental conditions, the spillover of NEFA SFA to the circulation from the LPL hydrolyzed TAG-rich lipoproteins appears to be higher than that of MUFA.

## 6.4. VLDL-TG synthesis

In the fed states, the liver helps to distribute lipid by esterification of NEFA into VLDL-TG [101, 102] for export into the circulation and ultimate storage of FA in the adipose TAG pool or oxidation in skeletal muscle. The packaging of the dietary NEFA released into circulation by LPL from CM into VLDL has been shown an early event of the postprandial state [103] as such VLDL formation plays a key role in dietary FA trafficking.

Comparing the TAG fractions, apoB48 and retinyl esters responses to test meals containing predominantly palm oil (SFA), or olive oil (MUFA), Jackson et al. [74, 75] did not report significant differences in the CM or large VLDL (Sf 60-400) nor in the VLDL and CM remnants (Sf 20-60) (Table 2). Similarly, after high palmitic or oleic acid test meals (43 weigth% of the test meal for each FA), Bysted et al. [100] showed that while the FA composition of the meal was reflected in the CM fractions, it was diminished in VLDL-TG, suggesting a dilution effect by the endogenous hepatic FA. Gill et al. [104] replaced dietary SFA with moderate or high amounts of MUFA (10.3% vs. 13.7%) for 6 weeks and did not observe significant differences in plasma small (Sf 20-60) and large size VLDL (Sf 60-400) concentrations nor VLDL production or catabolic rates. Only a decrease in the concentration of the LDL density class after the high-MUFA diet was observed. However, Aarsland and Wolfe [105] reported that free oleic acid was the predominant source of VLDL-TAG synthesis in the liver compared to other FA. This may suggest a discrimination in hepatocyte favoring MUFA incorporation during VLDL-TAG synthesis. Since the microsomal MTP is highly involved in the

assembly of particles to synthesize VLDL-TAG [106], a greater affinity for oleate may explain this discrimation. However, to our knowledge, no *in vitro* studies have investigated the effect of different FA varying in the degree of saturation in the VLDL-TAG biosynthesis in hepatocyte and clear conclusions can not be drawn.

Taken these previous results altogether, MUFA seem more likely to be packaged into CM and to a lesser extent into VLDL than SFA. This is associated with a higher retention of SFA in the liver. Moreover, MUFA are also less likely to be released into the NEFA pool from hydrolysis of TG-rich lipoproteins, thus suggesting a greater uptake by peripheral tissues compared to SFA.

# 7. CELLULAR METABOLISM OF MUFA AND SFA IN ADIPOSE TISSUE AND SKELETAL MUSCLE

## 7.1. Dietary SFA and MUFA transport into myocytes and adipocytes

A proportion of the FA released by LPL hydrolysed TAG-rich lipoproteins and NEFA is taken up by the peripheral tissues. Transport of FA into cells can occur to some extent via diffusion across the lipid bilayer of the plasma membrane, but the predominant routing is protein mediated. Two major classes of FA transported protein have been described: FAT/CD36, an integral membrane glycoprotein found on the surface of a variety of cells, including adipocytes and oxidative muscle fibers [107] and a family of fatty acid transporter proteins (FATP) 1-6 which are homologous to long chain FA-coenzyme A synthetases that catalyze CoA activation of long chain FA to varying degrees [107].

An early study [108] investigating the permeation of the long-chain FA into adipocytes found similar Km values for linoleate, palmitate and stearate, but lower Km for oleate and approximately similar Vmax for these four FA. As an increase in affinity for the transporters would result in a decrease in the Km for FA transport with no change in Vmax, this study indirectly suggests a greater transport of oleate into adipocytes compared to others FA.

In cultured human muscle cells [109], the uptake of palmitate and oleate were similar when the cells were incubated separately with the two FA. When, Luiken [110] and Bonen [111] studied uptake of palmitate in isolated giant sarcolemmal vesicles from rat heart and hindlimb muscles, they reported a reduced radioactive-labelled palmitate uptake (-65%) in the presence of excess oleate in muscle vesicles and heart, indicating that FAT at muscle level present a degree of discrimination between unsaturated and saturated FA. With the exception of competition between oleate and palmitate for uptake by isolated hepatocytes and perfused rat liver [112], few studies have directly tested whether FAT/CD36 and FATP have a differential affinity for FA with regard to degree of saturation by performing an incubation of adipocyte or myocyte with different FA being present in similar proportions.

Inside the cell, cytosolic FABP buffer intracellular FA and play an important role in shuttling FA between the plasma membrane and intracellular membranes or

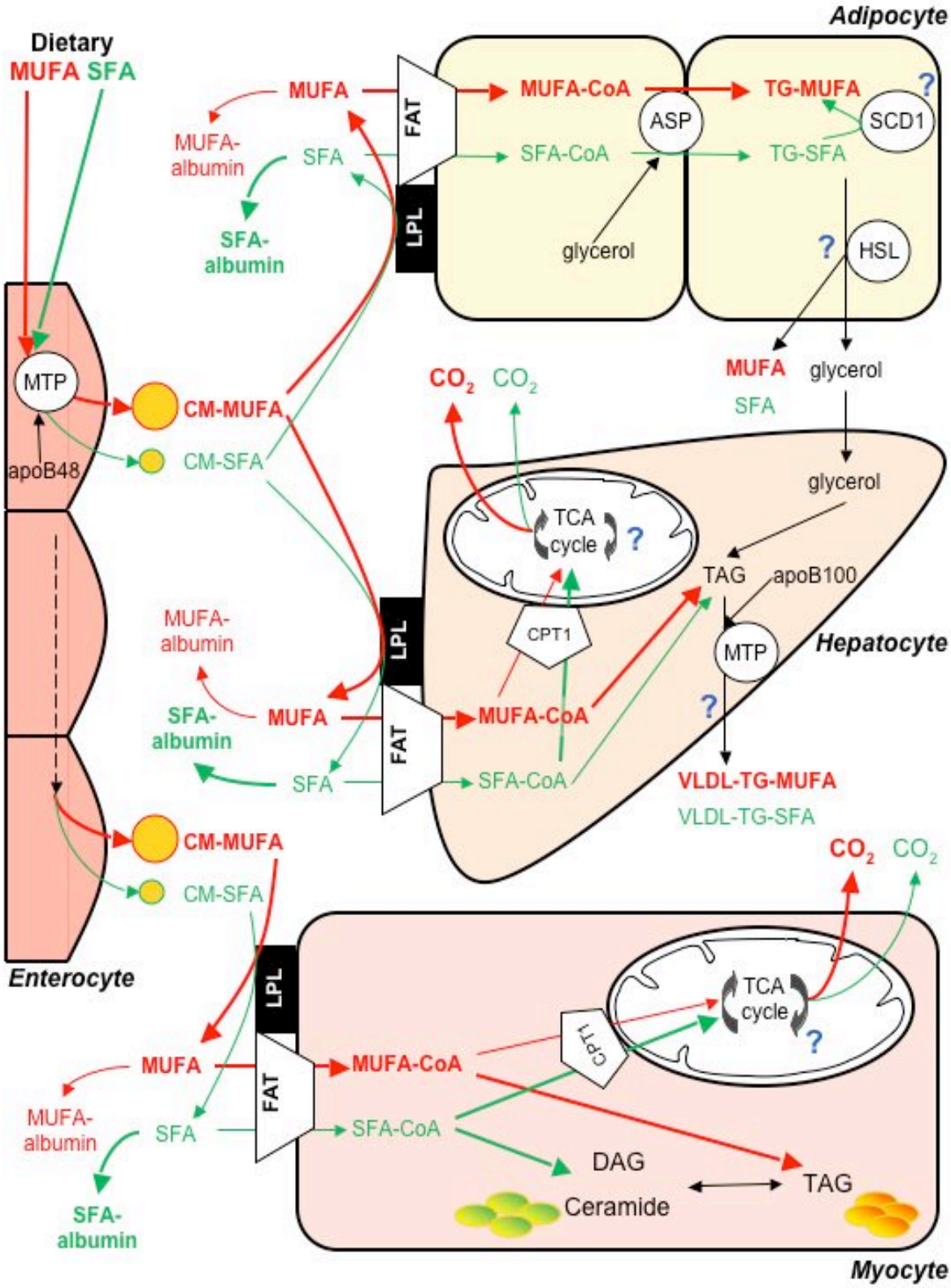
Adipocyte
Dietary
MUFA SFA
MUFA
MUFA-albumin
SFA
SFA-albumin
FAT
LPL
MUFA-CoA
SFA-CoA
ASP
TG-MUFA
TG-SFA
SCD1
glycerol
HSL
MUFA
glycerol
SFA
MTP
apoB48
CM-MUFA
CM-SFA
CO2
CO2
TCA cycle
glycerol
TAG
apoB100
CPT1
MTP
Hepatocyte
VLDL-TG-MUFA
VLDL-TG-SFA
Enterocyte
Myocyte
DAG
Ceramide
TAG

**Figure 2:** Dietary saturated (SFA) and mono-unsaturated (MUFA) fatty acids trafficking based on in vivo studies. The green and red arrows correspond to the MUFA and SFA channelling, respectively. After a similar digestion and assimilation, MUFA are greater packaged in chylomicrons (CM) of bigger size than SFA likely due to a differential affinity of the intestinal microsomal triacylglyceride transfer protein (MTP) towards fatty acids varying in the degree of saturation. Then, the large CM are better hydrolyzed by lipoprotein lipase (LPL) than the smaller CM. MUFA is more uptaked by the peripheral tissues via a greater affinity of the fatty acid transporters (FAT) for MUFA than SFA and in turn, SFA are spilled over in NEFA pool in higher proportions. Although MUFA may be released in priority, MUFA is the main fatty acid component of the adipose tissue either because of its greater entrance in adipocytes or because of a putative desaturation process of SFA by the steraoyl desaturase (SCD1). In the hepatocyte and myocyte, the carnitine palmitoyl transferase 1 (CPT1) presents a greater affinity for SFA than MUFA but MUFA are greater oxidised than SFA. This may be explained by a mass effect of MUFA on CPT1 and/or by a greater incorporation of SFA in complex lipids (ceramides and DAG) than SFA. This results in a higher retention of SFA in liver and in a possible greater synthesis of MUFA-rich very low density lipoproteins (VLDL) due to a better affinity of the liver MTP for MUFA than SFA. In the myocyte, MUFA are preferentially incorporated in triacylglycerol (TAG).

metabolic compartments [113]. FABP exhibit a greater degree of affinity for unsaturated than saturated FA [55]. However, studies, which have measured fasting fractional uptake of the FA by human heart [114], liver [115], forearm [115] or perfused rat liver [116] have failed to demonstrate any difference between palmitate and oleate in the fasting state. We can assume that results would be different in post-prandial state because of the regulation of FAT/CD36 by insulin [117] or because of a putative dietary FA feedback control on FAT activity.

Although only indirect data exist, it is possible that MUFA uptake by both adipocytes and myocytes is greater than that of SFA, which parallels the differential release of these two types of fatty acids into the NEFA pool after the hydrolysis by LPL of the TAG-rich lipoproteins (Figure 2). In the adipocytes or myocytes, MUFA and SFA also have different metabolic fate leading to different metabolic consequences.

## 7.2. SFA and MUFA metabolic fates in adipose tissue

In humans that are physically fit and near their ideal body weight, the primary fate of fatty-acyl-CoAs in adipose tissue is re-esterification to TAG. Adipose tissue has a characteristic profile in which the proportion of MUFA is greater than that in the diet [118-121]. For example, Garland et al. [121] showed that MUFA represented 56.0% of adipose tissue FA whereas, based on food questionnaires and 2 weeks of diet record for 140 by US women, MUFA intake was 39.6% of fat intake. This suggests a greater uptake or a lower release of MUFA by adipose tissue. As no clear difference in uptake was noted, the previous metabolic steps such as incorporation into CM-TAG and preferential hydrolysis of large CM by LPL may play a more important role than FA transport in the FA composition of the adipose tissue.

The greater MUFA proportion in adipose tissue may be the result of a differential mobilisation of FA varying in degree of saturation. There are well-defined preferences for particular FA in the process of mobilization from white adipose tissue in rats [122, 123] and rabbits [124]. *In vitro* studies, with rat adipocytes under conditions of stimulated lipolysis, show that for a given FA chain length, relative mobilization (calculated as the percentage of a particular FA of total NEFA released from the cells divided by the percentage of that FA in adipocyte TG) increases exponentially with increases in unsaturation degree [122]. However, within a given class of FA saturation, relative mobilization decreases as chain length increases [122]. The relative differences in relative mobilization were also shown in humans after an overnight fast [125]. However, these results would favour a greater proportion of SFA or PUFA in adipose tissue, which is contrary to the characteristic MUFA pattern of the adipose tissue. One possible explanation is that selective mobilization of FA from adipocytes was shown mainly *in vitro* under conditions of simulated lipolysis or *in vivo* in animals and humans after a fasting period. As humans spend most of the time in a postprandial state, such interprandial remodeling may not occur. Nevertheless, further studies are needed to reconcile these observations.

An alternative explanation may be a remodelling of adipose tissue composition during interprandial periods. Garland et al. [121] showed in 140 US women that an average fat intake composed of 39.6% of MUFA, 38.9% of SFA and 21.5% of PUFA resulted in the following average adipose tissue composition: 56.0% of MUFA, 21.6% of SFA and 21.7% of PUFA. Interestingly, adipose tissue oleic acid showed a greater correlation ($r$ = 0.25 vs. $r$ = 0.05, respectively) with SFA than MUFA intake [126]. These results suggest a desaturation of SFA in

adipose tissue leading to a greater proportion of MUFA and a lower proportion of SFA. However, we did not find relevant data concerning the role of desaturase in adipose tissue, such as desaturase steraoyl CoA 1, which has been extensively studied in liver and muscle (see below), to support this hypothesis.

FA seem also to exert depot-specific effects on lipid accumulation. After having differentiated subcutaneous and visceral pre-adipocytes from fat biopsies from pre-pubertal children, Sabin et al. [127] showed that palmitate consistently led to significant increases in lipid accumulation in visceral but not in subcutaneous adipocytes. Exposure to oleate, however, had the reverse effect by significantly increasing lipid accrual in subcutaneous only. A greater distribution of unsaturated and saturated FA in subcutaneous and visceral adipose tissues, respectively, have been confirmed in obese adults [128]. Moreover, studying the depot-specific differences in subcutaneous, perivisceral and omental adipose tissue in overweight and obese adults, Garaulet et al [129] reported that n-6 and n-3 PUFA content are related to a reduced adipocyte size while adipose tissue and dietary SFA significantly correlated with an increase in fat cell size and number. No data were found for dietary MUFA. These results are of particular interest considering that visceral adiposity is associated with the development of numerous metabolic complications including type 2 diabetes and cardiovascular diseases [130].

MUFA is the preponderant FA in adipose tissue. This may be explained by a greater uptake by adipocytes of MUFA compared to SFA and by a putative desaturation process of dietary SFA in adipocyte, rather than by a preferential release of SFA than MUFA, in particular in individuals who spent most of time in post-prandial conditions (Figure 2). Interestingly, the SFA-rich adipose tissue are related to metabolic disorders, suggesting that the process favouring the MUFA accumulation in adipose tissue may be considered adaptive response to alleviate the SFA storage related deleterious effects.

## 7.3. SFA and MUFA metabolic fates in muscle

### 7.3.1. Differential oxidation and incorporation into complex lipids

Upon entering mycocyte, FA are activated to acyl-CoA by the acyl-CoA synthetases and the resulting long-chain acyl-CoA enters numerous pathways, i.e., oxidation or incorporation into complex lipids as signalling molecules (Figure 3) and structural components of the membrane. The major fate of FA in muscles is oxidation to provide ATP for muscle contraction.

As Delany et al. [41] reported a greater oxidation of dietary short and unsaturated chain FA than of dietary long and saturated FA at the whole organism level in healthy subjects, we hypothesize that unsaturated FA are more channelled towards mitochondria for oxidation than SFA, which in turn would be preferentially stored in myocyte. Some indirect evidence, however, surprisingly suggests that palmitate oxidation would be favoured compared to oleate oxidation in myocyte. For example, it was found in rats that CPT1, the key protein responsible for the entrance of long-chain fatty acyl-CoA into mitochondria, has a lower affinity for oleyl-CoA than palmitoyl-CoA [131] and that enzyme activity towards oleyl-CoA was more sensitive to inhibition by malonyl-CoA than was activity towards palmitoyl-CoA [132]. In a recent study, Koves et al. [133] also found that an incubation of rat L6 myotubes with both oleate and palmitate at similar concentration (0.5mmol/l each) induces a 3-fold greater palmitoylcarnitine

content than oleylcarnitine, confirming that palmitate is greater uptake by mitochondria than oleate (Figure 3). Although the absolute values for oleate oxidation are not directly reported by Gaster et al.'s [134], the figures suggest palmitate oxidation was greater than oleate oxidation (9.5 ± 1.3 vs. approximately 7 nmol/mg protein, respectively) in myotubes from healthy donors cultured separately with 0.6mmol/l of [1-$^{14}$C]oleic acid or [1-$^{14}$C] palmitic acid. In contrast, they reported a reduced palmitate oxidation and no change in oleate oxidation in cultured myotubes from diabetic subjects compared to those from control subjects. Further studies are clearly required to better determine the differential oxidation of FA varying in degree of saturation in myocytes.

While the oxidation of the different FA in muscle cell has been poorly investigated, a growing body of data suggest that the incorporation of unsaturated and saturated FA into complex lipids markedly differ in cultured muscle cells. Using $^{14}$C radioactive isotope labelling, Gaster et al. [134] observed a greater radioactivity in DAG and TAG when muscle cells were exposed to palmitate and a higher radioactivity in FFA after incubation with oleate in cultured myotubes from both control and diabetic subjects. After a 20-hour incubation of muscle cells with $^{14}$C labelled palmitate (16:0), stearate (18:0) and oleate (18:1), Montell et al. [135] also found a preferential incorporation of SFA into DAG. But contrary to Gaster et al. [134], they observed a lower incorporation of SFA in TAG whereas unsaturated FA accumulate in TAG and in very minor proportions in DAG. This differential incorporation of the fatty acids leads to alterations of the different lipid pool sizes in muscle cells. In human C2C12 myotubes, an incubation of several hours with excess palmitate increased MAG [136], DAG [136-138] and ceramides [138] content. On the contrary, it has been reported that excess oleate incubation did not alter DAG and ceramides content but increased TAG accumulation [138].

Taken together these results suggest that TAG biosynthesis, in cultured conditions, is much lower in cells exposed to saturated compared to unsaturated FA and consequently palmitate has a low rate of incorporation into TAG, which in turn leads to abnormal accumulation of palmitoyl-CoA, DAG, and/or ceramide. The greater accumulation of unsaturated FA in TAG may be explained by the apparently 50% lower affinity of DGAT2, enzyme that controls the rate of TAG synthesis from DAG, for SFA compared to unsaturated FA [139, 140]. On the other hand, it has been reported that the rates of ceramide synthesis depend largely on the availability of long-chain saturated fat, which together with serine are the rate limiting substrates in the *de novo* ceramide synthesis by serine palmitoyltransferase (SPT) [141]. SPT is specific for SFA. In contrast, the second FFA incorporated in ceramides originates from either saturated or unsaturated FFA [141]. Figure 3 summarised the differential oxidation and incorporation into complex lipids of palmitate and oleate when myocytes are incubated separately with these two FA.

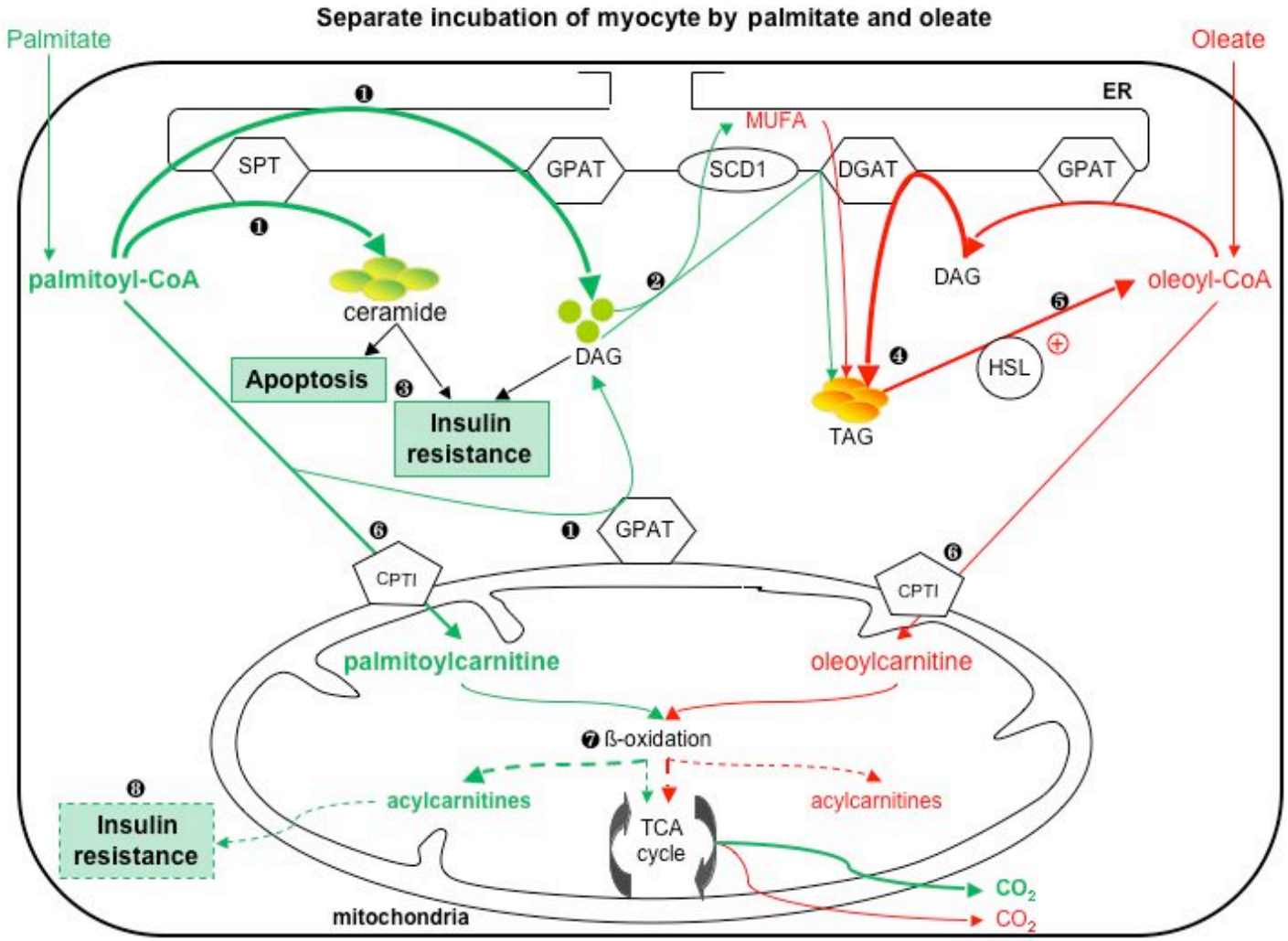


**Figure 3:** Differential oxidation and incorporation in complex lipids in myocyte incubated separately with either excess palmitate or oleate. The green and red arrows correspond to the oleate and palmitate channelling, respectively. The solid arrows represent results from relevant *in vitro* studies whereas dotted arrows are only hypothetical interpretation. ❶ Palmitate is preferentially incorporated in ceramide and diacyl glycerol (DAG) because of the high affinity of the serine palmitoyl transferase (SPT) and the mitochondrial glycerol-3-phosphate acytransferase (GPAT) and the lower affinity of the diacylglycerol transferase (DGAT) for saturated fatty acids. ❷ A small proportion of palmitate is incorporated in triacylglycerol (TAG) pool as mono-unsaturated fatty acyl-CoA (MUFA) after a previous desaturation process via the steraoyl desaturase (SCD1), which is close to the DGAT on the endoplasmic reticulum (ER) membrane. ❸ However, palmitate is mostly incorporated in ceramide and DAG, which triggers the development of an insulin resistance and apoptosis. ❹ On the other hand, oleate preferentially accumulates as triacylglycerol (TAG) due to the high affinity of DGAT for unsaturated fatty acids. However, in spite of the increased TAG content, ❺ TAG turnover may be high since oleate activates the gene expression of the hormone sensitive lipase (HSL). Nevertheless, ❻ because of the higher affinity of the carnitine palmitoyl transferase 1 (CPT1), the transport of palmitate into mitochondria is greater than of oleate, which results in a greater oxidation of palmitate than oleate in *in vitro* conditions. However, we assume that a greater proportion of palmitate are incompletely oxidised ❼ inducing accumulation of acylcarnitines, which have been recently proposed to alter the insulin signals ❽.

### 7.3.2. The associated insulin responsiveness and lipotoxicity

In addition to the differential incorporation of MUFA and SFA into complex lipids, it has been reported that palmitate and oleate have opposite effects on insulin signalling and cell viability (Figure 3). While incubation of human myotubes with excess palmitate increased intermediary lipid pool sizes [136-138] and triggered an insulin resistance of glucose metabolism [136, 138], excess oleate incubation did not alter the insulin responsiveness in spite of the greater TAG content. In rat L6 myotubes, palmitate exposures increase *de novo* ceramide synthesis, capspase 3 activation and apoptosis [142]. To our knowledge, only one study has been conducted so far *in vivo* in rats [143]. After 8 weeks of a chow control diet, a high SFA or a high PUFA diet was provided, the insulin sensitivity was assessed at the whole organism by an hyperinsulemic euglycemic clamp and then, animals were sacrificed to determine the muscle lipid content. High SFA and PUFA diets caused a resistance to the effects of insulin and an increase in insulin responsiveness, respectively, due to opposite accumulation into DAG and TAG pools. Ceramide content was unaffected by either high fat diets, which suggests that ceramides may not induce insulin resistance *in vivo*. No similar *in vivo* studies addressed the question of the effects of high MUFA vs. SFA diet on muscle lipid content and its related insulin responsiveness.

Taken together, the different effects of palmitate and oleate on insulin sensitivity and cell viability are likely related to their ability to promote ceramide and DAG accumulation. Although the respective role of these two intermediary lipids in insulin responsiveness alteration is still in debate, they are thought to engage stress-activated serine kinases that interfere with insulin signal transduction [144, 145]. DAG is a potent allosteric activator of both conventional and novel PKC isoforms. In skeletal muscle cells, PKCΘ is the most abundant PKC isoform [146-148]. PKCΘ phosphorylates IRS1 [149], the main mediator of insulin response in muscle [150], and decreases IRS1 associated PI3 kinase activity [145], leading to impaired insulin signalling. In addition, PKCΘ has the unique ability among the PKC isoforms to activate pro-inflammatory nuclear factor NFkB [146], which has been linked to FA-induced impairment of insulin action in skeletal muscle in rodents [151, 152]. Ceramides, on their part, may mediate their antagonistic effect on insulin signalling via inhibition of the phosphorylation of Akt [153, 154], independently of changes in the ability of insulin to stimulate IRS1 phosphorylation or PI3 kinase activation. Increased ceramide is also associated with processes such as oxidative stress, inflammation and apoptosis [153]. However, as it is well explained in Summers' review [153], a precise understanding of the molecular mechanism by which ceramides regulate insulin action and impact cell viability is still elusive. Lastly, Koves et al. [133] recently provided new results concerning additional factors that could be involved in muscle insulin resistance. In a context of a high-fat feeding, they showed that, while beta-oxidation was not altered, the TCA cycle was impaired, which results in an incomplete beta-oxidation and consequently, in the accumulation of acyl-carnitines in mitochondria and myocyte. Acylcarnitines are thought, via their role in the protein acetylation/acylation processes, to activate serine kinase that act on IRS1 in association with the reactive oxidation species. In the light of the relationship between palmitate and muscle insulin resistance, we propose that future research may ascertain whether palmitate is less completely oxidised than oleate and thus, form more acylcarnitines.

We previously reported that an excess oleate incubation induced high TAG content but no alteration in insulin responsiveness. This is in accordance with the paradoxical high intra-muscular TAG (IMTG) content observed by Goodpaster et al. [155] in both insulin resistant obese individuals and insulin sensitive endurance-trained subjects, suggesting that IMTG pool content *per se*

does not cause insulin resistance. Rather, they suggested that a low IMTG turnover (i.e. rate between lipolysis and lipid synthesis) may be the primary factor enhancing the accumulation of lipid intermediaries and thus, the key factor that affects the insulin responsiveness. Interestingly, using the rate of glycerol release as an indirect index of IMTG turnover, Koves et al. [133] showed that rat L6 myotubes exposed to 500μM of palmitate had a lower content of TAG and a lower turnover accompanied by increased cell death and insulin resistance compared to the cells exposed to a similar concentration of oleate. Future investigations applying the pulse [$^{14}$C]palmitate-chase [$^{3}$H]palmitate approach developed by Guo et al. [156] for both palmitate and oleate would be required to directly determine the influence of MUFA and SFA on IMTG turnover and the associated accumulation of lipid intermediates and insulin responsiveness.

In regard of all these results, the development of the insulin resistance in myocyte is likely the consequence of a low rate of incorporation of SFA into TAG associated with a low IMTG turnover, leading to a high accumulation of palmitoyl-CoA, DAG and ceramides, which in turn activates PKC and/or other kinases that serine phosphorylate IRS1 and results in insulin resistance. This significant difference in SFA and MUFA contribution to insulin-resistance observed in cultured cells is consistent with the results from observational epidemiological studies. Studies in rodents and numerous dietary epidemiological studies in human populations indicated that SFA markedly decrease insulin responsiveness in peripheral tissues [32, 157-159], whereas unsaturated fats and in particular MUFA oleic acid improves insulin sensitivity [160-162].

### 7.3.3. The protective effect of monounsaturated fatty acid

In a context of a rising prevalence of obesity and Type 2 diabetes worldwide, the improvement of insulin sensitivity associated to the Mediterranean diet due to a high intake of MUFA [163] is noteworthy. Recent findings from *in vitro* studies have provided new insights to better understand the mechanisms related to the MUFA healthy effects.

The presence of oleate has been reported to partially or fully rescue palmitate-induced cell apoptosis, which seems mediated via a low palmitate incorporation into DAG [133] and/or into ceramides [164], through an enhanced utilization of palmitate for beta-oxidation [133] and/or for TAG biosynthesis [133, 164] (Figure 4). Pickersgill et al. [138] have shown in human muscle cells that oleate co-incubation also completely prevented the insulin resistance induced by palmitate through a sequestration of palmitate into TAG pool, thus preventing its conversion to ceramide and/or DAG. Furthermore, Coll et al. [165] have recently provided further mechanistic evidences to explain the protective effect of oleate. In skeletal muscle cells, co-incubation of palmitate exposed cells with oleate promoted TAG accumulation and mitochondrial beta-oxidation, thus preventing DAG synthesis and activation of PKC/NFkB pathway, which protects the insulin signalling. The activation of the beta-oxidation in presence of oleate seems to be related to an enhanced expression of CPT1 and PGC1alpha, a transcriptional co-activator promoting oxidative capacity in skeletal muscle. The activated CPT1 and PGC1α expressions are themselves likely under the control of protein kinase A and PPARα. The last is expressed in tissues exhibiting high rates of FA oxidation and serves to suppress genes encoding proteins of lipid synthesis while inducing genes encoding proteins of FA oxidation [166]. PPARα is more stimulated by MUFA than SFA [166, 167] (Figure 4).

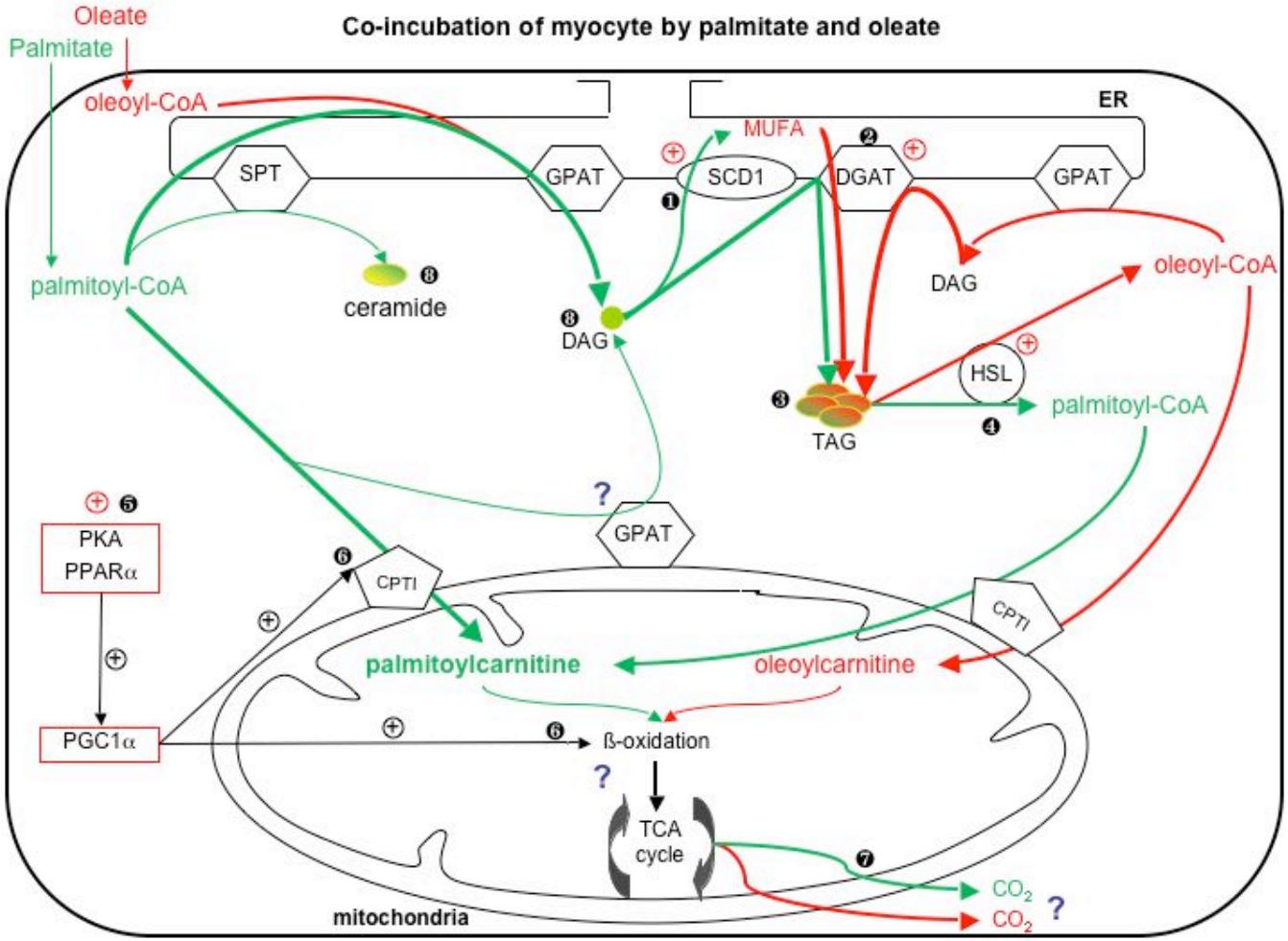


**Figure 4**: Protective effect of oleate against palmitate-induced apoptosis and insulin-resistance in myocyte co-incubated by equimolar concentration of palmitate and oleate. The green and red arrows correspond to the oleate and palmitate channelling, respectively. ❶ Oleate activates the gene expression of the steraoyl desaturase (SCD1) increasing the desaturation process of palmitate in mono-unsaturated fatty acyl-CoA (MUFA). ❷ Oleate also stimulates the gene expression of the diacylglycerol transferase (DGAT). ❸ Thus, MUFA from the desaturated palmitate and in a greater proportion palmitate-rich diacylglycerol (DAG) are highly incorporated into triacylglycerol (TAG) pool. ❹ Oleate also increases the TAG turnover by the activation of the hormone sensitive lipase (HSL). On the other hand, ❺ stimulating protein kinase A (PKA) and PPARα, which activate PGC1α expression, ❻ oleate enhanced expression of both the carnitine palmitoyl transferase 1 (CPT1) and the gene encoding for beta-oxidation. ❼ This oleate-induced transcriptional regulation results in an increase in palmitate oxidation. Finally, oleate, inducing an elevated incorporation of palmitate into TAG, a high TAG turnover and an increased palmitate oxidation, reduces the accumulation of ceramides and DAG pool ❽ and thus, prevents the muscle cell against the palmitate-induced insulin resistance and cell death. However, some points are still obscure when myocyte is co-incubated by oleate and palmitate, such as the mitochondrial glycerol-3-phosphate transferase (GPAT) transcriptional regulation by oleate, the proportion of palmitoyl- and oleoylcarnitines completely and incompletely oxidised in mitochondria and the relative oxidation of oleate vs. palmitate.

In myotubes, Montell et al. [135] observed that the accumulation of DAG was a positive dose-response to the concentration of palmitate. When a mixture of [1-$^{14}$C]palmitate and unlabeled oleate was applied to the muscle cells, FA incorporation in DAG decreased, whereas TAG labelling progressively increased in parallel with oleate concentration. Such observation could be partially explained by the oleate protective effect against the palmitate-induced down-regulation of DGAT2 gene expression [165]. In Chinese Hamster Ovary cells co-supplemented with palmitate and oleate, a similar increase in cellular TAG levels was observed. Importantly, the channelling of palmitate towards the TAG pool correlated with the level of desaturase activity [164], which was proportional to the percentage of unsaturated acyl chains. The steraoyl-desaturase (SCD1) is an endoplasmic reticulum enzyme that is responsible for the critical committed step in the *de novo* synthesis of MUFA [168-171]. The preferred substrates of SCD1 are palmitoyl- and steraoyl-CoA, which are converted into palmitoleoyl- and oleoyl-CoA [172]. These two MUFA are the major fatty acid found in TAG as well as in membrane phospholipids [173]. Interestingly, Man et al. [174], combining the methods of confocal microscopy, coimmunoprecipitation and fluorescence resonance energy transfer, reported that SCD1 and DGAT2 are located very close to each other on the endoplasmic reticulum membrane. Thus, SCD1 may participate in TAG synthesis by providing a more accessible pool of MUFA to DGAT2, which presents a greater affinity towards unsaturated than saturated FA. SCD1 may also promote the palmitate incorporation in TAG pool through this desaturation process (Figure 4).

However, if this process seems acceptable based on the above described experimental evidences, Listenberger et al [164] reported in SCD1-overexpressing Chinese hamster ovary (+SCD) cells that much of the exogenous palmitate is incorporated into TAG without a previous desaturation. Indeed, by supplementing +SCD and Chinese hamster ovary cells with deuterated palmitate for 6h and analyzing cellular TAG composition by mass spectrometry, they observed a greater total amount of C16:0 and C18:1 FA in the TAG pool of +SCD cells compared to that of normal Chinese hamster ovary cells. While about one-forth of the deuterated label in TAG was associated with 16:1 or 18:1 unsaturated acyl chains, the majority of the deuterated label (71%) in the TAG pool of +SCD cells remained associated with palmitate.

Although further studies are warranted to better understand the metabolic fate of SFA and MUFA into myocytes and their impact on insulin responsiveness, these previous studies provide relevant evidence regarding the muscle fat metabolism and the relationship between the fatty acids and the insulin responsiveness. The accumulated evidence indicates the presence of unsaturated FA, from endogenous or exogenous sources, facilitates channelling of exogenous palmitate to TAG stores, lowering the accumulation of cellular DAG and ceramides and enhances the utilization of palmitate for beta-oxidation. These adaptive metabolic processes result in a consequent rescue of palmitate-induced insulin-resistance [165] or apoptosis [164] (Figure 4). This also implies, as it has been previously hypothesized, that the cellular TAG biosynthesis, in which SCD1 plays a critical role, may be protective against lipotoxicity [164] and prevent insulin resistance in muscle [175]. Nevertheless, based on the paradoxical IMTG content observed between the insulin resistant obese and the insulin sensitive athletes, the TAG protective effect seems to be tightly related to the IMTG turnover rate. As such physical exercise represents an additional level of regulation that is likely to interact with the differential fate of dietary SFA and MUFA. Little recent evidence supports this view.

# 8. RELATIONSHIP BETWEEN DIETARY FATTY ACIDS FATE AND PHYSICAL ACTIVITY LEVEL

## 8.1. Effect of the physical activity level on dietary fatty acid oxidation

The initial portion of this review, emphasized the preponderant role of dietary fat in the development of obesity; however, epidemiological and cross-sectional studies also have shown a negative relationship between physical activity level among population and the body mass index or body fat [176-179] as well as improved insulin sensitivity [179]. 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 [180] and women [181]. A large body of data effectively reported that exercise promotes a preferential channelling of lipids towards oxidation associated with a decrease in lipid storage [182] [183]. Evenly, acute or chronic exercise was associated with improvement in insulin responsiveness [184, 185]. Nevertheless, our modern western societies have adopted a sedentary lifestyle [186]. For example in the US, 60% of the population currently does not participate in regular physical activity, and 25% are almost entirely sedentary [187]. More striking is the fact that physical inactivity is classified as the second cause of mortality in US [188] due to its role in the development of chronic diseases such as cardiovascular and coronary diseases, stroke, cancer, obesity or type 2 diabetes. In the obesity context, Smith et al. [180] showed that human capacity to adapt fat oxidation to a high-fat diet was dependent on energy turnover, i.e adaptation of inactive subjects takes longer than active individuals. Later, Shepard et al [189] observed in both men and women with stable body weight that one day of physical inactivity after 14 days of high-fat diet was sufficient to induce a positive fat balance. Interestingly, we showed in both men [190] and women (Bergouignan, unpublished data) that long-term bed rest induced-physical inactivity *per se*, i.e. independently of changes in energy balance, decreases fasting and post-prandial total lipid oxidation and triggers a development of insulin resistance. Therefore, while physical activity increases fat oxidation, physical inactivity reduces it. However, this positive relationship between fat oxidation and physical activity level seems to be subtler and dependent on the biochemical properties of the FA. Using a stable isotope double labelling method, Votruba et al. [191] reported that prior light, moderate or heavy exercise significantly increases the dietary [1-$^{13}$C]oleate oxidation but not dietary [$d_{31}$]palmitate oxidation. On the contrary, extreme physical inactivity induced by long-term strict bed rest does not alter the dietary [1-$^{13}$C]oleate oxidation, but decreases dietary [$d_{31}$]palmitate oxidation by 11% and 8% in healthy men [190] and women (Bergouignan personal data), respectively. Moreover, the reduced dietary palmitate oxidation correlated with the physical inactivity-induced increase in muscle fat content as measured by MRI. As we did not report an alteration of MUFA and SFA trafficking and uptake under physical inactivity conditions, these results indicate that physical inactivity-induced reduction in palmitate oxidation would be explained, at least in part, by a preferential channelling of palmitate towards muscle fat. Clearly, the physical inactivity-induced altered partitioning of SFA in muscle may be due to a reduced oxidative capacity of SFA at mitochondria level and/or an enhanced incorporation of

SFA into complex lipids for storage in myocyte. We did not find in the literature studies having investigated the effect of physical inactivity on the key proteins involved in the regulation of the partitioning of MUFA or SFA in muscle. Based on current knowledge on FA metabolism, however, we can propose a mechanistic hypothesis to explain the complex fate of FA within the physical activity continuum.

## 8.2. Potential mechanisms that might alter the fate of SFA and MUFA under different conditions of energy turnover.

The first committed step of glycerolipid biosynthesis is the acylation of glycerol-3-phosphate catalyzed by glycerol-3-phosphate acyltransferase (GPAT). Two isoforms have been described: a microsomal and a mitochondrial isoform (mtGPAT) [192]. In tissues other than liver, mtGPAT activity is approximately 10 % of the total GPAT activity [193]. Because both mtGPAT and CPT1 are located on the outer mitochondrial membrane, they can compete for acyl-CoA. Thus, they can regulate the partitioning of FA between degradative and biosynthetic fates [194]. The coordinated regulation of CPT1 and mtGPAT is mediated by AMP-activated kinase (AMPK) which reciprocally regulates TAG synthesis and fat oxidation in liver and muscle [194]. AMPK is a regulatory sensor of the energy stores, which protects cells against the consequences of ATP depletion by inhibiting biosynthetic pathways and stimulating energy-generating pathways [195]. In response to increase in the cellular AMP/ATP ratio, AMPK inactivates acetyl-CoA carboxylase by phosphorylation [196] resulting in a decrease in its product, malonyl-CoA, an intermediate in *de novo* synthesis of FA and an allosteric inhibitor of CPT1 [197]. By decreasing malonyl-CoA, AMPK relieves the inhibition on CPT1 and thereby increases fat oxidation [198, 199]. In both muscle and liver, activated AMPK also inhibits FA esterification into TAG by AMPK dependent inactivation of mtGPAT, but not DGAT or microsomal GPAT [194]. AMPK might also participate in the regulation of ceramide synthesis. Indeed, 5-aminoimidazole-4-carboxamide (AICA) riboside, which enters cells and is converted to AICA ribotide, an ATP analog, has been shown to inhibit palmitate-induced SPT activity and *de novo* ceramide synthesis in rat astrocytes [200] and bovine retinal pericytes [201]. AMPK was also reported to inhibit the palmitate-induced increase in NFkB pathway [202].

Interestingly, the AMPK is stimulated in human skeletal muscle during exercise, and the degree of activation is dependent on the exercise intensity [202-205]. Consequently, exercise training decreases the concentration of malonyl-CoA and increases the expression and activity of malonyl-CoA decarboxylase in human muscle [206] enhancing fat oxidation and inhibiting lipid biosynthesis. Furthermore, 4 weeks of endurance training in male Sprague-Dawley rats [207] and 8 weeks in obese humans [208], decreased DAG and ceramide content and increased insulin sensitivity but did not affect muscle TG content. In the other direction, muscle unloading down-regulates AMPK [209], which may induce opposite metabolic cascade. Interestingly, Kump et al. [210] showed that cessation of physical activity after 21 days of wheel running in rats increases epididymal fat mass in association with a concomitant increase in mtGPAT activity and protein levels. Clearly, a similar study on the effect of physical inactivity on muscle lipid content and mtGPAT activity is warranted.

Nevertheless, because mtGPAT has a 3- to 10-fold higher activity with palmitoyl-CoA than oleyl-CoA [192], the coordinated regulation of CPT1 and mtGPAT mediated by AMPK may be a good candidate pathway to explain the relationship between the physical activity level and the differential oxidation and

storage in muscle fat of dietary oleate and palmitate, i.e. the physical inactivity-induced reduced palmitate oxidation and the physical activity-induced increased oleate oxidation observed in our studies [190] and in Votruba et al. [191] ones, respectively (Figure 5). However, further studies are required to better determine the mechanism of regulation involved in the relationship between the physical activity level and the saturated fatty acid oxidation.

By its inhibitory effect on SPT, GPAT and the palmitate-induced NFkB pathway, AMPK also reduces the accumulation of ceramides, DAG and TAG and thus prevents the development of insulin resistance. Therefore, AMPK may also represent a good candidate to explain the relationship between insulin sensitivity and the physical activity level observed in epidemiological studies (Figure 5). However, further studies are required to clearly demonstrate this association between energy demand, i.e. physical activity or inactivity, AMPK levels, lipid intermediaries content and insulin responsiveness.

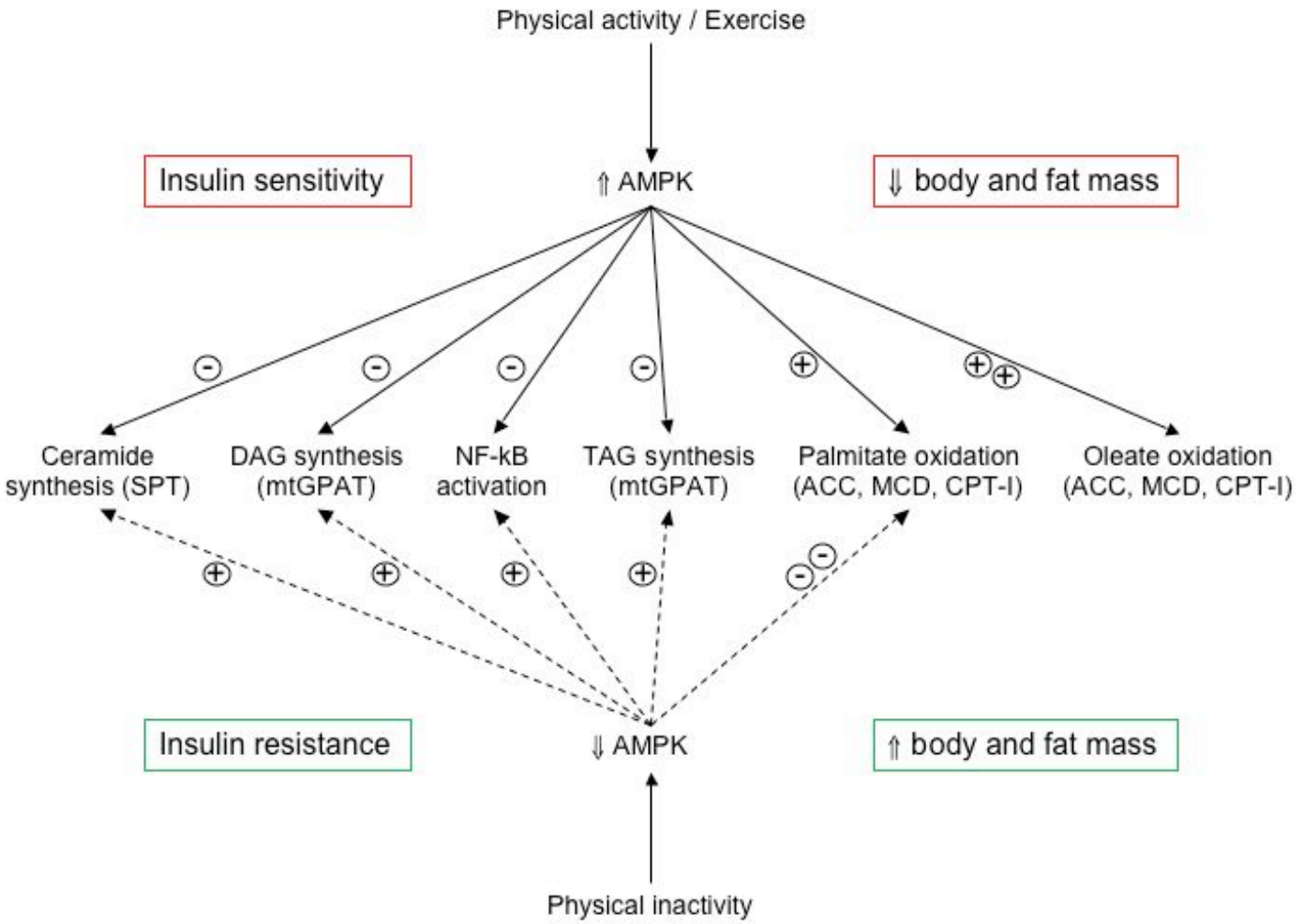


**Figure 5:** Effect of the physical activity level on the lipid biosynthesis and oxidation in muscle cell via the regulation of the AMPK concentration, and the ultimate consequences on insulin responsiveness and body weight regulation.

SPT, serine palmitoyl transferase; DAG, diacylglycerol; mtGPAT, mitochondrial glycerol-3-phosphate transferase; TAG, triacylglycerol; ACC, acetylCoA carboxylase; MCD, malonylCoA deshydrogenase; CPT1, carnitine palmitoyl transferase 1.

# 9. CONCLUSION

In summary, we propose that the metabolic steps such as the packaging into CM-TG, the lipolysis of lipoproteins by LPL, the VLDL biosynthesis from NEFA in the liver and the FA transport into peripheral tissues would favour the retention of SFA in the liver and the entrance of a greater proportion of MUFA than SFA into myocyte. Then, both the high rate of incorporation of SFA into complex lipids and the MUFA mass effect on CPT1 may explain the higher MUFA oxidation compared to SFA oxidation measured at the whole body level and thus, reconcile the results from *in vitro* and *in vivo* studies. Therefore, the Mediterranean diet usually considered with its high proportion in MUFA favors lipid oxidation, which as proposed by Flatt [33] reduces the risk of obesity development. MUFA are also of particular interest in their protective role against SFA-induced insulin resistance and cell death. However, further studies are warranted to better understand the role of lipid intermediaries such as acyl-CoAs, DAG, ceramides, and acylcarnitines as factors altering the insulin signal. In this regard, it would be interesting to investigate the effect of the fatty acid composition in these complex lipids on the insulin responsiveness. Lastly, although diet and physical activity patterns are known to be the two major environmental correlates in the increase of obesity observed over the past decades, most studies have only focused on the consequences of the high-fat diet. Although physical inactivity and high-fat diet induce similar metabolic deleterious consequences, we are convinced that the mechanisms involved are different. Therefore, future advances in the prevention and treatment of obesity and diabetes will necessarily require further research, which investigate these factors as well as their interaction.

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# ANNEXE 2

## La dépense énergétique : composantes & déterminants

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*Nutrition & Facteurs de risque : 3 : 19-22, 2005*

# 1. INTRODUCTION

La notion de balance énergétique appliquée aux êtres vivants découle des lois de la thermodynamique. La stabilité à long terme de la masse corporelle, malgré des apports fractionnés et variés, implique l'existence de mécanismes régulateurs précis entre les entrées (ration alimentaire) et sorties (dépense énergétique totale). Grâce au développement et à l'application, il y a moins de 20 ans, de l'eau doublement marquée chez l'homme (1), il est devenu possible de déterminer avec précision la dépense énergétique totale en conditions de vie libre i.e. sans le confinement confondant inhérent aux chambres calorimétriques. Dès lors, toutes les composantes de la dépense de 24h ont pu être dissociées avec précision tout comme leurs déterminants et leurs implications dans de nombreuses pathologies nutritionnelles. Ces composantes comprennent le métabolisme de base, le coût de l'homéothermie, la thermogenèse post-prandiale, l'activité physique et, transitoirement, l'énergie dépensée lors de la croissance ou de la lactation (**Figure 1**).

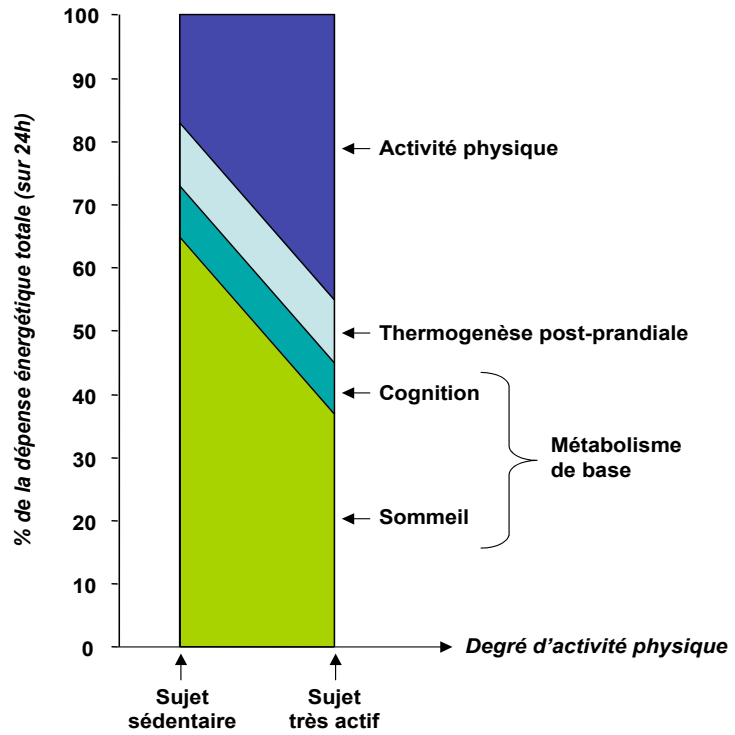


**Figure 1:** Représentation schématique des composantes de la dépense énergétique totale et de leurs variations relatives en fonction du degré d'activité des sujets.

# 2. LE METABOLISME BASAL ET DE REPOS

Le métabolisme basal correspond à l'énergie dépensée en post-absorptif i.e. après une nuit à jeun (minimum 12 à 14 heures), au repos sans mouvement, allongé, éveillé, et à thermoneutralité. Le métabolisme de repos correspond à une situation où exercice physique et alimentation exercent une influence minimale sur le métabolisme. En d'autres terme, cette dépense représente l'énergie requise pour maintenir *a minima* l'activité métabolique des tissus, l'énergie nécessaire pour assurer la circulation du sang, la respiration et les fonctions gastro-intestinales et rénales (2). D'un point de vue plus holistique, le métabolisme basal comprend l'énergie dépensée lors du sommeil et le coût des processus cognitifs. Il est généralement extrapolé sur 24h pour exprimer la dépense énergétique basale. Il est à noter que de nombreuses publications scientifiques ne rapportent que des mesures de métabolisme de repos ; ce dernier est moins contraignant en ce sens qu'il ne requiert pas de mesures au réveil du sujet.

La masse maigre, qui comprend les tissus métaboliquement actifs de l'organisme, est le principal déterminant du métabolisme basal comme de repos. La masse maigre peut expliquer de 70 à 80% de la variabilité du métabolisme de repos contre à peine 2% pour la masse grasse (**Figure 2**). Ceci explique que les femmes présentent en valeur absolue un métabolisme de repos plus faible que celui des hommes. L'existence d'une différence liée au sexe après ajustement pour les différences de masse maigre reste controversée. Une partie de cette controverse s'explique par le type de normalisation statistique appliqué. De nombreux auteurs expriment encore les taux métaboliques par kg de masse maigre pour s'affranchir de l'influence propre de la masse. Une telle normalisation est incorrecte en raison de la présence d'un intercepte significatif dans la relation masse maigre/métabolisme de repos et qui explique que la relation métabolisme de repos/kg masse maigre versus masse maigre soit négativement corrélée. Si l'on prend l'exemple indiqué sur la **Figure 3** nous sommes faussement amenés à conclure que les femmes ménopausées (70-79ans) ont une dépense de repos plus faible que des hommes de même âge. En fait cette relation montre simplement que la contribution relative des organes à haute demande énergétique (cerveau, foi et cœur) est moindre par rapport à celle des organes à faibles demandes (muscles) lorsque la masse corporelle augmente. En revanche, lorsqu'un ajustement statistique approprié, prenant en compte l'existence de l'intercepte, est réalisée e.g. analyse de covariance prenant la masse maigre en covariant, on s'aperçoit que la différence liée au sexe

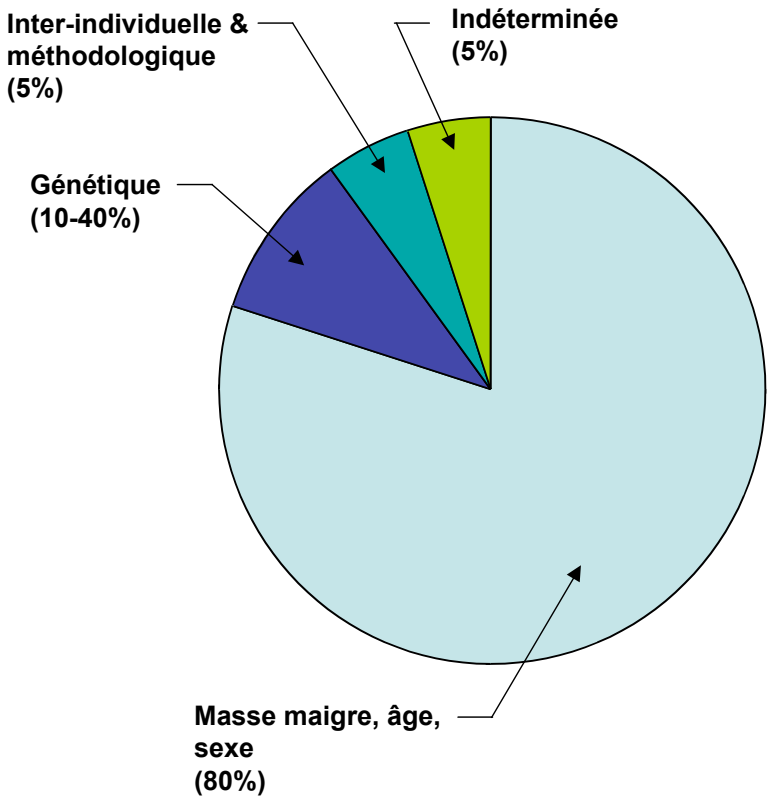


**Figure 2 :** Proportion de la variance du métabolisme de repos expliquée par ses principaux déterminants.

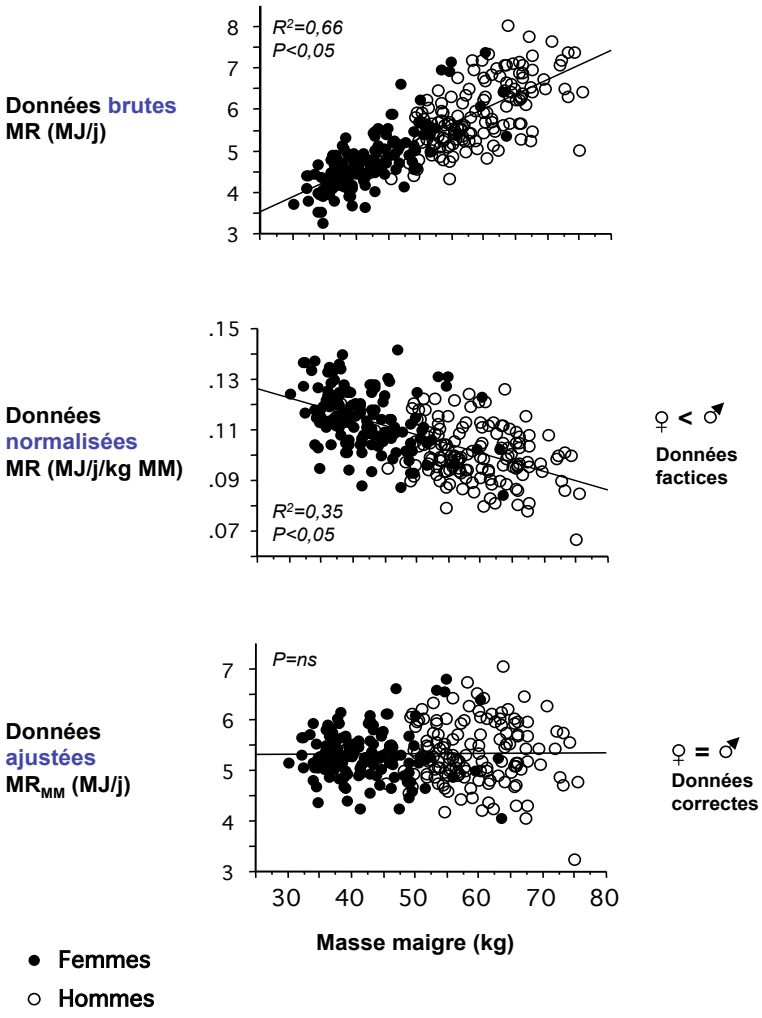


**Figure 3 :** Relation masse maigre et métabolisme de repos chez n=144 femmes et n=144 hommes âgés de 70 à 79 ans montrant l'importance du type de normalisation appliqué sur les conclusions que l'on peut tirer. Voir explications dans le texte. MM : masse maigre, MR métabolisme de repos.

disparaît. Lorsque ce type d'analyse est appliqué chez des femmes en préménaupose, une différence liée au sexe reste incertaine. Les femmes présentent un métabolisme de repos soit inférieur, soit similaire à celui des hommes. Le rôle du cycle hormonal féminin dans ces résultats ne peut être directement incriminé car les données sont également contradictoires : le métabolisme de repos étant soit augmenté de quelques pourcents lors de la phase lutéale soit similaire à la phase folliculaire. Lorsque des comparaisons hommes/femmes sont nécessaires, le métabolisme de repos moyen mesuré lors de chaque phase doit certainement être adopté.

Le métabolisme de repos varie également en fonction de l'âge (3). Chez le nouveau-né, ce dernier exprimé par kg de masse est deux fois plus important que chez l'adulte (210 kJ/kg/j) et le cerveau représente le principal contributeur (70%). Lors de l'enfance, puis de la puberté et jusqu'à l'âge adulte, l'augmentation de masse maigre dicte les variations du métabolisme de repos. A l'âge adulte, une diminution de 1 à 2% du métabolisme de repos est observé par décade jusqu'à l'âge de 40 ans chez l'homme et de 50 ans chez la femme. Passé ce cap, la diminution s'amplifie à 42kJ/an chez l'homme et à 29kJ/an chez la femme. Cette réduction du métabolisme liée au vieillissement est principalement due à la fonte musculaire (sarcopénie) mais environ 5% reste inexpliqués. Toutefois lorsque les personnes âgées maintiennent un degré d'activité physique important, la réduction du métabolisme de repos est mitigée. Enfin, il convient de constater que des différences ethniques existes entre caucasiens et noir-américains et qu'un métabolisme de repos plus bas (3 à 10%) chez ces derniers a été avancé comme l'un des facteurs expliquant leur plus grande propension à l'obésité (4). Cette implication requiert néanmoins de plus amples études ; surtout si l'on tient compte du fait qu'un faible métabolisme de repos dans d'autres populations prédisposées à prendre du poids ne semble avoir qu'un faible impact sur la prise pondérale.

25 à 50% de la variabilité interindividuelle dans la composition corporelle est expliquée par des facteurs génétiques (5). C'est pourquoi la contribution génétique à la variabilité du métabolisme de repos varie de 10 à 40% selon les études. On commence à s'intéresser aux gènes (UCP1, récepteurs ß-adrénergiques, UCP3) sous-tendant ces différences mais le peu de résultats est pour l'heure contradictoire et requiert des travaux complémentaires.

## 3. LA THERMOGENESE POSTPRANDIALE

Toute consommation de nourriture entraîne une augmentation de la dépense énergétique de repos dont l'intensité et la durée sont fonction de la ration alimentaire et de la composition en macronutriments (2). Cette dépense représente le coût de la digestion, du transport et du stockage des nutriments et à un plus faible niveau l'activation du système nerveux sympathique lors de l'ingestion de carbohydrates. Lors d'un repas mixte,

l'incrément de la dépense énergétique en sus du repos, et exprimé en pourcentage de l'énergie ingérée, varie de 5 à 10% pour les glucides, de 0 à 5% pour les lipides et de 20 à 30% pour les protéines (6). Cette valeur importante lors de l'ingestion de protéines représente le coût élevé de l'absorption des acides aminés, de la synthèse protéique, de l'uréogenèse et de la glucogenèse. Lors d'un repas mixte standard, la valeur de la thermogenèse postprandiale est stable aux alentours de 10% de l'énergie ingérée et n'est observée que quelques heures suivant un repas.

A l'inverse chez les nouveaux nés, nourris fréquemment, la thermogenèse postprandiale exerce une influence continue sur la dépense énergétique. L'amplitude de la thermogenèse postprandiale a été corrélée avec la prise pondérale suggérant qu'une partie traduit le coût de la croissance (21-33kJ/g). L'âge ne semble pas influencer la valeur classique des 10% de l'énergie ingérée. En revanche, une faible thermogenèse postprandiale a souvent été proposée comme un facteur favorisant la prise de poids et pouvant aboutir à un état d'obésité. Toutefois l'énergie économisée est probablement <105kJ/j ce qui pourrait expliquer une prise pondérale de 2kg au maximum. En fait lorsque les valeurs de la thermogenèse postprandiale sont extrapolées sur la dépense de 24h grâce à l'eau doublement marquée, sa contribution dans la genèse de l'obésité apparaît peu convainquante.

Une étude originale vient de mettre en évidence une thermogenèse liée à l'absorption d'eau, principalement liée au coût nécessaire pour ramener à 37°C l'eau ingérée (7). Pour un apport journalier de 2L (ce qui est un peu excessif par rapport aux besoins), cette thermogenèse s'élèverait à 400kJ soit 4% des dépenses de 24h. Cette dépense reste modeste car certainement incluse pour sa plus grande part dans la thermogenèse postprandiale.

# 4. THERMOREGULATION

L'homéothermie des oiseaux et des mammifères représente un coût non négligeable sur la dépense énergétique de 24h. L'augmentation du métabolisme est généralement plus importante quant la température ambiante est en deçà plutôt qu'en dessus de la zone de thermoneutralité. Toutefois l'homme ajustant ses vêtements et son environnement de manière à maintenir une zone de confort, le coût de la thermorégulation n'affecte pas significativement la dépense énergétique totale.

# 5. LE COUT DE L'ACTIVITE PHYSIQUE

La dépense énergétique liée à l'activité physique constitue l'élément le plus variable de la dépense de 24h. Chez un sujet sédentaire, le coût de l'activité physique peut représenter moins de la moitié du métabolisme de repos. Chez un sujet très actif ces proportions peuvent être inversées (**Figure 1**).

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. Bien que ce rapport soit extrêmement pragmatique, il n'est pas tout à fait satisfaisant puisque la majorité des dépenses liées à l'activité sont fonction de la masse alors que le métabolisme de repos est proportionnel à la masse$^{0.75}$. Ce PAL a permis de classifier les individus. Ainsi, 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 (8). Par exemple, un individu marchant 3,5 km par jour en 30 minutes, et en sus de ces activités quotidiennes, a un PAL d'environ 1,5. Pour augmenter ce PAL à 1,75 ce même individu devra courir 30 minutes en plus à 10,5 km/h. Il ne s'agit pas là de limites définies. On a estimé qu'en parcourant 295,03km en 24h, le grec Yannis Kouros avait développé une dépense énergétique totale de 14 x son métabolisme de repos.

Le rendement de la conversion énergie ingérée/travail mécanique est remarquablement constant lorsqu'il est mesuré dans des conditions où la masse corporelle et le degré d'entraînement ne sont pas confondants i.e. sur une bicyclette ergométrique (9). Pour les autres activités, le coût énergétique est globalement proportionnel au poids. Marcher –qui représente de loin l'activité physique la plus commune– à une vitesse de 7 km/h, soit une marche modérée, correspond à une dépense de 143kJ/km pour un homme de 70kg et 119kJ/km pour une femme de 57kg. L'impact de cette marche sur la dépense énergétique totale est en fait plus importante. En effet, l'augmentation de la dépense peut perdurer jusqu'à 24h en période post-exercice. Ce phénomène est appelé consommation d'oxygène post-exercice et est fonction de la durée et de l'intensité de l'exercice, des températures ambiantes et de l'état d'hydratation (9). Pour des exercices tels que la marche décrite précédemment, cette consommation représente environ 15% du coût de l´exercice. Si l'on tient également compte de la thermogenèse post-prandiale, le coût global de la marche pris comme exemple précédemment est de 179-153kJ/km pour des individus de 70-64kg, ce qui représente 2,6kJ/km/kg. La marche a été prise comme exemple mais ceci reste valable pour tout type d'activité structuré. A côté de l'activité physique volontaire, l'activité physique réalisée dans les activités de la vie de tous les jours apparaît également significative et de l'ordre de 420 à 2900kJ/j. 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 (10).

S'il apparaît évident que l'exercice aigu augmente la dépense énergétique totale, cela ne semble pas être le cas de l'entraînement à long terme. Ceci, observé chez l'enfant et la personne âgée, suggère une

interrelation complexe entre l'activité physique intentionnelle et non-intentionnelle. De plus, dans la mesure où la masse maigre est le principal déterminant du métabolisme de repos et où l'entraînement physique favorise l'accrétion de masse maigre, une augmentation du métabolisme de repos serait attendue en réponse à l'entraînement. Les données expérimentales ne vont néanmoins pas dans ce sens, probablement en raison de la contribution modeste (25%) des muscles squelettiques au métabolisme de repos.

Chez l'enfant, le coût énergétique de la maintenance et de la croissance sont inversement proportionnels au besoin croissant d'énergie pour l'activité physique. Au cours de la croissance le PAL augmente. Il passe de 1,2 à 3 mois à 1,4 à 24 mois et de 1,4-1,5 à 5 ans à 1,5-1,8 entre 6 et 18 ans chez des enfants de pays industrialisés. Chez l'adulte, le PAL est extrêmement variable est dépend du type d'emploi, de la localisation géographique, du niveau social, etc… 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 (8). Allant dans le même sens, les noir-américains présentent une dépense énergétique liée à l'activité physique moindre (10-20%) que des caucasiens témoins ; ce qui pourrait contribuer à leur plus grande prévalence de l'obésité.

Au cours du vieillissement, la dépense énergétique liée à l'activité physique diminue de manière équivalente à la réduction du métabolisme de repos. Chez des personnes de 90 ans, un PAL proche de 1,0 a même été observé. Toutefois cette réduction n'est pas systématique et représente essentiellement le fait que la plupart des études ont été réalisées sur des personnes âgées en institution de retraite, présentant *de facto* quelques handicaps et placés sous polypharmacie. En étudiant un groupe de 288 individus âgés de 70-79 ans, en conditions de vie libre et exempt de tout handicap, nous avons observé un PAL de 1.7 (4). En conséquence la réduction de PAL de la personne âgée ne semble pas être liée au vieillissement *per se* mais plutôt au changement de l'environnement.

# 6. NOTIONS D'ADAPTATIONS DE LA DEPENSE ENERGETIQUE

Le terme d'adaptation métabolique dérive des études de sur et sous nutrition. Ces dernières montrent que les changements de dépense énergétique sont supérieurs à ceux attendus en fonction des changements de composition corporelle. Ces changements compensatoires ont pour but de s'opposer aux modifications de masse corporelle imposées par le nouvel apport calorique (11). La notion de temps est particulièrement importante : les réponses différent selon que l'organisme est étudié en état de balance énergétique perturbé ou bien lorsqu'un nouvel équilibre est atteint. Prenons

par exemple le cas d'un sujet ayant une dépense énergétique totale de 10MJ/j, sous régime hypocalorique, chez lequel on veut provoquer une perte de masse d'environ 100g/semaine. Ces 100g sont équivalent une réduction d'environ 3MJ par jour de l'apport calorique (en considérant 75% de masse grasse à 38,7kJ/g et 25% de masse maigre à 4,2kJ/g). Sachant que 1) la perte de masse s'accompagne d'une réduction de 69,4 kJ/kg de la dépense énergétique totale et que 2) l'adaptation métabolique induit une réduction d'environ 8% de cette même dépense énergétique, on peut estimer que la réduction totale de la dépense énergétique après 10 semaines sera de 1,3MJ/j pour un sujet. Ces calculs simples indiquent que si l'on veut maintenir constant le taux de perte pondéral au bout de 10 semaines de diète la réduction de l'apport calorique devra passer de 3 à 4,3MJ/j. Une fois la balance énergétique rétablie, cette adaptation métabolique semble disparaître chez l'homme et ne peut expliquer la récidive importante de personnes post-obèses.

De tel processus sont également observés lors de surnutrition aigue mais également chronique. Toutefois l'amplitude de l'adaptation métabolique est modeste à long terme. Une étude longitudinale sur 3,6±2,7 ans suggère en effet qu'elle ne dépasse pas l'équivalent d'une demi pomme par jour (12).

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# TABLES DES MATIERES

Obesity is a fat storage disease and its aetiology is classically viewed as the result of an incapacity to use fat as fuel. However, the recent observation of the tight relationship between energy and lipid balances strongly suggests that the physical activity level, which represents the main variable component of the total energy expenditure, may play a key role in the dietary lipid fate. The direct corollary of this observation is that the incapacity to use fat as fuel observed in both obese and post-obese individuals may be secondary to the generalised adoption of sedentary behaviours in the general population. Nevertheless, our current knowledge on the physical inactivity physiology is very poor.

The main aim of this present thesis was to determine in normal-weight men and women whether the physical inactivity impairs the regulation of both energy and lipid balances, both involved in the body weight regulation and to delineate the related mechanisms. To do so, we used the long-term bed rest model to submit healthy lean women and men without any genetic predisposition to obesity to 2 and 3 months of physical inactivity, respectively.

We showed that during long periods of physical inactivity, energy intake is spontaneously decreased under the control of satietogenic signals (i.e. leptin) and matches the reduced energy expenditure. Energy balance can thus be maintained on the long-term. However, physical inactivity alters the macronutrients homeostasis. Indeed, it induces a preferential channelling of lipids towards storage associated with a reduced fat oxidation and a development of an insulin resistance. This decrease in total lipid oxidation is likely due, at least in part, to a reduced transport of fatty acids into myocyte and mitochondria. Physical inactivity also induces an increase in intestinal absorption and/or a drop in the plasmatic clearance of exogenous fatty acids, which results in a hypertriglyceridemia and an elevated 'spill-over' of the free fatty acids released by the hydrolysis of the triglyceride-rich lipoproteins by the lipoprotein lipase. Interestingly, physical inactivity decreases the oxidation of the dietary saturated fatty acids (palmitate) but not that of the dietary monounsaturated fatty acids (oleate). Since saturated and monounsaturated fatty acids have a similar absorption rate, trafficking and uptake by the peripheral tissues, physical inactivity seems to impact the partitioning of saturated fatty acids towards storage versus oxidation likely via a preferential accumulation of palmitate in muscle fat. This hypothesis is supported by a negative relationship between the physical inactivity-induced reduced palmitate oxidation and the muscle lipid content. This result is of particular interest when considering the clear relationship observed between intra-muscular triglycerides and the development of insulin resistance. Therefore, physical inactivity, independent of energy balance changes, triggers the development of metabolic features close to what is observed in both obese and diabetic individuals. These observations support our hypothesis, which places sedentary behaviours as one of the major causes responsible for the aetiology of obesity. Further studies on the mechanisms involved in the preferential storage of saturated fatty acids towards intra-muscular triglycerides and on the related insulin response under physical inactivity conditions are, however, required in order to gain a better understanding on the interaction between diet and physical activity in the development of both obesity and diabetes.

The second aim of this thesis was to test the efficiency of two physical exercise training programs (resistive vs. resistive and aerobic) to counteract the deleterious effects induced by physical inactivity. Our results highlighted the key role of the total energy expenditure in the regulation of total lipid oxidation. Nevertheless, we think that the inefficacy of the exercise trainings to maintain the exogenous fatty acid oxidation would be rather due to a low spontaneous physical activity (any bodily movement) than to an insufficient exercise-induced energy expenditure. Thus, structured and spontaneous physical activity may differently affect the lipid metabolism. This only represents a hypothesis and further studies on the physiology of exercise, of non-structured physical activity but also of physical inactivity are clearly required. The model of the long-term bed rest seems to be highly relevant for such investigations.

**Résumé en français voir au verso**

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L'obésité est une maladie du stockage des lipides et une vision classique de son étiologie repose sur une incapacité à utiliser les lipides qui serait causale dans la prise de poids. Or l'observation relativement récente d'un alignement étroit de la balance lipidique sur la balance énergétique suggère fortement que le niveau d'activité physique, en tant qu'élément le plus modulable de la dépense énergétique totale, pourrait jouer un rôle clef dans le devenir des lipides alimentaires. Le corollaire direct de cette observation est que l'incapacité à utiliser les lipides en tant que substrat, observée chez les sujets obèses et post-obèses, pourrait être secondaire à l'adoption généralisée d'un mode de vie sédentaire. Toutefois, nous disposons à ce jour de peu de données concernant la physiologie de l'inactivité physique.

Le but majeur de ce travail de thèse a été de déterminer chez l'homme et la femme normo-pondérés si l'inactivité physique altère la régulation des balances énergétique et oxydative des lipides impliquées dans la régulation du poids et de caractériser les mécanismes sous-jacents. Pour cela, nous avons soumis des sujets femme et homme sains sans aucune prédisposition génétique à l'obésité à 2 et 3 mois d'inactivité physique, respectivement, en utilisant le modèle de l'alitement prolongé.

Nous avons montré que lors de longues périodes d'inactivité physique, l'énergie ingérée est diminuée proportionnellement à la dépense énergétique totale sous l'effet de signaux satiétogènes dont la leptine. Ainsi la balance énergétique peut-être maintenue sur le long terme. Néanmoins, l'inactivité physique altère l'homéostasie des macronutriments avec une répartition des lipides au profit du stockage et entraîne le développement d'une insulino-résistance. Cette diminution de l'oxydation lipidique totale est probablement due, en partie, à la diminution du transport des acides gras dans le myocyte et la mitochondrie. L'inactivité physique induit aussi une augmentation de l'absorption intestinale et/ou une diminution de la clairance plasmatique des acides gras exogènes résultant en une hypertriglycéridémie et un "spill-over " accru des acides gras libres provenant de l'hydrolyse des lipoprotéines par la lipoprotéine lipase. De manière intéressante, l'inactivité physique diminue l'oxydation des acides gras alimentaires saturés (palmitate) mais pas celle des acides gras monoinsaturés (oléate). Puisque les acides oléique et palmitique alimentaires semblent avoir une absorption, un acheminement et une captation par les tissus périphériques similaires, l'inactivité physique altèrerait la répartition des acides gras saturés au profit d'un stockage au niveau musculaire. Cette hypothèse semble étayée par la relation négative obtenue entre la réduction d'oxydation du palmitate et la quantité de lipides au niveau musculaire. Ce résultat est d'autant plus intéressant lorsque l'on considère la relation établie entre l'insulino-résistance et les triglycérides intra-musculaires. Ainsi, l'inactivité physique, indépendamment des changements de la balance énergétique, induit chez des individus sains des caractéristiques physiologiques proches de celles que l'on observe chez des sujets obèses. Ces observations soutiennent notre hypothèse selon laquelle la sédentarité serait une des causes majeures de l'étiologie de l'obésité. De plus amples études sur les mécanismes impliqués dans le stockage préférentiel des acides gras saturés dans les triglycérides intramusculaires et l'impact sur la réponse à l'insuline en conditions d'inactivité physique sont cependant requises afin d'étendre notre compréhension sur l'interaction entre régime alimentaire et activité physique dans le développement de l'obésité et du diabète.

En testant l'efficacité de deux protocoles d'entraînement d'exercice physique (résistif versus résistif et aérobic) pour contrer les effets délétères de l'inactivité physique, nos résultats ont mis en avant le rôle clef de la dépense énergétique dans la régulation de l'oxydation lipidique totale. Toutefois, nous pensons que l'inefficacité de l'entraînement physique observée sur le maintien de l'oxydation des acides gras exogènes serait plutôt due à une faible activité de type spontanée (tout type de mouvement) qu'à une dépense énergétique insuffisante induite par l'exercice physique. Ainsi, l'activité physique de type structurée ou spontanée influencerait différemment le métabolisme lipidique. Ceci ne représente qu'une hypothèse à ce jour et des études supplémentaires sur la physiologie de l'exercice, de l'activité physique non structurée mais aussi de l'inactivité physique sont clairement requises. Le modèle de l'alitement prolongé semble approprié pour de telles investigations.

**Mots Clés :** Alitement prolongé – dépense énergétique – oxydation des substrats totaux – oxydation des lipides alimentaires – palmitate – oléate - isotopes stables – calorimétrie indirecte