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THÈSE présentée par :

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Effet de la microgravité réelle et simulée sur la flexibilité métabolique chez l'homme

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Enthusiasm is common.
Endurance is rare.

Angela Duckworth

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Liste des communications et publications

Communications

Présentations orales

- Le Roux E, Zahariev A, Garnotel M, Gauquelin-Koch G, Blanc S, Bergouignan A, and Simon C. **Effects of the exercise countermeasure to prevent the metabolic alterations induced by microgravity: evidence from ground-based and inflight studies.** *41st Annual ISGP Meeting, College Station, Texas, USA. Virtual meeting (May 24th – May 27th, 2021)*
- Le Roux E. **Relations entre inactivité physique, sédentarité et métabolisme : leçons des science spatiales.** *Journées Francophones de la Nutrition, Lille, France (November 10th, 2021), Virtual meeting*

Posters

- Le Roux E, Zahariev A, Garnotel M, Gauquelin-Koch G, Blanc S, Bergouignan A, and Simon C. **Effects of the exercise countermeasure to prevent the metabolic alterations induced by microgravity: evidence from ground-based and inflight studies**. *Doctoral School Days, Strasbourg, France (April 21st – April 22nd, 2021)*
- Le Roux E, Zahariev A, Bourdier P, Chery I, Schoeller DA, Maillet A, Thevenot C, Garnotel M, Gauquelin-Koch G, Zwart SA, Smith SM, Simon C, Bergouignan A, Blanc S. **Nutrient metabolism in male astronauts on the International Space Station: The ENERGY study.** *Recent Advances & Controversies in the Measurement of Energy Metabolism, RACMEM2022, Québec City, Canada (October 8th – October 10th, 2022)*

Publications

Journal avec comité de lecture

- Le Roux E*, De Jong NP*, Blanc S, Simon C, Bessesen DH and Bergouignan A (2022). **Physiology of physical inactivity, sedentary behaviors and non-exercise activity: insights from the space bed-rest model**. J Physiol, 600: 1037-1051. https://doi.org/10.1113/JP281064 (**Annexe 1**). *Equivalent contribution.

- Le Roux E, Zahariev A, Bourdier P, Chery I, Schoeller DA, Maillet A, Thevenot C, Garnotel M, Van Den Berghe L, Gauquelin-Koch G, Simon C, Blanc S and Bergouignan A. **Substrate oxidation and metabolic flexibility in male astronauts on the International Space Station: The ENERGY study.** Soumis le 30/01/2023 à *Journal of Nutrition*, en cours de révision.
- Bourdier P, Zahariev A, Schoeller DA, Chery I, Le Roux E, Thevenot C, Maillet A, Garnotel M, Gauquelin-Koch G, Beroguignan A, Blanc S, Simon C. **Effect of Exercise on Energy Expenditure and Body Composition in Astronauts Onboard the International Space Station: Considerations for Interplanetary Travel**. Sports Med (2022). https://doi.org/10.1007/s40279-022-01728-6 (ne fait pas partie intégrante du travail de thèse, **Annexe 2**)
- Guirado T, Bourdier P, Pereira B, Le Roux E, Bergouignan A, Birat A, Isacco L, Thivel D, Duclos M, Metz L. **Metabolic profile in women differs between high versus low energy spenders during a low intensity exercise on a cycle-desk.** Sci Rep 12, 9928 (2022). https://doi.org/10.1038/s41598-022-14002-6 (ne fait pas partie intégrante de mon travail de thèse, **Annexe 4**)

Journal sans comité de lecture

- Le Roux E, Simon C, Blanc S, Bergouignan A. **Inactivité physique et sédentarité : impact sur la santé métabolique, de quoi parle-t-on ?** Nutritions & Endocrinologie, Novembre-Décembre 2021, vol. 19, n° 98 p. 110-114 (**Annexe 3**)

Publications en préparation

- **Short-term physical inactivity triggers whole-body and skeletal muscle metabolic inflexibility and insulin resistance.** Le Roux E*, Lair B*, de Glisezinki I, Larrouy D, Harant I, Gauquelin-Koch G, Blanc S, Simon C, Moro C, Laurens C[#], Bergouignan A[#]. En preparation pour soumission à Metabolism journal

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Abréviations

4E-BP1	Eukaryotic translation initiation factor 4E-binding protein 1
8-OHdG	8-hydroxydésoxyguanosine
ACC	Acetyl-CoA carboxylase
ACS	Acyl-CoA synthetase
Akt	Protein kinase B
ALT	Alanine aminotransferase
AMP	Adenosine monophosphate
AMPK	Adenosine monophosphate kinase
AS160	Akt substrate of 160 kDa
ASC	Agence Spatiale Canadienne
AST	Aspartate aminotransferase
ATGL	Adipose tissue triglyceride lipase
ATP	Adenosine triphosphate
AUC	Area under the curve
BDC	Baseline data collection
BMI	Body mass index
CADMOS	Centre d'aide au développement des activités en micropesanteur des opérations spatiales
CALT	Chinese Academy of Launch Vehicle Technology
CaMKK	Ca^{2+}/calmodulin-dependent 20protein kinase kinase
CaMKII	Ca^{2+}/calmodulin-dependent p20rotein kinase II
CEVIS	Cycle ergometer with vibration isolation and stabilization system
CNES	Centre National des Etudes Spatiales
CNSA	China National Space Administration
COX4	Cytochrome c oxidase subunit 4
CPT1	Carnitine palmitoyltransferase 1
CPT2	Carnitine palmitoyltransferase 2
CSA	Cross Sectional Area
DGAT	Diacylglycerol acyltransferase
DLW	Doubly-labeled water
DRI	Dietary requirement intake
EAC	European astronaut center
EHC	Euglycemic hyperinsulinemic clamp
EPO	Erythropoïétine
ESA	European Space Agency
ETC	Electron transport chain
FAT/CD36	Fatty acid translocase/Cluster of differentiation 36
FATP	Fatty acid transport protein
FDPase	Fructose-1,6-biphosphatase
FFA	Free fatty acid
FOXO	Forkhead family of transcription factors
FQ	Food quotient
G6P	Glucose-6-phosphate
G6Pase	Glycose-6-phosphatase
GLUT4	Glucose transporter type 4
GP	Glycogen phosphorylase
GPAT	Glycerol phosphate acyltransferase
GS	Glycogen synthase
HDL	High density lipoprotein
HDT	Head down tilt period
HK2	Hexokinase 2
HOMA-IR	Homeostatic model assessment for insulin resistance
HRP	Human Research Program
HSL	Hormone sensitive lipase
IGF-1	Insulin Growth Factor-1
IBMP	Institut des problèmes biomédicaux de Russie
IMAT	Intermuscular adipose tissue
IMCL	Intramyocellular lipid
iRED	Interim resistive exercise device
IRS-1	Insulin Receptor Substrate-1
ISS	International Space Station
JAXA	Japan Aerospace Exploration Agency
LACS	Leg/Arm Cuff System
LBNP	Lower body negative pressure
LDL	Low density lipoprotein
LPA	Light intensity physical activity
LPL	Lipoprotein lipase

MAD	Mean amplitude deviation
MARES	Muscle Atrophy Research and Exercise System
MCD	Malonyl-CoA decarboxylase
MEDES	Institut de Médecine et de Physiologie Spatiale
MGL	Monoglyceride lipase
MPC	Mitochondrial pyruvate carrier
mTORC1	Mammalian target of rapamycin complex 1
MAFbx/Atrogin-1	Muscle atrophy F-box
mtGPAT	Mitochondrial glycerol-6-phosphate acyltransferase
MET	Metabolic equivalent task
MuRF1	Muscle RING-finger protein 1
MVPA	Moderate-to-vigorous physical activity
NASA	National Aeronautics Space Agency
NEFA	Non-esterified fatty acid
NF-κB	Nuclear factor-kappa B
OGTT	Oral glucose test tolerance
OMS	Organisation mondiale de la santé
ONAPS	Observatoire national de l'activité physique et de la santé
OXPHOS	Phosphorylation oxydative
p38 MAPK	p38 mitogen-actived protein
PA	Physical activity
PAL	Physical activity level
PC	Pyruvate carboxylase
PDH	Pyruvate dehydrogenase
PDK	Pyruvate deshydrogenase kinase
PEPCK	Phosphoenolpyruvate carboxykinase
PFK	Phosphofructokinase
PK	Pyruvate kinase
PFS	Pulmonary Function System
PGC1α	Proliferator-activated receptor-Y coactivator-α
PI3K	PhosphatidylInositol 3 Kinase
PIP2	Phosphatidylnositol-4,5-biphosphate
PKCζ	Protein kinase C ζ
QR	Quotient respiratoire
QRNP	Quotient respiratoire non protéique
RRAD	Ras-related associated with diabetes
RNS	Reactive nitrogen species
RONS	Reactive oxygen and nitrogen species
ROS	Reactive oxygen species
PIP3	Phosphatidylnositol-4,5-triphosphate
S6K1	S6 kinase 1
SB	Sedentary behaviours
SLAMMD	Space Linear Acceleration Mass Measurement Device
SWP	Sensewear Pro
T2	2nd generation treadmill
TBW	Total body water
TCA	Tricarboxylic acid cycle
TG	Triglyceride
TNF-α	Tumor necrosis factor α
TVIS	Treadmill with vibration isolation system
URSS	Union des Républiques Socialistes Soviétiques
VLDL	Very-low density lipoprotein
VCO_2	Volume de production de dioxyde de carbone
VO_2	Consommation d'oxygène
WHO	World health organization

Contribution aux travaux de thèse et impact de la pandémie COVID-19

Impact of the COVID-19 pandemic and contribution to thesis work

Initially, the objective of this thesis was to test preventative strategies against the negative effects of physical inactivity and sedentary lifestyles on metabolic health. Three strategies were supposed to be evaluated. The first study was the BURST (Breaking Up pRolonged Sedentary Time) study that aimed to test the effects of a one-month intervention consisting in breaking up prolonged sedentary time by brief episodes of physical activity spread throughout the day on metabolic health outcomes in male and female adults with overweight or obesity. To further test whether the effects were due to the active breaks per se or to the increases in total active time and/or energy expenditure, the effects of this intervention were compared to those elicited by an intervention consisting in single daily bouts of continuous moderate-intensity activity that was matched for total active time. The BURST study was carried out in Denver, Colorado in the United States within the framework of the CNRS International Research Program that has been established between the IPHC CNRS UMR7178 and the University of Colorado (Coordinator: A. Bergouignan). When I started my PhD defense the last 4 subjects out of 24 needed to be studied to complete the study, and I was supposed to go to the University of Colorado for about 3 months to participate in final data collection and then assist with data and samples analysis at the IPHC UMR7178. Second, the REMOVE (Remise En MOuVEment) study was initiated in collaboration with the AME2P laboratory (EA3533) in Clermont-Ferrand and aimed to test the impact on key cardiometabolic and psychological health outcomes of a three- and six-month physical activity intervention consisting in the use of cycle-desk during working days in sedentary and inactive employees of the tertiary sector. I was supposed to assist with data collection at both baseline and post-interventions. Third, using collected data and samples from the Cocktail bed-rest study completed in 2017, I was supposed to assess the effects of a novel nutritional supplementation with anti-oxidant and anti-inflammatory compounds to prevent the adverse effects of physical inactivity induced by bed-rest on key metabolic health outcomes.

The COVID-19 pandemic impacted all three projects. Six months after the beginning of my PhD in October 2019, all human biomedical research was stopped as of March 2020 and access to the IPHC was restricted. The BURST study resumed in August 2021 and it took 6 more months to complete it. Statistical power was not reached without those last 4 subjects. As planned, I participated in the

baseline data collection of the REMOVE study in January 2020, the protocol was stopped just before the second measurement session due to the pandemic. The data obtained during the first measurement session could be valorized in an article published in Scientific Reports which is presented in the appendix (Appendix 4). In regard of the last chapter of this thesis, I performed assays on blood samples of some markers of oxidative stress as well as hormones involved in the regulation of appetite. I was also supposed to assist with the preparation of blood samples for analysis by GC-MS for measuring ^{13}C-glucose, ^{2}H-glucose and D_5-glycerol and thus determine the glucose kinetics and whole-body lipolysis. In collaboration with I. Chery, engineer of the team, we worked on the development method of measuring the concentration and enrichment of glycerol in blood by GC-MS. In addition to a limited access to the laboratory for a long period of time due to confinement, technical difficulties in the preparation and analysis of the samples greatly reduced the possibility of finishing these analyses by the end of my thesis originally scheduled for September 2022.

Given these multiple and unexpected difficulties, the strategy proposed by my supervisors was to use data collected during previous experiments. Thus, I worked on the analysis of the data and the writing of two studies that required to change de context of my thesis. Those data will be presented in this thesis. This change of strategy required new literature search and time to understand the background and scientific questions associated with the new focus of my PhD work. In agreement with the members of the thesis follow-up committee and the Doctoral School ED414, a request for a thesis extension was made and the Mission pour les Initiatives Transverses et Interdisciplinaires (MITI) of the CNRS granted a 3-month extension to complete the work by December 2022. My supervisors also allowed me to extend the duration of my thesis by an additional extension allowing to defend my work on March 27th 2023.

Préambule

Preamble

It was in the 19th century that the greatest upheavals occurred in the study of the living world and in particular in medicine. Until this period, medicine and the use of drugs were based on empirical rules and traditions without rationality. François Magendie (1783-1855) and Claude Bernard (1813-1878), both French Professors of Medicine, were the first ones to express their skepticism about these theoretical speculations. In his book "Introduction to the study of experimental medicine", Claude Bernard developed the general rules of experimental reasoning applied to medicine, which until then had been used exclusively in the physical and chemical sciences. Claude Bernard revolutionized the knowledge of the time by being the first to question the established facts that were widely taught until the first half of the 19th century, particularly on energy metabolism. With his famous liver wash experiment, he demonstrated that the liver was a major organ in the metabolism of blood sugar and showed that it was able to release glucose into the bloodstream from glycogen. He was the first one to foresee the existence of a "law regulating glycogen variations" intended to maintain a stable blood glucose level. In 1865, in his writings, he postulated that "All the vital mechanisms, however varied they may be, always have only one aim, that of maintaining the unity of the conditions of life in the internal environment. It is the stability of the inner environment that is the condition of free and independent life". The American physiologist Walter Bradford Cannon named this concept "homeostasis" in 1932. He developed that "changes in the environment trigger reactions in the system or affect it directly, resulting in internal disturbances of the system. Such disturbances are normally kept within narrow limits because automatic adjustments within the system come into action and in this way wide oscillations are avoided; the internal conditions being kept more or less constant [...]. The coordinated physiological reactions which maintain most of the dynamic equilibrium of the body are so complex and so peculiar to living organisms that it has been suggested that a special designation be used for these reactions: that of homeostasis".

Physiology aims to study the homeostasis of living beings according to modifications of the environment. From this point of view, Man, like all species of the living world, is the result of evolution, which has been shaped by the constraints exerted by the environment, such as light, oxygen, temperature or gravity. The role of gravity, or rather the absence of gravity (microgravity) on human physiology began to interest scientists as early as the first manned space flights in the 1960s. With the context of space exploration, scientists have sought to characterize the adaptations of physiological systems induced by microgravity. While the study of cardiovascular, bone and muscle homeostasis in

microgravity conditions has been widely studied, the first studies on metabolism appeared around the 1980s. Among the major adaptations of metabolism to microgravity highlighted by studies conducted on Earth with the use of analogous models, the development of a metabolic inflexibility, defined as an inability to modulate the oxidation of substrates according to their availability, was recently noted. Metabolic inflexibility is notably one of the manifestations encountered in metabolic pathologies such as type 2 diabetes, obesity or metabolic syndrome. The study of metabolic flexibility in astronauts is an important axis knowing that it is likely interrelated with other metabolic alterations such as insulin resistance, muscle atrophy and ectopic lipid storage. However, its characterization in astronauts has never been done and the cellular and molecular mechanisms involved in the development of metabolic inflexibility remain unclear.

This thesis work sought to fill these current gaps in order to better understand the impact of microgravity on metabolic flexibility, a major feature of metabolic health, as well as the underlying cellular and molecular mechanisms. Specifically, the aim was to test the hypothesis that both simulated and real microgravity leads to a decrease in whole-body metabolic flexibility in humans, which is related to reduced metabolic flexibility of the muscle cell. The secondary goal was to evaluate the relationships between physical exercise performance, diet and body composition with metabolic flexibility. The pursuit of these goals was accomplished through two independent research studies that are presented in two separate chapters of this thesis. The thesis is composed as follows: The introduction is divided into five chapters: after a brief history of the conquest of space, **Chapter 1** presents the future objectives and challenges of the international space agencies, **Chapter 2** introduces the constraints imposed by space biomedical research, the different models to study the effects of microgravity as well as the adaptations of the major physiological systems induced by microgravity. **Chapter 3** focuses on the metabolic adaptations to microgravity, which are thought to play a central role in the development of other physiological alterations observed during spaceflights as presented in **Chapter 4**. Finally, **Chapter 5** aims to present the countermeasures developed so far by space agencies to mitigate those physiological alterations with a particular focus on exercise and nutrition. After the presentation of the objectives and hypotheses of this thesis will follow two chapters that will compose the experimental part of this thesis. **Chapter 6** aims to determine the role of muscle in the development of whole-body metabolic inflexibility and insulin resistance in healthy men immersed for 5 days during a dry immersion study. **Chapter 7** is devoted to characterize for the first time in astronauts substrate use and metabolic flexibility following a long stay (> 3 months) onboard the International Space Station (ISS). Finally, the last part of this work is devoted to the discussion of the results obtained from these studies, followed by perspectives.

Introduction

1 La conquête spatiale

1.1 Historique

1.1.1 La rivalité entre les Etats-Unis et l'URSS comme moteur de la course aux étoiles

La conquête spatiale a été marquée par de nombreux évènements depuis la deuxième moitié du XXe siècle (**Figure 1**). Les États-Unis et l'Union des Républiques Socialistes Soviétiques (URSS), sortis vainqueurs de la seconde guerre mondiale sur le plan économique vont s'affronter dans la course à l'espace dans un contexte de guerre froide. Grâce aux avancées technologiques dans le domaine des missiles acquises par les Allemands, les Américains et les Soviétiques annoncent leur projet d'envoyer des satellites dans l'espace au début des années 1950. L'envoi dans l'espace du premier satellite artificiel Spoutnik 1 en octobre 1957 depuis le cosmodrome de Baïkonour par l'URSS lancera officiellement la course de la conquête spatiale entre les deux superpuissances. Un mois plus tard, l'URSS réalise le premier vol habité dans l'espace en envoyant la chienne Laïka à bord de Spoutnik 2 qui décèdera quelques heures après le décollage. Les États-Unis envoient à leur tour en orbite leur premier satellite artificiel Explorer 1 en 1958. L'URSS va creuser son avance dans la conquête spatiale en réalisant le premier vol orbital habité par un homme en avril 1961 avec Youri Gagarine qui restera en orbite terrestre pendant 89 minutes à bord de Vostok 1. Sa compatriote Valentina Terechkova deviendra la première femme à voyager dans l'espace en juin 1963 à bord de Vostok 6. L'Union soviétique gardera son statut de leader dans la course vers la Lune jusqu'en juillet 1969 où les astronautes de la NASA Buzz Aldrin et Neil Armstrong au cours de la mission Apollo 11 fouleront pour la première fois la surface de la Lune.

1.1.2 La collaboration internationale au service de l'exploration spatiale

Les fonds engagés pour ces missions brèves étant très élevés, la course à l'espace entre les deux superpuissances va s'apaiser. S'engage alors une réflexion sur la nécessité d'allonger la durée des vols spatiaux avec la création de stations spatiales. La première station spatiale en orbite Salyut sera lancée par l'URSS en 1971, la station de la NASA Skylab sera mise en orbite deux ans plus tard. C'est à ce moment que naissent les premiers rapports établissant les impacts de la microgravité sur la santé des astronautes (Michel *et al.*, 1976). Toujours dans ce contexte de guerre froide, cette période va être marquée par un apaisement des relations entre Est et Ouest qui se traduira par une collaboration économique dont la coopération dans le domaine spatial servira de symbole. Le projet d'un rendez-vous orbital entre les Etats-Unis et l'URSS naît alors et sera concrétisé en juillet 1975 où la poignée de

main historique entre l'astronaute Stafford et le cosmonaute Leonov aura lieu à bord de l'ensemble Apollo-Soyouz. Dans un but d'établir une présence constante dans l'espace, le projet de la station Mir prend forme. La station sera mise en orbite en 1986 et accueillera d'abord des équipages soviétiques puis la chute de l'URSS rendra l'accès possible à des astronautes Nord-Américains, Européens et Japonais. En parallèle, le projet de la station spatiale internationale (ISS) est lancé par le président américain Reagan. Le programme fut initialement lancé et piloté par la NASA mais l'agence spatiale russe Roscosmos, grâce au succès de la station Mir, deviendra également un acteur majeur du projet. Avec la collaboration des agences spatiales européenne (ESA), japonaise (JAXA) et canadienne (ASC), la station spatiale sera occupée en permanence par un équipage international qui se consacre essentiellement à la recherche scientifique en micropesanteur. L'ISS est mise en orbite en novembre 1998 et est actuellement toujours en service. Des missions sont prévues jusqu'en 2030, date à laquelle l'ISS sera désassemblée. Alors que les Etats-Unis et l'URSS se sont affrontés pendant plusieurs décennies dans la course aux étoiles, la Chine vise à concurrencer les deux superpuissances. C'est le 24 avril 1970, date de la mise en orbite de leur premier satellite artificiel, que la Chine entre dans le cercle des puissances spatiales au rang de 5ème place derrière l'URSS, les États-Unis, la France et le Japon. En 2003, l'agence spatiale chinoise (CNSA) réussi à placer son premier taïkonaute en orbite à bord du vaisseau Shenzhou 5 et quelques années plus tard, en 2011 à lancer la première version de sa station spatiale (CSS pour China Space Station). Les Chinois réalisent une prouesse inédite en étant les premiers à faire alunir leur module Chang'e-4 sur la face cachée de la Lune en 2018. Cette nouvelle puissance entend donc faire partie des acteurs incontournables de la conquête spatiale, mais de façon isolée, avec notamment une présence chinoise permanente dans l'espace à bord de leur station spatiale.

1.1.3 L'émergence des compagnies privées et du tourisme spatial

Aujourd'hui des compagnies privées font partie des acteurs de la conquête spatiale au même titre que les agences spatiales nationales entre autres pour des raisons économiques. Pour réduire leurs coûts, les agences spatiales nationales ont signé des contrats avec les sociétés SpaceX, Boeing, Virgin Galactic ou encore Blue Origin qui mettent à disposition leur compétences pour développer et envoyer des engins dans l'espace. C'est dans ce contexte qu'en 2012 le cargo Dragon de SpaceX fut le premier engin ravitaillant l'ISS entièrement financé par une compagnie privée. La société dirigée par Elon Musk a depuis marqué la conquête spatiale de son empreinte en développant notamment un système de fusées réutilisables pour diminuer les coûts des lancements spatiaux. En mai 2020, SpaceX a réalisé le premier vol orbital avec un équipage à destination de l'ISS, permettant alors aux États-Unis

de marquer leur retour dans la course de l'exploration spatiale alors qu'ils dépendaient depuis une dizaine d'année de la Russie et de ses lanceurs Soyouz pour rejoindre l'ISS.

La diffusion en direct à la télévision des premiers pas sur la Lune et les nombreux films et séries d'aventure se déroulant dans l'espace ont nourrit les imaginaires et les rêves de voyages dans l'espace. L'émergence de sociétés privées a permis notamment à certaines personnes très fortunées de réaliser ce rêve. Alors que seuls les astronautes voyageaient dans l'espace (600 au total), l'ISS est maintenant accessible à des personnes non-astronautes, en échange de plusieurs dizaines de millions de dollars. À ce jour 13 touristes ont séjourné entre 7 et 12 jours à bord de l'ISS.

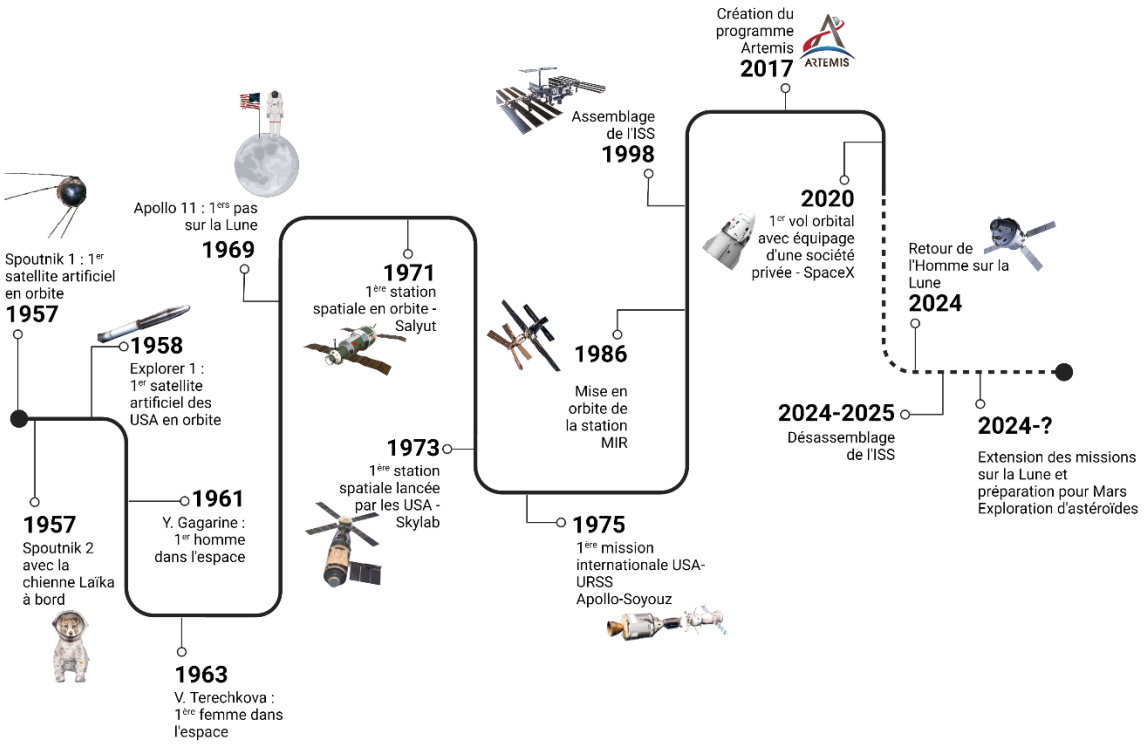


Figure 1 : Les grandes étapes de la conquête spatiale.

1.2 Prochains objectifs

1.2.1 Le projet Artemis : cap sur la Lune

La NASA et ses partenaires ont créé en 2017 le programme Artemis pour rediriger l'Homme, et plus particulièrement la femme vers l'exploration lunaire avec pour but préparer la prochaine grande étape de l'exploration spatiale vers la planète Mars. À ce jour, 24 personnes se sont rendues sur la Lune et seulement 12 astronautes ont pu fouler sa surface (National Aeronautics and Space Administration, 2022b). Depuis maintenant 50 ans, personne ne s'est rendu à nouveau sur la Lune. Le

plan actuel prévoit de lancer la fusée Space Launch System (SLS) et le vaisseau spatial Orion pour une première mission en novembre 2022, Artemis I, qui sera un vol d'essai sans équipage autour de la Lune. La deuxième mission Artemis II consistera à envoyer un équipage en orbite autour de la Lune à bord d'Orion lancé sur SLS au cours de l'année 2024. Une troisième mission, Artemis III, vise à envoyer des astronautes à la surface de la Lune et permettra notamment à la première femme astronaute de marcher sur la Lune. Le but est d'implanter une base lunaire au pôle Sud de la Lune, dont on sait maintenant qu'il contient de l'eau sous forme de glace. À terme, l'objectif est d'extraire l'eau du sol lunaire qui pourra être employée pour le refroidissement des équipements et la fabrication du carburant des véhicules pour les missions dans l'espace lointain. Grâce à Artemis, la NASA prévoit d'entretenir la diplomatie spatiale construite au fil des ans grâce à l'ISS en engageant un dialogue et une collaboration permanente avec les autres agences spatiales internationales. L'ESA, l'ASC et la JAXA ont finalisé des accords avec la NASA en vue de collaborer à la réalisation de la station lunaire Gateway, un avant-poste en orbite autour de la Lune. Ces accords marquent également l'intention de la NASA d'offrir aux futurs astronautes japonais et européens des possibilités de vol vers Gateway. L'accord entre l'ASC et la NASA comprend un engagement à offrir aux astronautes canadiens des vols vers la passerelle et sur la mission Artemis II.

1.2.2 Une vie extraterrestre bientôt possible pour l'Homme ?

Par la suite, l'ensemble des agences spatiales souhaitent étendre la conquête du système solaire notamment avec l'exploration de la planète Mars. Bien que cette vision ait nourri les imaginaires depuis des décennies, les agences spatiales promettent que ce projet sera bientôt à portée de main. Par exemple, le président de la Chinese Academy of Launch Vehical Technology (CALT) Wang Wiaojun a annoncé en 2021 à la Global Space Exploration Conference que la Chine sera en mesure d'effectuer le premier vol habité vers la planète Mars dès 2033 (Global Times, 2021). Voyager vers Mars constitue un challenge technique. La Terre et Mars étant toutes les deux en orbite autour du soleil mais selon des plans différents, la distance entre les deux planètes varie entre 55 et 400 millions de kilomètres. Parcourir ces 55 millions de kilomètres prendrait entre six et neuf mois (Space.com, 2022). S'élancer vers Mars ou revenir sur la Terre requiert d'être sûr que les deux planètes sont alignées, laissant alors une fenêtre de départ tous les 26 mois. Par conséquent, il sera nécessaire d'attendre 16 à 18 mois pour entamer le voyage de retour. Une mission entière vers Mars durerait donc en tout entre 30 et 35 mois, sans aucune possibilité de réapprovisionnement en nourriture, eau et énergie.

Concernant la nourriture, à l'heure actuelle aucun système alimentaire ne répond aux défis en matière de nutrition que ce soit en termes d'acceptabilité, de sécurité et de ressources que posent les

missions d'exploration prolongée (Douglas *et al.*, 2020; Smith *et al.*, 2021). La logistique pourrait imposer qu'un système alimentaire soit déjà présent dans l'espace (à bord d'une station spatiale en orbite autour de Mars par exemple) ou sur Mars même avant que l'équipage ne quitte la Terre. A la fin de la mission, le système alimentaire serait alors vieux de 5 à 7 ans. Plusieurs nutriments et facteurs de qualité critiques dans le système alimentaire de longue conservation pour les vols spatiaux se dégraderont à des niveaux inacceptables bien avant 5 ans de stockage. Des solutions pour garantir un système alimentaire nutritif et adéquat pour des durées prolongées sont nécessaires. Bien que les aliments de longue conservation (aliments lyophilisés, boites de conserve) présentent des avantages (familiers, faciles à préparer), de nouveaux traitements et emballages, de températures de stockage restent à évaluer. En revanche, ces aliments nécessitent une masse et un volume important lorsqu'ils sont lancés depuis la Terre. Bien que les compagnies privées aient permis de diminuer le cout de transport vers l'orbite basse, en moyenne 2720 euros par kilo à bord du Falcon 9 de SpaceX contre en moyenne 20000 euros par kilo entre les années 1970 et 2000 (Jones, 2018), ces chiffres pourraient exploser pour la planète Mars. Avec ces contraintes en tête, l'utilisation d'insectes semble prometteuse compte tenu de leur petite taille et de leur composition nutritionnelle en protéines élevée (Kok & van Huis, 2021). Alors que de nombreuses équipes se penchent sur le développement de plantes en gravité nulle ou modifiée (Carnero Diaz *et al.*, 2020), se nourrir de pommes de terre produites directement sur le sol martien comme le héros du film *Seul sur Mars* de Ridley Scott reste encore pour l'instant de la science-fiction.

Un système de production d'électricité efficace est également une condition *sine qua non* à toute mission sur une autre planète. Bien qu'à bord de l'ISS, l'électricité nécessaire à la station provienne exclusivement de l'énergie solaire, cette solution ne suffirait pas pour alimenter d'éventuelles bases extraterrestres. Mars par exemple présente un certain nombre de défis pour le fonctionnement des systèmes d'énergie solaire, notamment une atmosphère poussiéreuse qui modifie le spectre et l'intensité de l'éclairage solaire incident en fonction de l'heure de la journée. Des dépôts de poussières pourraient entrainer une dégradation des performances des panneaux solaires sans oublier la température très basse sur la planète (entre -95 et -4°C), qui pourrait également entacher le fonctionnement de ces systèmes (Landis *et al.*, 2004). Sur la Lune, des astronautes pourraient passer jusqu'à 16 jours dans l'obscurité pendant la nuit lunaire. Des recherches sont donc en cours pour trouver le meilleur système de production d'électricité et les chercheurs tendent à statuer que l'énergie nucléaire semble être une solution viable. La NASA a annoncé avoir signé un contrat avec trois sociétés pour développer des prototypes de systèmes de production par fission nucléaire sur la Lune dans le cadre du projet Artemis (National Aeronautics and Space Administration, 2022a). En revanche, implémenter ce type de système pose certaines questions, sachant que le

processus dégage de nombreux déchets radioactifs. Depuis la fin des années 1990, le potentiel de production d'énergie sur la Lune par fusion nucléaire à partir de tritium intéresse les scientifiques (National Aeronautics and Space Administration, 1988). Contrairement à la Terre qui est protégée par son champ magnétique, la Lune a été bombardée par de grandes quantité de tritium par le vent solaire. Les scientifiques suggèrent que cet isotope pourrait fournir une énergie nucléaire plus sûre dans un réacteur à fusion, car il n'est pas radioactif et ne produirait pas de déchets dangereux (European Space Agency, 2012). Bien que sur le papier cette technologie ne possède que des avantages, cela impliquerait d'exploiter la régolithe composant le sol lunaire.

La question de l'utilisation des ressources extraterrestre est épineuse. La Lune, Mars et les astéroïdes contenant de la matière première, il sera plus économique de les utiliser que de les ramener sur Terre. Concernant l'eau, nous savons que la Lune et Mars en possèdent sous forme solide. Cette eau pourrait être utilisable évidemment directement par les équipages mais après électrolyse elle deviendrait une source d'oxygène pour les équipages et d'hydrogène pour remplir les réservoirs de carburants. Des projets d'utilisation d'imprimantes 3D pour construire des bâtiments à partir du sol lunaire ou martien sont également évoqués (European Space Agency, 2019). Les astéroïdes et la Lune contiennent également des terres rares en quantité, qui sont des métaux indispensables à la haute technologie (composants de batteries de voitures électriques, dans les LED, les puces de smartphone etc). Alors que le Traité de l'espace établi en 1967 stipule qu'aucune nation ne peut prétendre détenir la Lune ou autre corps céleste, il est indiqué que leur exploration et utilisation est possible et doit être faite librement par tous les Etats sans aucune discrimination et dans des conditions d'égalité conformément au droit international (URSS *et al.*, 1967). Cependant, il est difficile d'envisager que l'exploitation industrielle et commerciale de ces ressources naturelles repose sur ces conditions d'égalité internationale (Mariez, 2020).

C'est dans ce contexte d'exploration spatiale que les durées des missions vont s'allonger. A titre d'exemple, une mission vers la Lune durerait plusieurs mois à un an alors qu'une mission vers la planète Mars durerait jusqu'à trois ans. Depuis les années 1960, la priorité des agences spatiale a été de caractériser les adaptations physiologiques à l'espace. Le vol de la chienne Laïka à bord de Spoutnik 2 en 1957 a montré pour la première fois la capacité de l'organisme à s'adapter à un voyage de courte durée en microgravité, il en est peut-être autrement pour les vols de durée plus longue. A ce titre, il est alors impératif de caractériser au mieux les adaptations physiologiques de l'exposition de longue durée à la microgravité afin et de développer des contremesures efficaces.

2 Adaptations physiologiques à la microgravité réelle et simulée

2.1 Étudier la physiologie humaine soumise à la microgravité réelle

Avant de permettre un vol habité par l'homme, la recherche animale dans l'espace a largement éclairé les scientifiques sur les adaptations induites par la microgravité. A la fin des années 1940, des drosophiles ont été les tous premiers êtres vivants à être envoyées dans l'espace à bord d'un missile lors d'un vol qui durera un peu plus de trois minutes. En 1949, le singe rhésus Albert II sera le premier mammifère à voyager dans l'espace. Depuis, la recherche animale a permis d'élargir considérablement les connaissances sur les effets de l'exposition aux radiations et à la microgravité sur les systèmes physiologiques, avec notamment le programme Bion érigé dans les années 1970 par les Russes, où 12 vols entre 5 et 30 jours se succéderont jusqu'en 2013.

Depuis le premier vol de Gagarine, les scientifiques ont mené de nombreux travaux pour préparer les astronautes à un séjour dans l'espace et pour comprendre les effets délétères de la microgravité sur les différents systèmes physiologiques. Entre 1998 et 2018, 3000 expérimentations scientifiques ont été réalisées à bord de l'ISS dont environ 300 qui portaient sur l'Homme (Witze, 2020). A titre d'exemple, le Human Research Program (HRP) de la NASA consacre 100 millions de dollars (0,4% de son budget total en 2023) à la recherche biologique et physique, dont fait partie la recherche humaine. Elle regroupe 22 axes de recherche différents sur l'Homme sur la physiologie de l'exercice, les capacités médicales, le comportement psychosocial, les effets des radiations, etc. (Milstead & Platts, 2022). A bord de l'ISS, les astronautes disposent de nombreux appareils de mesure pour conduire les expérimentations (**Figure 2**). Ils sont eux-mêmes les sujets des études ainsi que les expérimentateurs et travaillent en collaboration étroite avec les équipes scientifiques au sol comme le CADMOS, (Centre d'Aide au Développement des activités en Micropesanteur des Opérations Spatiales) dont le rôle est de préparer, organiser et assurer l'exécutions des missions scientifiques réalisées à bord de l'ISS.

En revanche, réaliser de la recherche biomédicale au cours des missions spatiales pose des difficultés pratiques, compte tenu des contraintes logistiques de ces missions et de leurs ressources limitées, tant sur le plan humain que sur le plan matériel. Il y a en effet peu de vols spatiaux vers l'ISS (entre 3 et 5 par ans) et le nombre d'astronautes par vol est restreint (entre 3 et 5 par lancement). Plusieurs vols spatiaux étalés sur plusieurs années seraient donc nécessaires pour obtenir une taille d'échantillon assez grande pour exploiter les données. De plus, les protocoles de recherche doivent s'insérer dans les emplois du temps très chargés des astronautes, qui occupent la majorité de leurs journées à l'entretien et la réparation du vaisseau spatial, à l'installations de nouveaux équipements,

et à la pratique de l'exercice physique pour assurer le maintien de leur santé et de leurs performances. Le volume des outils pour réaliser les examens et les protocoles de recherche est également un élément limitant, sachant que la place que va occuper chaque appareil doit être la plus petite possible. De plus, ces appareils doivent aussi répondre à des critères de sécurité très stricts. Ces caractéristiques font l'objet de longues études de développement pour adapter les appareils à l'environnement spatial (miniaturisation, résistance aux vibrations et aux radiations…). Les conditions expérimentales variants d'une mission à une autre, les données recueillies être peuvent modifiées ou interférer avec certains autres protocoles menés en même temps rendant ainsi très difficile la maitrise des protocoles.

<u>Figure 2</u> : Les appareils de recherche à bord de l'ISS.

A bord de l'ISS, les astronautes ont à disposition plusieurs appareils de mesure qui sont utilisés à la fois dans le cadre de leur suivi médical mais également dans des protocoles de recherche. En haut à gauche : l'astronaute Thomas Pesquet (ESA) en train d'utiliser le Pulmonary Function System (PFS) qui permet de mesurer les échanges gazeux (crédit NASA/ESA). En haut à droite : l'astronaute Tim Peake (ESA) sur le Muscle Atrophy Research and Exercise System (MARES) qui permer d'étudier les effets de la microgravité sur le système musculaire (crédit NASA/ESA). En bas à gauche : l'astronaute Karen Nyberg (NASA) en train d'utiliser le Space Linear Acceleration Mass Measurement Device (SLAMMD) qui permet de calculer la masse des astronautes en absence de gravité (crédit NASA). En bas à droite l'astronaute Chris Hadfield (ASC) photographié après avoir installé le PFS, le European Physiology Module (EPM), le Cardiolab (CDL) et le système Leg/Arm Cuff System (LACS) dans le cadre d'un protocole de recherche sur la régulation de la pression artérielle (crédit NASA).

Compte tenu des nombreuses difficultés pour étudier directement les adaptations physiologiques à la microgravité, les scientifiques ont mis au point des modèles simulant les effets de la microgravité sur Terre. Ces modèles permettent de tester les hypothèses scientifiques sur des échantillons plus importants. Ils offrent également la possibilité de mettre au point et tester des contremesures qui une fois validées seront testées en vol par les astronautes.

2.2 Les modèles d'étude de microgravité simulée

Les premiers modèles de simulation de microgravité sont apparus dès les années 1960. Bien que complètement supprimer la gravité soit impossible, le but de ces modèles est de recréer les principales adaptations à la microgravité qui sont l'hypokinésie (diminution des mouvements corporels), l'hypodynamie (diminution de la force et puissance musculaire) et le déplacement des fluides corporels vers le haut du corps due à la perte du gradient hydrostatique. La partie suivante vise à présenter les principaux modèles de simulation de microgravité chez l'Homme et l'animal (**Figure 3**) à savoir l'alitement prolongé, l'immersion sèche et les animaux suspendus par la queue.

2.2.1 Alitement prolongé

Dès les années 1970, l'alitement horizontal est pratiqué pour recréer les symptômes rencontrés par les astronautes au cours du vol spatial. Il est en revanche rapidement mis en avant qu'une des composantes essentielles de l'adaptation à la microgravité, à savoir la redistribution des fluides corporels du bas vers le haut du corps est absente. Différents angles d'inclinaison de la tête vers le bas de -15 à -4° ont été testés et selon les sensations rapportées par les astronautes il semble que les effets engendrés avec un angle de -6° soient les plus proches de ce que les astronautes vivent réellement dans l'espace. Depuis les années 1970, c'est donc l'alitement à tête déclive à -6° qui est le plus employé (**Figure X** en haut à gauche). En Europe, les études d'alitement prolongé financées en partie par l'ESA se déroulent sur trois sites : à Toulouse à la Clinique de l'espace gérée par l'Institut de Médecine et de Physiologie Spatiale (MEDES), à Cologne en Allemagne au Centre de Recherche Envihab et en Slovénie à l'Olympic Sport Centre Planica dans la ville de Ratece.

2.2.2 Immersion sèche

Contrairement aux protocoles d'alitement incliné où les participants sont en contact avec un support, l'immersion dans l'eau permet de réduire significativement l'effet de la gravité grâce à la poussée d'Archimède exercée sur le corps créant alors une flottaison totale. Cette approche a été

utilisée à partir des années 1960. L'immersion prolongée dans l'eau peut toutefois mener à des effets indésirables cutanés (Tsai & Maibach, 1999). Depuis, le modèle d'immersion sèche a été inventé dans les années 1970 par deux chercheurs russes de l'Institut des problèmes biomédicaux de Russie (IBMP) à Moscou (Navasiolava *et al.*, 2011). Ce système consiste à immerger le corps des volontaires jusqu'au cou tout en les maintenant au sec avec un tissu étanche élastique (**Figure 3** à droite). Ce modèle permet de mettre en place des protocoles plus longs (jusqu'à 56 jours) en reproduisant des effets similaires à ceux retrouvés dans les protocoles d'alitement prolongé (Tomilovskaya *et al.*, 2019). Pendant longtemps l'utilisation de ce modèle n'était faite qu'en Russie, et récemment grâce à la collaboration entre le Centre National des Etudes Spatiales (CNES) et l'IBMP, deux baignoires d'immersion sèches ont été installées en 2015 au MEDES à Toulouse. Comparé avec l'alitement prolongé, l'immersion sèche permet de recréer les effets de la microgravité plus rapidement. Par exemple, au bout d'une semaine d'immersion sèche, les pertes au niveau des fonctions contractiles musculaires sont similaires à celles observées après deux mois d'alitement (Shenkman & Kozlovskaya, 2019).

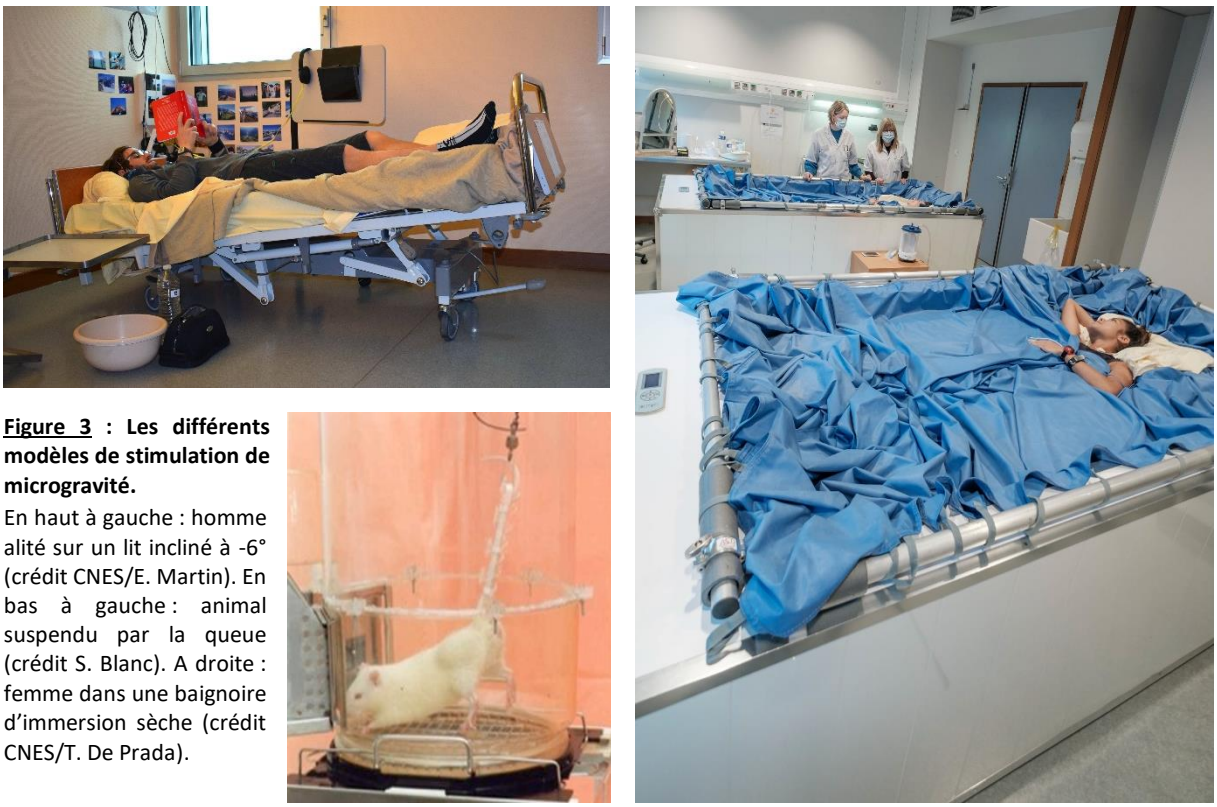


Figure 3 : Les différents modèles de stimulation de microgravité.
En haut à gauche : homme alité sur un lit incliné à -6° (crédit CNES/E. Martin). En bas à gauche : animal suspendu par la queue (crédit S. Blanc). A droite : femme dans une baignoire d'immersion sèche (crédit CNES/T. De Prada).

2.2.3 Animaux suspendus par le train arrière

Bien que les modèles de simulation de microgravité chez l'Homme cités précédemment permettant d'investiguer tous les systèmes physiologiques, les études chez le petit animal permettent des études plus invasives. Le modèle de suspension par la queue a été développé par la NASA dans les années 1970 et est la méthode la plus largement utilisée pour simuler les effets des vols spatiaux chez

les rongeurs (**Figure 3** en bas à gauche). L'animal est suspendu par le train arrière par un harnais au niveau du bassin ou une attache à la base de la queue et cette position permet de recréer l'hypodynamie et l'hypokinésie au niveau des pattes postérieures ainsi que le changement de répartition des fluides vers la tête induite par la microgravité.

Tous ces modèles d'études ont donc été indispensables pour caractériser les adaptations induites par l'exposition à la microgravité, qui sont présentées dans la partie suivante.

2.3 Réponses physiologiques à la microgravité

La force gravitationnelle, au même titre que la lumière ou l'oxygène a été une contrainte majeure de l'évolution sur les êtres vivants. Chez les Vertébrés, elle a notamment façonné la mise en place des systèmes cardiovasculaire, squelettique, musculaire, et neuro-vestibulaire. L'absence de gravité entraîne des dysfonctionnements de ces systèmes, c'est ce que l'on appelle le déconditionnement spatial. Ces adaptations sont résumées dans la **Figure 4** et seront développées dans les parties 2.3 et 3.

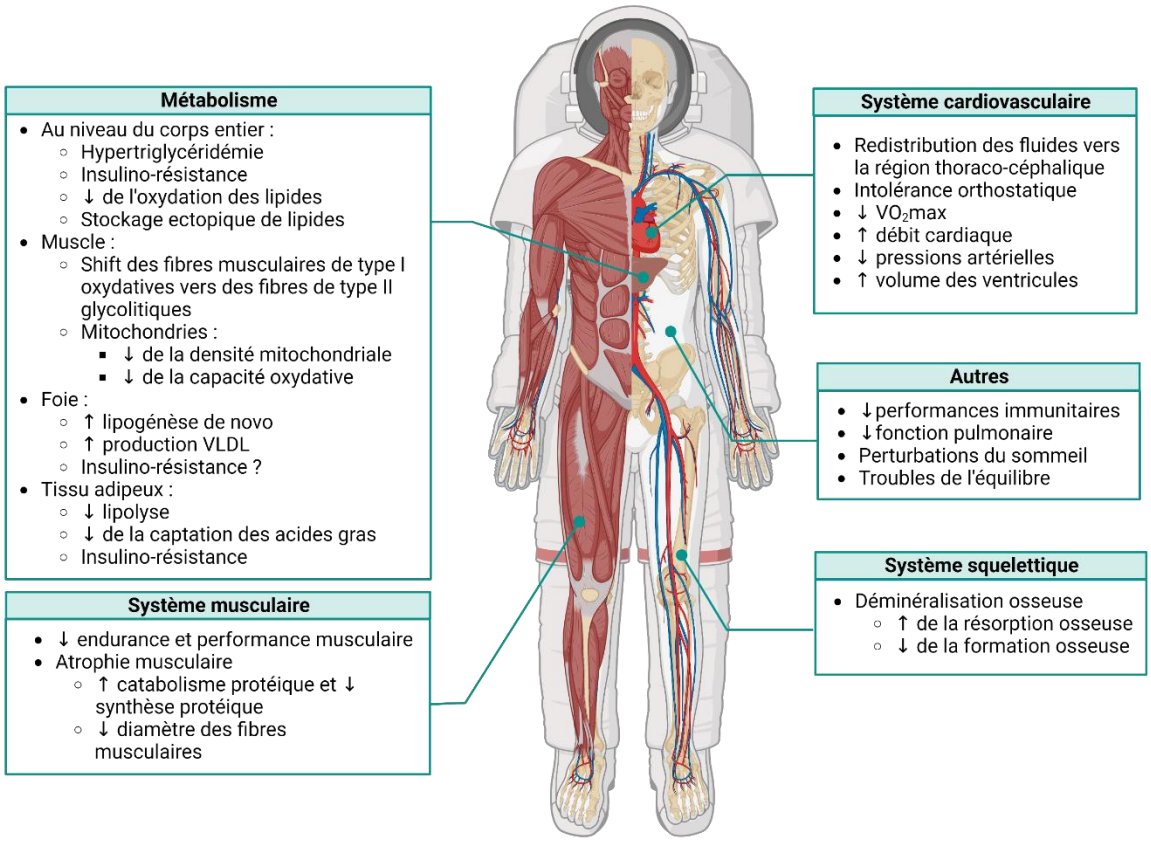


Figure 4 : Les principales adaptations induites par la microgravité.

2.3.1 Système cardiovasculaire : un déconditionnement général

L'évolution a fait que le système cardiovasculaire de l'homme est adapté à la position debout, *i.e.*, l'orthostatisme. L'orthostatisme induit une distribution inégale des liquides de l'organisme avec une accumulation des fluides au niveau des membres inférieurs. Nous parlons alors d'un gradient de pression du volume sanguin qui suit le vecteur de gravité, avec une pression plus faible au niveau de la tête et du thorax et qui augmente vers les pieds. Le système cardiovasculaire et son fonctionnement sont adaptés à cette position debout et le fait de supprimer la gravité entraine des modifications multiples de ses mécanismes de régulation. La microgravité induit une redistribution du volume sanguin immédiate vers la région thoraco-céphalique se traduisant par un gonflement du visage et un affinement des jambes (Kirsch *et al.*, 1993). Ces changements sont associés à une réduction de la pression intrathoracique par expansion du thorax et une augmentation du volume cardiaque (Aubert *et al.*, 2016). Cette redistribution des fluides notamment au niveau du cœur est interprétée par l'organisme comme une augmentation de la volémie ce qui entrainera des réponses régulatrices menant à une hypovolémie (Hughson *et al.*, 2018) par inhibition du système rénine-angiotensine-aldostérone et une augmentation de la synthèse du peptique atrial natriurétique. Ce phénomène est associé à une perméabilisation des capillaires en condition de microgravité entrainant un déplacement de fluides du milieu intravasculaire vers le milieu extravasculaire (Leach *et al.*, 1996). Cette hypovolémie se produit dans les 12 premières heures après l'arrivée dans l'environnement spatial et reste stable tout le long du séjour. En revanche, elle est une cause importante du déconditionnement cardiovasculaire (voir détails ci-dessous) et entraine des symptômes importants à gérer lors du retour en environnement avec de la gravité (Terre, Lune, Mars) comme de l'intolérance orthostatique et une réduction importante de la capacité d'exercice (Convertino, 2002; Hodkinson *et al.*, 2017).

Le système nerveux autonome, chef d'orchestre régulateur de la pression artérielle en position debout, est également sensible à la microgravité. En conditions physiologiques, ses deux composantes à savoir les systèmes nerveux sympathique et parasympathique agissent de façon antagoniste. L'exposition à la microgravité modifierait la balance entre le système sympathique et parasympathique se traduisant notamment par des modifications au niveau de la variabilité de la fréquence cardiaque (Otsuka *et al.*, 2016) ainsi qu'une altération du système baroréflexe après des voyages de courte ou longue durée dans l'espace (Eckberg *et al.*, 2010).

Des études ont montré que le muscle cardiaque subit un remodelage important en environnement de microgravité. Des hommes et des femmes en bonne santé alités de 60 à 70 jours ont présenté une diminution des volumes des ventricules entre 11 et 20% et une diminution de la masse des ventricules comprise entre 8 et 25% (Perhonen *et al.*, 2001; Dorfman *et al.*, 2007; Westby

et al., 2016; Scott *et al.*, 2018). Cette atrophie des ventricules est notée dès 7 jours d'alitement (Scott *et al.*, 2018). Ces observations ont été également mises en évidence chez des astronautes ayant passé entre 9 et 16 jours dans l'espace (Perhonen *et al.*, 2001; Summers *et al.*, 2005).

Le débit cardiaque, le rythme cardiaque ainsi que les pressions artérielles sont modifiés en conditions de microgravité. Bien que la fréquence cardiaque ne diminue pas systématiquement, les pressions artérielles diminuent et le débit cardiaque augmente chez des sujets exposés à de la microgravité réelle ou simulée (Baran *et al.*, 2021). D'autres études mettent également en évidence une altération de la structure et de la fonction des artères en réponse à la microgravité (Navasiolava *et al.*, 2020) ainsi que des anomalies du rythme cardiaque (Shen & Frishman, 2019). Une des conséquences de la diminution du débit cardiaque est la diminution de l'absorption d'oxygène. Il a été montré que la consommation maximale d'oxygène par le muscle squelettique (VO_2max) diminue lors de l'alitement, mais se stabilise lorsque l'alitement se prolonge, indépendamment de la diminution de la VO_2max et la diminution du poids corporel et de la masse maigre (Greenleaf *et al.*, 1989; Kramer *et al.*, 2017; Ried-Larsen *et al.*, 2017).

L'ensemble de ces données montrent effectivement un impact de la microgravité (réelle ou simulée) sur les fonctions cardiaques et vasculaires, représentant ainsi un risque non négligeable pour les astronautes à court terme pendant les missions.

2.3.2 Système musculaire : un remodelage structurel et fonctionnel

Immédiatement au retour sur Terre, il est très courant de voir les astronautes être portés en raison des difficultés à se tenir debout et de marcher. En situation de microgravité, la diminution de la sollicitation de la charge mécanique induit une atrophie musculaire associée à une diminution de la force et de l'endurance musculaire dès les premières semaines d'exposition à la microgravité. Tout comme le système squelettique, les muscles des membres inférieurs et posturaux sont particulièrement affectés (Mulder *et al.*, 2014; Parry & Puthucheary, 2015; Dirks *et al.*, 2016). Une diminution de la masse maigre totale de 0,57% en moyenne et une diminution de 1% de masse maigre au niveau des jambes par mois passé dans l'espace a été notée chez des cosmonautes ayant séjourné entre 4 et 14 mois à bord de la station Mir (LeBlanc *et al.*, 2000b). Les clichés d'imagerie par résonance magnétique (IRM) de trois astronautes ayant passé entre 8 et 15 jours dans l'espace montrent une diminution du volume du muscle extenseur du genou de 5,6% à 15,4% (Akima *et al.*, 2000). Une diminution du volume musculaire des grands groupes musculaires de l'ordre de 3 à 10% et de 5 à 17% a été remarquée chez des astronautes après 17 jours et 16 à 38 semaines de mission, respectivement (LeBlanc *et al.*, 2000a). L'exposition à la microgravité réelle lors de missions de durée plus longue à

bord de l'ISS (plus au moins six mois) induit des pertes de masse musculaire jusqu'à -20% au niveau du dos ainsi que des diminutions allant jusqu'à -12% pour le quadriceps. Cette perte de masse au niveau de la jambe peut mener alors jusqu'au une perte de 25 à 30% de la force de contraction du mollet (LeBlanc *et al.*, 2000a; Trappe *et al.*, 2009; Fitts *et al.*, 2010).

Plusieurs facteurs participent à la perte de masse musculaire. Premièrement, le diamètre des fibres musculaires diminue et cette diminution dépend du type de fibre musculaire. Au niveau du muscle soléaire et du vaste latéral, les fibres de type II qui correspondent aux fibres glycolytiques rapides présentent une diminution de leur surface de section transversale (en anglais CSA pour cross-sectional area) supérieure à celle des fibres de type I correspondant aux fibres oxydatives lentes après 5 à 17 jours de vol (Edgerton *et al.*, 1995; Widrick *et al.*, 1999). Dans une revue, Phillips et al. suggèrent que la section transversale des fibres diminue de 0,6% par jours d'alitement (Phillips *et al.*, 2009).

La masse musculaire est régulée par un équilibre entre synthèse et dégradation protéique qui constitue la balance protéique. La balance azotée est l'un des indices reflétant la balance protéique. Les protéines sont des polymères d'acides aminés qui comportent des groupements azotés. Elle est calculée en soustrayant l'excrétion en azote des urines aux apports en azote de l'alimentation. Une balance azotée négative indique une excrétion en azote supérieure aux apports ce qui reflète une dégradation des protéines. Au contraire, dans le cas d'une balance azotée positive, les apports en azote sont supérieurs à l'excrétion, traduisant une augmentation de la synthèse protéique. L'atrophie musculaire rencontrée systématiquement chez les astronautes suggère une dérégulation de cette balance protéique. Elle peut être le résultat d'une diminution de la synthèse protéique ou d'une augmentation du catabolisme protéique ou les deux. Une balance azotée négative a été mesurée chez des astronautes ayant séjourné dans l'espace entre 28 et 84 jours lors des missions Skylab (Lane, 1992) ainsi que chez des sujets ayant été alités pendant 14 jours (Ferrando *et al.*, 1996). Plusieurs études ont montré qu'au niveau du corps entier chez l'homme, l'origine de cette balance azotée négative était une diminution de la synthèse protéique (Stuart *et al.*, 1990; Ferrando *et al.*, 1996; Biolo *et al.*, 2004) plutôt qu'une augmentation du catabolisme protéique.

Des études chez l'animal ont permis d'aller plus loin dans la compréhension des mécanismes cellulaires à l'origine de l'atrophie musculaire induite par l'exposition à la microgravité notamment concernant le catabolisme protéique. Bien que certains mécanismes restent encore à élucider, il a été établit qu'une diminution de l'activation de la voie de signalisation IGF-1/Akt/mTOR est impliquée dans la diminution de la synthèse protéique en conditions d'immobilisation (Gao *et al.*, 2018). Cette voie est impliquée dans la régulation de la masse musculaire (**Figure 5**). Cet axe de signalisation régule notamment l'initiation de la translation (synthèse des protéines à partir d'ARNm) au niveau du muscle (Schiaffino & Mammucari, 2011). La fixation de l'hormone l'Insulin Growth Factor 1 (IGF-1) sur son

récepteur déclenche une cascade de signalisation stimulant l'activité tyrosine kinase de l'insulin receptor substrate-1 (IRS-1) activant alors le phosphatidylinositol 3 kinase (PI3K). PI3K peut alors phosphoryler phosphatidylinositol-4,5-biphosphate (PIP2) en phosphatidylinositol-3,4,5-triphosphate (PIP3). PIP3 recrute par la suite la protein kinase (PDK) qui active Akt par phosphorylation qui lui-même active mammalian target of rapamycin complex 1 (mTORC1). La protéine mTORC1 phosphoryle alors les deux protéines 4E-BP1 (eukaryotic translation initiation factor 4E-binding protein 1) et S6K1 (S6 kinase 1) entrainant ainsi l'activation de la synthèse protéique en initiant la translation. Alors que des études menées chez l'animal montrent une diminution de l'activation de cette voie, son implication dans la diminution de synthèse protéique en conditions d'immobilisation chez l'homme n'est pas claire (Gao *et al.*, 2018). Bien que 14 et 23 jours de suspension unilatérale de jambe aient entrainé une diminution du taux de synthèse protéique, aucun changement au niveau de l'activation de la voie IFG1/Akt/mTOR n'a été noté (de Boer *et al.*, 2007; Glover *et al.*, 2008). Ces résultats suggèrent que la diminution de la synthèse protéique observée en condition de microgravité pourrait être régulée par d'autres voies de signalisation (Gao *et al.*, 2018). En parallèle, le catabolisme des protéines semble être impliqué dans l'atrophie musculaire et plus particulièrement via la voie IGF-1/Akt/FOXO. En conditions normales, la phosphorylation de Akt inhibe l'action de la famille de facteurs de transcription FOXO (forkhead family of transcription factors). FOXO est connu pour activer la transcription de MuRF1 (muscle RING-finger protein 1) et MAFbx/atrogin-1 (muscle atrophy F-box), deux ubiquitines ligases impliquées dans l'atrophie musculaire (Bodine & Baehr, 2014). En conditions de microgravité ou d'immobilisation, plusieurs études montrent que les taux d'ARNm des protéines FOXO1 et/ou FOXO3 sont augmentés au niveau de muscles présentant une atrophie (Gao *et al.*, 2018). Il a été montré que les niveaux d'ARNm des gènes codants pour MuRF1 et atrogin-1 augmentent rapidement chez des animaux suspendus par la queue, dénervés ou ayant séjourné dans l'espace (Gao *et al.*, 2018). Il a également été montré que des souris MuRF1-KO suspendues par la queue pendant 10 jours présentent une atrophie nettement moins sévère du muscle soléaire comparée aux souris de type sauvage également suspendues (Labeit *et al.*, 2010). Une augmentation de la transcription de ces deux protéines a également été notée chez l'homme immobilisé pendant deux et cinq jours (Abadi *et al.*, 2009; Dirks *et al.*, 2014) ainsi qu'après 20 jours d'alitement prolongé (Ogawa *et al.*, 2006). En revanche, l'augmentation de l'expression de la protéine MuRF1 a été notée uniquement au niveau du muscle soléaire et non au niveau du vaste latéral chez des femmes alitées pendant 60 jours (Salanova *et al.*, 2008).

Le catabolisme protéique est également augmenté en condition de microgravité et d'immobilisation par le stress oxydant (Powers *et al.*, 2012) et l'inflammation qui seront développés dans la partie suivante. Rapidement, l'augmentation de la formation d'espèces réactives de l'oxygène

(ROS) augmenterait la transcription et l'activation de NF-κB (nuclear factor-kappa B) qui participe également à l'augmentation de la transcription de MuRF1 (Dodd *et al.*, 2010). En parallèle, l'augmentation de la concentration plasmatique de TNF-α (tumor necrosis factor α), cytokine pro-inflammatoire, en condition d'alitement (Schmitt *et al.*, 1996) augmenterait également la transcription de NF-κB et par cascade également MuRF1.

En parallèle, plusieurs études suggèrent que la lipotoxicité induite par l'accumulation de composés lipidiques (diacylglycérols, céramides) au sein du muscle (développé dans la partie 3.4) pourrait jouer un rôle dans l'atrophie musculaire (Meex *et al.*, 2019). Dans des modèles de culture cellulaire l'accumulation d'acide palmitique sous forme de céramides a été associée à une augmentation de l'expression de MAFbx et de FOXO3 (Turpin *et al.*, 2006; Bryner *et al.*, 2012). Au contraire, bloquer la formation de céramides a permis d'augmenter la signalisation issue de mTOR et de diminuer les expressions de MAFbx et FOXO3 (Rivas *et al.*, 2016). De plus, des analyses de contenu en céramides spécifiques du muscle squelettique de l'homme âgé ont montré que le contenu des céramides C16 était négativement associée à la masse maigre de la jambe et associée à une réponse atténuée de certaines molécules impliquées dans la synthèse musculaire normalement activées par l'exercice de haute intensité comme Akt, FOXO1 et S6K1 (Rivas *et al.*, 2012).

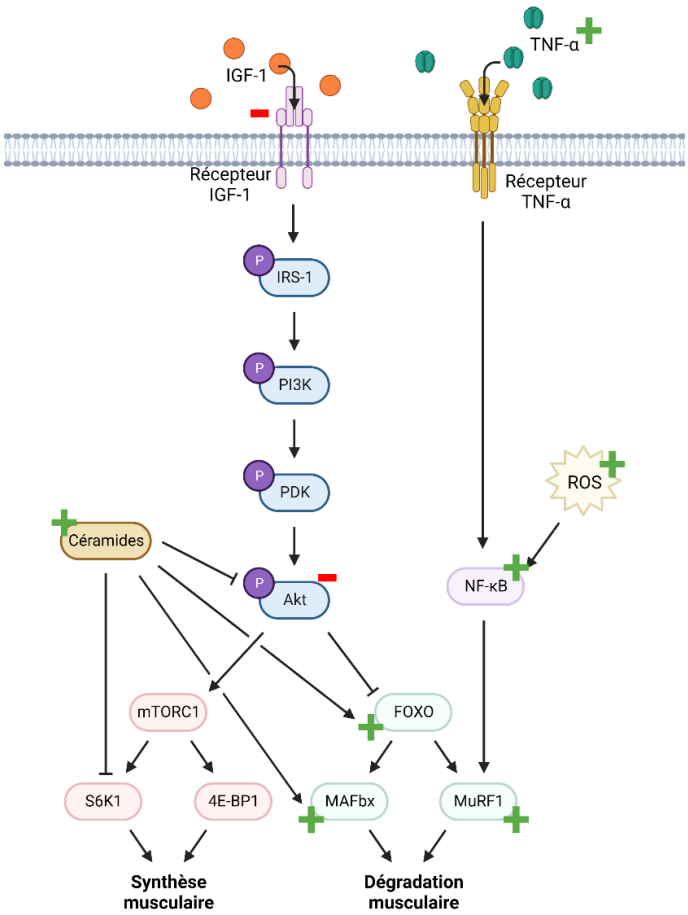


Figure 5 : Représentation schématique de la voie de signalisation IGF/PDK/Akt dans la synthèse et la dégradation protéique et effets potentiels de la microgravité.

Le signe moins en rouge désigne une diminution de l'expression génique, une diminution de l'activité en condition de microgravité ou une diminution de la concentration. Le signe plus en vert désigne une augmentation de l'expression génique, une augmentation de l'activité ou une augmentation de la concentration. 4E-BP1, Eukaryotic translation initiation factor 4E-binding protein1; Akt, Protein kinase B; IGF-1, Insulin growth factor-1; IRS-1, Insulin receptor substrate 1; FOXO, forkhead family of transcription factors; MAFbx, Muscle atrophy F-box, aussi appelé Atrogin-1; mTORC1, Mammalian target of rapamycin complex 1; MuRF1, Muscle ring finger-1; NF-κB, Nuclear factor-kappa B; PDK, Protein kinase; PI3K, phosphatidylinositol 3 kinase; ROS, Reactive oxygen species; S6K1, S6 kinase 1; TNF-α, Tumor necrosis factor alpha.

2.3.3 Système squelettique : modifications de la masse, de la structure et de la résistance de l'os

Le manque de sollicitation des membres en raison de la suppression du stress gravitationnel a pour principale conséquence d'induire une perte de masse osseuse. Les premières observations de pertes osseuses ont été notées chez des astronautes ayant séjourné à bord de Skylab dans les années 1970 présentant une diminution de la masse osseuse de 1 à 2% par mois passés dans l'espace (Whedon *et al.*, 1976; Rambaut & Johnston, 1979; Tilton *et al.*, 1980). Cette variation est cliniquement significative ; elle est équivalente à celle observée de façon annuelle chez les femmes ménopausées (Bloomfield, 2010). Plus précisément, les os dits porteurs seraient les plus sensibles à la perte de masse osseuse en condition de microgravité (Collet *et al.*, 1997) via une diminution de la densité minérale (masse osseuse par unité de volume) après voyage dans l'espace (Lang *et al.*, 2004; Sibonga *et al.*, 2007; Dana Carpenter *et al.*, 2010).

La compréhension des mécanismes cellulaires à l'origine de la perte osseuse des astronautes se base exclusivement sur des analyses de sang ou d'urine qui donnent accès à des biomarqueurs reflétant les activités de formation et de résorption osseuse. Ainsi, une diminution des marqueurs de formation osseuse tels que l'alkaline phosphatase, le collagène de type 1, et l'ostéocalcine sont observées. En parallèle, une augmentation des marqueurs de résorption osseuse comme le N-telopeptide et un métabolisme calcique perturbé (augmentation de l'excrétion urinaire et diminution de l'absorption de calcium) sont notées en vol (Caillot-Augusseau *et al.*, 1998; Smith *et al.*, 1999). Ces modifications reflètent l'homéostasie perturbée du système squelettique qui ont été également été observées au cours des protocoles d'exposition à la microgravité simulée. Des sujets sains alités avec la tête déclive pendant 6 à 90 jours ont présenté une augmentation des marqueurs de résorption osseuse (Watanabe *et al.*, 2004; Heer *et al.*, 2005; Smith *et al.*, 2008; Morgan *et al.*, 2012), une diminution des marqueurs de formation osseuse (Smith *et al.*, 2008; Smith *et al.*, 2009), une perturbation de la balance calcique (Heer *et al.*, 2005; Morgan *et al.*, 2012), une diminution de la densité minérale de la hanche et des os des membres inférieurs (Watanabe *et al.*, 2004; Rittweger *et al.*, 2005; Smith *et al.*, 2008; Armbrecht *et al.*, 2011) ainsi qu'une diminution de l'épaisseur corticale (Armbrecht *et al.*, 2011). Certains de ces changements ont été observés à des stades très précoces d'alitement avec notamment une augmentation des marqueurs de résorption osseuse dès 24h après le début de l'immobilisation (Heer *et al.*, 2005). Ces protocoles d'alitement associés aux études d'immobilisation de l'arrière-train chez l'animal ont permis d'élucider les mécanismes à l'origine de cette fragilité osseuse et montrent que les modifications de la balance formation/résorption osseuse sont le résultat de l'activité diminuée des ostéoblastes et à l'opposé d'une augmentation de l'activité

des ostéoclastes, qui sont les cellules cheffes d'orchestre de l'homéostasie osseuse (Carmeliet *et al.*, 2001).

Ces altérations sont préoccupantes sachant qu'elles ne semblent pas être réversibles. Effectivement, un an après le retour sur Terre, la densité minérale et l'épaisseur corticale d'astronautes ayant séjourné au moins 6 mois sur l'ISS étaient significativement inférieures aux valeurs d'avant le vol (Gabel *et al.*, 2022). Sur les 17 astronautes inclus dans cette étude, 9 n'avaient pas recouvert leur densité minérale au niveau du tibia un an après la fin de la mission à bord de l'ISS. Les études au sol montrent que les hommes et les femmes ayant été alités pendant 60 et 90 jours ont récupéré l'épaisseur corticale distale du tibia un an après la fin des protocoles mais le contenu trabéculaire lui ne semble pas récupérer même après un à deux ans après la fin de l'alitement (Armbrecht *et al.*, 2011; Belavy *et al.*, 2011; Cervinka *et al.*, 2014). À terme, ces détériorations pourraient se superposer à l'ostéopénie liée au vieillissement et ainsi augmenteraient le risque de fracture. Ce risque est probablement encore plus élevé pour les femmes qui en raison des changements hormonaux liés à la ménopause sont plus exposées au risque d'ostéoporose (Grimm *et al.*, 2016).

2.3.4 Perturbation de la balance oxydative et inflammation de bas grade chronique

La balance rédox définit un équilibre entre les molécules pro-oxydantes et anti-oxydantes. Les molécules pro-oxydantes peuvent être de deux types : les espèces réactives de l'oxygène (reactive oxygen species : ROS, souvent appelés radicaux libres ; exemple O_2^-, H_2O_2) et de l'azote (reactive nitrogen species : RNS ; exemple NO^-, NO_2^-). Bien que ces molécules soient indispensables pour le bon fonctionnement cellulaire, une haute concentration peut provoquer des dommages aux composants cellulaires tels que l'ADN, les lipides et les protéines (Di Meo *et al.*, 2016). Les RONS (reactive oxygen and nitrogen species) peuvent être de nature exogène (pollution, pesticides, métaux lourds, radiations) ou endogène c'est-à-dire produits au sein même de l'organisme. Au sein de la cellule, la majorité des ROS sont dérivés de la mitochondrie et plus particulièrement au niveau de la membrane mitochondriale où les complexes I et III de la chaîne de transport des électrons produisent des ROS (Turrens, 1997). Les molécules anti-oxydantes, ont pour rôle de contrecarrer les effets des RONS en prévenant notamment leur production et en bloquant leurs effets. Elles peuvent être d'origine enzymatique (exemple xanthine oxydase, superoxyde dismutase) ou non-enzymatique (exemple acide α-lipoique, coenzyme Q10, vitamine E, polyphénols). Le stress oxydant est alors défini par un « déséquilibre existe dans la balance rédox en faveur des pro-oxydants, menant à des perturbations des signaux rédox et/ou des dommages moléculaires » (Sies & Jones, 2007). Le stress oxydant non

contrôlé et chronique est impliqué dans l'étiologie de la résistance à l'insuline (Kim *et al.*, 2008), de nombreux cancers et autres pathologies chroniques et aigues ainsi qu'un facteur de vieillissement (Pizzino *et al.*, 2017). Les premières données évoquant une perturbation de la balance rédox chez l'astronaute datent des années 1990 où les molécules marqueurs du stress oxydant comme la 8-isoprostane, la 8-hydroxydésoxyguanosine (8-OHdG) et les protéines carbonylées (marqueurs des dommages oxydatifs sur les lipides, l'ADN et les protéines, respectivement) ont été mesurées chez des astronautes exposés à de la microgravité réelle ou simulée. Concernant les 8-isoprostanes, aucun changement n'est constaté dans les urines après 17 jours d'alitement ni après 17 jours passés à bord de Space Shuttle (Stein & Leskiw, 2000). Une diminution de la concentration dans les urines a été notée après le vol à bord de Space Shuttle et pendant le vol dans la station Mir. En revanche, une augmentation significative de l'excrétion des 8-isoprostanes dans les urines est notée après 3-4 mois à bord de la station Mir et après 60 jours d'alitement (Stein & Leskiw, 2000; Dolopikou *et al.*, 2020). L'excrétion urinaire de 8-OHdG augmente uniquement après le vol de longue durée à bord de Mir (Stein & Leskiw, 2000). Stein et ses collaborateurs ont attribué les changements de concentration à une diminution de production de radicaux libres en raison d'une diminution de l'apport énergétique en vol et ont suggéré que l'augmentation notée après le vol serait due à une augmentation de l'activité métabolique et la diminution de défenses anti-oxydantes au cours du vol. Les données concernant les protéines carbonylées et la microgravité sont mitigées dans la littérature. Alors qu'une augmentation de la concentration des protéines carbonylées est notée au niveau musculaire ou dans le plasma lors d'alitement prolongé de 35 et 60 jours chez l'homme (Dalla Libera *et al.*, 2009; Dolopikou *et al.*, 2020) ainsi qu'après des immobilisations de 7 à 14 jours chez l'animal (Ota *et al.*, 2011; Derbre *et al.*, 2012; Vitadello *et al.*, 2014), aucune évolution n'est observée après 14 jours d'immobilisation du genou, ni après 7 à 14 jours de suspension de l'arrière train chez le rat ou la souris (Cannavino *et al.*, 2014; Cannavino *et al.*, 2015; Dupre-Aucouturier *et al.*, 2015). Des études chez l'animal ayant séjourné dans l'espace suggèrent que les défenses anti-oxydantes, elles, sont diminuées au cours du vol (Indo *et al.*, 2016).

Le muscle squelettique est un organe endocrine produisant et relarguant dans la circulation systémique de nombreuses cytokines appelées myokines. Ces molécules sont particulièrement sécrétées pendant la contraction musculaire et constituent un réseau de communication inter-organes (Severinsen & Pedersen, 2020). Parmi leurs nombreux rôles, certaines sont connues pour réduire l'inflammation. Le manque de contraction musculaire est associé à une inflammation chronique qui est impliquée dans l'étiologie de nombreuses altérations physiologiques comme la résistance à l'insuline, la dyslipidémie, la dysfonction endothéliale, l'atrophie musculaire etc. Ceci augmente alors le risque de développer des pathologies chroniques dont les maladies cardiovasculaires, le diabète de

type 2, la stéatose hépatique non-alcoolique chez les gens ne pratiquant pas d'activité physique (Booth *et al.*, 2012). Des données suggèrent que l'inactivité physique induite par la microgravité réelle ou simulée perturbe le profil inflammatoire des astronautes. En vol, les concentrations de molécules pro-inflammatoires mais également anti-inflammatoires augmentent (Crucian *et al.*, 2014; Voorhies *et al.*, 2019; Trudel *et al.*, 2022). Au cours de l'étude TWIN, les signatures omiques d'un astronaute ayant séjourné un an dans l'espace et son jumeau resté à terre ont été examinées. En plus d'une perturbation du profil inflammatoire, une augmentation de l'abondance dans le plasma d'acides gras pro et anti-inflammatoires a été noté, suggérant une augmentation du statut inflammatoire au cours des missions de longue durée dans l'espace (Garrett-Bakelman *et al.*, 2019). Au sol, le profil inflammatoire a été investigué dans plusieurs protocoles d'alitement prolongé et d'immersion sèche de 3 à 90 jours (Hamburg *et al.*, 2007; Biolo *et al.*, 2008; Mazzucco *et al.*, 2010; Rudwill *et al.*, 2013; Brooks *et al.*, 2014; Mutin-Carnino *et al.*, 2014; Jurdana *et al.*, 2015; Linossier *et al.*, 2017). Ces études montrent des variations très hétérogènes mais soulignent en effet une modification du statut inflammatoire sans tendance claire vers la mise en place d'une inflammation chronique ou d'un profil anti-inflammatoire.

Bien que les liens entre stress oxydant, inflammation et microgravité ne soient pas tout à fait évident, nous savons qu'inflammation et stress oxydant sont intimement liés (Di Virgilio, 2004). Ces processus ne sont pas à négliger d'autant qu'ils sont tous les deux impliqués dans de nombreuses altérations physiologiques associées à la microgravité comme les dysfonctions cardiovasculaires (Takahashi *et al.*, 2017), l'atrophie musculaire (Powers *et al.*, 2012) mais aussi des troubles liés au métabolisme intermédiaire.

2.3.5 Balance énergétique : une perte de poids systématique

Pendant la plupart des vols spatiaux, une perte de poids a été observée. Des pertes de masse corporelle de plus de 5% ont été observées chez des astronautes ayant séjourné 4 mois à bord de la station Mir (Smith *et al.*, 1999; Smith *et al.*, 2001), après 4 à 19 jours à bord de la fusée Shuttle (Stein *et al.*, 1999a; Wade *et al.*, 2002) ou encore plus récemment après des missions de 4 à 6 mois à bord de l'ISS (Smith *et al.*, 2005; Matsumoto *et al.*, 2011) malgré la présence de nourriture en quantité suffisante. Cette perte de masse ne présente pas de relation claire avec la durée de la mission (Laurens *et al.*, 2019). Dans certains cas, la perte de masse dépasse même les 10% de masse initiale, devenant ainsi cliniquement signifiant. A l'opposé, la masse corporelle a été maintenue au cours des missions SLS1 et SLS2 dans les années 1990 à bord du vaisseau Spacelab (Stein *et al.*, 1996) ou encore plus récemment à bord de l'ISS (Smith *et al.*, 2005; Smith *et al.*, 2012). Les rapports les plus récents montrent qu'à bord de l'ISS les astronautes perdent encore entre 2 à 5% de leur masse initiale avec

une large variabilité inter-individuelle (Matsumoto *et al.*, 2011; Zwart *et al.*, 2014). Notre équipe a fait l'hypothèse que cette diminution de masse corporelle était la conséquence d'une perturbation du couplage entre les apports énergétiques et la dépense énergétique totale journalière (Laurens *et al.*, 2019), mais peu de données existent à ce jour pour supporter cette hypothèse.

La dépense énergétique totale avec la méthode de référence de l'eau doublement marquée (DLW pour doubly labelled water en anglais) a été mesurée seulement sur des vols de courte durée (Stein *et al.*, 1996; Lane *et al.*, 1997; Stein *et al.*, 1999a). Deux de ces études montrent une balance énergétique négative quand de l'exercice est pratiqué car l'augmentation des apports énergétiques n'est pas suffisante pour compenser l'augmentation de la dépense énergétique (Stein, 2001). Au contraire, lorsque la pratique d'exercice n'était pas prescrite (pendant les missions SLS1 et SLS2), les astronautes maintenaient une balance énergétique stable avec seulement une balance azotée modérément négative en raison d'un maintien des apports en protéines malgré une diminution adaptée des apports énergétiques (Stein *et al.*, 1996). L'exercice physique étant la contremesure la plus largement utilisée depuis plus de 50 ans, il est important de prendre en compte le coût énergétique lié à l'exercice physique pour comprendre son rôle dans la régulation de la balance énergétique et plus largement les atteintes du métabolisme, notamment dans le cadre de missions de longue durée.

3 Réponses métaboliques associées à l'exposition à la microgravité

3.1 Intolérance au glucose et résistance à l'insuline

L'insuline est une hormone secrétée par le pancréas en réponse aux augmentations de glycémie et a pour rôle de permettre l'entrée du glucose dans les cellules. Une résistance aux effets de l'insuline a été observée chez des astronautes ayant séjourné dans l'espace. Dans les années 1980, un test de tolérance au glucose (OGTT) a été fait pour la première fois chez un astronaute avant et après avoir passé 150 jours à bord de la navette Salyut-Soyuz mais aucune modification de la concentration du glucose 2h après l'ingestion de glucose n'a été notée (Alexandrov *et al.*, 1985). Depuis, d'autres études se sont intéressées à l'impact de la microgravité réelle sur la tolérance au glucose et la sensibilité à l'insuline par OGTT ou en mesurant l'insulinémie à jeun et l'excrétion dans les urines du peptide C (précurseur de l'insuline et indice de sécrétion de l'insuline) chez des astronautes dont le séjour dans l'espace a duré entre 4 jours et 6 mois (Grigoriev *et al.*, 1987; Smirnov *et al.*, 1991; Stein *et al.*, 1994; Maaß *et al.*, 1997; Stein *et al.*, 1999b; Macho *et al.*, 2003; Hughson *et al.*, 2016). L'ensemble de ces études montre un développement d'une résistance aux effets de l'insuline en réponse à l'exposition à la microgravité.

Les études menées au sol de différentes durées d'alitement sont nombreuses à montrer une diminution de la sensibilité à l'insuline. Certaines ont permis d'investiguer les mécanismes conduisant à une résistance à l'insuline systémique, en se focalisant notamment sur les trois principaux organes périphériques impliqués dans la régulation de l'homéostasie énergétique à savoir le muscle squelettique, le foie et le tissu adipeux. Pour étudier la contribution du muscle dans la résistance à l'insuline, plusieurs études incluant des protocoles de clamp hyperglycémique (Mikines *et al.*, 1989) et euglycémique hyperinsulinémique (Mikines *et al.*, 1991; Dirks *et al.*, 2016) ont été menées. Ces études ont montré qu'après 7 jours d'alitement, une diminution de l'action de l'insuline sur la captation du glucose par le muscle et son stockage sous forme de glycogène ont été notés, traduisant ainsi une diminution de la sensibilité à l'insuline du muscle squelettique. Une diminution du contenu en glycogène au niveau du muscle a même été observée dès 3 jours d'alitement, suggérant que l'insulino-résistance se développe à un stade très précoce (Shur *et al.*, 2022). Après 7 et 20 jours d'alitement, une diminution du contenu des principaux transporteurs de glucose au niveau du muscle, les protéines GLUT4, a été soulignée (Tabata *et al.*, 1999; Bienso *et al.*, 2012). Ces changements sont associés à une diminution de l'activité de protéines impliquées dans la régulation du métabolisme du glucose (hexokinase II, Akt, glycogène synthase) appuyant alors le développement d'une résistance à l'insuline

consécutive à l'exposition à la microgravité (Kiilerich *et al.*, 2011; Bienso *et al.*, 2012; Mortensen *et al.*, 2014).

Les modèles animaux ont permis d'élucider certains mécanismes impliqués dans la résistance à l'insuline. Ces adaptations sont résumées dans la **Figure 6**. L'insuline augmente la captation du glucose dans les cellules musculaires via une cascade de phosphorylations de protéines. Cette cascade résulte dans la translocation des GLUT4 au niveau de la membrane plasmique. Cette voie est dite insulino-dépendante. A côté, le transport du glucose peut se faire de façon non insulino-dépendante. Les contractions musculaires permettent également la translocation des GLUT4 du cytosol vers la membrane cellulaire. Il a été montré lors d'un protocole d'immobilisation chez le rat que la suspension menait à une diminution de la tolérance au glucose et une réduction de la captation du glucose. (O'Keefe *et al.*, 2004). Bien qu'aucun changement ne soit remarqué au niveau de la voie IRS-1/PI3K/Akt, une augmentation de la phosphorylation de la protéine p38 MAPK (p38 mitogen-activated protein kinase), connue pour inhiber IRS-1 (Fujishiro *et al.*, 2003) et associée au développement du diabète de type 2 (Henriksen *et al.*, 2011) a été notée après 3 et 7 jours de suspension (O'Keefe *et al.*, 2004; Machida & Booth, 2005). D'un autre côté, une diminution de la phosphorylation de la protéine IRS-1 ainsi qu'une diminution de l'activité de la protéine PI3K ont été observées chez des rats à qui une patte arrière a été immobilisé pendant 7 jours par rapport à l'autre patte contrôle non immobilisée (Hirose *et al.*, 2000). Un autre modèle a été utilisé pour induire de l'inactivité physique chez le rongeur, le modèle de la roue bloquée. Lors de ces protocoles, il a été montré que l'altération du transport du glucose est associée notamment à une diminution de la liaison de l'insuline à son récepteur, à une diminution de la phosphorylation de la sous-unité β du récepteur à l'insuline ainsi qu'une diminution de phosphorylation de l'Akt (Kump & Booth, 2005a). Alors qu'à l'état basal, c'est-à-dire sans stimulation par l'insuline, la protéine AS160 inhibe la translocation des GLUT4 sur la membrane plasmique, en présence d'insuline l'Akt phosphorylée permet de phosphoryler AS160, la rendant ainsi moins active et permettant in fine la translocation des GLUT4. Au cours d'un protocole de stimulation insulinique de cellules musculaires de rats soumis à une immobilisation unilatérale de la patte arrière pendant 6h, une diminution la phosphorylation de l'Akt et de AS160 ont été notées (Kawamoto *et al.*, 2016). Autrement dit, l'inactivité physique induit une diminution de l'activation de Akt diminuant ainsi sa capacité à inhiber AS160, qui inhibe constitutivement la translocation des GLUT4. La diminution

drastique de l'activité physique induite par la microgravité participe également à la diminution de la captation du glucose.

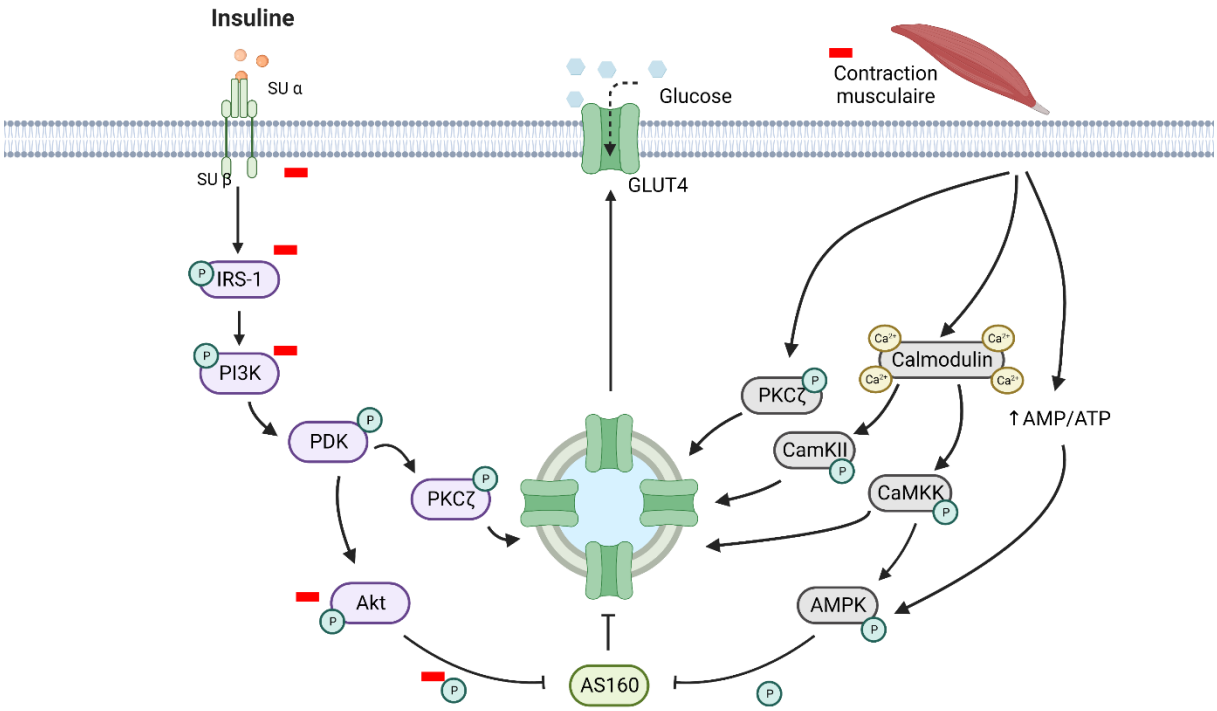


Figure 6 : Représentation schématique des voies insulino- et non insulino-dépendantes impliquées dans le transport du glucose au sein de la cellule musculaire et effets de la microgravité.
La partie gauche représente la voie insulino-dépendante et la partie droite la voie insulino-dépendante. Le signe moins rouge indique une diminution d'activité induite par la microgravité. Akt, protein kinase B ; AMP, adenosine monophosphate ; AS160, Akt substrate of 160 kDa ; ATP, adenosine triphosphate ; CaMKII, Ca^{2+}/calmodulin-dependent protein kinase II ; CaMKK, Ca^{2+}/calmodulin-dependent protein kinase kinase, IRS-1, insulin receptor substrate-1 ; PDK, pyruvate deshydrogenase kinase ; PI3K, phosphatidylinositol 3 Kinase ; PKCζ, protein kinase C ζ.

Chez l'homme, les mécanismes impliqués dans le développement de résistance à l'insuline ont notamment été étudiés lors de protocoles d'alitement prolongé. Dix jours d'alitement induisent une diminution de l'expression de 34 voies métaboliques au niveau du vaste latéral dont la plupart sont impliquées dans le fonctionnement mitochondrial. Plus précisément, l'expression du peroxisome proliferator-activated receptor-Y coactivator-α (PGC1α) et des gènes de la phosphorylation oxydative (OXPHOS) au niveau de la mitochondrie ont significativement diminué après l'alitement (Alibegovic *et al.*, 2010b). L'expression de l'hexokinase 2, enzyme catalysant la première réaction de la glycolyse après l'entrée du glucose au sein de la cellule a également diminué et celle de Ras-related associated with diabetes (RRAD), généralement surexprimée chez les patients atteints de diabète de type 2, a doublé après l'alitement. Ces altérations soulignent que l'inactivité physique induite par l'alitement entraine des altérations similaires à celles observées chez des personnes atteintes de diabète de type 2. Dans cette étude, les auteurs ont également mis en avant une augmentation de l'expression de gènes impliqués dans l'inflammation et le stress du réticulum endoplasmique en réponse à une

stimulation à l'insuline. Ces données, résonnent avec celles exposés précédemment suggérant le développement d'une inflammation et la perturbation de la balance oxydative lors des différents modèles de simulation de microgravité. Il est désormais bien établi que l'inflammation et le stress oxydant sont des composantes à part entière du développement de la résistance à l'insuline du muscle squelettique mais également au niveau du foie et du tissus adipeux (Savage *et al.*, 2005; Samuel & Shulman, 2012).

Alors qu'aucune modification de la production hépatique de glucose n'ait été notée après 7 jours d'alitement (Stuart *et al.*, 1988; Mikines *et al.*, 1991), l'utilisation de traceurs isotopiques a permis de révéler qu'après 7 jours d'alitement le foie développait également une résistance à l'insuline mais a priori uniquement chez les femmes (Blanc *et al.*, 2000). Plus récemment, la même technique a été utilisée chez des personnes âgées (7 femmes et un homme) avant et après 10 jours d'alitement. Cette étude a révélé que le foie était moins capable de supprimer sa production endogène de glucose pendant un stimulation insulinémique traduisant une résistance aux effets de l'insuline (Coker *et al.*, 2014). Nous ne savons que peu de choses concernant la sensibilité à l'insuline du tissu adipeux en conditions de microgravité. Alors qu'une résistance à l'insuline au niveau du tissu adipeux induirait une diminution de la capacité à diminuer la lipolyse sous stimulation insulinique et donc un taux de lipolyse inchangé, une diminution du taux de lipolyse a été mise en avant après 9 jours d'alitement (Alibegovic *et al.*, 2009; Alibegovic *et al.*, 2010a). En parallèle, Ward et ses collaborateurs ont utilisé un indice incluant comme paramètres la concentration d'acides gras plasmatique et l'insulinémie à jeun, permettant d'évaluer la résistance à l'insuline du tissu adipeux. Après 60 jours d'alitement, une augmentation significative de cet indice suggère que le tissu adipeux deviendrait résistant à l'action de l'insuline (Ward *et al.*, 2020). Il est important de noter que dans cette étude, les sujets étaient en balance énergétique comme l'indique le maintien de la masse grasse au cours de l'alitement (Zahariev *et al.*, 2005). Effectivement, certaines études ne contrôlant pas le régime alimentaire des participants pourraient avoir masqué les effets de l'inactivité physique seule en les confondant avec les effets d'une balance énergétique positive. Dans ce contexte, Bergouignan et ses collaborateurs ont lors de deux études d'alitements de 60 et 90 jours chez des femmes et des hommes contrôlé la balance énergétique en monitorant régulièrement la masse grasse de ces sujets. Ils ont démontré chez ces sujets alités que l'inactivité physique induite par la microgravité induisait bien une résistance à l'insuline systémique à jeun comme en condition postprandiale (Bergouignan *et al.*, 2006; Bergouignan *et al.*, 2009).

3.2 Hypertriglycéridémie

L'insulino-résistance induite par la microgravité s'accompagne du développement d'une hypertriglycéridémie chez des sujets alités (Blanc *et al.*, 2000; Bergouignan *et al.*, 2006; Hamburg *et al.*, 2007; Bergouignan *et al.*, 2009; Ward *et al.*, 2020). D'un point de vue mécanistique, cette augmentation peut être la conséquence d'une augmentation de la lipolyse, d'une diminution de la clairance des acides gras ou les deux. Peu d'étude ont étudié la lipolyse en condition d'alitement et montrent qu'au contraire, le taux de lipolyse diminue chez des sujets sains avec ou sans prédisposition au diabète de type 2 alités pendant 9 jours (Alibegovic *et al.*, 2009; Alibegovic *et al.*, 2010a). Cette diminution a été associée à une diminution de l'action de l'hormone sensitive lipase (HSL), enzyme catalysant l'hydrolyse des triglycérides au niveau du muscle (Alibegovic *et al.*, 2010a). La lipolyse a également diminué au niveau du tissu adipeux fémoral après alitement mais pas au niveau abdominal (Højbjerre *et al.*, 2010). Ces études écartent donc le rôle de l'augmentation de la lipolyse dans l'hypertriglycéridémie observée lors d'alitements prolongés et suggèrent l'implication d'une altération de la clairance des acides gras.

Notre laboratoire s'est penché sur la captation des acides gras venant de l'alimentation par les tissus périphériques en utilisant des traceurs isotopiques. Ces travaux révèlent qu'après 60 jours d'alitement, le relargage des acides gras non-estérifiés après hydrolyse des chylomicrons et VLDL augmente de façon significative (Bergouignan *et al.*, 2009). Cette diminution de la clairance des acides gras serait en partie expliquée par la diminution de l'expression génique de la lipoproteine lipase (LPL) et de la protéine FAT/CD36, responsables respectivement de l'hydrolyse des lipoprotéines et du transport des acides gras vers la cellule. L'atrophie musculaire contribuerait également à la diminution de la clairance des acides gras et indirectement à l'hypertriglycéridémie. Une diminution de l'activité de la LPL de l'ordre de 80% au niveau du muscle a été montrée dans des protocoles d'inactivité physique chez l'animal, mettant alors également en avant une réduction de l'absorption des acides gras induite par l'inactivité physique (Bey & Hamilton, 2003; Kump & Booth, 2005b; Kump *et al.*, 2006). Les auteurs ont noté une augmentation du poids de la graisse épididymaire et omentales dans les heures consécutives à l'arrêt de l'activité physique. De façon intéressante, ce phénomène est accompagné d'une augmentation de l'incorporation de palmitate dans les triglycérides et de façon concomitante à une augmentation de l'activité de la protéine mtGPAT, molécule régulatrice de la synthèse de triglycérides (Kump & Booth, 2005b; Kump *et al.*, 2006). De plus, deux études d'alitement de 10 jours montrent une augmentation de l'expression de la LPL au niveau du tissu adipeux sous-cutané, suggérant une augmentation de la captation des acides gras au sein du tissu adipeux sous-cutané (Højbjerre *et al.*, 2010; Hojbjerre *et al.*, 2011). Enfin, il semblerait également que bien que la

masse grasse augmente de façon générale, une augmentation plus marquée du tissu adipeux viscéral a été notée par rapport au tissu adipeux des jambes, des bras et du tronc après 60 jours d'alitement (Belavy *et al.*, 2014). L'ensemble de ces données soulignent que l'inactivité physique induite par la microgravité détourne l'acheminement des lipides plasmatiques du muscle vers le tissu adipeux et stimulerait, au moins chez le rat, la lipogenèse. Cette modification est probablement conjointe à une modification du métabolisme lipidique entre oxydation et stockage au sein du muscle.

3.3 Diminution de l'oxydation des lipides

En conditions physiologiques, l'oxydation des nutriments dépend de leur type et disponibilité au sein de la cellule. A jeun, les lipides sont majoritairement oxydés pour produire de l'énergie. L'exposition à la microgravité induit un changement dans l'utilisation des substrats à jeun. Il a été en effet observé chez des rongeurs ayant séjourné dans l'espace et lors de plusieurs protocoles d'alitement prolongé chez l'homme que l'oxydation des glucides augmente au détriment des lipides, indépendamment de la balance énergétique (Baldwin *et al.*, 1993; Ritz *et al.*, 1998; Blanc *et al.*, 2000; Bergouignan *et al.*, 2006; Bergouignan *et al.*, 2009). Une diminution d'oxydation du palmitate de 37% a été observée chez des rats ayant séjourné 9 jours à bord de la fusée Columbia (Baldwin *et al.*, 1993). Chez l'homme, l'oxydation des substrats n'a jamais été mesurée dans l'espace mais une augmentation du quotient respiratoire non-protéique (QRNP) de 4 à 14% a été notée après alitement de 42, 60 et 90 jours (Blanc *et al.*, 2000; Bergouignan *et al.*, 2006; Bergouignan *et al.*, 2009). Une diminution de l'utilisation des lipides à jeun de 33 à 90% et une augmentation de celle des glucides de 20 à 40% par rapport aux valeurs de base ont en effet été relevée dans ces protocoles. De façon similaire, l'alitement induit en condition postprandiale une diminution de 40% de l'oxydation des lipides contre une augmentation de 6% en faveur des glucides (Bergouignan *et al.*, 2006). La microgravité induit donc un changement dans l'utilisation des substrats en conditions postabsorptives et postprandiales.

Seulement, la réduction d'oxydation des lipides est plus complexe car elle dépend de la longueur et du degré de saturation des acides gras. Ainsi, plus la chaine carbonée sera courte et le nombre d'insaturations élevé, plus l'oxydation sera facile (DeLany *et al.*, 2000). L'utilisation de traceurs isotopiques a permis de montrer élégamment que la réduction de l'oxydation des lipides observée en cours d'alitement concernait les lipides saturés et non les lipides mono-insaturés (Bergouignan *et al.*, 2006; Bergouignan *et al.*, 2009). Cela suggère que les lipides saturés non oxydés sont détournés de la voie oxydative au profit du stockage.

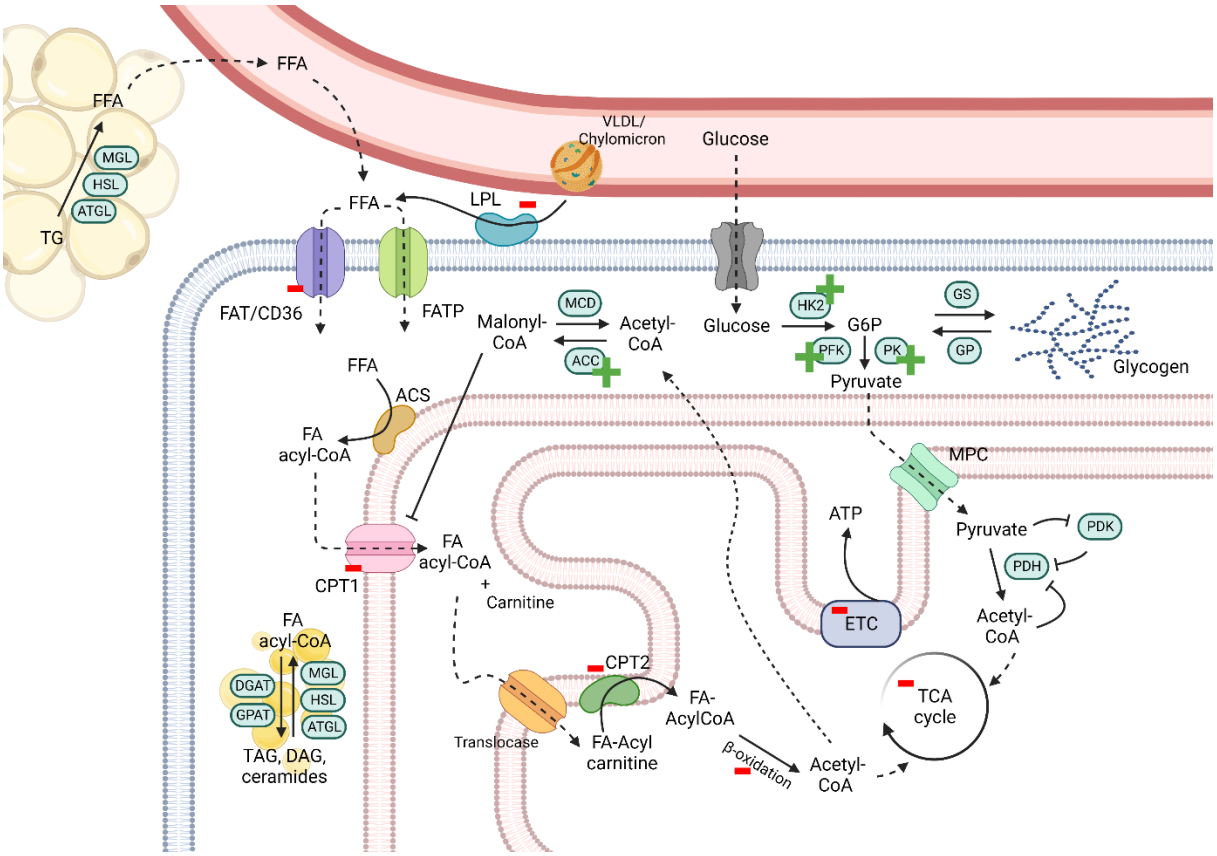
Il est important de noter que les changements dans l'oxydation des substrats sont indépendants de l'atrophie musculaire et des changements de composition corporelle. Néanmoins, cette modification dans l'utilisation des substrats peut être lié au changement de typologie des fibres musculaires observées après exposition à la microgravité réelle ou simulée. Effectivement, une augmentation de la proportion des fibres rapides glycolytiques (appelées également fibres de type II) et une diminution des fibres lentes dites oxydatives (fibres de type I) ont été largement documentés chez des souris et des singes lors de séjours dans l'espace de 11 à 91 jours (Kischel *et al.*, 2001; Harrison *et al.*, 2003; Sandona *et al.*, 2012; Ulanova *et al.*, 2015; Gambara *et al.*, 2017a; Gambara *et al.*, 2017b; Tascher *et al.*, 2017) et également chez des hommes et femmes alités en tête déclive entre 55 et 84 jours (Trappe *et al.*, 2004; Trappe *et al.*, 2007a; Salanova *et al.*, 2008; Moriggi *et al.*, 2010). Les fibres glycolytiques sont connues pour avoir un taux d'oxydation des lipides et une sensibilité à l'insuline inférieurs aux fibres oxydatives qui ont une plus grande densité mitochondriale et une activité des enzymes oxydatives plus élevée (Scott *et al.*, 2001).

En plus de la modification de typologie des fibres musculaires, des altérations transcriptionnelles participent également au changement dans l'utilisation des substrats pour produire de l'énergie. Ces modifications sont résumées dans la **Figure 7**. En parallèle à la diminution de captation des acides gras circulants par les cellules musculaires, une diminution de l'expression des gènes codants pour les protéines de transport des acides gras du cytoplasme au sein de la mitochondrie a été notée chez le rat suspendu par la queue et la femme alitée. Les expressions géniques de CPT1 (passage de la membrane externe mitochondriale) et CPT2 (passage de la membrane interne mitochondriale) ont diminué respectivement de 36 et 37% chez des rats immobilisés pendant 21 jours (Stein *et al.*, 2002). Soixante jours d'alitement chez la femme ont conduit à la diminution d'expression génique de CPT1 au niveau du muscle vaste latéral de près de 50% (Bergouignan *et al.*, 2009). Des analyses omiques faites sur des biopsies de muscle soléaire de souris ayant séjourné 30 jours dans l'espace et 38 jours à bord de l'ISS ont montré une diminution de l'abondance de protéines impliquées dans le transport et de la β-oxydation des acides gras (Tascher *et al.*, 2017; da Silveira *et al.*, 2020) ainsi que chez des sujets alités pendant 21 jours (Kenny *et al.*, 2020). En parallèle, les voies impliquées dans la régulation du métabolisme du glucose semble être activées chez des souris exposées à la microgravité réelle (Gambara *et al.*, 2017a; Tascher *et al.*, 2017; da Silveira *et al.*, 2020) et chez des rats suspendus comme l'attestent les augmentations d'expression d'enzymes impliquées dans la glycolyse comme la phosphofructokinase (PFK) et la pyruvate kinase (PK) (Stein *et al.*, 2002).

Le métabolisme de la mitochondrie, siège de la production d'énergie, est également profondément altéré par la microgravité réelle ou simulée. Ces modifications sont également

représentées dans la figure 7. Le cycle de Krebs (appelé TCA cycle en anglais dans la **Figure 7**), étape qui précède celle de la respiration mitochondriale semble être perturbé en condition de microgravité comme l'indique la diminution de l'activité de la citrate synthase (enzyme impliquée dans le cycle de Krebs) chez des souris ayant séjourné 11 jours à bord de la fusée Endeavour (Harrison *et al.*, 2003) et chez des hommes alités pendant 21 jours (Kenny *et al.*, 2020). Des réponses omiques altérées des mécanismes impliqués dans le cycle de Krebs ont également été notées chez des souris pensionnaires de l'ISS pendant 38 jours (da Silveira *et al.*, 2020). La respiration mitochondriale est également sensible aux conditions de microgravité. Une diminution de l'ordre de 30% de l'expression du gène de la cytochrome oxydase (appelée également COX4, protéine du complexe de la chaine respiratoire) a été notée après deux mois d'alitement (Bergouignan *et al.*, 2009). Une diminution de l'activité de la cytochrome oxydase a également été notée chez cinq astronautes ayant séjourné 6 mois à bord de l'ISS (Fitts *et al.*, 2013). Plus globalement, des analyses omiques ont mis en évidence une diminution de l'abondance de protéines impliquées dans la chaine respiratoire chez des souris ayant séjourné 30 jours en orbite terrestre (Tascher *et al.*, 2017). Sur terre, Kenny et ses collaborateurs ont montré que 21 jours d'alitement menaient à une diminution globale de la respiration mitochondriale non pas due à une altération de la fonction mitochondriale mais plutôt à une diminution de la densité mitochondriale (Kenny *et al.*, 2017). Alors que ces changements peuvent être perçus comme un stress de la mitochondrie (da Silveira *et al.*, 2020), ils pourraient représenter des adaptations de l'organite à un nouvel état énergétique à savoir un besoin énergétique moindre dû à l'inactivité physique, qui se reflèterait notamment par un changement dans la fonction et le nombre de mitochondries. En revanche, dans le cadre de variations importantes de besoins énergétiques (surnutrition ou exercice physique) importants, certaines des adaptations de la mitochondrie citées précédemment limiteraient la capacité de la mitochondrie à faire face à ces changements.

Au-delà des modifications transcriptionnelles au sein des voies métaboliques de la cellule squelettique, plusieurs études menées chez la souris et le rat suspendus par l'arrière-train suggèrent une modification du métabolisme des substrats au niveau du foie. Une première étude s'est intéressée à l'expression de gènes d'enzymes clé impliquées dans la néoglucogénèse c'est-à-dire la capacité de production de glucose à partir de composés non glucidiques (phosphoenolpyruvate carboxykinase [PEPCK], glucose-6-phosphatase [G6Pase], fructose-1,6-biphosphatase [FDPase], pyruvate carboxylase [PC]) chez le rat suspendu pendant 21 jours. Une augmentation de l'ARNm de ces enzymes a été effectivement notée (Stein *et al.*, 2005). Ces données ont été confirmées par une augmentation de l'activité enzymatique de PEPCK chez des souris suspendues pendant 11 jours par rapport à des souris contrôles (Ramirez *et al.*, 2014). Une autre étude utilisant des isotopes stables a permis de montrer une augmentation du taux de néoglucogenèse de 13% chez des souris suspendues pendant 3 jours



<u>Figure 7</u> : Résumé des connaissances actuelles sur les adaptations du métabolisme des glucides et des lipides à la microgravité.

Le signe moins en rouge et le signe plus en vert désigne représentent une diminution et une augmentation respectives de l'expression génique et/ou une diminution de l'activité des protéines impliquées dans les voies de régulation du métabolisme lipidique et glucidique au sein de la cellule musculaire en condition réponse à la microgravité. Le signe plus en vert désigne une augmentation de l'expression génique ou une augmentation de l'activité. ACC, acetyl-CoA carboxylase; ACS, acyl-CoA synthetase; ATGL, adipose tissue triglyceride lipase; ATP, adenosine triphosphate; CPT1, carnitine palmitoyltransferase 1; CPT2, carnitine palmityoltransferase 2; DGAT, diacylglycerol acyltransferase; ETC, electron transport chain; FAT/CD36, fatty acid translocase CD36; FATP, fatty acid transport protein; FFA, free-fatty acid; G6P, glucose-6-phosphate; GP, glycogen phosphorylase; GPAT, glycerol phosphate acyltransferase; GS, glycogen synthase; HK2, hexokinase 2; HSL, hormone-sensitive lipase; LPL, lipoprotein lipase; MCD, malonyl-CoA decarboxylase; MGL, monoglyceride lipase; MPC, mitochondrial pyruvate carrier; PDH, pyruvate dehydrogenase; PDK, pyruvate dehydrogenase kinase; PFK, phosphofructokinase; PK, pyruvate kinase; TCA, tricarboxylic acid; TG, triglyceride.

(Bederman *et al.*, 2013). A ce jour, seule une étude d'alitement chez l'homme a investigué la production endogène de glucose en soulignant une diminution significative chez l'homme mais pas la femme (Blanc *et al.*, 2000). Une étude de cessation d'activité physique progressive chez des rats naturellement gras a montré une diminution de l'oxydation des lipides ainsi qu'une diminution des capacités oxydatives mitochondriales. De plus, une augmentation de l'expression du gène codant pour l'ACC a été notée associée à une augmentation de la concentration de malonyl-CoA (Rector *et al.*, 2008). Des analyses protéomiques réalisées sur les foies de rat suspendus par la queue pendant 3 et 21 jours révèlent également une modification profonde de l'abondance de protéines impliquées dans le métabolisme du glucose et des lipides, et soulignent également une augmentation de l'abondance de la protéine ACC (Chen *et al.*, 2020). Cette étude suggère donc que le foie présenterait les mêmes altérations que le muscle. Ainsi chez l'animal la hausse de la néoglucogénèse augmente la conversion

d'acétyl-CoA en malonyl-CoA par l'ACC, ce dernier étant connu pour exercer une fonction inhibitrice de CPT1. In fine, l'entrée des acides gras au sein de la cellule et de la mitochondrie diminuent, créant alors un déséquilibre entre l'absorption et l'oxydation des acides gras. Ces lipides non-oxydés vont s'accumuler ailleurs que dans le tissu adipeux, alimentant alors un stockage ectopique de graisses.

3.4 Stockage ectopique de lipides

Le stockage ectopique de lipides désigne le dépôt de lipides dans des tissus autres que le tissu adipeux comme au niveau du tissu musculaire, du foie ou encore des os. En conditions physiologiques, les tissus non-adipeux contiennent peu de triglycérides. Le dépôt d'acides gras peut se faire au sein des cellules musculaires principalement sous la forme de triacylglycérols, diacylglycérols et/ou de céramides qui sont regroupées au sein des lipides intramyocellulaires (intramyocellular lipid en anglais, IMCL), mais également entre les cellules musculaires formant ainsi le tissu adipeux intermusculaire (intermuscular adipose tissue en anglais, IMAT). Plusieurs études menées au sol décrivent que l'inactivité physique entraine la formation d'accumulations lipidiques au sein du muscle. Dans un protocole d'immobilisation unilatérale de la jambe chez l'homme pendant 12 jours, l'utilisation de palmitate marqué au ^{14}C n'a pas permis de mettre en évidence une augmentation de son incorporation au sein de triacylglycérols et de diacylglycérols au niveau de la jambe inactive (Bilet *et al.*, 2020). En revanche, des analyses par IRM du *tibialis anterior* et des coupes histologiques de *vastus lateralis* ont montré une augmentation d'IMCL de l'ordre de 23% et 53% respectivement au niveau de la jambe suspendue (Bilet *et al.*, 2020). Vingt-huit et soixante jours d'alitement ont respectivement conduit à une augmentation moyenne des IMCL au niveau du muscle soléaire de 76% (Cree *et al.*, 2010) et de 3% au niveau des muscles lombaires (De Martino *et al.*, 2021). Dans un protocole d'alitement de 60 jours, les lipides intramusculaires au sein du soléaire augmentent de près de 2,7% chez la femme. Cette augmentation de contenu de lipides intramusculaire a été corrélée à la réduction d'oxydation de palmitate induite par l'alitement prolongé (Bergouignan *et al.*, 2009). Ceci suggère que les lipides alimentaires non oxydés sont incorporés au sein des lipides intramyocellulaires. En ce qui concerne les IMAT, 3 jours d'immersion sèche n'ont pas suffi pour observer une augmentation des IMAT mais une augmentation significative des marqueurs associés aux IMAT a été mise en avant (Pagano *et al.*, 2018). Des études de plus longues durées sont nécessaires pour mieux comprendre l'effet de la microgravité simulée sur les IMAT.

Le foie s'avère également être un organe susceptible à l'accumulation de lipides à l'inactivité physique induite par la microgravité. Des marqueurs systémiques suggèrent la formation de réserves lipidiques au sein du foie. Il a en effet été montré que deux indices de stéatose hépatique, l'aspartate

aminotransférase (AST) et l'alanine aminotransférase (ALT), augmentent significativement après 60 jours d'alitement (Rudwill *et al.*, 2015). Dans un protocole de roue bloquée chez des rats physiquement actifs, 4 semaines d'inactivité physique ont suffi pour induire le développement d'une stéatose hépatique (Linden *et al.*, 2013). En microgravité réelle, des analyses histologiques montrent en effet que le contenu en gouttes lipidiques au niveau du foie augmente de près de 3,5 fois chez des souris ayant voyagé 13 jours dans l'espace par rapport aux souris contrôles restées sur Terre (Jonscher *et al.*, 2016). Ces mêmes analyses ont été menées sur des foies de souris de trois missions différentes et mettent en évidence une augmentation du contenu en gouttelettes lipidiques après 14, 21 et 37 jours passés en orbite indépendamment de la durée du vol (même si une tendance semble se dégager). Il est probable que l'augmentation des transaminases pendant l'alitement reflète le développement d'une stéatose hépatique chez l'humain.

La moelle osseuse peut également être un lieu de stockage ectopique de graisses. La fraction lipidique au sein de la moelle hématopoïétique des vertèbres lombaires augmente de près de 2,5% chez la femme après 60 jours d'alitement (Trudel *et al.*, 2009). Cette augmentation correspond à l'équivalent de quatre années d'évolution physiologique (Meunier *et al.*, 1971; Gurevitch *et al.*, 2007) et était toujours visible un an après la fin du protocole.

Cette accumulation de lipides n'est pas sans conséquence pour les tissus concernés. Au niveau du muscle, alors que les IMCL sont une source importante d'énergie notamment pendant l'exercice (Badin *et al.*, 2013), l'augmentation des IMCL induit une accumulation de lipides intermédiaires lipotoxiques comme les diacylglycérols et les céramides. Ces composés interfèrent directement avec la voie de signalisation à l'insuline (Savage *et al.*, 2005), entretenant ainsi l'insulino-résistance musculaire. On parle alors de lipotoxicité. Comme exposé dans la partie 2.3.3, les diacylglycérols et céramides joueraient un rôle dans l'atrophie musculaire (Meex *et al.*, 2019). Au niveau du foie organe pivot du métabolisme du glucose et des lipides, la lipotoxicité induit également des effets sur son fonctionnement normal. Alors qu'en situation postprandiale l'augmentation de la production d'insuline supprime la production endogène de glucose du foie par l'inhibition de la glycogénolyse et de la néoglucogénèse, une diminution de la capacité à supprimer la néoglucogénèse est notée chez les individus insulino-résistants. Cette situation favorise alors l'hyperglycémie systémique. Plus encore, la production de lipides à partir d'acetyl-CoA provenant majoritairement du catabolisme des glucides, *i.e.* lipogenèse de novo, est maintenue voire augmentée, ce qui participe à entretenir l'hypertriglycéridémie systémique (Rector & Thyfault, 2011). De plus le stockage ectopique de lipides entretiendrait des relations complexes avec certaines voies menant à du stress oxydant (Hauck & Bernlohr, 2016) et de l'inflammation (Savary *et al.*, 2012) qui exacerbent à leur tour notamment la résistance à l'insuline et l'atrophie musculaire.

Les études mentionnées dans les parties 3.1 à 3.4 suggèrent ainsi que l'inactivité physique induite par la microgravité réelle ou simulée entraine, indépendamment des changements de balance énergétique, le développement d'une atrophie musculaire, un changement de typologie des fibres musculaires au profit des fibres rapides et glycolytiques, une résistance à l'insuline, une hypertriglycéridémie et un stockage ectopique de graisses. Il y a 12 ans, l'hypothèse de la séquence d'évènements suivante a été proposée pour expliquer le développement d'adaptations métaboliques induites par l'inactivité physique et menant au développement d'une inflexibilité métabolique (Bergouignan *et al.*, 2011) et est représentée sur la **Figure 8**.

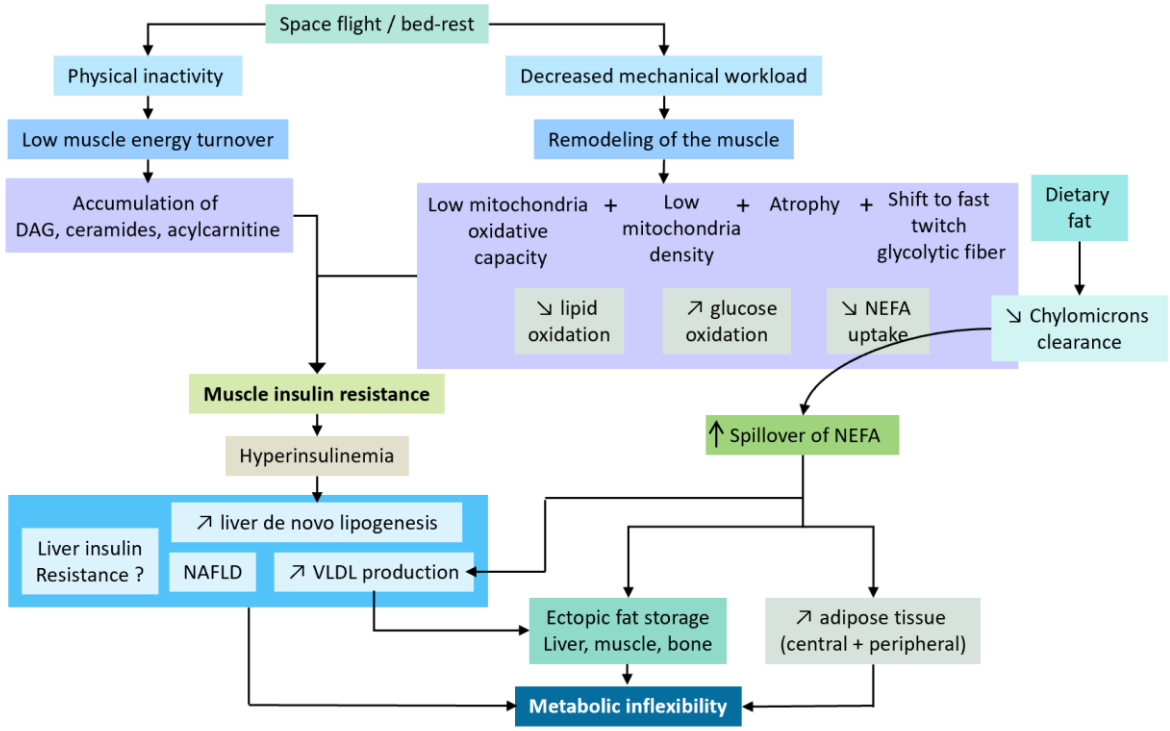


Figure 8 : Cascade d'adaptations métaboliques induites par la microgravité menant au développement d'une inflexibilité métabolique.

Adapté de (Bergouignan *et al.*, 2011). La microgravité induit une hypokinésie (diminution des mouvements corporels) et une hypodynamie (perte de force et de puissance musculaire) qui mènent à des modifications de l'ensemble des systèmes physiologiques (Bergouignan et al., 2011). La microgravité induit une perte de fonction et de masse musculaire qui se caractérise par une atrophie musculaire, une transition des fibres lentes oxydatives vers des fibres rapides glycolytiques, une diminution du contenu mitochondrial, de la capacité oxydative ainsi qu'une altération de l'expression de gènes impliqués dans la fonction mitochondriale. Cette situation induit également une diminution de la sensibilité à l'insuline au niveau du muscle qui est associée à une diminution du contenu et de l'activité des protéines clé impliquées dans le transport, la phosphorylation et le stockage du glucose. Ce phénomène induit notamment une hyperinsulinémie pour maintenir une clairance du glucose normale. De plus, l'expression et l'activité de gènes des enzymes impliquées dans le métabolisme oxydatif diminuent, en association avec une diminution de l'oxydation des lipides en faveur des glucides. Ces changements sont particulièrement critiques en situation postprandiale car ils mènent à une diminution de la clairance des lipides alimentaires ce qui contribue à l'hyperlipémie. Malgré une diminution de la lipolyse du tissu adipeux, l'excès de lipides circulants entraine l'accumulation de graisses au niveau du tissu adipeux viscéral et au niveau du muscle, du foie et des os. Cette situation entretiendra le développement d'une résistance à l'insuline. L'accumulation de lipides au niveau du foie stimule la de novo lipogenèse et augmente ainsi la synthèse des VLDL. Ces particules participent également à l'hypertriglycéridémie et au stockage ectopique de graisses. De façon concomitante, le foie n'est pas capable de supprimer sa production endogène de glucose, ce qui résulte en une augmentation de la néoglucogénèse qui aggrave elle-même l'hyperinsulinémie. L'ensemble de ces adaptations du métabolisme participent à l'installation d'une inflexibilité métabolique.

3.5 Inflexibilité métabolique

3.5.1 Définitions de la flexibilité métabolique

Le corps oscille constamment entre des périodes de jeûne et des périodes postprandiales ce qui entraîne de larges variations dans la disponibilité des nutriments. Le concept de flexibilité métabolique apparait à la fin des années 1990 lorsque Kelley et ses collaborateurs montrent qu'à jeun le muscle squelettique d'individus sains oxyde majoritairement des lipides fournis par une captation élevée des acides gras circulants et est capable d'augmenter l'oxydation des glucides lors de stimulation d'insuline. Dans cette étude, ils ont comparé la réponse de changement de quotient respiratoire (ΔQR) des individus sains à celle d'individus en surcharge pondérale ou atteints d'obésité. Bien qu'un même taux de captation des acides gras ait été noté, les personnes en surcharge pondérale oxydent moins de lipides à jeun et sont moins capables de supprimer l'oxydation des lipides au profit de l'oxydation des glucides lors du protocole d'infusion d'insuline (Kelley *et al.*, 1999). Ceci a été qualifié comme un état d'inflexibilité métabolique. Depuis, différentes définitions de la flexibilité métabolique ont été érigées. Goodpaster et Sparks la définissent comme la capacité d'un organisme à répondre ou à s'adapter en fonction des changements de la demande énergétique (Goodpaster & Sparks, 2017). Smith et ses collaborateurs la définissent comme une réponse adaptative d'un organisme à maintenir l'homéostasie énergétique en faisant correspondre la disponibilité et la demande de carburant pendant une période de jeûne, après un repas, en réponse à un épisode d'activité physique et aux fluctuations environnementales (Smith *et al.*, 2018). Plus récemment, Galgani et Fernandez-Verdejo ont proposé que la flexibilité métabolique est la capacité de l'organisme à adapter l'oxydation des substrats en fonction de leur disponibilité pour que la synthèse d'ATP soit adéquate à la demande (Galgani & Fernandez-Verdejo, 2021). Dans un contexte plus holistique, notre équipe de recherche postule que toute réponse métabolique à un stress, environnemental ou physiologique, donne des informations sur la flexibilité métabolique (Rynders *et al.*, 2018). La régulation de l'homéostasie énergétique et des substrats étant complexe et interconnectée, le concept de flexibilité métabolique permet de considérer tous les systèmes impliqués dans l'homéostasie énergétique, à savoir le foie, le tissu adipeux et le muscle squelettique. En ce sens, la flexibilité métabolique fait intervenir trois composants : un stress, un régulateur et un effecteur. C'est la relation entre le régulateur et l'effecteur en réponse au stress qui apporte des informations sur la flexibilité métabolique (Rynders *et al.*, 2018). Par exemple dans le cadre de la définition originelle de Kelley, le stress – le clamp euglycémique hyperinsulinémique – permet d'examiner la relation entre le régulateur – l'infusion d'insuline – et l'effecteur – le ΔQR.

3.5.2 Comment mesurer la flexibilité métabolique ?

Évaluer la flexibilité métabolique requiert de faire varier la demande énergétique et/ou la biodisponibilité des nutriments par un stress physiologique pour mesurer la capacité de l'organisme à moduler la relation entre le régulateur et l'effecteur. Dans cette optique, plusieurs challenges métaboliques ont été utilisés. Le clamp euglycémique-hyperinsulinémique a été largement utilisé par les chercheurs par la possibilité de contrôler très précisément les conditions du test. Ce protocole consiste à administrer de l'insuline par intraveineuse pour augmenter et maintenir une hyperinsulinémie stable. Le glucose, également perfusé par voie intraveineuse, est infusé à des taux variables pour maintenir une euglycémie à savoir une glycémie stable. Le taux de perfusion de glucose à l'état d'équilibre est en corrélation directe avec la sensibilité à l'insuline (Kim, 2009). Ainsi, l'hyperinsulinémie inhibe la production hépatique de glucose et permet de tester la capacité du muscle squelettique à augmenter l'oxydation de glucose exogène (Galgani & Fernandez-Verdejo, 2021). Bien que cette technique soit considérée comme la technique de référence pour mesurer la sensibilité à l'insuline, ce protocole est supraphysiologique. D'autres conditions plus physiologiques ont été envisagées comme le changement d'utilisation des substrats au cours au jeûne pendant la nuit, pendant un épisode aigu d'exercice physique ou encore en réponse à un repas qui peut varier au niveau de la composition en macronutriments (repas hyperglucidique ou hyperlipidique). La capacité à alterner l'utilisation des substrats peut être mesurée par l'intermédiaire du quotient respiratoire (QR) qui est le rapport entre le volume de dioxyde de carbone libéré (VCO_2) et le volume d'oxygène consommé (VO_2) tous les deux mesurées par respirométrie. Chez l'homme ce rapport varie entre 0,7 qui indique une oxydation majoritaire de lipides, 0,8 pour une oxydation majoritaire des protéines et 1,0 pour une oxydation des glucides dominante. L'oxydation des protéines étant stable et directement associée à l'ingestion de protéines, il est possible de retrancher l'excrétion d'azote urinaire au QR pour ainsi obtenir le quotient respiratoire non-protéique (QRNP ou NPRQ pour non-protein respiratory quotient en anglais) qui reflète uniquement l'utilisation des glucides et des lipides.

La transition de l'état de jeûne à l'état de stimulation énergétique quel qu'il soit peut être déterminé par l'amplitude de changement entre le QR pendant une période t et le QR à jeun t_0 ($\Delta QR = QR(t) - QR(t_0)$) (Galgani & Fernandez-Verdejo, 2021). Dans le cas de la flexibilité métabolique en réponse à un repas, un indice ΔQR élevé représentera une transition élevée entre l'oxydation des lipides à jeun et l'oxydation des glucides en conditions postprandiales et traduira une flexibilité métabolique élevée. Au contraire, une faible amplitude entre le QR à jeun et le QR après un repas reflètera une flexibilité métabolique réduite (**Figure 9**).

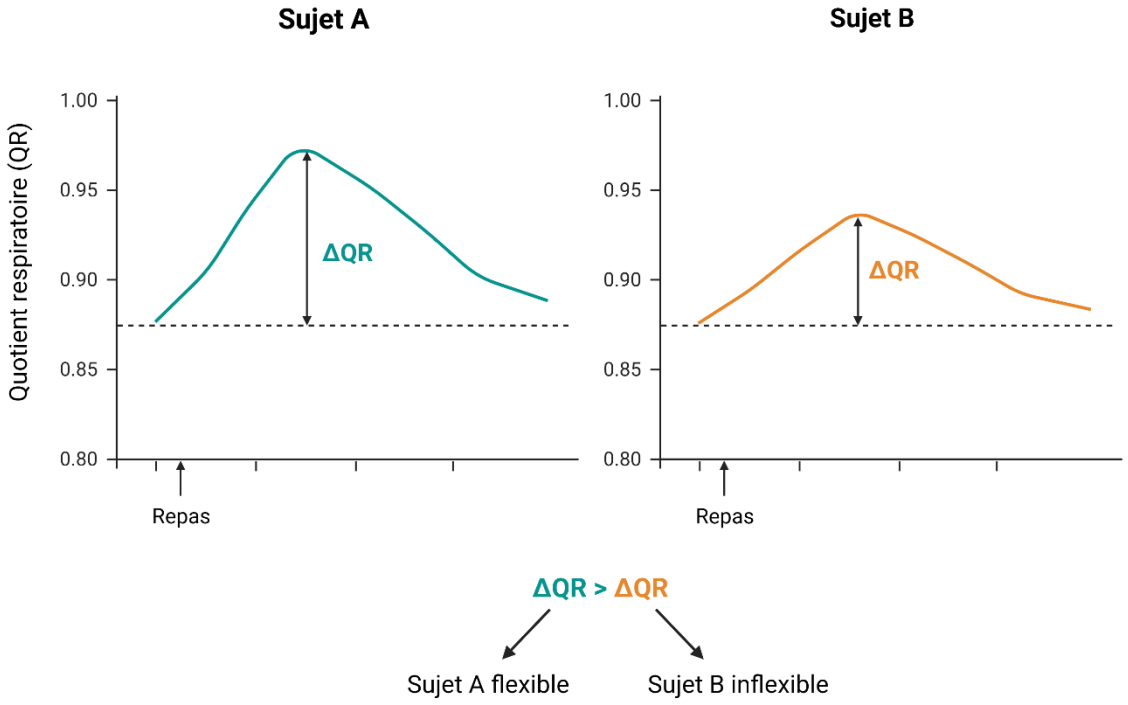
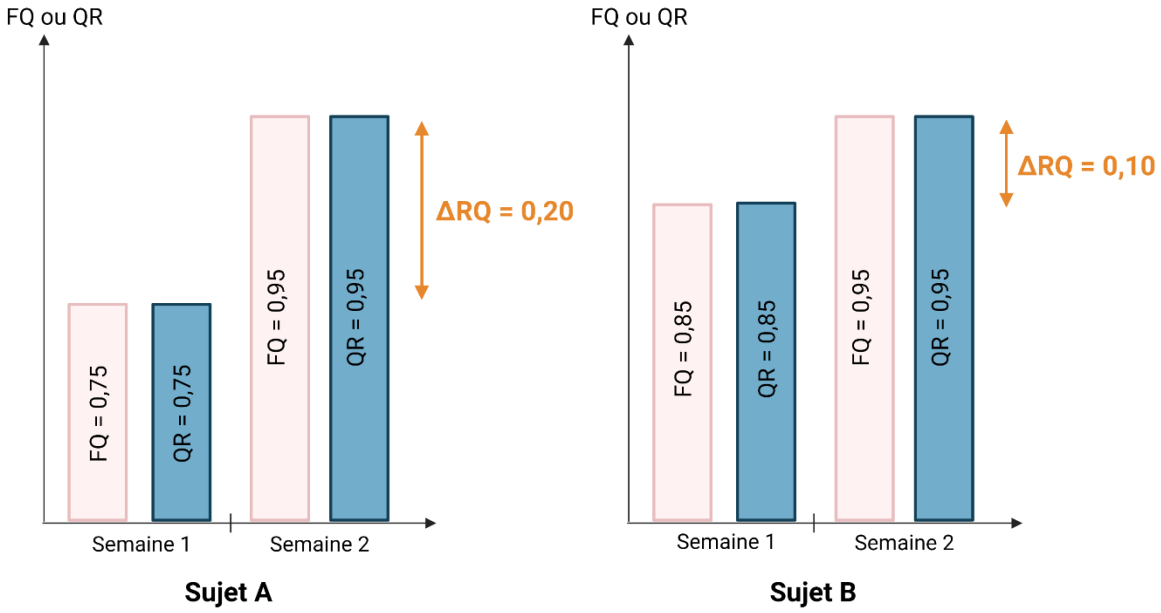


Figure 9 : ΔRQ comme indice de flexibilité métabolique.

Dans cet exemple, le sujet A présente une amplitude plus importante entre le QR à jeun et le QR postprandial maximal que le sujet B. Le sujet A sera alors considéré flexible et le sujet B inflexible.

Toutefois, l'évaluation de la flexibilité avec cet indice nécessite au préalable un contrôle de la balance énergétique et de l'alimentation. Dans leur récente revue, Galgani et Fernandez-Verdejo exposent qu'effectivement la balance énergétique et l'alimentation peuvent influencer le QR à jeun et donc le ΔQR (Galgani & Fernandez-Verdejo, 2021). Ils relatent ainsi que lors d'un clamp euglycémique-hyperinsulinémique, la valeur du QR à jeun était inversement associée au ΔQR et expliquerait entre 15% et 39% de la variance du ΔQR. De plus, une balance énergétique négative (Schutz, 1993) ou une alimentation riche en lipides aurait tendance à diminuer le QR à jeun (McNeill *et al.*, 1988; Miles-Chan *et al.*, 2015). Ainsi, interpréter une valeur mesurée de QR à jeun nécessite de le mettre en regard du QR théorique venant de l'alimentation que l'on appelle quotient alimentaire (ou food quotient en anglais, FQ). Le FQ correspond au rapport théorique entre la VCO_2 et la VO_2 quand tous les macronutriments disponibles amenés par l'alimentation sont oxydés (Black *et al.*, 1986) et sa valeur varie en fonction de la composition en macronutriments (glucides, lipides et protéines) du régime alimentaire. En condition de balance énergétique stable, tous les nutriments disponibles sont en théorie oxydés ce qui signifie que 24h FQ = 24h QR. L'influence du régime alimentaire habituel sur le QR à jeun et donc sur ΔQR peut être expliquée très simplement en utilisant un exemple théorique illustré dans la **Figure 10** (Galgani *et al.*, 2008; Galgani & Fernandez-Verdejo, 2021). Deux sujets A et B sont placés en chambre métabolique et maintenus en balance énergétique. Le FQ du régime



Qui de A et B est le plus flexible ?

- En se basant uniquement sur ΔRQ: $\Delta RQ_A > \Delta RQ_B$ → A est plus flexible que B
- En prenant en compte le FQ: FQ=QR chez A et B → A et B sont tous les deux flexibles

Figure 10 : Intérêt de standardiser le régime alimentaire pour l'étude de la flexibilité métabolique.
Exemple théorique illustrant l'importance du contrôle du régime alimentaire dans la mesure de la flexibilité métabolique.

alimentaire pendant la semaine 1 de A est de 0,75 et celui du sujet B est de 0,85. Après une semaine ils reçoivent tous les deux un nouveau régime avec un FQ de 0,95. A la fin de la semaine, le FQ et le QR sont égaux. Si nous voulons évaluer la flexibilité métabolique en réponse au changement d'alimentation correspondant au régime de la semaine 2, ΔQR sera la différence entre le QR à la fin de la semaine 2 et le QR du début de la semaine 2. Le sujet A sera alors considéré comme flexible car ΔQR = 0,95-0,75 = 0,20 alors que B sera considéré comme inflexible car ΔQR = 0,95-0,85 = 0,10. En revanche, à la fin de la semaine 2 les QR des deux sujets sont égaux à leur FQ respectifs ce qui veut dire que la totalité des nutriments apportés par l'alimentation a été oxydée. En d'autres termes, les sujets A et B ont été parfaitement capables d'adapter l'oxydation des nutriments par rapport au changement de leur disponibilité et devraient donc être tous les deux être considérés comme flexibles. Dans la mesure du possible, il est donc recommandé de contrôler la balance énergétique et l'alimentation avant tout protocole visant à évaluer la flexibilité métabolique.

3.5.3 L'activité physique est un modulateur de la flexibilité métabolique

Dans une revue récente, notre équipe relate les données suggérant l'activité physique comme facteur modulateur de la flexibilité métabolique (Rynders *et al.*, 2018). En combinant des résultats d'études en diminuant ou augmentant le niveau d'activité physique, notre groupe de recherche a montré que le niveau d'activité physique (PAL pour physical activity level qui correspond au rapport entre la dépense énergétique totale journalière et le métabolisme de repos), avec ou sans changement de composition corporelle module la flexibilité métabolique (Bergouignan *et al.*, 2011; Bergouignan *et al.*, 2013a; Laurens *et al.*, 2020). Plus récemment, la flexibilité métabolique testée lors d'un OGTT a diminué suite à un protocole de diminution du nombre de pas journalier pendant 20 jours (Damiot *et al.*, 2019). Enfin, une autre étude d'alitement prolongé a montré que 21 jours d'inactivité ont induit une inflexibilité métabolique sans altération de la tolérance au glucose (Rudwill *et al.*, 2018), indiquant ainsi que l'inflexibilité métabolique est un élément précurseur à l'intolérance au glucose qui a été régulièrement observée après exposition à la microgravité réelle ou simulée.

De nombreuses études montrent par ailleurs que l'entrainement physique améliore les composants de la flexibilité métabolique. Par exemple, il est connu que l'exercice redirige les lipides venant de l'alimentation, du tissu adipeux et du stockage ectopique dans les tissus périphériques vers le muscle pour être oxydé (Calles-Escandón *et al.*, 1996; Friedlander *et al.*, 1998) et augmente la sensibilité à l'insuline (Bruce *et al.*, 2006). La valeur de ΔQR pendant un clamp euglycémique-hyperinsulinémique est augmentée après 12 semaines d'entrainement de type aérobie (60 min/jour, 5 fois par semaine à 85% de la fréquence cardiaque maximale) chez des adultes âgés atteints de diabète de type 2 et d'obésité (Malin *et al.*, 2013). Chez des jeunes adultes atteints d'obésité présentant une capacité faible à augmenter l'oxydation des lipides en réponse à un repas riche en lipides, seulement 10 jours consécutifs d'exercice aérobie (1 h/jour à 70% de la VO_{2peak}) ont suffi pour augmenter l'oxydation des lipides après un repas riche en lipides (Battaglia *et al.*, 2012). L'ensemble de ces données montrent le rôle de l'exercice et de l'activité physique sur la flexibilité métabolique. Ces effets pourraient être médiés par l'intermédiaires de facteurs circulants comme les adipokines, myokines et hépatokines (Smith *et al.*, 2018) dont leur sécrétion varie en réponse à l'exercice. Ces molécules agissent de façon endocrine et/ou paracrine et pourraient affecter la flexibilité métabolique (Laurens *et al.*, 2020; Thyfault & Bergouignan, 2020).

L'inflexibilité métabolique apparait donc comme un mécanisme central dans le développement des adaptations métaboliques à la microgravité. En revanche d'autres études sont nécessaires pour déterminer le délai de développement de l'inflexibilité métabolique, la contribution

de chaque organe impliqué dans l'homéostasie énergétique à savoir le tissu adipeux, le foie et le muscle ainsi que les mécanismes sous-jacents.

4 Rôle du métabolisme intermédiaire dans les adaptations physiologiques à la microgravité

Bien que l'aspect morphologique et fonctionnel des cellules du corps diffèrent en fonction de leur spécialisation cellulaire au sein des tissus (hépatocytes, myocytes, neurones, adipocytes etc), elles ont toutes besoin d'énergie pour fonctionner. La cellule utilise l'énergie contenue dans les macronutriments (exogènes ou endogènes) qui est libérée au fur et à mesure des réactions chimiques du métabolisme sous forme d'ATP. En ce sens, le métabolisme énergétique est au carrefour du fonctionnement de tous les systèmes physiologiques. Les modifications du métabolisme en réponse à la microgravité peuvent donc contribuer aux altérations des autres grands systèmes physiologiques, et ainsi influencer la santé et les performances des astronautes notamment lors de vols de longue durée (**Figure 11**).

Le cœur produit et consomme en moyenne 6 kg par jour d'ATP, ce qui représente 15 fois son poids (Ingwall, 2002). Pour satisfaire la haute demande énergétique, il est important que le cœur maintienne une haute flexibilité métabolique pour être capable d'utiliser tous les substrats et ainsi produire de l'ATP (Ingwall, 2002; Doenst *et al.*, 2013; Kolwicz *et al.*, 2013; Smith *et al.*, 2018). Au repos les lipides sont principalement oxydés en raison du rendement énergétique supérieur à celui des glucides. En cas de demande énergétique importante par exemple pendant un exercice physique ou après stimulation β-adrénergique lors d'une augmentation du débit cardiaque (Griffin *et al.*, 2016), le glucose et les lactates sont oxydés prioritairement (Stanley *et al.*, 2005). Il a été montré que l'accumulation de lipides dans les cardiomyocytes pouvait mener à une augmentation de la production de ROS et une dysfonction mitochondriale (Pascual & Coleman, 2016). Sachant qu'une insulino-résistance a été mise en avant à plusieurs reprises chez des astronautes après un séjour dans l'espace et dans des protocoles de simulations de microgravité (voir partie 3.1), que la lipotoxicité entretient la résistance à l'insuline (voir partie 3.4) et que sur Terre, la sensibilité à l'insuline et la VO_2max sont directement associées (AlZadjali *et al.*, 2009; Nadeau *et al.*, 2009; Byrkjeland *et al.*, 2014), l'ensemble de ces données suggèrent que les adaptations du métabolisme peuvent influencer la capacité à l'exercice. Or, l'endurance musculaire et cardiovasculaire est une capacité primordiale pour accomplir plusieurs activités physiques sur la Lune ou Mars comme les tâches de construction ou les procédures d'urgence (Hackney *et al.*, 2015). La préservation du métabolisme énergétique et de la flexibilité métabolique est donc importante dans le cadre de la recherche exploratoire et le retour des astronautes en bonne santé.

Nous avons vu dans la partie 3.4 que les os étaient également le siège de conséquences d'atteintes du métabolisme induites par la microgravité avec notamment l'accumulation de lipides dans la moelle osseuse qui entraîne des conséquences sur la résistance de l'os. Les études menées chez l'adulte en bonne santé relatent que l'adiposité de la moelle osseuse est négativement associée à la densité minérale osseuse (Di Iorgi *et al.*, 2008; Di Iorgi *et al.*, 2010; Wren *et al.*, 2011). Ce stockage ectopique de lipide augmente alors le risque de développer une ostéoporose et donc de fracture osseuse (Schwartz *et al.*, 2013). Cette accumulation de lipides dans la moelle hématopoïétique n'est pas non pas sans effet sur une des fonctions primordiales de la moelle rouge, la production de cellules sanguines. L'adiposité de la moelle osseuse est négativement associée avec l'hématopoïèse chez les personnes saines et dans le cas de pathologies du sang comme l'anémie aplasique (arrêt de production de globules rouges, globules blanc et plaquettes) et dans le cas de myélome (accumulation incontrôlée anormale de plasmocytes dans la moelle osseuse) (Naveiras *et al.*, 2009). De plus, cette accumulation pourrait être préoccupante d'autant qu'elle est associée à des niveaux anormalement bas de l'hormone érythropoïétine (EPO) (Trudel *et al.*, 2009). Ceci pourrait avoir un impact significatif car une hémopoïèse anormale affecterait la capacité aérobie, le système immunitaire ou encore le contrôle de l'inflammation.

La lipotoxicité a notamment été associée à l'inflammation systémique dans le contexte de pathologies métaboliques comme le syndrome métabolique et l'obésité (Hotamisligil, 2006; Gregor & Hotamisligil, 2011). Cette accumulation de lipides peu mener à une augmentation de la sécrétion de cytokines inflammatoires comme le TNFα et une diminution de la sécrétion d'adipokines anti-inflammatoires comme l'adiponectine, pouvant alors mener au recrutement de cellules immunitaires. Cette lipotoxicité peut donc générer des intermédiaires de signalisation qui peuvent interférer avec les réponses immunitaires locales et systémiques (Ertunc & Hotamisligil, 2016). Bien que cet aspect n'ait pas été développé dans cette introduction, ces effets additionnés à ceux induits par les vols spatiaux sur le système immunitaire des astronautes peuvent présenter une menace pour le système immunitaire des astronautes qui pourrait être affaibli. La moelle osseuse et le thymus, deux organes lymphoïdes primaires majeurs, sont manifestement affectés par les changements gravitationnels pendant les vols spatiaux (Akiyama *et al.*, 2020). Les modifications des micro-environnements de ces organes nuisent à la lymphopoïèse et peuvent donc avoir un impact indirect sur l'immunité acquise. Les réponses immunitaires acquises peuvent également être perturbées par les fluctuations gravitationnelles, les facteurs de stress et les rayonnements spatiaux, à la fois directement et de manière dépendante des hormones de stress. Ces changements peuvent affecter les réponses immunitaires acquises en réponse à des agents pathogènes, aux allergènes et aux tumeurs. Il a même été récemment évoqué que le développement de la résistance à l'insuline consécutive au vol spatial

pourrait possiblement altérer les capacités de cicatrisation après une blessure ou une chirurgie faite dans l'espace (Strollo *et al.*, 2022).

Collectivement, ces données suggèrent que les adaptations du métabolisme à la microgravité pourraient participer/amplifier les effets directs de la microgravité sur les différents systèmes évoqués en présentant de réelles contraintes fonctionnelles pour les astronautes (**Figure 11**).

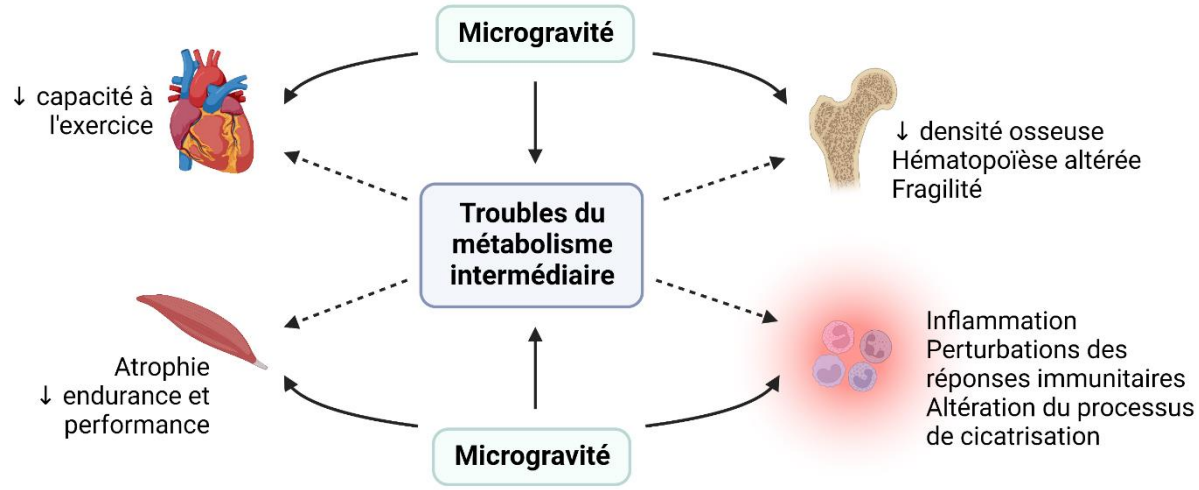


Figure 11 : Les potentielles conséquences fonctionnelles des adaptations du métabolisme intermédiaire induite par la microgravité.
Les adaptations du métabolisme consécutives à l'exposition à la microgravité pourraient s'ajouter aux effets directs de la microgravité sur le système cardiovasculaire, musculaire et osseux. Ces effets peuvent présenter des contraintes fonctionnelles importantes pour les astronautes.

De par sa position centrale dans la régulation ses systèmes physiologiques, l'étude des adaptations du métabolisme à la microgravité est importante pour mieux comprendre les mécanismes sous-jacents des altérations physiologiques induites par la microgravité et la mise en place de contremesures efficaces. Dans les parties précédentes, nous avons vu que la plupart des systèmes physiologiques sont impactés par la microgravité. Il est important de souligner que ces adaptations ne sont pas invalidantes pour l'astronaute lorsqu'il est en microgravité. En revanche, ces adaptations peuvent se révéler préoccupantes quand l'astronaute revient dans un environnement avec de la gravité, même faible, comme sur la Lune (0,2G) ou sur Mars (0,4G). A titre d'exemple, sur les vidéos des astronautes ayant été sur la Lune, les astronautes tombaient au sol et rencontraient des difficultés à se remettre debout. Comme les études de simulation de microgravité le suggèrent, la transition microgravité-gravité pourrait présenter une période particulièrement critique où les astronautes pourraient manifester de l'intolérance orthostatique, des troubles de l'équilibre, de la désorientation et des risques de blessures et de fractures. Ceci combiné aux potentielles tâches d'exploration du sol lunaire ou martien et de construction demandant un effort musculaire et cardiovasculaire important, ces altérations pourraient se révéler critique. En ce sens, le développement de contremesures efficaces

pour prévenir ces adaptations devient un enjeu crucial afin de préserver au mieux la santé et la performance des astronautes, et ainsi assurer le succès des missions.

5 Prévenir les adaptations induites par la microgravité

Si un des principaux enjeux des agences spatiales internationales est de comprendre comment le corps humain s'adapte à la microgravité, un second objectif est de tester et implémenter des contremesures efficaces pour prévenir ou mitiger ces adaptations.

5.1 Bref historique des contremesures développées

Depuis les années 1960, plusieurs contremesures ont été testées. Le développement des contremesures débute sur Terre où leur efficacité est testée lors de protocoles de microgravité simulée. Ces modèles sont effectivement indispensables afin de caractériser les effets de ces contremesures. Les contremesures validées sur Terre sont par la suite testées par les astronautes et implémentées lors des missions spatiales. La contremesure idéale doit protéger le plus de systèmes physiologiques possibles sans impacter négativement les fonctions non-visées et être facile à implémenter. Parmi les études d'alitement prolongé menées par l'ESA entre 2000 et 2017, pas moins d'une dizaine de contremesures ont été testées, seules ou associées (Demontis *et al.*, 2017).

La « lower body negative pressure » (LBNP, image en haut à gauche de la **Figure 12**) a été l'une des premières contremesures à être développée dans le but de limiter la redistribution des fluides vers le haut du corps (Campbell & Charles, 2015). La pression négative au niveau des membres inférieurs et du pelvis stimule les mécanismes de régulation cardiovasculaire permettant alors de recréer le gradient de pression hydrostatique que l'on observe en position debout sur Terre. Le premier prototype de LBNP a été développé dans les années 1960 par Duane Graveline, chirurgien américain également astronaute. Ce système était constitué d'un caisson hermétique dans lequel les sujets y étaient insérés des pieds à la taille en position allongée. Une dizaine d'années plus tard, les soviétiques ont mis au point la combinaison Chibis (image en haut à droite de la **Figure 12**) qui sera testée pendant le programme Salyut (Gazenko *et al.*, 1981). Différents modèles de LBNP ont été testés au cours des différentes missions mais cette contremesure montre une contrainte majeure par la nécessité d'utiliser ce système sur de longues périodes, ce qui est incompatible avec les emplois du temps des astronautes. Pour s'affranchir de cette contrainte de temps, les Russes ont mis au point un système différent permettant de limiter la redistribution des fluides : le brassard de cuisses (thigh cuffs en anglais, image en bas à droite de la **Figure 12**). En étant placé autour de la cuisse et constitué de bandes élastiques et non élastiques, la perte de liquides était effectivement ralentie mais le brassard ne permettait pas de prévenir l'intolérance orthostatique (Robin *et al.*, 2020).

<u>Figure 12</u> : Les différentes contremesures développées pour prévenir la distribution des fluides thoraco-céphalique induite par la microgravité.

En haut à gauche : caisson de Low-body Negative Pressure (LBNP), crédit CNES/E. Grimault. En haut à droite : combinaison Chibis développée par les Russes qui repose sur une technologie LBNP, crédit CNES/IMBP. En bas à gauche : centrifugeuse à bras court dans les locaux du MEDES, crédit CNES/R. Barranco. En bas à droite : brassard de compression en cours d'installation autour de la cuisse d'un volontaire, crédit CNES/R. Benoit.

La microgravité étant l'une des sources majeures à l'origine de nombreuses adaptations physiologiques dans l'espace, recréer une gravité artificielle serait en toute logique l'exemple type de la contremesure idéale. Cette idée est très plébiscitée dans les films et séries de science-fiction traitant de la conquête spatiale comme Interstellar de Christopher Nolan, Seul sur Mars de Ridley Scott ou encore la série For All Mankind développée par la plateforme de streaming Apple TV. Dans ces images, une partie du vaisseau spatial est constitué d'une roue qui tourne autour d'un axe, recréant ainsi une gravité artificielle par force centrifuge. Ce genre d'installation se limite pour l'instant à la fiction. Sur Terre, les effets de la gravité artificielle comme contremesure sont testés à l'aide de centrifugeuses à bras court (image en bas à gauche de la **Figure 12**). Les protocoles ont testé des gravités artificielles de 0,4G (équivalent de la gravité sur Mars) à 2G avec une moyenne de 1G. La durée d'exposition varie de 25 minutes à 4 heures, de façon continue ou intermittente. Le défi est de trouver le meilleur

compromis entre temps d'utilisation et les effets de prévention. Ces protocoles se sont avérés être efficaces pour lutter contre le développement de l'intolérance orthostatique. De plus, ils ont permis de réduire la perte de volume plasmatique et de maintenir la capacité d'exercice et limiteraient également le déconditionnement osseux (Clément *et al.*, 2016). Bien que la centrifugation pour recréer une gravité artificielle semble être une contremesure prometteuse, son encombrement physique et son coût de transport rendent son implémentation à bord de l'ISS laborieuse et quasi impossible pour l'instant. D'autres contremesures comme l'exercice, la nutrition, les supplémentations pharmacologiques ont été développées et les parties suivantes se consacrent à détailler plus en avant les contremesures d'exercice physique et la nutrition. Bien qu'historiquement l'exercice mérite un développement en premier, la nutrition sera d'abord développée ci-après.

5.2 La nutrition : un enjeu pour tous les systèmes physiologiques

Le rôle principal du système alimentaire spatial est de fournir les nutriments nécessaires aux astronautes pour couvrir leurs besoins. Définir et satisfaire les besoins nutritionnels des astronautes est un défi permanent depuis les tous premiers vols spatiaux. Pour les missions de courte durée, de quelques jours à quelques semaines, un apport nutritionnel inadéquat n'aurait que peu de conséquences. Pour les missions de longue durée en revanche, un déséquilibre nutritionnel pourrait entraîner des conséquences importantes (perte de poids cliniquement significative, problèmes hormonaux, fragilité osseuse etc.). En ce sens, la NASA a établi les besoins nutritionnels des astronautes pour la première fois en 1991 dans le cadre de la préparation des missions visant à envoyer des équipages à bord de la Space Station Freedom qui deviendra l'ISS après association avec les Russes (Lane *et al.*, 1991). Pour ces vols de 90 à 180 jours, les experts recommandaient les besoins nutritionnels classiques établis sur Terre avec des modifications spécifiques basées sur les pertes connues de tissus osseux et musculaires. Il a été également stipulé que le statut nutritionnel des astronautes devait être évalué avant, pendant et après la mission. Ces recommandations ont été plusieurs fois mises à jour en collaboration avec les agences spatiales canadienne, européenne, japonaise et russe et cette fois en considérant les vols de longue durée, avec pour objectif les missions vers la Lune et Mars (National Aeronautics and Space Administration, 1996, 2005, 2020). L'évolution de ces recommandations concernant les apports énergétiques totaux et les macronutriments est résumée dans la **Table 1**.

Table 1 : Evolution des recommandations alimentaires pour les astronautes.

Nutriments	Besoins pour des séjours à bord de l'ISS (1996)	Besoins pour les missions d'exploration (2005)	Besoins pour les missions d'exploration (2020)
Apports énergétiques	Basé sur les équations de l'Organisation Mondiale de la Santé (1985)	Basé sur les équations des apports nutritionnels de référence (Trumbo *et al.*, 2002)	Basé sur les équations des apports nutritionnels de référence (Trumbo *et al.*, 2002)
Protéines	Entre 12 et 15% des apports énergétiques journaliers totaux, le ratio des protéines animales/végétales ne doit pas être supérieur à 60/40	0,8 g/kg de poids de corps, inférieur à 35% des apports énergétiques totaux. Le ratio des protéines animales/végétales doit être de 2/3 et 1/3 respectivement	1,2 à 1,8 g/kg de poids de corps, le ratio des protéines animales/végétales ne doit pas être supérieur à 60/40
Glucides	Entre 50 et 55% des apports énergétiques journaliers totaux, les sucres ajoutés doivent être inférieurs à 10% des apports journaliers totaux	Entre 50 et 55% des apports énergétiques journaliers totaux	Entre 45 et 65% des apports énergétiques journaliers totaux, les sucres ajoutés doivent être inférieurs à 10% des apports journaliers totaux
Lipides	Entre 30 et 35% des apports journaliers totaux	Entre 25 et 35% des apports journaliers totaux	Entre 20 et 35% des apports journaliers totaux

Adapté de (Smith *et al.*, 2021) et élaboré à partir de (National Aeronautics and Space Administration, 1996, 2005, 2020).

Pendant les premières années de mise en service de l'ISS, les agences spatiales de la NASA et Roscosmos fournissaient chacune la moitié des provisions alimentaires. Pendant cette période, la NASA avait opté pour un menu dit standard au lieu de menus individualisés. Il était plus simple ainsi de faire face aux aléas des calendriers de lancement qui demandaient de s'assurer que la nourriture spécifique de chaque membre d'équipage soit à bord en même temps qu'eux. L'équipage recevait alors par cargo un assortiment d'aliments standardisés. Il a été souvent observé que les astronautes mangeaient moins dans l'espace que sur Terre, avec notamment une diminution des apports entre 20 et 25% (Stein *et al.*, 1999a; Heer *et al.*, 2000; Stein, 2000; Smith *et al.*, 2005; Zwart *et al.*, 2014). Le manque d'attractivité, de saveurs et de diversité de la nourriture disponible à bord de l'ISS a souvent été cité comme l'une des raisons pouvant expliquer cette diminution. En effet, la majorité des aliments consommés sont sous forme lyophilisés et de boîtes de conserve. Les agences spatiales ont fait de véritables efforts pour améliorer la qualité de la nourriture disponible dans l'espace (**Figure 13**). Le CNES a été un acteur important de cette amélioration, notamment en collaborant avec plusieurs chefs cuisiniers et pâtissiers (Pierre Hermé, Thierry Marx, Alain Ducasse) permettant ainsi aux astronautes de pouvoir déguster des plats améliorés pour les occasions particulières comme pour les anniversaires et les fêtes. La familiarité et le choix des aliments, ainsi que les aspects psychosociaux entourant la

nourriture (temps et espace pour se réunir et manger ensemble) deviennent en effet plus importants avec l'isolement prolongé, le confinement et l'éloignement de la Terre (Stuster, 2000).

Figure 13 : La nourriture à bord de l'ISS.

La nourriture occupe une place importante à bord de l'ISS. Photo en haut à gauche : l'astronaute Thomas Pesquet (ESA) très heureux de pouvoir manger des macarons pour son anniversaire (Crédit ESA/NASA/T.Pesquet). Photo en haut à droite : Tea time à bord de l'ISS (Crédit ESA/NASA). Photo en bas à gauche : la quintessence de la gastronomie à bord de l'ISS, thon-olives-cheddar-tortilla (Crédit ESA/NASA). Photo en bas à droite : de gauche à droite les astronautes Tim Kopra (NASA) et Franck De Winne (ESA), le cosmonaute Roman Romanenko (Roscosmos) et l'astronaute Michael Barratt (NASA) qui partagent un repas (Crédit ESA/NASA).

Pour les voyages spatiaux de longue durée au-delà de l'orbite terrestre, il sera essentiel que la nutrition non seulement prévienne les carences en nutriments mais serve également de contremesures aux nombreux effets négatifs induits par la microgravité sur les systèmes physiologiques. A ce jour, ceci constitue une lacune majeure dans notre compréhension du rôle de la nutrition dans les vols spatiaux. Une contremesure nutritionnelle semble prometteuse car elle permettrait de minimiser la charge de lancement car ne nécessite pas de matériel important, elle

pourrait être bénéfique pour de nombreux systèmes physiologiques et ne serait pas chronophage pour les astronautes.

Sur Terre, la supplémentation en acides aminés a été évaluée pour contrebalancer la perte de masse et de force musculaire observée au cours des vols spatiaux et des études d'alitement prolongé. Dans leur revue, Stein et Blanc relatent que sur les six études ayant testé cette contremesure, seules trois ont mis en évidence des effets positifs (Stein & Blanc, 2011). Il semblerait que les résultats dépendraient de l'apport en protéines de base, avec l'efficacité de la supplémentation qui diminue lorsque l'apport journalier en protéine dépasse les 1,2 g/kg/j. D'autres stratégies ont été testées avec notamment des supplémentations à base de composés à propriétés anti-oxydantes et anti-inflammatoires. Plusieurs études menées chez l'homme et le rat ont effectivement montré les effets positifs de nutriments comme les polyphénols, certaines vitamines et les acides gras essentiels sur les adaptations induites par la microgravité (Zwart *et al.*, 2010; Momken *et al.*, 2011). Dans une étude préliminaire financée par le CNES, un cocktail de composés anti-oxydants et anti-inflammatoires (acides gras ω-3, vitamine E, polyphénols, sélénium) a été testé dans un protocole de sédentarisation où des hommes sains physiquement actifs ont diminué drastiquement leur activité physique pendant 20 jours (Damiot *et al.*, 2019). Cette étude a montré que la supplémentation a permis de prévenir l'augmentation de la *de novo* lipogenèse, la diminution de l'oxydation des lipides postprandiale et l'augmentation de la triglycéridémie et la diminution de la CSA des fibres musculaires de type IIa induits par la diminution d'activité physique (Damiot *et al.*, 2019). Ce cocktail a été testé par la suite en 2017 dans un protocole d'alitement prolongé de 60 jours chez des hommes sains. La première étude ayant réalisé des analyses protéomique et d'expression de gènes sur des biopsies musculaires a montré que l'augmentation de la nitrosation des protéines (marqueur des dommages induits par les RNS sur les protéines) et de l'atrophie musculaire ont été prévenues par le traitement et la diminution des défenses antioxydantes a également été atténuée par le cocktail (Blottner *et al.*, 2021). Alors qu'une seconde étude menée dans le cadre de ce protocole a montré une diminution du taux de protéines carbonylées (indicateur des dommages induits par le stress oxydant sur les protéines) dans le groupe supplémenté, aucun effet sur la diminution de la force musculaire, l'atrophie des fibres musculaires et la diminution de la capacité oxydative n'ont été notés (Arc-Chagnaud *et al.*, 2020). Les résultats sur le métabolisme sont en cours de valorisation.

5.3 L'exercice physique : la pierre angulaire des contremesures spatiales

5.3.1 Historique de la contremesure d'exercice

L'exercice physique reste à ce jour la pierre angulaire des programmes de contremesure. L'exercice permet effectivement de lutter contre l'hypokinésie et l'hypodynamie imposées par la microgravité. Le programme Apollo (1961-1967) a été le premier à inclure une contremesure d'exercice pendant le vol. A l'époque, aucun protocole d'exercice précis était utilisé mais les astronautes disposaient de l' « Apollo Exerciser », un système basé sur un appareil de friction à corde à résistance variable. Cet appareil sera abandonné à cause du risque de générer un incendie, sachant que la friction des cordes générait de la chaleur et que l'air à bord des vaisseaux était composé à 100% d'O_2 (Scheuring *et al.*, 2007). Au cours de ce programme, un rapport de la NASA indique que les astronautes pratiquaient sur ce dispositif pour la récupération et pour la relaxation. Les astronautes ont en revanche soulevé la nécessité d'établir des consignes de pratique, alors que les planificateurs de missions stipulaient que ce n'était pas nécessaire pour des vols de courte durée (inférieurs à 14 jours) (Scheuring *et al.*, 2007). Pour la première mission Skylab (28 jours), 30 minutes par jour étaient au début allouées à la pratique d'exercice sur un cyclo-ergomètre avec un protocole recommandé mais pas imposé (Sawin *et al.*, 1975). Pour les deux missions SLS2 et SLS3 qui suivirent (56 et 84 jours), le temps d'exercice est passé à 60 puis 90 minutes par jour pour lesquels un tapis de course très rudimentaire a été mis à disposition des astronautes (Thornton & Rummel, 1977). Ces protocoles montraient déjà des effets intéressants avec une perte de force d'extension des jambes de l'ordre de 25% après les deux premières missions et inférieure à 10% pour la troisième mission Skylab. Les membres d'équipage pouvaient tenir debout et marcher sans difficultés après le retour sur Terre. La fréquence cardiaque à 75% du travail maximal était maintenue au niveau de pré-vol, et la capacité aérobie des membres du troisième équipage a même été améliorée (Scott *et al.*, 2019). Dans les années 1990 à bord du vaisseau Space Shuttle (entre 2 et 17 jours de vol), les équipages des 135 vols avaient à disposition un cyclo-ergomètre, deux tapis de course et un rameur. Les règles de vol stipulent que l'exercice devait être pratiqué au moins une fois tous les deux jours pour le commandant de bord, le pilote et l'ingénieur de vol, et tous les trois jours pour les spécialistes de mission et les spécialistes de charge utile, mais l'intensité et la durée n'étaient pas prescrites. La VO_2peak était maintenue, mais diminuait immédiatement après atterrissage, probablement en raison de la diminution du volume sanguin, du volume cardiaque et du débit cardiaque (Levine *et al.*, 1996).

Figure 14 : Les différents équipement pour la pratique d'exercice à bord de l'ISS.

A gauche : l'astronaute Alexander Gerst (ESA) courant sur le T2 (crédit NASA/ESA). Au milieu : l'astronaute Catherine Coleman (NASA) sur le CEVIS (crédit NASA). A droite : l'astronaute Steve Lindsey (NASA) s'exerçant sur l'ARED (crédit NASA).

Depuis, les protocoles d'exercice physique ont été optimisés (Hackney *et al.*, 2015; Korth, 2015; Loehr *et al.*, 2015; Scott *et al.*, 2019). Actuellement, à bord de l'ISS 2,5 heures par jour sont dédiées à la pratique d'exercice 6 jours par semaines. Ces 2,5 heures incluent le temps d'installation des équipements (harnais et élastiques) et le temps dédié à l'hygiène. Deux sessions par jours, 30 à 45 minutes d'activité aérobie et 45 minutes de résistif sont prévues. Plusieurs dispositifs d'exercice ont été implantés et ont évolué au cours du temps. Initialement, le Cycle Ergometer with Vibration Isolation and Stabilization System (CEVIS, **Figure 14**), permettant d'obtenir des charges de 350 W à 120 rpm, le treadmill with vibration isolation system (TVIS) et l'Interim Resistive Exercise Device (iRED) ont été mis en place dès 1993. L'iRED a été remplacé par l'Advanced Resistive Exercise Device (ARED, **Figure 14**) à partir de 2009 en raison du manque de stimuli généré par l'iRED pour amener des effets bénéfiques sur la force et la masse musculaire et la densité minérale osseuse. L'ARED permet donc de combler les limitations de l'iRED en reproduisant les forces exercées par de l'exercice résistif avec des poids libres sur Terre (English *et al.*, 2008; Loehr *et al.*, 2011). De plus, l'appareil permet de produire une force plus constante tout au long du mouvement avec une résistance générée par des cylindres à vide. L'ARED permet d'engager tous les grands groupes musculaires avec une charge maximale de 272 kg. Le TVIS a été remplacé en 2010 par un tapis de course de seconde génération (T2, **Figure 14**). Le TVIS ne permettait de courir que jusqu'à 16 km/h alors que le T2 permet maintenant de courir jusqu'à 20,4 km/h (Korth, 2015).

5.3.2 Les effets de la contremesure d'exercice sur les grandes fonctions physiologiques

Cette section est adaptée de la revue publiée en janvier 2022 dans Journal of Physiology (Le Roux et al., 2022).

Estimer l'efficacité de la contremesure d'exercice dans l'espace est un challenge sachant que tous les astronautes pratiquent de l'exercice en vol. Il serait en effet éthiquement difficilement acceptable de demander à des astronautes d'être des sujets contrôles. En ce sens, les études de microgravité simulée peuvent nous apporter ce genre d'information en comparant un groupe alité pratiquant de l'exercice selon différentes modalités et intensité (exercice résistif seul ou combiné avec de l'exercice aérobie) et un groupe alité strict (sans exercice). L'exercice aérobie s'est notamment révélé être efficace pour mitiger les adaptations cardiovasculaires menant à de l'intolérance orthostatique et la diminution de la capacité à l'exercice (Pavy-Le Traon *et al.*, 2007). Il a été montré que la diminution de volume plasmatique induite par 30 jours d'alitement a été totalement prévenue par la pratique quotidienne de 60 min/jour d'exercice isotoniques sur cyclo-ergomètre (Greenleaf *et al.*, 1992). À la suite d'un alitement de 16 jours, une seule session d'exercice maximal sur cyclo-ergomètre a montré des bénéfices sur l'intolérance orthostatique (Engelke *et al.*, 1995), ce qui était associé à l'augmentation ou la restauration du volume sanguin et des fonctions de baroréflexe (Convertino, 1991, 1996). De façon évidente, la pratique d'exercice pendant l'alitement permet de lutter contre la diminution de la capacité d'exercice. Cinq sessions d'exercice résistif sur presse associée à une plateforme de vibration ont permis de prévenir la diminution de la VO_2max mais n'a pas permis de mitiger la diminution de volume de plasma et l'intolérance orthostatique induits par 21 jours d'alitement (Guinet *et al.*, 2020; Kenny *et al.*, 2020). Pendant un alitement de 14 jours, des sessions d'exercice résistif (3 fois par semaine) et aérobie (6 fois par semaine) ont suffi à maintenir la VO_2peak au niveau d'avant alitement (Ploutz-Snyder *et al.*, 2014). Concernant le système squelettique, plusieurs régimes d'exercice ont été testés pour maintenir la masse osseuse et l'exercice résistif associé ou non avec d'autres formes d'exercice (aérobie, avec plateforme de vibration) semble être le plus efficace. De l'exercice résistif sur plateforme de vibration pratiquée à hauteur de 11 sessions par semaines a permis de prévenir la diminution de perte osseuse chez des hommes alités pendant 56 jours (Rittweger *et al.*, 2010). Globalement, l'exercice résistif combiné à l'exercice aérobie permet de maintenir les systèmes cardiovasculaires et osseux.

A ce jour, 10 études d'alitement ont testé les effets de l'exercice comme contremesure contre les adaptations métaboliques induites par l'alitement. Ces études s'étendent sur des périodes de 14 à 90 jours et les prescriptions d'exercice varient en termes de type (résistif ou résistif et aérobie), de durée, de fréquence et d'intensité. Les protocoles sont résumés dans la **Table 2** issue de l'article en **Annexe 1**. Les résultats sont reportés dans la **Figure 15**.

Table 2 : Résumé des différents protocoles d'exercice testés pendant des alitements prolongés (Le Roux et al. 2022).

Publication	Study name Duration of BR Sample size	Exercise modalities	Estimated duration of MVPA	Estimated energy cost
Resistance exercise				
(Bergouignan *et al.*, 2006) (Fernandez-Gonzalo *et al.*, 2020) (Irimia *et al.*, 2017) (Rudwill *et al.*, 2013) (Trappe *et al.*, 2004)	LTBR 2001-2002 90 d HDT-BR n=18 ♂	35 min every 3 d during BR on flywheel ergometer. Progressive warm-up + 4x7 max concentric/eccentric squat + 4x14 in calf press. 2 min rest between sets and 5 min between EX.	82 min MVPA/wk 12 min MVPA/d	8 MET.h/wk 1.2 MET.h/d
(Brooks *et al.*, 2014)	28 d HDT-BR n=31 ♂	1 h/d, 6 d/wk. Target intensity: 70-80% of 1RM as estimated by the OMNI rating of perceived EX 10 category scale. 7 to 8 REX targeting major muscle groups during each session. Lower body (squats, single leg squats, diagonal jump, calf raise, single-leg hip extension, leg curl, single-leg hip abduction) and upper body (pull-ups, pull-over, triceps press, chest fly, shoulder press, biceps curl, upright row, lateral arm raise) EX were performed on alternating days.	360 min MVPA/wk 51 min MVPA/d	36 MET.h/wk 5.2 MET.h/d
(Ferrando *et al.*, 1997)	14 d HDT-BR n=6 ♂	Squat on horizontal leg-training device every 2 d. 3x12 squats, training volume progressively increased to reach 5x8 squat at session 3 till the end of BR.	?	?
(Guinet *et al.*, 2020) (Kenny *et al.*, 2017) (Kenny *et al.*, 2020)	MNX 21 d HDT-BR n=12 ♂	5 sessions of EX, on leg press machine with a vibration platform (8 mm peak-to-peak, 25 Hz): bilateral squats (10 rep, 75% 1-RM, 8 s/rep), single heel raises (×1.3 body weight, contractions performed as fast as possible until fatigue) and bilateral heel raises (×1.8 body weight, contractions performed as fast as possible until fatigue). A 5% load adjustment was made based on the ability of volunteers to complete the set of EX.	5-15 min MVPA/wk 0.7-2.1 min MVPA/d	0.5-1.5 MET.h/wk 0.01-0.2 MET.h/d
(Moriggi *et al.*, 2010)	BBR1 55 d HDT-BR n=12 ♂	2 bouts/d of EX (6min each) of RVE at preset frequencies ranging from 19 at the beginning to 25Hz. Total of 89 sessions. 1. Squatting EX: Knees were extended from 90° to almost full extension in cycles of 6s for each squat (knee extensors).	36 min MVPA/wk 5.1 min MVPA/d	3.6 MET.h/wk 0.5 MET.h/d

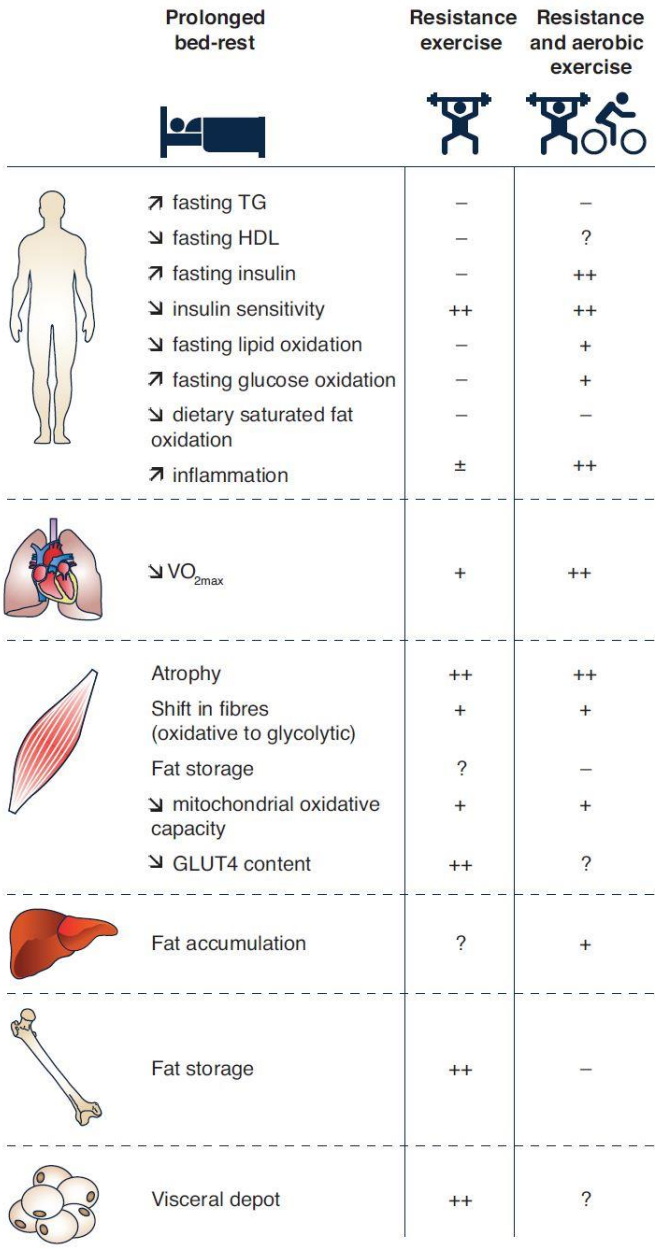
		2. Heel raises: With knees almost extended, heels were raised to fatigue. Only then, brief rests (< 5s) were allowed with the entire foot on the vibration platform in order to recover, and subjects started to raise their heels again (foot plantar flexors). 3. Toe raises: Similar to 2, but toes were raised instead of heels (foot dorsi-flexors). 4. "Kicks": With the same loading as in 1–3, knees were extended as quickly and forcefully as possible. The platform was struck with the balls of the feet, and legs rested on the Galileo Space framework in between the kicks. This was done 10 times with 10s of rest inserted.		
(Belavy *et al.*, 2014) (Trudel *et al.*, 2012)	BBR2-2 60 d HDT-BR n=24 ♂	3 d/wk: 1. Bilateral leg press (~75–80% of pre-bed-rest max voluntary contraction); 2. Dingle-leg heel raises (~1.3 times body weight); 3. Double leg heel raises (~1.8 times body weight); 4. back and forefoot raise (performing hip and lumbar spine extension against gravity with ankle dorsiflexion, but with ~1.5 times body weight applied at the shoulders). The RVE group performed the same exercises as the REX group, except that whole body vibration was applied. The corresponding vibration parameters were as follows: 1. frequency 24 Hz, amplitude 3.5–4 mm, and peak acceleration ~8.7 g, where g ~ 9.81 ms^{-2}; 2. frequency 26 Hz, amplitude 3.5–4 mm, and peak acceleration ~10.2 g; 3. frequency 26 Hz, amplitude 3.5–4 mm, and peak acceleration ~10.2 g; 4. frequency 16 Hz, amplitude 3.5–4 mm, and acceleration ~3.9 g	15.8 min MVPA/wk 2.3 min MVPA/d	1.6 MET.h/wk 0.2 MET.h/d
Combined resistance and aerobic exercise				
(Bergouignan *et al.*, 2009) (Bergouignan *et al.*, 2010) (Lee *et al.*, 2014) (Mutin-Carnino *et al.*, 2014) (Rudwill *et al.*, 2015) (Salanova *et al.*, 2008) (Trappe *et al.*, 2007b) (Trappe *et al.*, 2007a)	WISE 60 d HDT-BR n=16 ♀	REX: 35min every 3 d, 4x7 max concentric/eccentric squat + 4x14 in calf press. AEX: 50min every 2 d, 50min in lower body negative pressure vertical treadmill at 40-80% pre-bed-rest VO_2max	247 min MVPA/wk 35.3 min MVPA/d	33.1 MET.h/wk 4.7 MET.h/d

(Trappe *et al.*, 2008) (Trudel *et al.*, 2009)				
(Krainski *et al.*, 2014)	35 d HDT-BR n=27 ♂/♀	REX: 25-30min 2 d/wk. 2x8–12 of lower body exercises (leg press, plantar flexion, knee flexion, hip flexion, and hip abduction) and 1x8–12 of upper body EX (shoulder press, elbow flexion and extension, chest press, pullovers, and abdominal crunches) were performed in the supine position, loads were adjusted weekly to reach muscle fatigue during each set of EX. After 5 wk of BR, 2x20 plantar flexion exercises on each leg 2/d (6-8min) against an elastic band were added for all remaining subjects in EX group. AEX: 6 d/wk. During each week of BR, subjects completed 1 recovery (low intensity, typically <70% max HR), 2 base (moderate intensity, between 70-80% max HR), 1 MSS (vigorous intensity, 80–90% maximal HR), and 2 interval sessions (high intensity, 90–95% max HR or above), each lasting a total of 30 – 46 min and separate warm-up/cool-down phases lasting 5 min each. Intervals consisted of 6 cycles of 3 min at 90–95% of max HR, followed by 3 min at recovery pace.	381 min MVPA/wk 54.4 min MVPA/d	49.5 MET.h/wk 7.1 MET.h/d
(Ploutz-Snyder *et al.*, 2018)	70 d HDT-BR n=26 ♂	REX: 3 d/wk. 3x4 supine lifts (squat, leg press, unilateral leg curl, and heel raise); squats and leg press were each performed using a standard shoulder-width stance, single-leg stance, or wide-leg stance on a rotating basis. Training followed a nonlinear periodized model in which load and repetitions were varied on a daily basis to optimize adaptations. AEX: 6 d/wk. Alternating days of continuous cycle EX for 30 min at 75% of VO_2peak (3 d/w) with interval treadmill sessions of 30s, 2min, or 4min intervals (3 d/wk) at nearly max intensity.	314.5 min MVPA/wk 45 min MVPA/d	40 MET.h/wk 5.7 MET.h/d
(Ward *et al.*, 2020)	RSL 60 d HDT-BR n=23 ♂	48 sessions including 4 types of training sessions based on varying CMJ and repetitive hops between 80-90% of BW during 1.5-3min preceded by a warm-up and 3 max CMJ at 80% of BW.	17.5 min MVPA/wk 2.5 min MVPA/d	2.6 MET.h/wk 0.4 MET.h/d

HDT-BR, 6° head-down tilt bed-rest; d, days; wk, week; max, maximal; BW, body weight; CMJ, countermovement jump; EX, exercise; REX, resistive exercise; AEX, aerobic exercise.

L'exercice résistif seul a permis de prévenir la perte de fonction et de masse musculaire y compris la réduction du diamètre des fibres pendant l'alitement (Trappe *et al.*, 2004; Moriggi *et al.*, 2010). Cependant, les mécanismes qui sous-tendent les effets protecteurs de l'exercice contre l'atrophie musculaire ne sont pas totalement élucidés. Il a été montré que l'exercice résistif prévenait la diminution de la synthèse de protéines musculaires induite par l'alitement (Ferrando *et al.*, 1997) et de l'expression de la myostatine (Irimia *et al.*, 2017), une myokine connue pour contribuer à la perte musculaire. Alors que 21 jours d'alitement ont conduit à l'augmentation des voies métaboliques impliquées dans l'autophagie et la dégradation protéique, l'exercice résistif sur plateforme de vibration a permis d'atténuer ces effets (Kenny *et al.*, 2020). Malgré ces effets positifs sur le muscle squelettique, l'exercice résistif n'empêche que partiellement les altérations métaboliques induites par l'alitement. Il protège contre l'augmentation de la visfatine (Rudwill *et al.*, 2013), une adipokine qui imite les effets de l'insuline mais n'empêche pas l'augmentation de l'interleukine 6 et de la protéine C-reactive, deux marqueurs pro-inflammatoires, ou la diminution de l'adiponectine (Brooks *et al.*, 2014) dont les changements sont associés à l'inflammation, aux troubles du métabolisme lipidique et à l'obésité. Même lorsqu'il est pratiqué à haute intensité, l'exercice de résistance ne permet pas de prévenir la diminution du cholestérol-HDL (Brooks *et al.*, 2014; Guinet *et al.*, 2020), le développement d'une résistance à l'insuline, l'hyperlipidémie, ou le changement d'oxydation des substrats au détriment des lipides (Bergouignan *et al.*, 2006). Cette dernière observation est surprenante sachant que l'exercice résistif permet de prévenir le changement de typologie des fibres musculaires du type oxydatif au type glycolytique (Trappe *et al.*, 2004), compense les altérations transcriptomiques dans le muscle liées au métabolisme énergétique aérobie (chaine de transport d'électron, la β-oxydation des acides gras et le cycle de Krebs) (Fernandez-Gonzalo *et al.*, 2020), et maintient partiellement l'activité et l'expression génique d'enzymes contrôlant le métabolisme oxydatif (par exemple la citrate synthase et la succinate déshydrogénase) au niveau mitochondrial (Irimia *et al.*, 2017). Bien que l'exercice de résistance seul ne rétablisse pas les niveaux d'oxydation des acides gras aux valeurs de base (Bergouignan *et al.*, 2006), aucune accumulation de lipides dans les os (Trudel *et al.*, 2012) ou au niveau viscéral n'a été signalée (Belavy *et al.*, 2014). Il n'existe pas de données sur les effets de l'exercice résistif sur le stockage ectopique de lipides dans le foie ou les muscles pendant l'alitement. Dans toutes ces études, la séance d'exercice était effectuée en une seule fois de manière continue. Cependant, lorsque l'exercice est effectué sous forme de squats de façon intermittente tout au long de la journée, la perte de masse musculaire est évitée, mais pas la réduction de la sensibilité à l'insuline au niveau du corps entier et au niveau périphérique (Ward *et al.*, 2020). Dans l'ensemble, la faible dépense énergétique associée à l'exercice de résistance (**Table 2**) peut être responsable des effets protecteurs partiels ou limités sur les indices de santé métabolique.

Les études d'alitement ayant combiné l'exercice résistif à de l'aérobie ont induit probablement une dépense énergétique liée à l'exercice plus importante comparé aux protocoles d'exercice résistif seul. Cette approche préserve ou du moins atténue la structure et la fonction musculaire, le volume et la puissance musculaire des muscles de la jambe, la force et l'endurance musculaire, la composition et le diamètre des fibres musculaire ainsi que le contenu mitochondrial et la capacité oxydative (Trappe *et al.*, 2007a; Trappe *et al.*, 2007b; Salanova *et al.*, 2008; Bergouignan *et al.*, 2009; Krainski *et al.*, 2014; Lee *et al.*, 2014; Ploutz-Snyder *et al.*, 2018). Bien que les altérations musculaires aient été prévenues par tous les protocoles d'exercice de résistance et d'aérobie indépendamment du type, de la durée, de l'intensité et de la fréquence d'entrainement, les effets protecteurs sur le métabolisme étaient variables. Un entrainement combiné aérobie et résistif prévient le développement d'un état pro-inflammatoire (Mutin-Carnino *et al.*, 2014), la résistance à l'insuline et le changement d'oxydation des nutriments au profit des glucides et au détriment des lipides (Bergouignan *et al.*, 2009). Cependant, l'exercice ne permet pas de lutter contre l'augmentation des triglycérides à jeun, la réduction du taux d'oxydation des acides gras alimentaires, probablement due à une altération du transport des acides gras dans le myocyte, et l'accumulation de lipides dans le muscle squelettique (Bergouignan *et al.*, 2009) et les os (Trudel *et al.*, 2009). L'accumulation de lipides au niveau hépatique, mesurée par l'intermédiaire de marqueurs hépatiques, induite par la microgravité simulée semble être cependant compensée (Rudwill *et al.*, 2015).



<u>Figure 15</u> : Effets préventifs de l'exercice (résistif combiné ou non à de l'aérobie) sur les adaptations métaboliques induites par l'alitement prolongé (Le Roux et al. 2022).

-, pas d'effet; +, partiellement protégé; ++, totalement protégé; ?, pas de données; ±, pas de consensus. TG, triglycerides; HDL, high-density lipoproteins. Utilisation autorisée par John Wiley and Sons, licence 5486670832494.

Bien qu'étant une composante essentielle du programme de contremesures, l'exercice physique ne semble pas prévenir l'ensemble des adaptations physiologiques induites par la microgravité simulée. Bien qu'une relation effet-dose semble se dessiner (Le Roux *et al.*, 2022), les protocoles ont encore besoin d'être optimisés.

Les agences spatiales ont testé des contremesures associées à la pratique d'exercice (supplémentation biphosphonates, injections de testostérone), nous ne développerons pas cet aspect ici.

Objectifs et hypothèses

Le métabolisme intermédiaire et la flexibilité métabolique jouent un rôle majeur dans la régulation de santé métabolique mais aussi d'autres fonctions physiologiques. Les effets de la microgravité sur ces derniers entrainent des adaptations importantes pouvant impacter la performance et la santé des astronautes et ainsi compromettre les missions. Dans le contexte de la nouvelle phase exploratoire spatiale à venir, ce travail de thèse a cherché à combler les lacunes actuelles afin de mieux comprendre l'impact de la microgravité sur la flexibilité métabolique, ainsi que les mécanismes cellulaires et moléculaires sous-jacents. Spécifiquement, l'objectif général était de tester l'hypothèse selon laquelle l'exposition de courte et longue durée à la microgravité, aussi simulée que réelle, entraîne une diminution de la flexibilité métabolique chez l'homme. Le but secondaire était d'évaluer la relation entre la performance de l'exercice physique en vol et la flexibilité métabolique. La poursuite de cet objectif principal s'est réalisée à l'aide de deux études de recherche indépendantes qui sont présentées dans deux chapitres.

Le muscle squelettique étant un organe majeur dans le métabolisme, la première étude avait pour objectif de déterminer le rôle du muscle dans le développement d'une inflexibilité métabolique et d'une résistance à l'insuline au niveau du corps entier, chez des hommes sains immergés pendant cinq jours dans une baignoire d'immersion sèche, modèle de déconditionnement rapide sur Terre. L'hypothèse était que la microgravité induite par l'immersion sèche entraîne très tôt un changement de l'utilisation des substrats à jeun et après un repas, une baisse du contrôle du glucose ainsi qu'une diminution de la flexibilité métabolique ; ces altérations au niveau du corps entier sont associées à une réduction de la flexibilité métabolique et à une altération de la voie de signalisation de l'insuline du muscle squelettique.

La seconde étude avait pour objectif de caractériser pour la première fois chez des astronautes le métabolisme des substrats et la flexibilité métabolique en réponse à un séjour de longue durée (> 3 mois) à bord de l'ISS. L'hypothèse spécifique était que l'exposition de longue durée à la microgravité réelle induit un changement dans l'oxydation des substrats au profit de l'utilisation des glucides et au détriment de celle des lipides aussi bien à jeun qu'en condition postprandiale, et cela en association avec le développement d'une inflexibilité métabolique chez des astronautes. En l'absence d'un groupe contrôle lors des vols spatiaux, c-à-d d'un groupe qui ne pratique pas d'exercice, nous avons évalué les effets de l'exercice sur le métabolisme des substrats en examinant les relations entre la pratique de la contremesure d'exercice et le métabolisme des nutriments et la flexibilité métabolique. L'hypothèse était que les changements de l'oxydation des substrats et de la flexibilité métabolique seraient plus faibles chez les astronautes qui pratiquent le plus d'exercice à bord de l'ISS.

6 Short-term physical inactivity triggers whole-body and skeletal muscle metabolic inflexibility and insulin resistance

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Résumé

Introduction

Bien que les résultats de cette étude s'insèrent dans les sciences spatiales directement en lien avec les effets de la microgravité sur la flexibilité métabolique, nous avons choisi d'intégrer ces résultats dans un contexte différent. L'inactivité physique est reconnue comme une cause importante de la mortalité mondiale et constitue un facteur de risque majeur pour de nombreuses pathologies chroniques. L'inflexibilité métabolique est récemment apparue comme participant aux altérations du métabolisme induites par l'inactivité physique et précèderait le développement de l'intolérance au glucose dans un contexte de résistance à l'insuline. En revanche, les mécanismes cellulaires et moléculaires à l'origine de l'inflexibilité métabolique induite par l'inactivité physique restent encore inconnus. Le muscle squelettique est l'organe majeur responsable de la clairance du glucose en conditions post-prandiales. Il a été montré que l'inactivité physique induit des changements structuraux, transcriptionnels et moléculaires menant à une diminution de la sensibilité à l'insuline, une hyperlipidémie, une diminution de l'oxydation des lipides en faveur des glucides, et un stockage ectopique de lipides. Ces changements suggèrent que des altérations métaboliques du muscle squelettique pourraient être responsable de la diminution de la flexibilité métabolique et de la sensibilité à l'insuline observées au niveau du corps entier. Par ailleurs si les effets à long-terme de l'inactivité physique ont été bien étudiés lors d'études d'alitement prolongés, les effets à court-termes sont beaucoup moins connus.

Objectifs et hypothèses

Lors d'une étude d'immersion sèche, un modèle analogue de microgravité sur Terre, nous avons cherché à déterminer l'effet de 5 jours d'inactivité physique sur :

- La flexibilité métabolique et la sensibilité à l'insuline au niveau du corps entier ;
- Les propriétés métaboliques intrinsèques du muscle et notamment la sensibilité à l'insuline et la flexibilité métabolique ;
- La régulation moléculaire de protéines clé impliquées dans la régulation de la voie de signalisation à l'insuline et l'utilisation des substrats.

Nous faisons les hypothèses que :

- L'inactivité physique induite par l'immersion sèche entraîne un changement dans l'utilisation des substrats à jeun et après un repas en faveur de l'utilisation des glucides et au détriment de celle des lipides, une diminution de la sensibilité à l'insuline et de la flexibilité métabolique

- Les altérations au niveau du corps entier sont associées à une inflexibilité métabolique et à une altération de la voie de signalisation de l'insuline au niveau du muscle squelettique,
- Ces altérations métaboliques sont associées à un stockage ectopique des lipides, notamment au niveau du muscle squelettique et du foie.

Matériel et Méthodes

Dix-huit hommes sains et physiquement actifs ont été recrutés (âge 36,6 [SD 5,5] ans ; IMC 23,3 [1,8] kg/m²) et soumis à 5 jours d'immersion sèche. Avant et après 4 jours d'immersion sèche, l'oxydation des substrats a été mesurée par calorimétrie indirecte à jeun et après un repas riche en glucides standardisé. La flexibilité métabolique au niveau du corps entier a été déterminée comme la différence entre la valeur de quotient respiratoire (QR) 30 minutes après ingestion du repas standardisé et la valeur de QR à jeun (ΔQR_{30}) et par les variances mathématiques du QR et de l'insuline au cours du test métabolique. La capacité cardiorespiratoire maximale a été mesurée pendant un test d'exercice incrémental, le tissu adipeux intramusculaire, le contenu hépatique en lipides et la section transversale du quadriceps ont été mesurés par imagerie à résonance magnétique (IRM) et la composition corporelle par absorptiométrie biphotonique à rayon X. Des biopsies musculaires ont été prélevées avant et après 5 jours d'immersion et immédiatement mises en culture. Des expériences *in vitro* ont mesuré la sensibilité à l'insuline (synthèse de glycogène à partie du ^{14}C-glucose) et la capacité à augmenter ou à diminuer l'oxydation de ^{14}C-palmitate en présence croissance d'acide palmitique ou de glucose, respectivement. Des analyses moléculaires ont complété ces études.

Résultats

Cinq jours d'inactivité physique induite par immersion sèche entraîne une diminution de la masse maigre, de la capacité aérobique maximale, une réduction de la sensibilité à l'insuline associée à une hyperinsulinémie postprandiale et une augmentation du contenu lipidique hépatique. Si aucun changement dans l'utilisation des substrats à jeun ou dans les 5 heures suivant un repas n'a été détecté, une incapacité à augmenter l'utilisation des glucides en phase postprandiale aigue a été notée en lien avec une oxydation lipidique plus élevée. En parallèle, les expériences *in vitro* ont montré que les propriétés métaboliques intrinsèques du muscle squelettique sont modifiées avec une altération de la voie de signalisation de l'insuline et une diminution de la capacité à réduire l'oxydation des lipides en présence croissante de glucides.

Discussion

L'utilisation du modèle spatial d'immersion sèche a permis de mettre en avant que 5 jours d'inactivité physique aigue induit un déconditionnement métabolique rapide au niveau du corps entier

caractérisé par une diminution de la sensibilité à l'insuline et de la flexibilité métabolique au glucose. Ces adaptations sont probablement dues à des adaptations des propriétés métaboliques des cellules musculaires squelettiques. L'altération du métabolisme lipidique au niveau du corps entier n'est pas accompagnée par une diminution de la flexibilité métabolique aux lipides et induit le développement d'un stockage ectopique de lipides. Cette étude permet également de mettre en avant qu'une empreinte épigénétique des cellules satellites se produit rapidement en réponse à une inactivité physique de courte durée.

Abstract

Background: Physical inactivity is a major risk factor for the development of metabolic disease. Physical inactivity impairs metabolic flexibility (MF), a core component of metabolic health defined as the capacity of the body to adjust substrate use to changes in fuel availability. This inflexibility was shown to precede the development of glucose intolerance in the pathophysiology of insulin resistance under inactive conditions but the mechanistic underpinnings are poorly known.

Objective: To determine the role of skeletal muscle, the largest consuming glucose organ of the body, alterations in the development of whole-body decreased metabolic flexibility and insulin sensitivity and determine the underpinning cellular and molecular mechanisms following 5 days of dry immersion

Methods: Using a space analog model of rapid deconditioning, glycemia, insulinemia and respiratory quotient (RQ), carbohydrate and fat oxidation were measured by indirect calorimetry before and after a standardized carbohydrates-rich meal in 18 active, normal-weight men (age=33.6 [SD 5.5] years, BMI=23.3 [1.8] kg/m²) before and after five days of dry immersion. MF was determined as the difference between RQ 30min after meal ingestion and fasting RQ (ΔRQ_{30}). Liver fat content was assessed by magnetic resonance imaging (MRI) and body composition by absorptiometry. MF to glucose (suppressibility), to palmitate (adaptability), and insulin-stimulated Akt phosphorylation was assessed in vitro in isolated muscle cells differentiated into myotubes from muscle biopsy.

Results: Short-term physical inactivity induced decreases in lean and fat mass ($P<0.001$), fasting ($P<0.01$) and postprandial ($P<0.001$) hyperinsulinemia, a delay in the adjustment of postprandial carbohydrate oxidation ($P<0.001$) without change in fasting nor postprandial lipid use and ΔRQ_{30}. Concomitantly, intrinsic metabolic properties of skeletal muscle were impaired with altered insulin signaling ($P=0.02$), suppressibility ($P=0.01$) and intact adaptability. This was associated with increased fat accumulation in liver ($P<0.001$).

Conclusion: Our findings suggest that intrinsic skeletal muscle cell changes may precede alterations of whole-body MF and insulin sensitivity and contribute to physical inactivity-induced metabolic alterations.

Key words: substrate oxidation, metabolic flexibility, suppressibility, adaptability, physical inactivity.

Introduction

Insufficient physical activity is a public health concern and a major risk factor for common chronic diseases including obesity, metabolic syndrome, insulin resistance, and type 2 diabetes (T2D) (Booth *et al.*, 2012; Booth *et al.*, 2017). However, the mechanistic underpinnings of the associations between physical inactivity and metabolic diseases have not been fully elucidated. This is partly because studying the physiology of physical inactivity in humans is challenging. Dry immersion and bed-rest studies are traditional ground-based space analog models (Pavy-Le Traon *et al.*, 2007) that have provided key insights on the pathophysiology of physical inactivity over the past two decades. Using these models, we and others showed that physical inactivity *per se* triggers insulin resistance, hyperlipidemia, decreased clearance of dietary lipids, reduced fasting and post-prandial lipid oxidation in favor of greater use of carbohydrate as fuel, and favors ectopic fat storage (Bergouignan *et al.*, 2011; Le Roux *et al.*, 2022). Collectively, these abnormalities define the main tenants of the metabolic inflexibility concept.

Metabolic flexibility is the capacity of the body to adjust the level of daily substrate use to changes in fuel availability and energy demand (Kelley & Mandarino, 2000; Rynders *et al.*, 2018; Galgani & Fernandez-Verdejo, 2021). Impairments in metabolic flexibility are commonly observed in people with metabolic diseases (Kelley *et al.*, 1999; Galgani & Fernandez-Verdejo, 2021). The metabolically inflexible state in these chronic metabolic disorders is characterized by reduced capacity to burn fat as fuel in the fasting state and inability to increase carbohydrate oxidation following meal consumption. Although the regulation of metabolic flexibility is still an area of investigation, it was demonstrated that habitual physical activity predicts metabolic flexibility and the transition from an active to an inactive lifestyle triggers the development of metabolic inflexibility (Bergouignan *et al.*, 2011). We further showed in lean healthy men that 21 days of bed rest trigger metabolic inflexibility even when energy balance is maintained (Rudwill *et al.*, 2018). While decreased insulin sensitivity and increased fat deposition were observed at skeletal muscle level, systemic glucose intolerance was detected only in response to acute overfeeding. This indicated that metabolic inflexibility precedes systemic glucose intolerance in the pathophysiological context of insulin resistance. The molecular and cellular mechanisms leading to metabolic inflexibility under inactive conditions are however unknown.

Skeletal muscle is the largest glucose consuming organ in the body accounting for more than 80% of the insulin-stimulated glucose clearance (Baron *et al.*, 1988), and therefore has a key role in determining systemic insulin sensitivity. Impaired glucose utilization in muscle determines the severity of systemic insulin resistance in such common metabolic diseases as type 2 diabetes and obesity. Skeletal muscle is also quantitatively the predominant tissue during physical activity (Egan &

Zierath, 2013). Medium and long-term physical inactivity induced by bed-rest leads to structural, transcriptional and molecular changes of skeletal muscle including muscle atrophy, a shift from oxidative to glycolytic muscle fibers, reduced mitochondrial volume and oxidative capacity, and altered expression of genes involved in mitochondrial function (Bergouignan *et al.*, 2011). In parallel, gene expression and activity of enzymes coupled with oxidative metabolism are down-regulated. This reduced skeletal muscle oxidative capacity was associated with lipid accumulation in the muscle and potentially in the liver (Bergouignan *et al.*, 2009; Rudwill *et al.*, 2015; Rudwill *et al.*, 2018). In contrast, gene expression and activity of proteins involved in carbohydrate metabolism are up-regulated (Stein *et al.*, 2002; Stein & Wade, 2005; Bergouignan *et al.*, 2009). Bed-rest also reduces skeletal muscle insulin action on glucose uptake and glycogen storage (Mikines *et al.*, 1991; Alibegovic *et al.*, 2009; Alibegovic *et al.*, 2010b; Dirks *et al.*, 2016; Shur *et al.*, 2022). Altogether, these changes suggest that metabolic alterations of skeletal muscle could be responsible for the metabolic inflexibility and decreased insulin sensitivity observed at the whole-body level in response to inactivity.

Prior studies found that both characteristics of muscle insulin resistance (Henry *et al.*, 1995) and properties of metabolic flexibility in the transition between fat and glucose oxidation that are manifested *in vivo* (Ukropcova *et al.*, 2005) are retained in myocyte cultures. In this 5-day dry immersion study, we tested in healthy and physically active male adults the hypothesis that short-term inactivity reduces metabolic flexibility and insulin sensitivity at the whole-body level in association with intrinsic muscle defects including decreased capacity to adapt fuel oxidation to changes in their availability and altered insulin signaling pathway.

Material and methods

Participants

Twenty healthy and physically active men were recruited from the local community (**Figure 16**). Eligibility criteria included being aged between 20 and 45 years, being normal weight (body mass index [BMI] = 20-26 kg/m²), physically active, and free of any known diseases and in a good state of general health on the basis of medical history, physical and psychological examination, and routine urine and blood biochemical screening. None had a family history of diabetes mellitus or gastrointestinal disease or was taking any medication for 3 months before the study. All subjects were informed about the experimental procedures and gave their written consent. The experimental protocol conformed to the standards set by the Declaration of Helsinki and was approved by the local Ethic Committee (CPP Est III: October 2, 2018, n° ID RCB 2018-A01470-55) and French Health Authorities (ANSM: August 13, 2018). The study was registered at ClinicalTrials.gov (NCT03915457). Two subjects withdrew before the pre ambulatory period for reasons unrelated to the protocol. A total of eighteen subjects were included in the study.

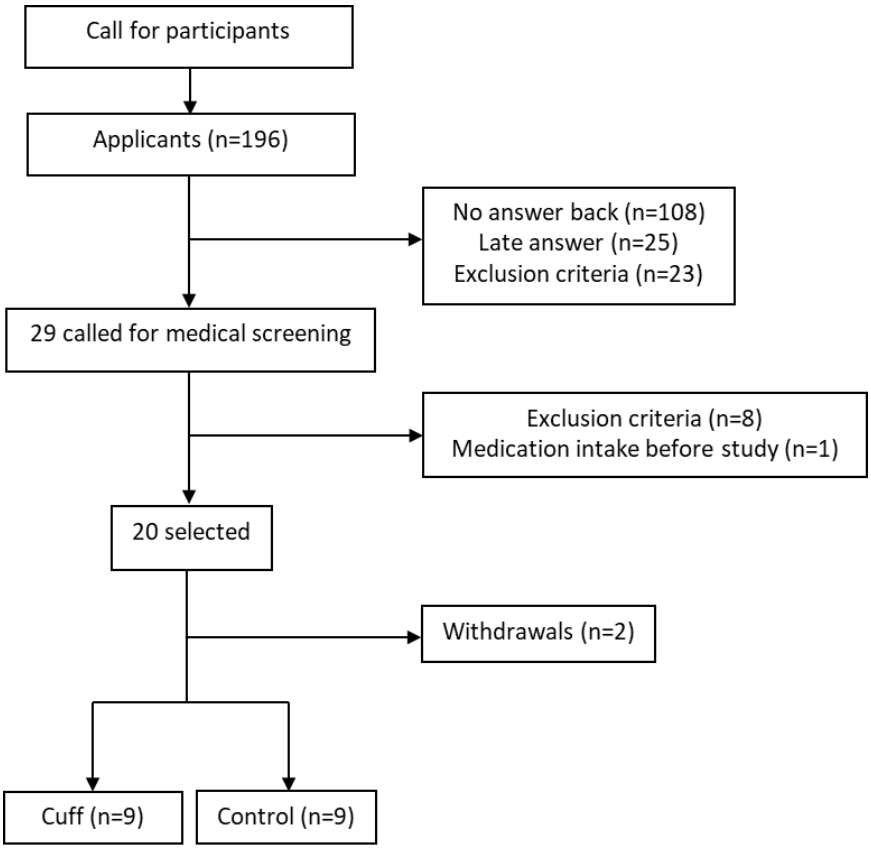


Figure 16: Flow Chart of general selection of the dry immersion study at the MEDES Space Clinic.

Overall study design

Details of the dry immersion protocol were previously published (De Abreu *et al.*, 2017). The study was conducted at the MEDES Space Clinic located at the Hôpital Rangueil in Toulouse, France from November 19, 2018, to March 23, 2019. The study design is presented in **Figure 17**. The experimental protocol included four days of ambulatory baseline measurements followed by five days (120 hours) of dry immersion and two days of ambulatory recovery. Participants arrived in the evening of the first day of the ambulatory period and left in the morning of day two of recovery. They were also asked to report to the MEDES a week prior to the beginning of the study for pre-immersion muscle biopsy and resting metabolic rate (RMR) measurement to estimate the individual energy needs.

The study was organized and funded by the French Space Agency (CNES). Most ground-based studies supported by the CNES have a dual objective, *i.e.*, 1) to better understand the physiological effects of simulated microgravity on the body and the associated mechanistic underpinnings, and 2) develop and test countermeasures to mitigate these adverse effects. In this study, the effects of thigh cuffs were tested. To understand the rationale behind this countermeasure, it is important to know that the loss of gravity induces a redistribution of body fluids through the upper part of the body leading to decreased left ventricle end-diastolic volume and stroke volume, remodeling and enlargement of arteries and veins, and heart deconditioning (Herault *et al.*, 2000). Thigh cuff compression was suggested to be a promising countermeasure to limit the body fluids redistribution (Yao *et al.*, 2008). By consequence, two days before the start of the study, participants were randomly divided into two groups: Control group (strict dry immersion) and Cuffs group (countermeasure group). Subjects of the Cuffs group were asked to wear thigh cuffs during the five days of dry immersion, from 1000h to 1800h on day one of dry immersion and from 0800h to 1800h on days two to five. Thigh cuffs are elastic strips, adapted to each subject to have the same effects on lower-limb distensibility as at counterpressure of about 30 mmHg. Individual adjustment was determined for each subject with calf plethysmography, performed in the supine position two days before immersion. On day one of dry immersion, thigh cuffs were put on immediately prior to the onset of immersion at 1000h.

Two subjects, one Control and one Cuffs, underwent dry immersion simultaneously in the same room, in two separate baths. Thermoneutral water temperature was continuously maintained. Lights were turned off from 2300h to 0700h. Daily hygiene, weighing and some specific measurements required extraction from the bath. During these out-of-bath periods, subjects were placed in -6° head-down position to maintain the shift in blood fluid from the lower part to the upper part of the body. Total out-of-bath supine time for the 120 h of immersion was on average 9.7±1.3 h. Otherwise, subjects remained immersed in a supine position for all day activities and were continuously observed by video

monitoring during the five days of dry immersion. Body weight, blood pressure, heart rate and tympanic body temperature were measured daily. The day before dry immersion and on day four, a metabolic test was run to assess whole-body metabolic flexibility and insulin sensitivity. A week before the start of the study, participants were asked to report to the MEDES for the collection of the baseline muscle biopsy.

Body mass and composition

Body mass was measured daily in supine position with a calibrated balance. Fat-mass and lean body mass were measured after an overnight fast by dual-energy X-ray absorptiometry (DXA, HOLOGIC QDR 4500W, USA) four days before and after five days of dry immersion.

Cardiorespiratory fitness

The assessment of aerobic capacity was performed on a cycle ergometer in upright position, before and during the first day of recovery following the dry immersion period. Subjects were connected to the metabolic cart (Oxycon Pro, Jegger) for gas exchange determination in the breath-by-breath mode. After 5 minutes of baseline data collection, subjects cycled for 5 minutes at 25, 50 and 75 % of the VO_2max at selection. At completion of the third stage, the workload increased by 25W every minute until they could no longer maintain the desired cycling cadence (75 rpm) and/or they wanted to stop and/or the test termination criteria were met.

Diet

Diet was tightly controlled during the study by registered dieticians and provided by the MEDES metabolic kitchen. Participants were fed with conventional foods calculated to provide 1-1.2 g/kg/day of protein, 35-38% of energy as fat and the rest as carbohydrates. Energy intake was assessed to provide 160% of estimated RMR using the World Health Organization (WHO) equation (1985) during the control and recovery periods and 130% of estimated RMR during the dry immersion. No extra food was allowed between the three daily meals taken at set times. All leftovers on the trail were weighted by the staff of the Metabolic kitchen. Actual energy intake was calculated using the Nutrilog software (Version 3.11b). Daily nutrient intake is presented in **Table 3**.

Table 3: Dietary intakes during ambulatory control period and dry immersion.

	n	Ambulatory period	Dry immersion
Energy intake (kcal)	18	2604 (241)	2137 (207)
Carbohydrates (kcal)	18	1274 (128)	1000 (101)
Carbohydrates (%)	18	49 (1)	47 (1)
Lipids (kcal)	18	921 (83)	751 (77)
Lipids (%)	18	35 (1)	35 (1)
Proteins (kcal)	18	409 (41)	386 (41)
Proteins (%)	18	16 (1)	18 (1)

Data are means (SD).

Metabolic test

A metabolic test was performed before and after four days of dry immersion **(Figure 18)**. The day before the test, participants meals were standardized. They were composed of 49% carbohydrates, 35% lipids and 16% protein for a total of about 2639 Kcal and 2185 Kcal intake during the ambulatory control and dry immersion periods, respectively. More than twelve hours after the last evening meal and an overnight fast, intravenous catheters were inserted into a forearm vein for blood sampling. Substrate oxidation was measured in fasting state (60 and 30 minutes before meal ingestion) and after a mixed carbohydrate-rich breakfast meal challenge. Participants ingested a standard breakfast at 1000h in less than 20 minutes that represented 35% of RMR and was composed of 74% carbohydrates, 14% lipids and 12% protein for a total of 574 (SD 53) Kcal energy intake in average. Blood samples were collected in fasted state to measure plasma non-esterified fatty acids (NEFA), glycerol, glucose, triglycerides, and insulin. At 30, 60, 120, 180, 240 and 300min post-meal consumption blood was drawn for plasma glucose and insulin measurement. Blood was centrifuged immediately after collection at 3500 rpm and 4°C for 15 minutes, and plasma was then separated and frozen at -80°C until analysis.

During the protocol, subjects were not allowed to sleep and were under constant supervision of the investigators. Urine samples were collected at baseline and from the 5h cumulative urines for measurement of urinary nitrogen excretion. Gas exchange was measured by indirect calorimetry for 20-min periods that started 30, 60, 90, 120, 180, 240 and 300 minutes after meal ingestion (T0) using canopy dilution respirometry (Quark, Cosmed, Italy).

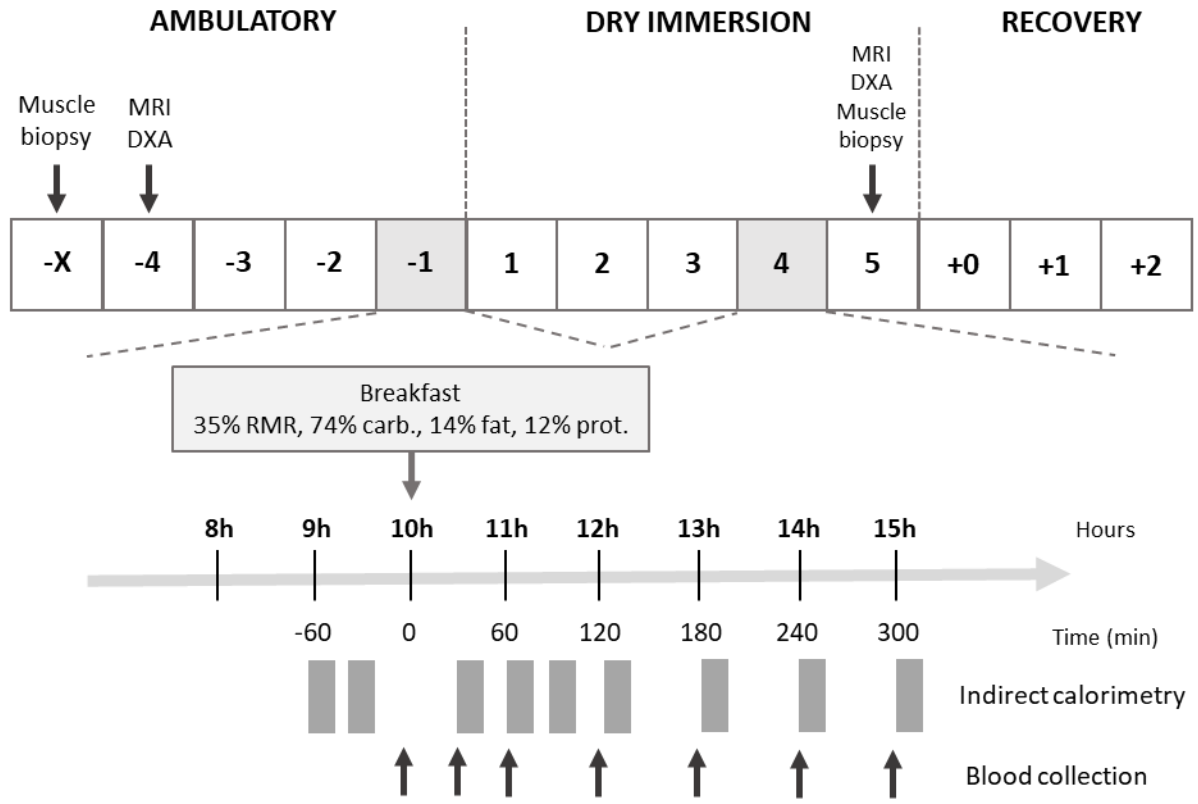


Figure 17: Experimental design of the dry immersion study.

Muscle biopsy during the ambulatory phase was performed a week prior to the first day of the stay at MEDES (BDC-X). Carb, carbohydrates; DXA, dual-energy X-ray absorptiometry; MRI, magnetic resonance imaging; Prot., proteins; RMR, resting metabolic rate.

Metabolites

Plasma glucose (Glucose GOD FS, DiaSys), NEFA (NEFA-HR(2), Fujifilm WAKO), free glycerol and triglycerides (TR0100, Sigma-Aldrich) were measured by colorimetric assays and chemiluminescence methods following supplier's instructions. Insulin was measured by enzyme-linked immunosorbent assay (ELISA) (80-INSHU-E10.1, ALPCO). Plasma AST, GGT and alkaline phosphatase are indices of liver health and were measured by enzymatic test using phosphate pyridoxal, as routinely done in hospital laboratories. Homeostasis model assessment for insulin resistance (HOMA-IR) was calculated as $HOMA - IR = fasting\ glucose\ (mmol/L) \times fasting\ insulin\ (\mu UI/ml)\ /22.5$ (Matthews *et al.*, 1985). Insulin sensitivity index (IS) was calculated as $IS = 1.89 + 2690/(I_b G_b I_m G_m)$ with I_b, G_b and I_m, G_m the values of insulin (µUI/ml) and glucose (mmol/L) in fasting condition and 180min after meal ingestion. This index was developed to assess insulin sensitivity during a high-carbohydrate meal and was validated against values obtained during an intravenous glucose tolerance test (Aloulou *et al.*, 2006). Postprandial insulinemia and glycemia were assessed by calculating the incremental areas under the curve (iAUC) from 0 to 300 min using the trapezoidal method.

Substrates oxidation and metabolic flexibility

Respiratory quotient (RQ), total carbohydrate and lipid oxidation were calculated from VO_2 and VCO_2 using classical equations of indirect calorimetry (Frayn, 1983). Postprandial carbohydrate and lipid oxidation were assessed as the total area under the curve (tAUC) calculated with the trapezoidal method from breakfast ingestion to 5h post-meal ingestion. The early and late postprandial phases were also assessed by calculating tAUC from 0 to 90 min and from 90 min to 300 min, respectively. Metabolic flexibility was assessed using the difference between RQ measured at T+30min post-meal and fasting value (ΔRQ_{30}). A decrease in ΔRQ_{30} indicates a decrease in metabolic flexibility, *i.e.*, a lower capacity to shift from fat oxidation to carbohydrate oxidation following meal ingestion.

Muscle cross sectional area, intramuscular adipose tissue and liver fat content

Quadriceps cross sectional area (CSA) and intramuscular adipose tissue (IMAT) were assessed by 1.5T magnetic resonance imaging (MRI; CHU Rangueil, Toulouse) according to the Dixon I/O technique, four days before and after five days of dry immersion. IMAT content was calculated by ImageJ software (1.52k, Java 1.8.0_172, 64-bit, NIH, USA). Three-point Dixon MRI was used to measure IMAT as a percentage inside the anterior muscle compartment from the images. IMAT was measured in the same region of interest (ROI) on images centered on the same landmark. The measurements were repeated until agreement was within the 3% threshold, and the values were averaged. Due to technical issues, we have data for only 16 subjects.

Two methods were used to calculate the liver fat content with two different softwares MRQuantif 2019.05.12 version and OleaSphere 3.0.16 to improve the analysis. One method by the MRQuantif was validated in previous studies and used for the first time on liver iron concentration by the team of Professor Yves Gandon (Rennes University, France) (Alústiza *et al.*, 2004; Gandon *et al.*, 2004). The second method commonly used was previously described as advanced chemical-shift based gradient-echo MRI technique that estimates the proton density fat fraction (PDFF). It is a standardized and objective measure of fat content (Kühn *et al.*, 2014; Patel *et al.*, 2015; Hetterich *et al.*, 2016). The multiple echoes acquisition at different 10 echo times with fat and water signals nominally in phase or out of phase with each other and applies an algorithm to generate a PDFF parametric map depicting fat quantity and distribution throughout the liver. This method was shown to reliably measure liver fat content when compared to other magnetic resonance techniques and histology-determined steatosis (Noureddin *et al.*, 2013; Zhong *et al.*, 2014). In order to estimate PDFF across the liver, three ROI 500mm² to 1500mm² in area were placed in each different parts of the liver with a homogeneous signal

avoiding large vessels and enlarged bile ducts. The liver fat content percentage is considered as the mean of three ROIs for three echo times in/out phases. Due to technical issues, data are available for 17 subjects only.

Skeletal muscle cell culture

Muscle biopsies of *vastus lateralis* were obtained in fasting state, using the Bergstrom technique, a week before and after five days of dry immersion. Muscle tissue (60-80mg) was collected in DMEM (Dulbecco's modified Eagles's medium) low glucose-Glutamax™/penicillin-streptomycin 2% (PS)/ fungizone 0.5 µg/ml and minced until muscle was in tiny pieces. Then, tissue was washed and digested in a trypsin 0.25%, collagenase type IV 0.068%, EDTA 0.05%, BSA 0.1% solution for 30 min (37°C, mild agitation) to isolate the stromal fraction. The tissue slurry was centrifuged (350g, 10min, room temperature), resuspended in proliferation medium 1 (DMEM low glucose-Glutamax™, FBS 16%, human epithelial growth factor [hEGF] 10ng/mL, dexamethasone 0.39µg/mL, BSA 0.05%, fetuin 0.5mg/mL, gentamycin 50ng/mL, fungizone 50ng/mL) to reach sub-confluence. Medium was changed every three days until 80% confluence and then cells were transferred into two T75 flasks in proliferation medium 2 (DMEM low glucose-Glutamax™, FBS 10%, human epithelial growth factor (hEGF) 10ng/mL, dexamethasone 0.39µg/mL, BSA 0.05%, fetuin 0.5mg/mL, gentamycin 50ng/mL, fungizone 50ng/mL). At 80% confluence, cells were trypsinized (0.05% trypsin-EDTA) and frozen (1.10^6 cells/cryotube) in a cryopreservation medium (DMEM, fetal bovine serum 16%, dimethylsufoxide 10%) until further use for *in vitro* experiments.

Cells from thawed primary cultures were amplified in T75 flasks in proliferation medium 2 until subconfluence. Cells were then trypsinized (0.05% trypsin-EDTA) and divided into two groups: 1) unsorted fraction, and 2) immunosorted fraction in which quiescent and activated satellite cells (*i.e.* myoblasts) were purified using PE-Vio770 mouse anti-human CD56 (Miltenyi Biotec) and fluorescent-activated cell sorting analysis (FACS, BD Influx, BD Biosciences), as previously described (Ukropcova *et al.*, 2005).

CD56+ cells were plated in 6, 12 or 24-well plates (10 000 cells/cm²) and grown in proliferation medium 2. Differentiation into myotubes was initiated at approximately 80% confluence, by switching to myogenic medium (AlphaMEM, FBS 2%, PS 2%, fetuin 0,5mg/ml). The medium was changed every two days. To study myogenic differentiation, unsorted, CD56+ or CD56- fractions were lysed in RLT/2-Mercaptoethanol 1% lysis buffer and stored at -80°C for mRNA extraction or fixed in 4% paraformaldehyde and stored at 4°C for cytochemical analysis. Finally, differentiated myotubes from

both fractions were used for lipid and glucose metabolism assays as well as western blotting analysis of insulin signaling pathway.

Immunocytochemistry

Primary myotubes (day 3 of differentiation) were permeabilized for 20 min in PBS with 0.5%Triton. After rinsing, cells were incubated in PBS with glycine (100 mM, 15 min), then in PBS with 3% BSA (30 min) and finally in PBS with 0.1% BSA, 0.2% Triton and 0.05 % Tween containing mouse primary antibody directed against human sarcomeric myosin (MF20, DSHB, 1/4, overnight, 4°C). Cells were rinsed and incubated with the corresponding fluorescence-labeled second antibody (goat anti-mouse-AlexaFluor 546, Invitrogen, 1/250, 90 min, 4°C). Cells were washed and incubated with 5 µg/ml Hoechst 33242 to stain nuclei, then washed again before direct observation with a fluorescence microscope (Nikon Eclipse Ti). Representative images were recorded (original magnification x100, Image analysis system LUCIA).

Reverse transcription and real-time quantitative PCR

Total RNA was isolated primary myotubes (day 3 of differentiation) with Qiagen RNeasy mini kit according to manufacturer instructions (Qiagen GmbH, Hilden, Germany) and quantified on a Nanodrop ND-1000 (Thermo Scientific, Rockford, IL, USA). RNA was reverse-transcribed using the Multiscribe Reverse Transcriptase method on a GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA, USA). Real-time qPCR was performed to determine cDNA content. Probes were bought from Applied Biosystems (MYF5 Hs00271574_m1, PAX7 Hs00242962_m1, MYOD Hs00159528_m1, MYH1 Hs00428600_m1, MYH2 Hs00430042_m1, MYH7 Hs01110632_m1, TBP Hs00427620_m1). The amplification reaction was performed in duplicate on 10 ng of the cDNA samples in a final volume of 10 µL in 384 reaction plates on a Viia7 system (Applied Biosystems). All expression data were normalized by the 2(ΔCt) method using TBP rRNA as internal control.

Skeletal muscle cells protein content

Primary myotubes (day 3 of differentiation) were exposed to α-MEM low glucose in the absence or presence of 100 nM of insulin for 20 min (37°C). They were then scrapped in RIPA buffer supplemented with protease and phosphatase inhibitor cocktail (Sigma-Aldrich) and snapped frozen. Equal amount of proteins (25 µg) were run on a 4-20% SDS-polyacrylamide gel electrophoresis (Biorad), transferred onto nitrocellulose membrane (Bio-Rad) and washed with Tris-buffered saline

Tween 0.2%(TBS-T)/5% milk (1h, 4°C). Membranes were then incubated overnight at 4°C with primary antibodies against total Akt (1/1000 CST #4691) and Phospho-Akt (Thr308) (1/1000, CST #4060), washed in TBS-T and finally incubated with anti-rabbit horseradish peroxidase-linked secondary antibody (1/10000, 1h, 4°C). Immunoreactive proteins were revealed by enhanced chemiluminescence reagent (Clarity Western ECL Substrate, Bio-Rad), visualized using the ChemiDoc MP Imaging System and data analyzed using the ImageLab 4.2 version software (Bio-Rad Laboratories, Hercules, USA).

In vitro palmitate oxidation

Primary myotubes (day 3 of differentiation) were preincubated for 3h with [1-^{14}C] palmitic acid (1 µCi/ml; PerkinElmer, Boston, MA, USA) and non-labeled (cold) palmitic acid with or without glucose. Palmitate was coupled to fatty acid–free BSA in a molar ratio of 5:1. To determine the relationship between fatty acid oxidation and palmitate concentration in the medium (*i.e.*, adaptability assay), cells were incubated with a mixture of cold and radiolabeled palmitate at final concentrations of 20, 100 and 200 µM of total palmitate. To determine the relationship between fatty acid oxidation and glucose concentration (*i.e.*, suppressibility assay), cells were incubated with radiolabeled palmitate at 100 µM final concentration and 5 mM of cold glucose. Following incubation, $^{14}CO_2$ was measured as previously described for glucose oxidation assay (Laurens *et al.*, 2016). In parallel, medium was centrifuged and the supernatant was counted to quantify ^{14}C-ASM (acid soluble metabolites). All assays were performed in triplicate and data were normalized to cell protein content measured with Pierce BCA protein assay kit (Thermo Scientific).

In vitro suppressibility was calculated as $suppressibility\ (\%) = (1 - [palmitate\ oxidation\ at\ 5\ mM\ glucose / palmitate\ oxidation\ of\ 100\ \mu M\ palmitate]) \times 100$. In vitro adaptability was calculated as $adaptability\ (fold\ increase) = palmitate\ oxidation\ at\ 200\ \mu M\ palmitate / palmitate\ oxidation\ at\ 20\ \mu M\ palmitate$ (Ukropcova *et al.*, 2005).

In vitro glucose oxidation and glycogen synthesis

Primary myotubes (day 3 of differentiation) were preincubated with a glucose- and serum-free medium for 90 min. This incubation was followed by a 3h incubation with D[U-^{14}C]glucose (1 µCi/ml, PerkinElmer) and 5.5 mM of non-labeled (cold) glucose in the presence or absence of 100 nM of insulin. Following incubation, medium was transferred into a 24-well trapping plate. NaOH 1N was added to adjacent wells and the plate was sealed. Using a Hamilton syringe, 70% perchloric acid was added in each well containing culture medium. After agitating on shaker during 1h, NaOH was transferred to a

scintillation vial and radioactivity was counted on a β-counter (PerkinElmer) to determine $^{14}CO_2$. All assays were performed in triplicates, and data were normalized to protein content.

To study basal and insulin-stimulated glycogen synthesis, the cells were solubilized by the addition of KOH 30%. The samples were added of glycogen (Sigma-Aldrich) 60 mg/ml in distilled water and heated at 80°C for 20 min. Following incubation, ice-cold absolute ethanol was added to precipitate glycogen. The tubes were then centrifuged (10000 rpm, 20 min, 4°C), and the supernatant was immediately removed and discarded. After one wash with ethanol 70%, the glycogen precipitate was re-suspended in distilled water, dissolved under shaking for 20 min and counted by liquid scintillation. All assays were performed in triplicates, and data were normalized to protein content.

Western blot analysis

Muscle tissues were homogenized in a buffer containing 50mM HEPES, pH 7.4, 2 mM EDTA, 150 mM NaCl, 30 mM NaPPO4, 10 mM NaF, 1% Triton X-100, 1.5 mg/ml benzamidine HCl and 10 µl/ml of each: protease inhibitor, phosphatase I inhibitor and phosphatase II inhibitor (Sigma-Aldrich). Tissue homogenates were centrifuged for 25min at 15,000 g and supernatants were stored at −80°C.

Protein lysates were prepared for automated capillary immunoblotting using a Wes System (ProteinSimple) according to the manufacturer's instructions. PLIN5 (#GP31, Progen), and OXPHOS Complex V (#ab110413, Abcam) and GAPDH (#2118, Cell Signaling Technology Inc.) primary antibodies were used. Secondary HRP-linked anti-mouse and anti-guinea pig antibodies were used. Data were analyzed using Compass for Simple Western 6.1.02 version software.

Data and statistical analysis

Between-group differences on anthropometric characteristics were tested with unpaired T-test at baseline. Linear mixed-effect models accounting for repeated measurements with time as fixed effects and subjects as random factor with the best covariance structure (CS or VC based on the smallest information criteria) were used to test the overall effect of dry immersion on body composition, fat distribution, fasting and postprandial metabolites, substrate use, indices of metabolic flexibility, myogenic differentiation, *in vitro* skeletal muscle suppressibility, adaptability, and glycogen synthesis, and protein and gene expressions from muscle biopsies and primary myotubes. Changes in substrate oxidation were also tested with adjustments on lean body mass and fat mass. Linear mixed-effect models with time, treatment (insulin stimulation, glucose concentration or palmitate concentrations) and time-by-treatment interaction as fixed effects and subjects as random factor with

the best covariance structure were used test the overall effect of dry immersion on the intrinsic characteristics of the skeletal muscle cells, *i.e.,* palmitate oxidation in presence of incremental concentration of palmitate or glucose and glycogen synthesis. Because no *a priori* hypothesis was stated on the effects of the thigh cuffs countermeasure on our metabolic outcomes of interest, no group effect or group-by-intervention interaction were considered in the analyses. Baseline values (age and anthropometric characteristics) are presented as means (standard deviation, SD) and results of linear mixed-models are presented as least square means (standard error, SE). Statistical analyses were performed using SPSS (v26.0, IBM, SPSS Statistics, Chicago, IL) with a significance level set up at 5% and graphs were prepared using GraphPad Prism 9 (GraphPad Software, San Diego, USA).

Results

Participants' baseline characteristics

Except for the height that was smaller in the Control group compared to the Cuffs group (1.76 (0.06) vs 1.80 (0.04) m; P=0.04, **Table 4**), baseline characteristics were not different between the two groups. On average, participants were 33.6 (5.5) years, normal-weighted with weight of 74.1 (8.0) kg and BMI of 23.3 (1.8) kg/m². They had an average lean body mass of 55.6 (5.6) kg, fat mass of 17.6 (3.4) kg, and percent of body fat of 24.0 (2.9) %. Baseline VO_2max was 46.7 (6.9) ml/min/kg, indicating a relatively high cardiorespiratory fitness.

Table 4: Participants' baseline anthropometric characteristics.

	All subjects (n=18)	Control group (n=9)	Cuffs group (n=9)	Group difference P-value
Age (yr)	33.6 (5.5)	33.8 (4.0)	33.3 (7.0)	0.45
Height (m)	1.78 (0.06)	1.76 (0.06)	1.80 (0.04)	0.04
Body mass (kg)	74.1 (8.0)	74.3 (7.4)	74.5 (9.3)	0.48
BMI (kg/m²)	23.3 (1.8)	24.1 (1.6)	22.8 (2.0)	0.08
Lean body mass (kg)	55.6 (5.6)	55.5 (4.7)	55.8 (6.7)	0.46
Fat mass (kg)	17.6 (3.4)	17.7 (3.3)	17.6 (3.7)	0.49
Fat mass (%)	24.0 (2.9)	24.0 (2.8)	23.9 (3.2)	0.93
VO_2max (ml/min/kg)	46.7 (6.9)	46.5 (8.1)	46.9 (5.8)	0.45

Data are means (SD). Between-group differences are tested by unpaired t-tests. BMI, body mass index.

Short-term physical inactivity reduced cardiorespiratory fitness and fat-free mass, and promoted fat accumulation in the liver

As shown in **Table 4,** body mass and BMI decreased during dry immersion compared to the ambulatory period (P<0.001). The loss of body mass was due to a decrease in both lean body mass and fat mass (P<0.001 for all), the latter indicating a negative energy balance. Five days of dry immersion also reduced MRI-derived quadriceps CSA (from 7826 [238] to 7591 [239] pixel, P<0.001) and cardiorespiratory fitness (from 46.7 [1.4] to 43.2 [1.4] mL/min/kg, P<0.01). No change in IMAT was detected by MRI measurement (4.68 [0.08] vs 4.82 [0.08] %, P=0.22). However, liver fat fraction increased (from 5.7 [0.2] to 6.9 [0.2] %, P<0.001). This was associated with increases in fasting plasma ALT concentrations (23.7 [1.3] vs 27.4 [1.3] µUI/L, P<0.05; **Table 5**), although values remained within the normal range (29-33 µUI/L). No change in fasting AST, GGT and alkaline phosphatase was observed (**Table 5**).

Table 5: Body composition, cardiorespiratory fitness, quadriceps CSA and ectopic fat storage before and during dry immersion.

	n	Ambulatory period	Dry immersion	P-value
Body composition and cardiorespiratory fitness				
Body mass (kg)	18	73.8 (1.9)	72.2 (1.8)	<0.001
Body mass index (kg/m²)	18	23.3 (0.4)	22.7 (0.4)	<0.001
Lean body mass (kg)	18	55.6 (1.3)	54.2 (1.3)	<0.001
Fat mass (kg)	18	17.6 (0.8)	17.2 (0.8)	<0.001
Fat mass (%)	18	24.0 (0.2)	23.9 (0.2)	0.81
VO_2max (ml/min)	18	3433 (83)	3120 (83)	0.001
VO_2max (ml/min/kg)	18	46.7 (1.4)	43.2 (1.4)	<0.01
Muscle CSA and ectopic fat storage				
Liver fat content (%)	17	5.7 (0.2)	6.9 (0.2)	<0.001
Quadriceps CSA (pixel)	16	7826 (238)	7591 (239)	<0.001
IMAT (%)	16	4.7 (0.1)	4.8 (0.1)	0.22

Data are lsmeans (SE) and P-value from mixed-effect model for repeated measurements. CSA, cross-sectional section area; IMAT, intramuscular adipose tissue.

Short-term physical inactivity reduced *in vivo* insulin sensitivity

Figure 18A and **18B** represent kinetics of plasma insulin and glucose levels following the consumption of the high-carbohydrate meal test. Dry immersion increased fasting plasma insulin (3.9 [0.7] to 5.5 [0.7] µUI/mL, P<0.01; **Table 6**), but not fasting plasma glucose (0.73 [0.02] vs 0.74 [0.02] g/L, P=0.46; **Table 6**). Although the HOMA-IR index remained within the range of normal clinical values for insulin sensitive adults (0.5-1.4), it increased from 0.71 [0.10] in pre-immersion to 1.00 [0.14] after 4 days of dry immersion (P=0.01; **Table 6**). Postprandial insulinemia rose after dry immersion (4948 [929] vs 9119 [929] µUI/mL, P<0.001; **Figure 18C**) but postprandial glycemia only tended to increase (47 [7] vs 64 [7] g/L, P=0.06; **Figure 18D**). However, the insulin sensitivity index including both fasting and postprandial values of insulinemia and glycemia significantly decreased after dry immersion (3.86 [0.23] vs 2.56 [0.23], P=0.001; **Table 6**), indicating a decrease in insulin sensitivity.

Table 6: Fasting metabolic parameters and hepatic biomarkers before and during dry immersion.

	n	Ambulatory period	Dry immersion	P-value
Metabolic characteristics				
Insulin (µUI/mL)	18	3.9 (0.7)	5.5 (0.7)	<0.01
Glucose (g/L)	18	0.73 (0.02)	0.74 (0.02)	0.46
NEFA (µmol/L)	18	438.5 (38.6)	394.4 (38.6)	0.36
Triglycerides (µmol/L)	18	682.2 (68.1)	780.2 (68.1)	0.01

Glycerol (µmol/L)	18	38.8 (6.4)	50.1 (6.4)	0.02
HOMA-IR	18	0.71 (0.12)	1.00 (0.12)	<0.01
Insulin sensitivity index	18	3.86 (0.23)	2.56 (0.23)	0.001
Resting metabolic rate (MJ/day)	18	1.21 (0.01)	1.20 (0.01)	0.82
Hepatic biomarkers				
AST (µUI/L)	18	24.2 (1.7)	24.2 (1.7)	0.97
ALT (µUI/L)	18	23.7 (2.0)	27.4 (2.0)	<0.05
GGT (µUI/L)	18	16.7 (0.9)	15.3 (0.9)	0.17
Alkaline phosphatase (µUI/L)	18	55.6 (3.1)	53.8 (3.1)	0.14

Data are lsmeans (SE) and P-values from mixed-effect model for repeated measurements. NEFA, non-esterified fatty acids; HOMA-IR, homeostasis model assessment of insulin resistance; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyl transferase.

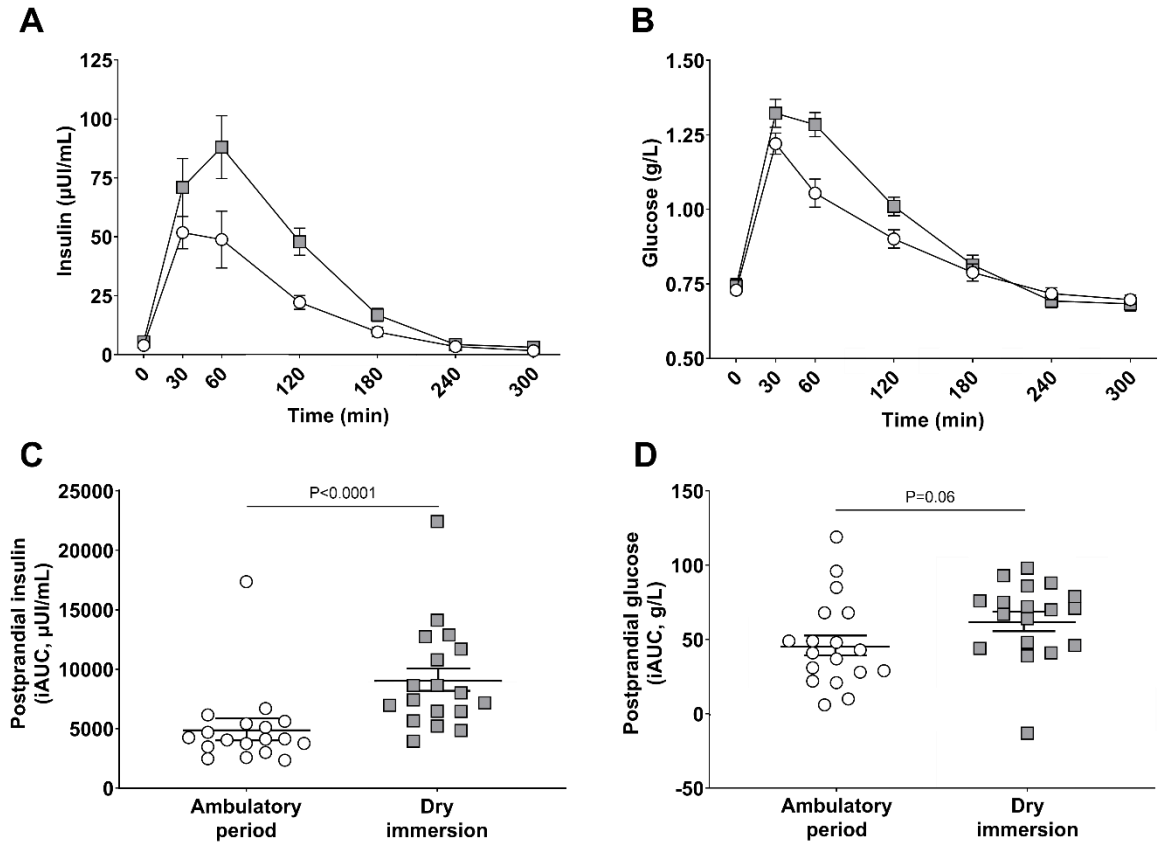


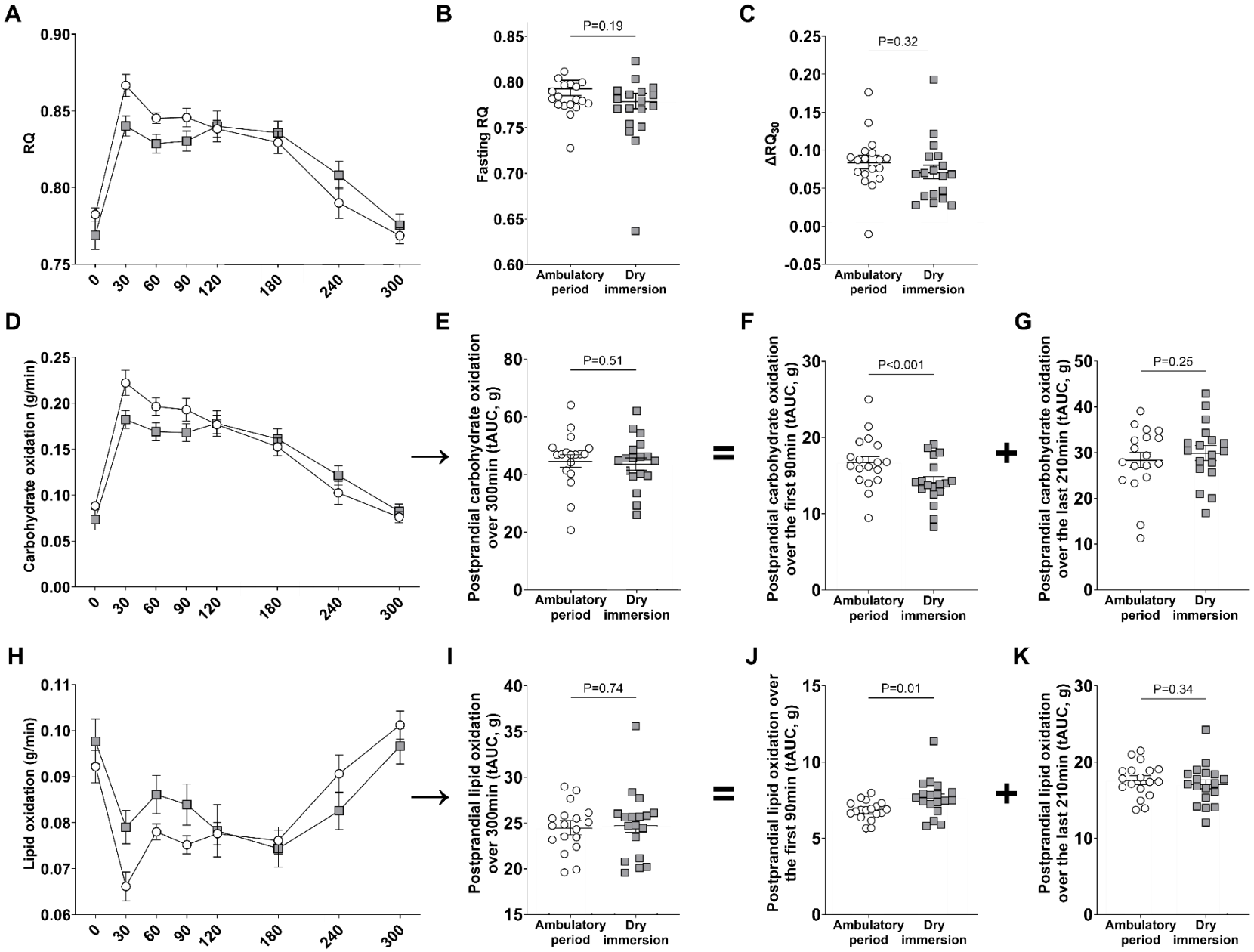
Figure 18: Plasma insulin and glucose after high-carbohydrate mixed meal before and during dry immersion.

Plasma insulin (A) and glucose (B) kinetics before and for 300 min following ingestion of mixed-meal rich in carbohydrates (T0) during ambulatory period (white circles) and dry immersion (grey squares) measured in 18 healthy male adults. Postprandial insulin (C) and glucose (D) incremental area under the curve (iAUC) measured over 300 min following meal ingestion over the total postprandial period. Values are lsmeans (SE) and P-values from mixed-effects models accounting for repeated measurement.

Short-term physical inactivity did not impact *in vivo* metabolic flexibility, but induced hypertriglyceridemia and reduced the postprandial increase in carbohydrate use as fuel

Although concentration of fasting plasma NEFA did not change after four days of dry immersion (438.5 [38.6] vs 394.4 [38.6] µmol/L, P=0.36; **Table 6**), fasting glycerol increased (38.8 [6.4] vs 50.1 [6.4], P=0.02; **Table 6**) suggesting greater mobilization of the adipose tissue. Concentration of fasting plasma triglycerides (682.2 [68.1] vs 780.2 [68.1] µmol/L, P=0.01) also rose, indicating the development of hypertriglyceridemia.

No change in fasting RQ was noted after four days of dry immersion (0.78 [0.01] vs 0.77 [0.01], P=0.19; **Figure 19B**). Similarly, no change in postprandial carbohydrate oxidation (45.22 [2.19] vs 44.13 [2.19] g, P=0.51; **Figure 19E**) and fat oxidation (24.49 [0.77] vs 24.77 [0.77] g, P=0.74; **Figure 19I**) was observed over the 5 hours of the test. However, the kinetics of substrates use were different. A reduction in carbohydrate oxidation was noted in the early postprandial phase *(i.e.*, first 90 minutes, 16.78 [0.76] vs 14.16 [0.76] g, P<0.001; **Figure 19F**) along with a concomitant increase in lipid oxidation (6.83 [0.23] vs 7.68 [0.23] g, P=0.01; **Figure 19J**). No change occurred over the last 210 minutes of the postprandial phase for neither carbohydrate (28.44 [1.65] vs 29.97 [1.65] g, P=0.16; **Figure 19G**) nor lipid (17.66 [0.58] vs 17.09 [0.58] g, P=0.34; **Figure 19K**) oxidation. Adjusting for lean body mass and fat mass did not modify the results. ΔRQ_{30}, an index of metabolic flexibility, was not different in dry immersion compared to baseline values (0.08 [0.01] vs 0.07 [0.01], P=0.32; **Figure 19C**). These results suggest that short-term physical inactivity does not impact fasting and post-prandial fuel selection, and metabolic flexibility. However, it reduces the capacity to increase carbohydrate oxidation in response to the consumption of a high-carbohydrate mixed-meal.

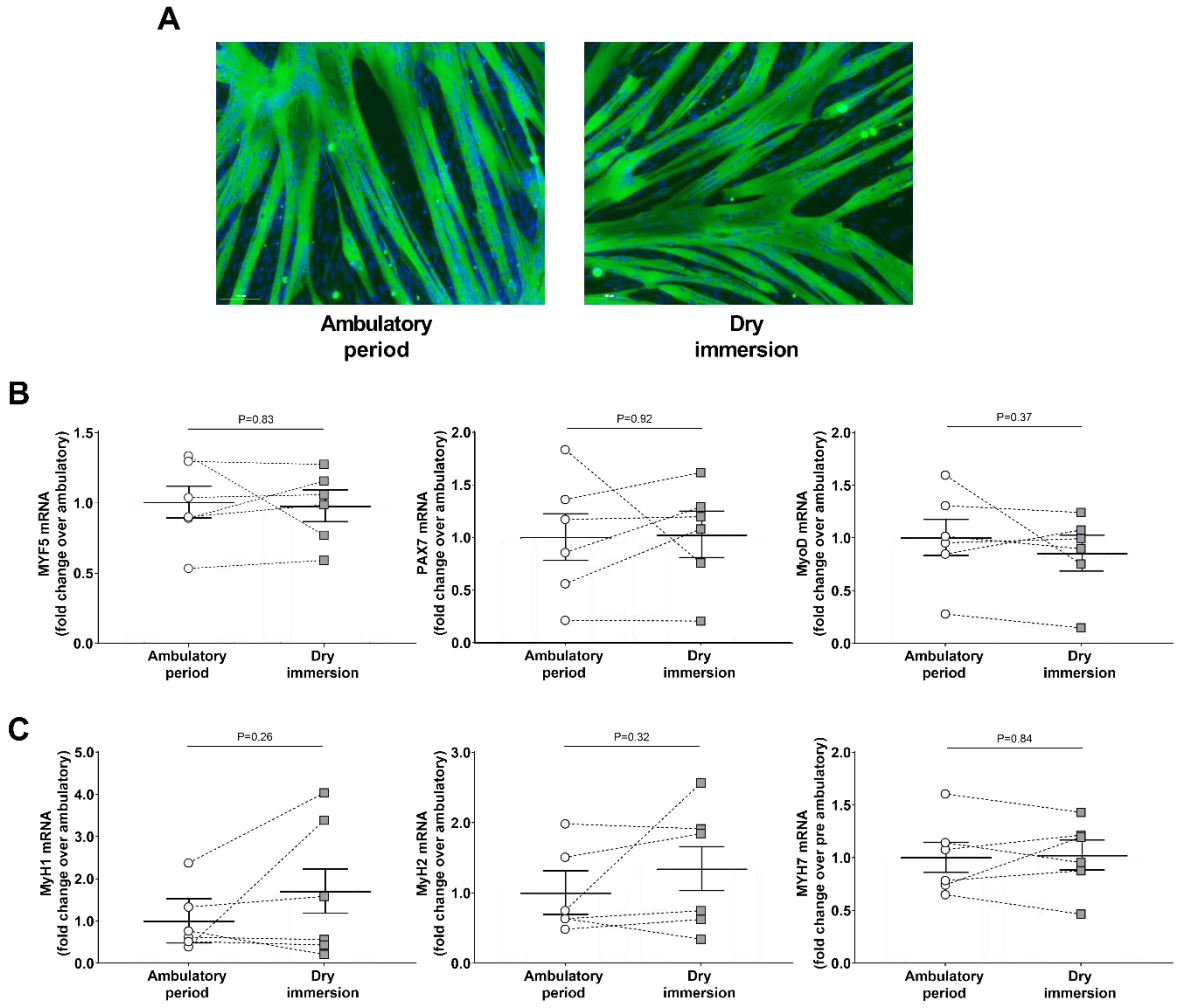


<u>Figure 19</u>: Whole-body metabolic flexibility and postprandial substrate oxidation during ambulatory period and dry immersion.

Respiratory quotient (RQ, A), carbohydrate oxidation (D) and lipid oxidation (E) before and for 300 min after ingestion of high-carbohydrate breakfast challenge (T0) during ambulatory period (white circles) and dry immersion (grey squares) in 18 healthy male adults. Fasting RQ (B), ΔRQ_{30} (value of RQ at T30 min minus fasting RQ value, C). Postprandial carbohydrate and lipid oxidation total area under the curve (tAUC) measured during the whole 300 min (E and I), the first 90 min (F and J) and the last 210 minutes (G and K) of the test. Values are lsmeans (SE) and P-values from unadjusted mixed-effects models accounting for repeated measurement.

Short-term physical inactivity did not impact myogenic differentiation in cultured myotubes

Multinucleated myotubes displayed the same phenotype before and after dry immersion as indicated by the DAPI/myosin immunocytological staining (**Figure 20A**). In addition, gene expression of progenitor cell markers (Myf5, Pax7, MyoD; **Figure 20B**) and myogenic differentiation markers (MYH1, MYH2, MYH7; **Figure 20C**) was similar between myotubes isolated before and after 5 days of dry immersion. Altogether, these results indicate that the *in vitro* myogenic potential of the muscle cells was not affected by dry immersion-induced physical inactivity.



<u>Figure 20</u>: Primary myotubes phenotype and gene expression of markers of myogenic differenciation during ambulatory period and dry immersion.

Fixed CD56+-derived myotubes phenotypes in pre ambulatory period and after dry immersion (A). Progenitor cell markers (Myf5, Pax7, MyoD; B) and myogenic differentiation markers (MYH1, MYH2, MYH7; C) measured in muscle from 6 healthy male adults. Values are lsmeans (SE) and P-values from unadjusted mixed-effects models accounting for repeated measurement.

Short-term physical inactivity reduced skeletal muscle cells *in vitro* insulin sensitivity

No change in basal glucose oxidation level in cultured myotubes was noted after five days of dry immersion compared to baseline values (1.00 [0.20] vs 0.99 [0.20] respectively, P=0.97; **Figure 21A**). As expected, glycogen synthesis was increased by insulin stimulation compared to basal conditions both before and during dry immersion (0.88 [0.10] vs 1.55 [0.10] for basal and insulin conditions respectively, treatment effect P<0.0001; **Figure 21B**). Although the dry immersion lowered the rate of glycogen synthesis compared to the ambulatory period in both basal and insulin-stimulated conditions (1.36 [0.10] vs 1.07 [0.10] for ambulatory and dry immersion period respectively, dry immersion effect P=0.04; **Figure 21B**), it did not affect the amplitude of this increase (1.00 [0.14] to 1.72 [0.14] vs 0.77 [0.14] to 1.38 [0.14] for ambulatory and dry immersion period respectively, dry immersion-by-treatment interaction: p=0.67). Whereas five days of dry immersion did not significantly modify the ratio of insulin-induced Akt phosphorylation (1.00 [0.20] vs 0.58 [0.20], P=0.10; **Figure 21C**), the expression of phosphorylated Akt was reduced after dry immersion (0.95 [0.20] vs 0.59 [0.20], P=0.02; **Figure 21D**), indicating an alteration of the insulin signaling pathway.

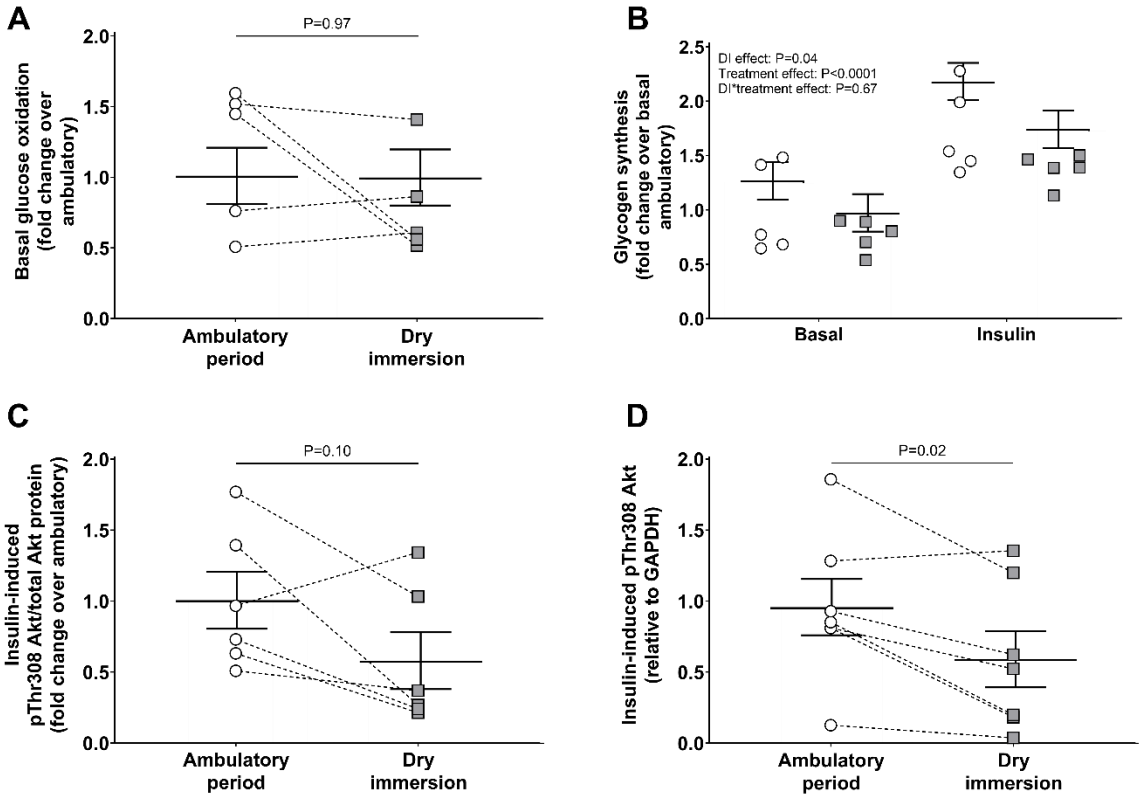


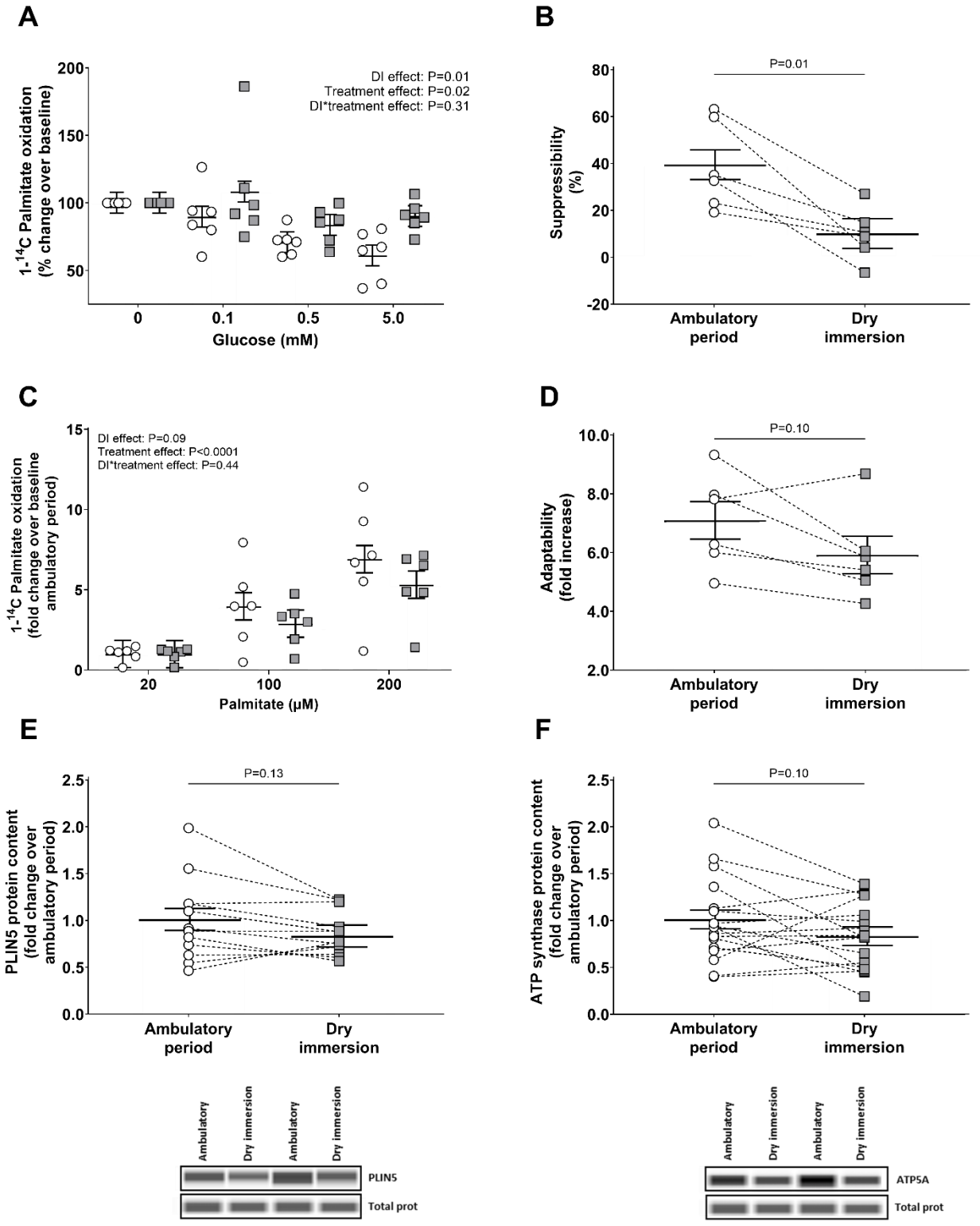
Figure 21: Insulin sensitivity of primary myotubes collected and cultured before and after five days of dry immersion.

Basal glucose oxidation (A, n=6), glycogen synthesis under basal and insulin-stimulated condition (B, n=5), insulin-induced ratio of phosphorylated Thr308 Akt over total Akt (C, n=6) and insulin-stimulated phosphorylation of Thr308 Akt (D, n=7) during ambulatory period (white circles) and after five days of dry immersion (grey squares) measured in muscles collected from healthy male adults. Values are lsmeans (SE) and P-values from unadjusted mixed-effects models accounting for repeated measurement.

Short-term physical inactivity diminished isolated skeletal muscle cells metabolic flexibility

As expected, *in vitro* palmitate oxidation was reduced by incremental increases in glucose concentration in the medium both before and after five days of dry immersion (treatment effect P=0.01; **Figure 22A**). Palmitate oxidation was higher at all glucose concentrations in the cultured skeletal muscle cells collected during dry immersion compared to those collected before (dry immersion effect P=0.02; **Figure 22A**). The lower suppressibility after dry immersion compared to before (38.9 [6.2] % vs 9.8 [6.2] %, respectively, P=0.01; **Figure 22B**) further indicates an impaired capacity to suppress palmitate oxidation in presence of increased glucose availability. In parallel, palmitate oxidation rose in response to increases in palmitate availability in cultured skeletal muscle cells collected both before and during the dry immersion (treatment effect: P<0.001). Both the level of palmitate oxidation (dry immersion effect P=0.09; **Figure 22C**) and the capacity to increase palmitate oxidation following increases in medium palmitate concentrations indicated by the adaptability index (7.1 [0.6] vs 5.9 [0.6] fold increase, P=0.10; **Figure 22D**) were not significantly affected by short-term inactivity. This being said the adaptability index was lower in the cultured primary myotubes collected during dry immersion compared to before in five subjects but was greater in one participant. Altogether, these results indicate that primary myotubes metabolic flexibility to glucose is decreased by short-term physical inactivity.

To decipher the molecular mechanisms involved in this altered metabolic flexibility, we measured the expression of proteins involved in muscle oxidative metabolism. No change in perilipin 5 (1.00 [0.12] vs 0.82 [0.12], P=0.13; **Figure 22E**) and ATP synthase (1.00 [1.00] vs 0.82 [1.00], P=0.10; **Figure 22F**) protein content was observed after dry immersion.



<u>Figure 22</u>: Primary myotubes metabolic flexibility, muscle PLIN5 and ATP synthase protein content during ambulatory period and dry immersion.

Palmitate oxidation measured in primary myotubes collected from 6 healthy male adults and incubated with increasing glucose concentrations (A) during ambulatory period (white circles) and dry immersion (grey squares). The suppressibility index (B) is the associated ability of the primary myotubes to decrease palmitate oxidation in presence of glucose 5 mM compared to baseline (no glucose). Palmitate oxidation measured in primary myotubes collected from 6 healthy male adults and incubated with increasing concentrations of palmitate in the medium (C) during ambulatory period (white circles) and dry immersion (grey squares). The adaptability index (D) is the associated ability to increase palmitate oxidation when the concentration of palmitic acid in the medium increases ten-fold from 20 mM to at 200 mM. Protein content of perilipin 5 (PLIN5, E) and ATP synthase (F) measured in skeletal muscle samples collected before and after five days of dry immersion in 12 healthy adult males. Values are lsmeans (SE) and P-values from unadjusted mixed-effects models accounting for repeated measurement, treatment effect and time-by-treatment interactions for the assessment of palmitate oxidation in presence of incremental concentrations of glucose (A) and palmitic acid (C). For all the other outcomes, P-values from unadjusted mixed-effects models accounting for repeated measurement.

Discussion

Using the space analog dry immersion model, we showed that short-term physical inactivity decreases muscle cells insulin signaling, glycogen synthesis and metabolic flexibility to glucose. This was associated with reduced *in vivo* insulin sensitivity but not with decreases in *in vivo* metabolic flexibility, supporting that muscle cells metabolic defects likely precede whole-body metabolic inflexibility. However, four days of inactivity were sufficient to induce hypertriglyceridemia and accumulation of fat in the liver, suggesting that alterations in lipid metabolism are already at play. Along with published data from this dry immersion protocol, this study brings news insight on the cascade of events triggered by physical inactivity and leading to the onset of metabolic dysfunctions and highlights a rapid imprinting of physical inactivity-induced muscle metabolic defects in satellite cells.

Short-term physical inactivity triggers a rapid metabolic deconditioning but is insufficient to induce a shift in fasting and postprandial substrate use

Five days of dry immersion were sufficient to reduce fat-free mass and muscle CSA, as well as cardiorespiratory fitness. This reduction in VO_2max may be associated with the decrease in *in vivo* insulin sensitivity, given that the two are known to be directly linked (AlZadjali *et al.*, 2009; Byrkjeland *et al.*, 2014). Contrary to bed-rest studies of 7, 42, 60 and 90 days (Ritz *et al.*, 1998; Blanc *et al.*, 2000; Bergouignan *et al.*, 2006; Bergouignan *et al.*, 2009), few days of dry immersion was not sufficient to trigger a shift in substrate use in fasting and postprandial states in favor of carbohydrate oxidation. In addition to energy balance and diet macronutrient composition (Peronnet & Haman, 2019), prior studies showed that type I muscle fiber content influences fasting RQ (Goedecke *et al.*, 2000). In all the previous long-term bed-rest studies, the shift in substrate use was indeed associated with a shift in muscle fibers typology from oxidative type I fibers to glycolytic type II fibers (Trappe *et al.*, 2004; Trappe *et al.*, 2007a; Salanova *et al.*, 2008; Moriggi *et al.*, 2010) the latter being characterized by lower mitochondrial density, oxidative enzyme activity, rates of lipid oxidation and insulin sensitivity (Scott *et al.*, 2001). In contrast, no detectable change in fiber type was noted in the muscle of our participants (Fovet *et al.*, 2021). This unaffected fuel selection in fasting state was further paralleled by primary myotubes basal glucose oxidation that remained unchanged by dry immersion. However, Fovet et al. reported a decrease in the expression of PGC1a (Fovet *et al.*, 2021), a known dominant regulator of mitochondrial function, biogenesis and respiration (Rohas *et al.*, 2007) that associates with insulin resistance (Mootha *et al.*, 2003; Patti *et al.*, 2003). This observation suggests that while five days of physical inactivity are not sufficient to trigger a remodeling of skeletal muscle and affect fuel selection,

impairments of mitochondrial oxidative capacity may be initiated, which may influence insulin sensitivity and other metabolic outcomes.

Short-term physical inactivity reduces *in vivo* and *in vitro* insulin sensitivity, but only *in vitro* metabolic flexibility to glucose

Prior studies reported metabolic inflexibility in healthy adults after several days (*e.g.* 10 days) (Damiot *et al.*, 2019), weeks (Rudwill *et al.*, 2018) and months (Bergouignan *et al.*, 2011; Shur *et al.*, 2022) of inactivity. This study showed that four days of exposure to enforced physical inactivity are not sufficient to alter whole-body metabolic flexibility, as indicated by the unchanged ΔRQ_{30} following the ingestion of a mixed meal rich in carbohydrates. Nevertheless, the increase in carbohydrate oxidation was blunted in the first 90 min of the postprandial period. This may be due to a delay in insulin secretion and/or altered glucose uptake by peripheral tissues. In this line, drops in whole-body glucose disposal were observed after 3 days of bed-rest and in absence of concomitant changes in *in vivo* insulin-stimulated carbohydrate and fat oxidation (Shur *et al.*, 2022). A decrease in total and membrane protein content of GLUT4 was also noted after 7 and 20 days of bed-rest (Tabata *et al.*, 1999; Bienso *et al.*, 2012). Altogether, these data suggest that impaired glucose disposal may precede the onset of metabolic inflexibility, and hence the subsequent development of glucose intolerance in the pathophysiological context of insulin resistance as shown previously (Rudwill *et al.*, 2018). Although glucose disposal was not measured in our study, the changes in both HOMA-IR, postprandial insulinemia and insulin sensitivity index indicate decreases in *in vivo* insulin sensitivity.

This reduction in *in vivo* insulin sensitivity was paralleled with alterations of the intrinsic metabolic properties of skeletal muscle. The rates of glycogen synthesis also dropped after dry immersion, which goes along with the observation of lower glycogen content in skeletal muscle of healthy participants after 3 days of bed-rest (Shur *et al.*, 2022). Prior research showed that transcriptional responses linked to impairment of muscle insulin signaling were occurring within 9 days of bed-rest (Alibegovic *et al.*, 2010b) but needed at least more than one day to be apparent (Dirks *et al.*, 2018) in healthy male adults. In this study, we observed a decrease in insulin-stimulated phosphorylated Akt protein content in primary myotubes, which supports the onset of insulin signaling alteration within five days of transition to inactivity.

Although no change was detected in *in vivo* metabolic flexibility, was associated *in vitro* metabolic flexibility to glucose was diminished. The decreased suppressibility, *i.e.*, capacity of muscle cells to decrease palmitate oxidation when glucose availability increases, observed after five days of physical inactivity suggests that the mechanisms of inhibition of fatty acid oxidation by glucose are

altered. This is mediated by malonyl-CoA, which inhibits the entry of long-chain fatty acyl into mitochondria (Randle *et al.*, 1963). Of note, the concentration of malonyl-CoA is regulated by acetyl-CoA carboxylase (ACC), whose activity is inhibited by AMPK activation produced by muscle contractions. In other words, muscle disuse likely removes the inhibition of ACC activity, which dampens fat oxidation and maintains carbohydrate oxidation. Future studies will need to better elucidate the mechanisms underpinning metabolic inflexibility to glucose, including the contribution of insulin-dependent and independent pathways of regulation of glucose uptake, transcriptomics events to insulin sensitivity, glucose and lipid metabolism, and mitochondrial dysfunctions.

An early onset in whole-body lipid metabolism alterations despite no change in the whole-body and skeletal muscle oxidative capacity

Contrary to what was previously observed in response to medium- and long-term exposure to physical inactivity (Bergouignan *et al.*, 2006; Bergouignan *et al.*, 2009; Bergouignan *et al.*, 2013a; Damiot *et al.*, 2019), five days of dry immersion did not impact postprandial total fat oxidation, independent of changes in body composition. Similarly, no significant changes in the ability to promote fat oxidation in response to increased fatty acid availability were detected in the primary myotubes. However, the fact that a reduction in the adaptability index was observed in five out of six subjects suggests that alterations in lipid metabolism may slowly take place in skeletal muscle. Other mechanisms may also be at play. Prior bed-rest (Bergouignan *et al.*, 2006; Bergouignan *et al.*, 2009) and clinical studies on physical inactivity (Bergouignan *et al.*, 2013a; Damiot *et al.*, 2019) showed that lower postprandial fat oxidation and hypertriglyceridemia are associated with transcriptional alterations linked to fatty acid uptake by muscle cells and mitochondria. Although this was observed after several weeks or months of inactivity, Shur et al. reported that transcriptional changes linked to fuel selection were more pronounced after 3 days of bed-rest rather than after 56 days (Shur *et al.*, 2022). Altogether this suggests that transcriptional events at skeletal muscle level may precede skeletal muscle metabolic flexibility to lipids but be sufficient to trigger some alterations of lipid metabolism.

Increases in fasting triglycerides concentrations were previously observed in response to medium and long-term exposure to inactivity (Bergouignan *et al.*, 2006; Bergouignan *et al.*, 2009; Damiot *et al.*, 2019). This study showed that hypertriglyceridemia is an early metabolic defect induced by physical inactivity. This was associated with accumulation of hepatic fat content as detected by MRI after five days of dry immersion. Even if no impaired oxidative capacity was detected with the methods

used in this study, this greater lipid content in the liver likely resulted from imbalance between lipid availability and lipid oxidation thus leading to accumulation of non-oxidized lipids elsewhere than in adipose tissue. The observed increase in the concentrations of ALT, a liver enzyme known to be associated with steatosis, steatohepatosis and NAFLD-related metabolic features (Zderic & Hamilton, 2006; Harrison *et al.*, 2008), supports the onset of a deterioration of the liver metabolism. Similar increases in ALT but also of AST and other hepatic biomarkers were previously observed in female adults subjected to a 60-day bed-rest (Rudwill *et al.*, 2015). In addition, the greater mobilization of adipose tissue, as suggested by the increase in fasting plasma glycerol and associated with the negative energy balance status of the participants may have fostered this accumulation of hepatic fat. Contrary to our hypothesis, no increases in IMAT were detected, like previously reported by other investigators involved in this protocol (Guilhot *et al.*, 2022). Of note, no change in muscle lipid droplets were noted after 3 days (Shur *et al.*, 2022), 7 days (Dirks *et al.*, 2016) and 56 days (Shur *et al.*, 2022) of bed-rest despite a decreased whole-body insulin sensitivity, while other investigators did report increases in intramuscular lipid content (Bergouignan *et al.*, 2009). These discrepancies may be linked to the methods used to assess lipid accumulation in the muscle. In addition, we now know that it is more important to assess the lipid species and their location in the muscle than the total lipid content. Information that we unfortunately could not gather.

Strengths and limitations

Strengths and limitations need to be acknowledged. Thanks to the use of the rapid deconditioning model of dry immersion we highlighted the processes of metabolic adaptations to physical inactivity at a very early stage, and this helped clarifying the series of events that led to these alterations. We were able to evaluate the skeletal muscle's intrinsic metabolic characteristics in response to short-term physical inactivity. Contrary to other techniques used, such as the hyperinsulinemic euglycemic clamp, which is considered as a supraphysiological challenge, the use of high carbohydrate meal challenges to test metabolic flexibility at whole body level and the suppressibility and adaptability tests at skeletal muscle level allowed to emphasize the regulation mechanisms of energy homeostasis under physiological conditions. However, skeletal muscle cells analysis was performed on biopsies from only few subjects due to the limited quantity of muscle samples collected. In absence of a clamp or an intravenous glucose tolerance test, we could not investigate changes in whole-body glucose disposal and insulin secretion. Finally, like mentioned above, the lack of lipidomic analysis preclude our understanding of the short-term effects of physical inactivity on the partitioning between storage and oxidation of lipid species.

Conclusions

Using the space analog dry immersion model, we showed that short-term enforced inactivity induces muscle atrophy, declines in cardiorespiratory fitness and metabolic disruptions both at the skeletal muscle and whole-body levels including altered insulin signaling, reduced rates of glycogen synthesis, and decreased metabolic flexibility to glucose,

The observed alterations of the skeletal muscle cells metabolic properties highlight that an epigenetic imprinting of satellite cells rapidly occurs in response to short-term physical inactivity. The *in vitro* altered insulin signaling likely precedes the decreases in *in vivo* insulin sensitivity. This latter along with the *in vitro* reduced rates of glycogen synthesis, and decreased metabolic flexibility to glucose, and a future reduction in whole-body metabolic flexibility. Although no change in fasting and postprandial fuel selection was noted, the development of hypertriglyceridemia and hepatic fat accumulation suggest that alterations in lipid metabolism are rapidly occurring in response to short)-term inactivity.

Future studies will need to further establish the cascade of events using an integrative approach going from the whole-body level to the tissue, cell, organite, protein and gene level. Understanding what are the very first metabolic outcomes to be affected by physical inactivity may help preventing or mitigating the development of metabolic diseases.

Contributorship: AB, SB, and CL designed the study. ELR, BL, CL and AB drafted the manuscript. CL, BL, IG, DL, IH, and MPB collected data. ELR, BL, CL, LT, CS and AB analyzed and interpreted the data. ELR and CS realized the statistical analysis. All the authors mentioned significantly contributed to the realization of this study and approved this version of the manuscript.

7 Substrate oxidation and metabolic flexibility in male astronauts onboard the International Space Station: The ENERGY study

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Résumé

Introduction

La microgravité mène à de nombreuses adaptations de tous les systèmes physiologiques dont le métabolisme énergétique. D'après les études menées sur des modèles analogues, 7, 42, 60 et 90 jours d'alitement prolongé induisent un changement d'oxydation des substrats caractérisé par une diminution de l'oxydation des lipides au profit des glucides pour fabriquer de l'énergie, indépendamment de la durée de l'étude et de la balance énergétique. Ce changement a été associé au développement d'une inflexibilité métabolique, définie comme une diminution de la capacité à ajuster l'oxydation des substrats en fonction de leur disponibilité et de la demande énergétique. Dans l'espace, les réponses métaboliques pourraient différer de celles observées sur Terre. Les astronautes pratiquent de l'exercice comme contremesure pour prévenir les adaptations liées à la microgravité ; et l'exécution d'un protocole d'entrainement combiné aérobie et résistif a partiellement prévenu le changement d'oxydation des substrats. De plus, nous avons précédemment observé une grande variabilité inter-astronaute concernant les réponses de métabolisme énergétique et de composition corporelle au cours de missions de 6 mois à bord de la station spatiale internationale (ISS).

Objectifs et hypothèses

Les objectifs de l'étude ENERGY sont :

- Déterminer si un changement dans l'oxydation des substrats et une diminution de flexibilité métabolique se manifeste chez des astronautes pendant un vol spatial de longue durée
- Déterminer si ces changements sont médiés par l'alimentation, l'activité physique et la composition corporelle.

Nous faisons les hypothèses que :

- Le vol spatial induit une augmentation de l'oxydation des glucides au détriment des lipides indépendamment des changements de composition corporelle à jeun et en post-prandial. Ce changement est accompagné par une diminution de la flexibilité métabolique.
- L'amplitude des changements d'oxydation des substrats à jeun, post-prandial et de flexibilité métabolique sont associés à l'alimentation, l'activité physique et à la composition corporelle en vol

Matériel et Méthodes

Onze hommes astronautes ont été recrutés entre 2011 et 2017 (âge 45,7 [SD 7,7] ans ; IMC 24,3 [2,1] kg/m²). Avant et après au moins trois mois à bord de l'ISS, l'oxydation des glucides et des

lipides a été mesurée par calorimétrie indirecte à jeun et après un repas standardisé. La flexibilité métabolique a été déterminée comme la différence entre la valeur maximale post-prandiale du quotient respiratoire (QR) moins la valeur de QR à jeun. Le quotient alimentaire a été estimé à partir d'enquêtes alimentaires remplies par les astronautes au sol et en vol. La masse grasse et la masse maigre ont mesurées par dilution isotopique et l'activité physique mesurée par un brassard multi-senseur d'activité physique et d'après des journaux d'activité physique.

Résultats

Les vols spatiaux de longue durée sont associés à un changement métabolique en faveur de l'oxydation des glucides, principalement en raison de changements dans la disponibilité des substrats alimentaires. Les vols spatiaux de longue durée ne modifient pas la capacité à ajuster l'oxydation des substrats en réponse au repas standardisé. Les changements induits par le vol dans l'utilisation des substrats en post-prandial et de flexibilité métabolique sont associés aux changements de composition corporelle et à l'exercice en vol.

Discussion

Comme il l'a été souligné au cours des études d'alitement sur les 30 dernières années, un changement dans l'oxydation des substrats en faveur des glucides a été noté après séjour dans l'espace. Cependant, le fait que ce changement soit fortement associé aux apports alimentaires pendant le vol suggère que l'alimentation pendant le vol, plutôt que la microgravité, influence principalement l'oxydation des substrats pendant les vols spatiaux. Contrairement à ce qui a été montré au sol, la flexibilité métabolique est restée intacte. En revanche, la variabilité dans les réponses métabolique a permis de mettre en avant des associations entre les changements d'oxydation des substrats en post-prandial et de flexibilité métabolique avec les changements de composition corporelle et l'exercice aérobie en vol. Ces relations sont en accord avec des études menées au sol suggérant des associations entre masse maigre, masse grasse et flexibilité métabolique et suggèrent que l'exercice physique pourrait en être le médiateur.

Abstract

Background: Simulated microgravity induces a shift in fasting substrate utilization in favor of carbohydrate use and in detriment of lipids, and reduces metabolic flexibility (MF), *i.e.*, the capacity to adjust fuel oxidation to changes in substrate availability and energy demand. These metabolic alterations can affect astronaut's performance and health.

Objective: To determine whether a shift in substrate oxidation and reduced MF occur during long-term spaceflights and explore the associations with diet, physical activity and body composition.

Methods: Before and after at least three months onboard the International Space Station, respiratory quotient (RQ), carbohydrate and fat oxidation were measured by indirect calorimetry before and following a standardized meal in 11 male astronauts (age=45.7 [SD 7.7] years, BMI=24.3 [2.1] kg/m²). MF was determined by 0-to-260 min postprandial incremental areas under the curve (iAUC) of nutrient oxidation and the difference between maximal postprandial and fasting RQ (ΔRQ). Food quotient (FQ) was calculated from diet logs. Fat (FM) and fat-free mass (FFM) were measured by hydrometry and physical activity by accelerometry and diary logs.

Results: Three months in space increased fasting RQ (P=0.01) and carbohydrate oxidation (P=0.04), and decreased fasting lipid oxidation (P<0.01). An increase in FQ (P<0.001) indicated a shift in diet composition. Spaceflight-induced changes in RQ adjusted on ground RQ were associated with inflight FQ (P<0.01). No changes were noted in mean postprandial nutrient oxidation and ΔRQ. Individual postprandial lipid oxidation iAUC was negatively associated with changes in FFM and inflight aerobic exercise, and positively with changes in FM. The opposite was observed with postprandial carbohydrate oxidation. Changes in ΔRQ were negatively and positively related to FM and FFM changes, respectively.

Conclusion: The shift in fasting substrate oxidation observed during spaceflight is essentially driven by dietary modifications. Between-astronauts variability in postprandial substrate oxidation depends on body composition changes and inflight physical activity.

Key words: substrate oxidation, metabolic flexibility, exercise, body composition, spaceflight.

Introduction

Microgravity leads to numerous physiological alterations including dysfunctions of metabolism (Vernikos, 1996). Metabolism being at the crossroad of multiple physiological functions (Kim *et al.*, 2017; Ritterhoff & Tian, 2017) such as cardiovascular and musculoskeletal functions, it can affect astronauts' performance and health. However, the consequences of long-term spaceflights on nutrient metabolism are poorly known.

Space analog models on Earth, *i.e.* the head down tilt bed-rest (Pavy-Le Traon *et al.*, 2007), showed in healthy male and female adults, that exposure of 7, 42, 60 and up to 90 days to simulated microgravity induces a shift in substrate use characterized by a decrease in fat oxidation in favor of the use of carbohydrates as fuel (Ritz *et al.*, 1998; Blanc *et al.*, 2000; Bergouignan *et al.*, 2006; Bergouignan *et al.*, 2009). This was observed in both fasting and postprandial states and, so far, it was seen independent of changes in body composition and diet. Bed-rest studies also showed that microgravity reduces metabolic flexibility, which is defined as the ability of the body to adjust substrates use to changes in substrates availability and energy demand (Kelley & Mandarino, 2000). In a 21-day bed-rest study, we further showed that the development of metabolic inflexibility preceded the onset of glucose intolerance in the pathophysiological context of insulin resistance (Rudwill *et al.*, 2018). Decreases in metabolic flexibility are of significance as they favor the development of ectopic fat storage (Bergouignan *et al.*, 2009; Fernandez-Verdejo *et al.*, 2018; Petersen & Shulman, 2018). Lipid accumulation in skeletal muscle has been associated with systemic and muscle insulin resistance (Moro *et al.*, 2008) and muscle atrophy (Meex *et al.*, 2019), which are key physiological changes classically observed during bed-rest studies (Mikines *et al.*, 1989; Mikines *et al.*, 1991; Tabata *et al.*, 1999; Blanc *et al.*, 2000; Bergouignan *et al.*, 2006; Bergouignan *et al.*, 2009; Bienso *et al.*, 2012) as well as in astronauts after 4 days and 6 months in space (Grigoriev *et al.*, 1987; Smirnov *et al.*, 1991; Stein *et al.*, 1994; Maaß *et al.*, 1997; Stein *et al.*, 1999b; Macho *et al.*, 2003; Hughson *et al.*, 2016).

Changes in substrate oxidation and metabolic flexibility in response to actual microgravity in space may differ to what was observed under simulated microgravity conditions using space models analogs on Earth. Indeed, all astronauts are asked to observe an exercise training protocol aiming to mitigate the microgravity-induced body alterations (Hackney *et al.*, 2015), and no true control (no exercise) group exists in space. This being said, a combined aerobic and resistance exercise training protocol similar to the one applied on the International Space Station (ISS) only partially prevented the shift in substrate oxidation towards carbohydrate oxidation in both fasting and postprandial states during a 2-month bed-rest study (Bergouignan *et al.*, 2009). It is also important to note that a large between-subjects variability in body composition and energy metabolism in response to long-term

spaceflights was recently reported in the ENERGY experiment (Bourdier *et al.*, 2022); similar individual variations may exist in the responses of nutrient metabolism. Therefore, whether the previously observed inflight insulin resistance (Grigoriev *et al.*, 1987; Smirnov *et al.*, 1991; Stein *et al.*, 1994; Maaß *et al.*, 1997; Stein *et al.*, 1999b; Macho *et al.*, 2003; Hughson *et al.*, 2016) is associated with a shift in substrate use and metabolic inflexibility remains a question to be answered.

In addition to investigate energy expenditure, energy intake and body composition in relation to the physical exercise countermeasure (Bourdier *et al.*, 2022), the ENERGY experiment included a one-day metabolic test combining fasting and post-meal response in substrate oxidation. The standard meal was not high in carbohydrates like it is commonly used for measuring metabolic flexibility (Rynders *et al.*, 2018), but rather moderately high in fat and protein due to meal selection by the astronauts. Despite this difference in macronutrients composition, we used substrate oxidation data to infer changes in metabolic flexibility induced by a 6-month spaceflight.

This study aimed to determine whether a shift in substrate oxidation and reduced metabolic flexibility are developed in space like it was repeatedly reported during bed-rest studies. We further used the large between-subjects variability observed in the ENERGY experiment to examine the associations with diet, physical activity and body composition.

Material and methods

Subjects

Eleven male astronauts from eight countries (United States, Japan, Italy, Canada, France, Germany, United Kingdom and The Netherlands), who flew for six months onboard the ISS, voluntary took part in the ENERGY experiment. None had history of chronic disease, and all were healthy throughout the missions. The study was approved by NASA Institutional Review Board (IRB) under NASA 7116301606HR, and by the European Space Agency (ESA) Medical Board and Japanese Space Agency (JAXA) Institutional Review Board for human experiment. Written informed consent was obtained from all astronauts.

Overall study design

Each astronaut completed two strictly identical metabolic tests, one on Earth (ground) and one onboard the ISS (inflight). Both sessions were realized under the supervision of ESA and French Space Agency (CNES) science officers and the investigators from Toulouse Space Center (CADMOS, Toulouse, France). They were preceded by dry runs of the experiments conducted at the European Astronaut Center (EAC) in Cologne (Germany). On average, the ground session was conducted 99 days (SD 78) before the flight at EAC with the astronauts being deemed in energy balance. The flight session started before the last month onboard the ISS and after at least three months in space (mean of 108 days [SD 18]). The 3-to-5-month inflight window was selected to provide long-term spaceflight data while avoiding the period of preparation for return to Earth.

To explore potential contributors of substrate oxidation changes induced by long-term spaceflights, body composition was determined using isotopic dilution on the same day and physical activity was estimated using a Sensewear Pro (SWP) multi-sensor activity monitor (Body media Inc®, Pittsburgh, PA USA) worn for 10 days following the metabolic test day. Inflight exercise training during the two months preceding the research session was also obtained from the NASA exercise diary logs. Finally, food quotients were calculated from self-reported inflight food intake during the same 10-day period of physical activity measurement.

Test meal challenge

On the ground and inflight test days, energy expenditure and substrate oxidation were measured by indirect calorimetry in fasting state and after a mixed-breakfast challenge. The

measurements were performed after an overnight fast, at least 16hr after the last exercise session, early after morning waking, after experimental devices calibration (for inflight session) and after resting for at least 45 minutes. The breakfast challenge was ingested in less than 20 minutes and designed to cover about 30% of the theoretical daily ground needs of the subjects, based on individual body weight and an estimated physical activity level of 1.8. It represented a mean (SD) energy intake of 54 kJ/kg (6), and was composed of 37% carbohydrates (9), 35% lipids (4) and 28% proteins (8). Gas exchanges were measured for 45 minutes before breakfast (*i.e.*, fasting state) and during seven 20-min periods that started 20, 50, 100, 160, 210 and 240 minutes after meal ingestion (T0).

To minimize the confounding effect of recent diets, subjects were provided with standardized meals designed to cover subject's theoretical needs the day prior to the metabolic test. It represented a mean (SD) energy intake of 187 kJ/kg/day (25) composed of 33% carbohydrates (4), 38% lipids (3) and 29% proteins (3) with a repartition of about 30%, 40% and 30% for breakfast, lunch, and diner, respectively.

The breakfast-test meal and the standardized pre-test meals were dishes prepared by the French Chef Ducasse, which allowed to maximize astronauts' compliance to the research protocol. Dishes were individually adapted to subject's preferences and were identical for the ground and flight sessions. Dishes examples are presented in **Supplemental Table 1**.

Indirect calorimetry

Throughout the test, O_2 consumption (VO_2) and CO_2 production (VCO_2) were measured by the pulmonary function system (PFS, manufactured by Danish Aerospace Company [DAC], Odense, DK]) (Jensen *et al.*, 2002; McCleary *et al.*, 2007). Subjects were connected to the PFS by a custom-designed two-way nonrebreathing valve coupled to a mixing reservoir for expired gases. The PFS uses a photo-acoustic method for CO_2 analysis (operating range from 0-12%) and an Oxigraf^{TM} model X2004 sensor (Oxigraf, Mountain View, CA), a laser diode absorption spectroscopy sensor, to measure O_2 (operating range from 0 –100% O_2 with a resolution of 0.01%). A differential pressure flowmeter (pneumotach; range 0-900 L/min) is used for ventilation measurement on the inspired side of the non-rebreathing valve rather than on the expired side. Indeed, reduction of contamination from moisture contained in expired gas usually rely on gravity, which obviously could not work in space. Temperature, humidity, and inspired gas concentrations are measured on the inspired side of the respiratory circuit because inspired gas concentrations on the ISS slightly deviate from normal atmospheric values. A proprietary software package (DAC) was used to compute minute-by-minute VO_2 and VCO_2 from raw data signals measured by the PFS (VE, FeO_2 and $FeCO_2$) considering the delay time for the sensors to detect expired

gas and the time shift to match the gas fraction data to ventilation. As inspired ventilation is measured, VO_2 is calculated using the Haldane transformation. Before each experimental session, the PFS underwent acceptance tests to validate instrument performance, the gas analyzer module was calibrated using reference gases, and the flowmeter was calibrated with a 3-L volumetric syringe. On the ground, a technician from the DAC calibrated the PFS and showed the participants how to proceed in space.

Energy expenditure, substrate oxidative rates and proxies of metabolic flexibility

For each of the eight periods of indirect calorimetry measurement (one in fasting and seven in post-prandial state), the first five minutes were excluded, and stability of the data was visually checked. VO_2 and VCO_2 were then averaged over each of the eight time-periods of gas exchange measurements. The amount of protein oxidized daily was calculated from total urinary nitrogen excretion, approximated from measures (normalized for weight) obtained in 130 astronauts in preflight conditions and 25 astronauts after 4 months in the ISS (Smith SM & Zwart SR, unpublished data) and assuming that 1 g of nitrogen comes from approximately 6.25 g of protein. Respiratory quotient (RQ) was calculated as VCO_2/VO_2 and the equations of Weir (Weir, 1949) and Frayn (Frayn, 1983) were used to respectively calculate energy expenditure and the total oxidative rates of carbohydrates and fat.

The post-breakfast substrate oxidation response was expressed as 0-to-260 min incremental areas under the curve (iAUC) calculated by the trapezoidal method. Fasting and post-breakfast substrate oxidations were expressed both in absolute values (grams per minute and grams over 260 min, respectively) and relative to the corresponding energy expenditure, *i.e.*, respectively fasting and post-breakfast energy expenditure iAUC. The difference between maximal post-breakfast RQ and fasting RQ (ΔRQ) was used as a proxy of metabolic flexibility (Rynders *et al.*, 2018; Galgani & Fernandez-Verdejo, 2021). Inflight data from one subject were not included in the data analysis because his fasting RQ was > 1.2, which is very unlikely and suggests a technical or other issue.

Body mass and composition

Body mass (BM) was assessed on the first day of both sessions. A calibrated scale was used on the ground and subjects were in undergarments. The SLAMMD (Space Linear Acceleration Mass Measurement Device) device was used inflight. Fat-free mass (FFM) was obtained by isotopic dilution from doubly-labeled water (DLW) measurement as previously detailed (Schoeller, 1988; Bourdier *et al.*, 2022). Briefly, on the ground the astronauts ingested 3.0 g/kg total body water (TBW) of a pre-mixed dose of DLW providing 0.3g/kg TBW from 10% enriched $H_2{}^{18}O$ and 0.15 g/kg TBW from 99%

enriched $^{2}H_2O$. Inflight, the same DLW dose was used for all astronauts to simplify the inflight handling of materials. The dose was calculated to provide 0.45g/kg TBW of $H_2{}^{18}O$ and 0.35 g/kg TBW of $^{2}H_2O$ for an 80 kg astronaut (Bourdier *et al.*, 2022). FFM was calculated assuming a hydration coefficient of 73.2% (Blanc *et al.*, 2002). Fat mass (FM) was calculated as the difference between BM and FFM. Body mass index (BMI), FFM index (FFMI), FM index (FMI) were calculated by dividing BM, FFM and FM (in kg) by squared height (in squared m).

Physical activity and inflight physical training

The SWP, an activity multi-sensor armband, worn on the non-dominant arm for the 10 days of ground and inflight sessions, was used to measure total exercise and non-exercise physical activity as previously detailed (Bourdier *et al.*, 2022). Its companion software (modified professional version 8.0) that incorporates a proprietary machine-learning activity classification algorithm based on heat flux, galvanic skin response, skin and near-body ambient temperature, and accelerometry measure patterns was used to identify non-wear periods and, after exclusion of non-valid days, minutes of physical activity, walking and running. The norm of the 1-min acceleration-signal mean amplitude deviation (MAD) (Vähä-Ypyä *et al.*, 2015) aggregated over activity periods was used as a proxy of physical activity workload (in milli-g, *i.e.* 0.001g). MAD removes the static component due to gravity from the acceleration signal to only keep its dynamic component related to body movements; it is therefore poorly influenced by microgravity. Valid data were obtained in 11 astronauts on ground and 9 astronauts inflight.

Inflight physical training sessions, prescribed 6 days/week, were composed of aerobic exercises performed on either a cycle ergometer (CEVIS) or a second-generation treadmill (T2) with vibration isolation system, and of resistive exercises performed on the advanced resistive exercise device (ARED). Inflight exercise training achieved by each astronaut was obtained from exercise dairy logs provided by NASA. Exercise parameters were calculated over a period beginning 2 months before the inflight research session as previously detailed (Bourdier *et al.*, 2022). For this study, weekly aerobic exercise duration and weekly resistance exercise repetitions were considered.

Food quotient

A 10-day food record completed by photos of meals, snacks and drinks was used to assess macronutrients intake during the sessions on ground and inflight. Inflight, it was associated with a systematic scanning of the barcode of nonperishable food portion-packages provided on the ISS. This allowed a quite precise estimation of both the quantity and quality of the food consumed by all the

subjects. On the ground, exploitable food records for at least 6 days were obtained in 7 subjects. Corresponding energy and macronutrient contents were obtained from food tables provided by NASA, ESA, JAXA, the Russian Space agency and CADMOS for inflight data and from CIQUAL food table (Anses, 2020) for ground data. Food quotients (FQ) were calculated using the equations of Black (Black *et al.*, 1986) .

Statistical analysis

Linear mixed-effects models accounting for repeated measurements with subjects as random effect were used to test the effect of spaceflight on the different anthropometric, activity and metabolic variables. Heterogenous variances models were used in the case of variance inhomogeneity across ground and inflight periods. FFM and FM were additionally introduced as fixed effects in the models testing for the effect of spaceflight on energy expenditure and substrates oxidation. Our statistical inference was on the net changes between ground and inflight in fasting and post-breakfast energy expenditure, substrates oxidation and RQ with their 95% confidence interval (CI). Models without adjustment on FFM and FM are provided in supplemental table 2 for information.
Similar linear mixed-effects models accounting for repeated measurements were used to examine the associations of specific individual metabolic data (*e.g.*, fasting and post-breakfast RQ and substrate oxidation variables) measured on ground and inflight, as dependent outcomes, with corresponding FQ values as explanatory variables. Whether the effect of spaceflight on RQ was mediated by FQ was further investigated using multilevel mediation analysis (Bauer *et al.*, 2006).

Finally, general linear models were used to examine the associations of changes (difference between inflight and ground values) in RQ and fasting- and post-breakfast substrates oxidation as dependent outcomes with successively 1) changes in BM, FFM, FM and physical activity, 2) inflight exercise training, and 3) inflight FQ controlling for baseline values of the dependent variable.

Baseline values and inflight physical activity training and diet-related variables are presented as means (standard deviation, SD). Unless otherwise noted, results of the linear mixed-effects models are presented as least square means (standard error, SE, or 95% confidence interval [CI]). LSmeans estimated from models adjusting for FFM and FM were calculated using whole group mean baseline FFM and FM values (63.77 and 15.59 kg, respectively). Statistical analyses were performed using SAS 9.4 (SAS Institute, Cary, North Carolina) with a significance level at 5%. Figures were realized with Prism 9 (GraphPad, San Diego, California).

Results

Subjects' ground characteristics

At baseline (**Table 7**), astronauts had a mean age of 45.7 years (SD 7.7), were normal-weight with a mean BMI of 24.3 kg/m² (2.1) and presented a normal-to-high FFMI (19.6 kg/m^2 [1.9]). This latter agreed with a quite high daily time spent physically active; overall SWP-derived physical activity time was estimated at 162 min/d (56), and included 10,077 daily steps (2834), 67 min/d walking (34) and 8 min/d running (10).

Changes in body composition and physical activity during flight

Compared to preflight values (**Table 7**), after at least 3 months onboard the ISS, BM slightly decreased by 1.20 kg (95%CI -2.3 to -0.1, P=0.04) mainly due to a non-significant reduction in FFM of 0.9 kg (95%CI -2.3 to 0.4, P=0.14). However, these numbers masked a large inter-individual variability with changes in FFM ranging from -5.4 kg to + 1.3 kg and changes in FM from -3.5 kg to + 5.3 kg.

As expected, spaceflight was associated with a dramatic drop in SWP-derived walking time, which represented only less than 4 min/day. Mean daily SWP-derived overall physical activity time was not significantly modified, partly due to the exercise countermeasure program (**Table 7**). The astronauts reported a total of 168 min/week (SD 44) of exercise on either the CEVIS or T2, and 4.5 resistance training sessions/week (1.8) on the ARED leading to a total of 1481 repetitions/week (834). Like for anthropometric data, a high inter-individual variability in the adherence to the exercise prescription was noted with time spent in aerobic exercise ranging from 87 to 258 min/week and number of repetitions on the ARED ranging from 468 to 3044/week.

Table 7: Ground and inflight body composition and physical activity characteristics of the 11 astronauts.

	Ground	Inflight	Changes from ground[1]	
	Mean (SD)	**Mean (SD)**	**Lsmeans (95%CI)**	**P value**
Anthropometry and body composition				
Body mass (kg)	79.4 (10.6)	78.2 (11.0)	-1.2 (-2.3 to -0.1)	0.04
Body mass index (kg/m^2)	24.3 (2.1)	23.9 (2.1)	-0.4 (-0.8 to -0.0)	0.04
Fat-free mass (kg)	63.8 (8.8)	62.8 (9.0)	-0.9 (-2.3 to +0.4)	0.14
Fat-free mass index (kg/m^2)	19.6 (1.9)	19.3 (2.1)	-0.3 (-0.7 to +0.1)	0.13
Fat mass (kg)	15.6 (3.9)	15.3 (5.5)	-0.3 (-2.2 to +1.7)	0.77
Fat-free mass index (kg/m^2)	4.8 (1.1)	4.7 (1.5)	-0.1 (-0.7 to +0.5)	0.70
Physical activity				

***SWP-derived*[2]**				
Total activity & exercise (min/day)	162 (56)	175 (48)	13.2 (-36.8 to +63.2)	0.56
Walking (min/day)	66.7 (34.2)	3.6 (2.6)	-63.2 (-87.3 to -39.1)	<.001
Running (min/day)	8.11 (10.49)	14.4 (6.4)	6.3 (-2.1 to +14.7)	0.13
Inflight training				
Aerobic exercise (min/week)		168 (44)		.
Resistive exercise (repetitions/week)		1481 (834)		.

SWP, Sensewear Pro activity device; aerobic exercise, T2+CEVIS; resistive exercise, ARED; FQ, food quotient.
[1]Estimated LSmeans (95%CI) and P value from mixed-effects models accounting for repeated values.
[2]inflight data from 9 subjects.

Energy expenditure and substrate oxidative rates

Long-term spaceflight is associated with a metabolic shift towards carbohydrate oxidation, mainly due to changes in food substrate availability

Values of energy expenditure and substrate oxidation were all controlled for FM and FFM. As illustrated in **Figure 23** and detailed in **Table 8**, three months in space did not significantly alter fasting energy expenditure compared to ground values (**Figure 23A**)**.** However, it was associated with a pronounced increase in carbohydrate oxidation (+0.06 g/min [95%CI 0.00 to 0.11], P=0.04; **Figure 23B**) along with a reduction in fasting fat oxidation (-0.03 g/min [95%CI -0.05 to -0.01], P<0.01; **Figure 23C**). Consequently, fasting RQ significantly increased by 0.06 (95%CI 0.02 to 0.11, P=0.01; **Figure 23D**). Carbohydrates represented 65% (SE 5) of fasting energy expenditure inflight as compared to 44% (5) on the ground (P=0.01), while the contribution of fat oxidation to fasting energy expenditure decreased from 34% (6) to 10% (3) (P<0.01).

Concomitantly to this fasting metabolic shift, changes in diet composition were observed with an increase in the proportion of carbohydrates in the diet energy intake at the expense of fat contribution. These dietary changes translated into a mean inflight FQ of 0.87 (SD 0.01) as compared to a FQ of 0.84 [0.01] obtained in 7 subjects on ground (P<0.001; **Table 8** and **Figure 23E**). Individual inflight and ground RQ were significantly associated with corresponding FQ (R^2=0.52; P<0.01; **Figure 23F**). Mediating analyses indicated that FQ explained 69% of the changes in RQ induced by long-term spaceflight (P<0.01). Multivariable analysis indicated that 1) changes in RQ adjusted for ground RQ were significantly associated with inflight FQ (partial R^2=0.72; P<0.01; **Figure 23G**), and 2) inflight FQ and baseline RQ explained together 90% of the variance observed in the spaceflight-induced RQ changes.

No relation was noted between outcomes related to the fasting metabolic shift and inflight anthropometric or physical activity data (data not shown).

Table 8: Ground and inflight nutrition and substrate oxidation data of the astronauts.

	Ground	Inflight	Changes from ground	
	LSmeans (SE)	**LSmeans (SE)**	**LSmeans (95%CI)**	**P Value**
Fasting state				
Energy expenditure (kJ/min)	4.73 (0.20)	4.58 (0.21)	-0.16 (-0.48 to 0.16)	0.30
Glucose oxidation (g/min)	0.13 (0.02)	0.18 (0.02)	0.06 (0.00 to 0.11)	0.04
Lipid oxidation (g/min)	0.04 (0.01)	0.01 (0.01)	-0.03 (-0.05 to -0.01)	<0.01
Respiratory quotient (RQ)	0.85 (0.02)	0.91 (0.02)	0.06 (0.02 to 0.11)	0.01
Glucose oxidation (%)	44 (5)	65 (5)	21 (6 to 36)	0.01
Lipid oxidation (%)	34 (6)	10 (3)	-23 (-37 to -9)	<0.01
Protein oxidation (%)	22 (1)	24 (1)	2 (0 to 4)	0.02
Post breakfast-challenge				
Energy expenditure iAUC (kJ)	302 (29)	452 (45)	150 (36 to 264)	0.01
Glucose oxidation iAUC (g)	16.8 (6.4)	22.3 (2.5)	5.5 (-9.5 to 20.5)	0.44
Lipid oxidation iAUC (g)	0.1 (2.3)	3.1 (0.8)	2.1 (-3.3 to 7.5)	0.41
ΔRQ	0.06 (0.02)	0.05 (0.02)	-0.01 (-0.07 to 0.04)	0.66
Diet				
Food quotient (FQ)[1]	0.84 (0.00)	0.86 (0.00)	0.03 (0.01 to 0.04)	<0.001

Data are estimated Lsmeans (SE), LSmeans (95%CI) and P values from mixed-effects models accounting for repeated measurements and adjusted for FFM and FM. Data for FQ are unadjusted.
iAUC, incremental area under the curve over the 260 min period of measurement; FFM, fat-free mass; FM, fat mass; ΔRQ, maximal postprandial RQ – fasting RQ.
[1]ground data from 7 subjects.

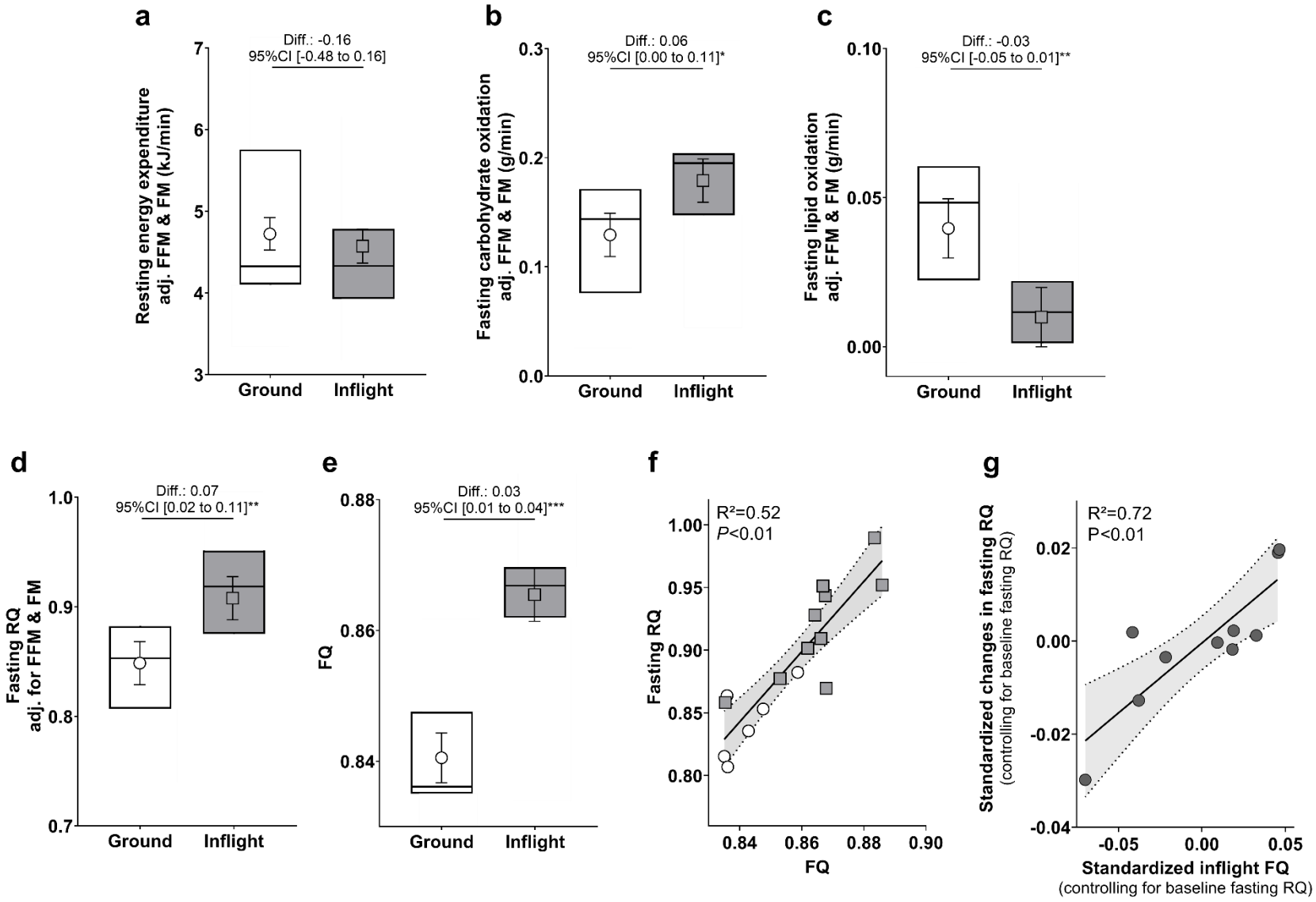


Figure 23: Fasting energy expenditure, substrate oxidation rates, respiratory quotient and food quotient on the ground and inflight.

Ground and inflight fasting resting energy expenditure (A), carbohydrate oxidation (B), lipid oxidation (C), respiratory quotient (RQ, D) and food quotient (FQ, E). Values are interquartile range with median and lsmeans (SE) from mixed-effects models accounting for repeated measurements adjusted for FFM and FM with differences (Diff.) presented with their 95% confidence interval. Values of FQ are unadjusted. *P<0.05, **P<0.01, ***P<0.001.

Scatterplot of individual ground (white circles) and inflight (blue squares) fasting RQ with corresponding FQ (F). Illustration of the partial relationship of fasting RQ with inflight FQ adjusted for ground RQ using the scatterplot of the relationships of standardized fasting RQ (controlling for ground RQ) with standardized inflight FQ (G). Least square regression lines are plotted with their 95% confidence interval (grey area)

FFM, fat-free mass; FM, fat mass; RQ, respiratory quotient; FQ, food quotient.

Long-term spaceflight does not alter postprandial substrate oxidation in response to a breakfast challenge.

Figures 24A-D represent unadjusted kinetics for energy expenditure, carbohydrates and fat oxidation, and RQ after ingesting the breakfast challenge (time=0-260 min) during the ground and inflight sessions. Post-breakfast iAUC of energy expenditure, and of carbohydrate and fat oxidation, adjusted for FFM and FM, are presented in **Figures 24E-G** and **Table 8**.

Post-breakfast energy expenditure iAUC adjusted for FFM and FM was higher inflight than on the ground by 150 kJ (95%CI 36 to 264, P=0.01; **Figure 24E**). RQ following ingestion of the breakfast challenge remained higher inflight than on the ground, which is in line with the metabolic shift observed towards the use of carbohydrate as fuel in fasting state.

Metabolic responses to the breakfast-challenge were characterized by a lower inter-individual variability inflight than on the ground (**Table 8**; **Figures 24F** and **24G**) for both carbohydrate oxidation iAUC (interquartile range [IQR] 8.7 g inflight vs 20.1 on the ground) and fat oxidation iAUC (IQR 3.1 g inflight vs 7.2 on the ground). However, long-term spaceflight did not impact mean iAUC of carbohydrate oxidation (+5.5 g [95%CI -9.5 to 20.5], P=0.44; **Figure 24F**) and fat oxidation (+2.1 g [95%CI -3.3 to 7.5], P=0.41; **Figure 24G**), both being adjusted for FFM and FM. Similarly, adjusted ΔRQ, a proxy of metabolic flexibility, was not modified inflight compared to ground values (-0.01 [95%CI -0.07 to +0.04], P=0.66; **Figure 24H**).

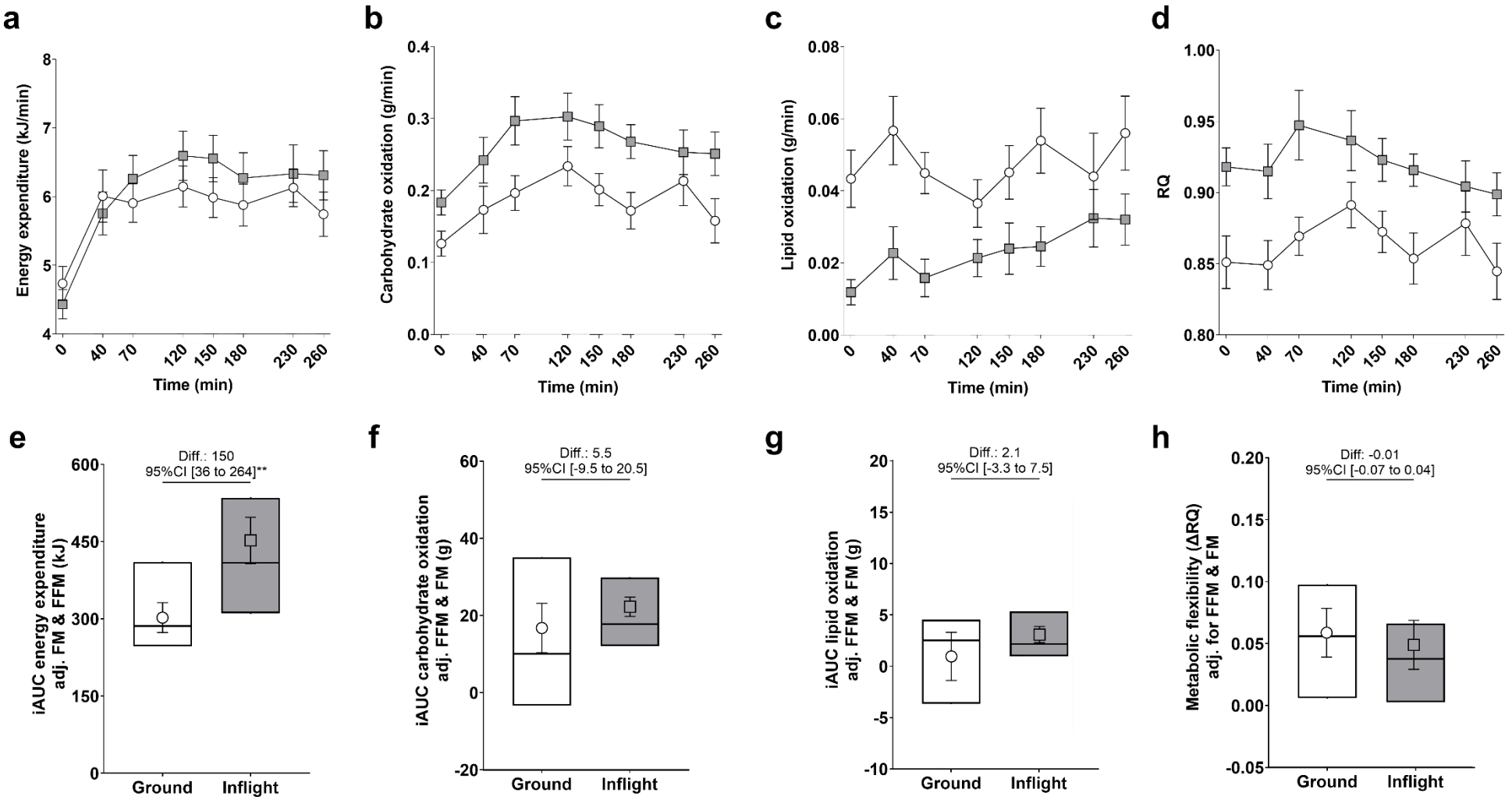
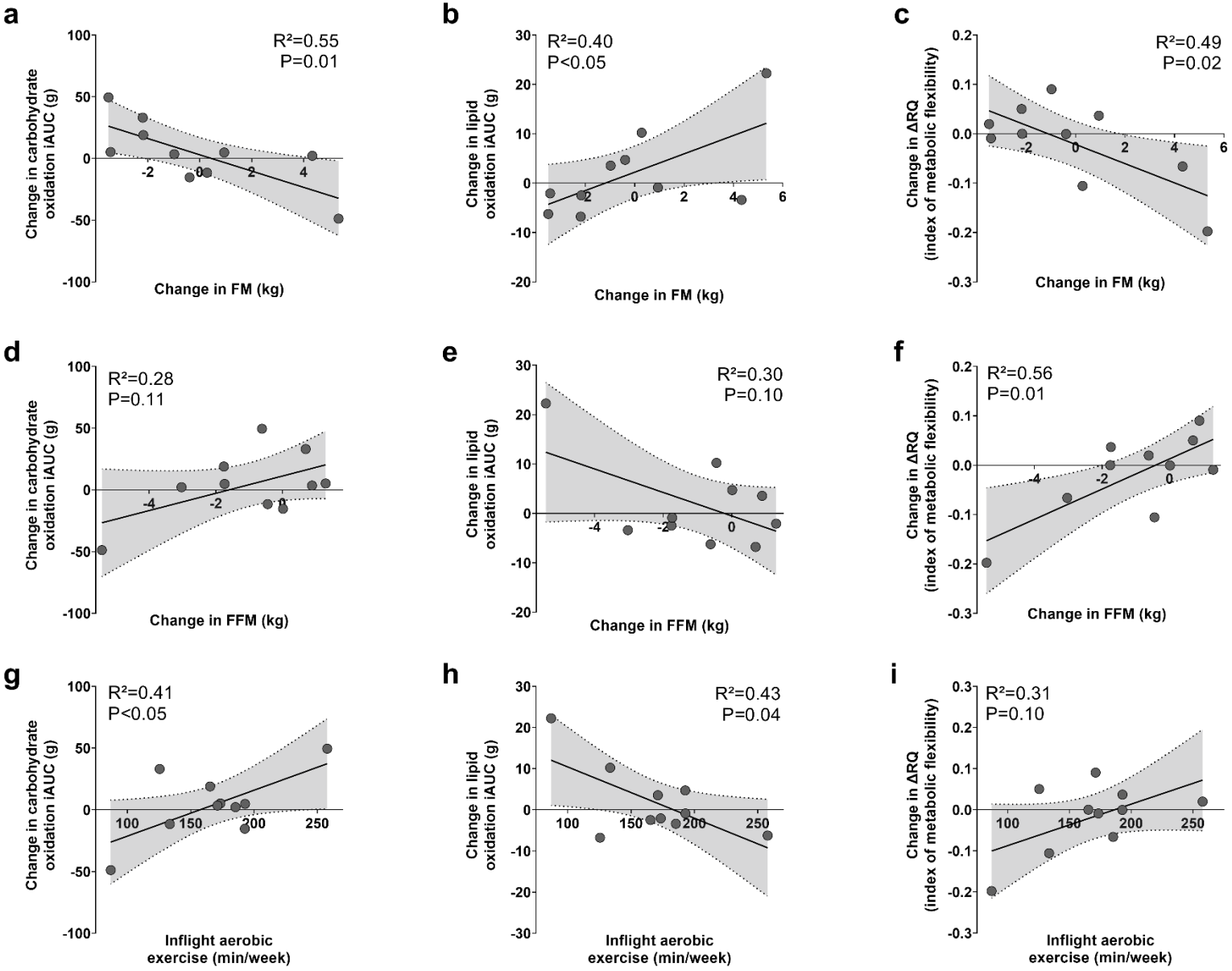


Figure 24: Response of energy expenditure, substrate oxidation and respiratory quotient to the breakfast-challenge on the ground and inflight.

Kinetics and corresponding iAUC over the 260-min postprandial period of the breakfast-meal challenge of energy expenditure (A and E), carbohydrate oxidation (B and F) and lipid oxidation (C and G) on the ground and inflight. Kinetics of RQ during the breakfast-meal challenge (D) and ΔRQ (H), used as an index of metabolic flexibility, on the ground and inflight.
Kinetics are presented by ground (white circles) and inflight (blue squares) unadjusted means (SE). Incremental AUCs and ΔRQ are interquartile range with median and lsmeans (SE) from mixed-effects models accounting for repeated measurements adjusted for FFM and FM with differences (Diff.) presented with their 95% confidence interval; *P<0.05, **P<0.01, ***P<0.001.
FFM, fat-free mass; FM= fat mass; RQ, respiratory quotient; ΔRQ, difference between maximal post meal RQ and fasting RQ; iAUC, incremental area under curve over the 260 min postprandial period.

Associations of changes in post-breakfast substrate oxidation and metabolic flexibility with changes in body composition and exercise

Spaceflight-induced changes in the metabolic response to the breakfast challenge were associated with spaceflight-induced changes in FFM and FM, and inflight aerobic exercise (**Figure 25**). FM changes were positively associated with changes in post-breakfast lipid oxidation iAUC (R^2=0.40, P<0.05; **Figure 25B**), but negatively with changes in carbohydrate oxidation iAUC (R^2=0.55, P=0.01; **Figure 25A**). Changes in ΔRQ were negatively and positively associated with changes in FM (R^2=0.49, P=0.02; **Figure 25C**) and FFM (R^2=0.56, P=0.01; **Figure 25F**), respectively. Finally, changes in carbohydrate and lipid oxidation iAUC were positively (**Figure 25G**) and negatively (**Figure 25H**) associated with inflight time spent in aerobic exercise, respectively.



<u>Figure 25</u>: Associations between spaceflight-induced changes in post-breakfast substrate oxidation and metabolic flexibility and both body composition changes and inflight exercise.

Scatterplots for the relationships of spaceflight-induced changes in post-breakfast carbohydrate lipid oxidation iAUC and ΔRQ with FM changes (A, B, and C, respectively), FFM changes (D, E, F, respectively) and self-reported inflight exercise (G, H, I, respectively).

Least square regression lines are plotted with their 95% confidence interval (grey area).

iAUC, incremental area under curve over the 260min period; ΔRQ, difference between maximal post meal RQ and fasting RQ.

Discussion

The aim of the ENERGY study was to investigate energy and nutrient metabolism in astronauts during a 6-month flight onboard the ISS. The effects of long-term exposure to microgravity on energy expenditures and body composition during the ENERGY study were previously published (Bourdier *et al.*, 2022). Here are reported the effects on nutrient uses and metabolic flexibility measured in fasting state and in response to a standardized mixed-meal. Like it has been repeatedly reported in bed-rest studies for the past 30 years, a shift in total substrates use in favor of carbohydrates and in detriment to lipid oxidation was observed in space. However, this shift seemed to be primarily influenced by changes in dietary intakes. Contrary to what was observed in response to simulated gravity on Earth, no decrease in metabolic flexibility was noted. However, the large inter-individual variability noted on the ground and to a lesser extent inflight allowed to detect associations between changes in both post-breakfast nutrient oxidation and metabolic flexibility, and both changes in body composition and inflight exercise performance.

Inflight diet may predict the shift in substrates use observed during space flight more than microgravity per se

To date, four bed-rest studies conducted in healthy adults and lasting between 7 and 90 days showed that simulated microgravity induces a shift in nutrient oxidation in favor of carbohydrates use as fuel in both fasting and fed states (Ritz *et al.*, 1998; Blanc *et al.*, 2000; Bergouignan *et al.*, 2006; Bergouignan *et al.*, 2009). This shift seemed to be unrelated to energy balance as it was observed in conditions of positive, stable and negative energy balance (Stein & Wade, 2005). However, it was prevented by high volumes of exercise, at least partially (Bergouignan *et al.*, 2011; Le Roux *et al.*, 2022). For example, a 60-day bed-rest study reported that an average of 30 min/day of moderate-to vigorous intensity aerobic exercise combined with an average of 12 min/day of resistive exercise significantly mitigated the increase in fasted and postprandial NPRQ induced by strict bed rest in healthy women adults (Bergouignan *et al.*, 2009). In the present study, astronauts self-reported on average 24 min/day of aerobic exercise using both the T2 and CEVIS devices and 1481 repetitions per week using the ARED device (Bourdier *et al.*, 2022), which is approximately equivalent to 29 min/day of resistive exercise. Despite having performed volumes of exercise about equivalent to what was tested on the ground, the exercise countermeasure was not sufficient to prevent the shift in nutrient use during the spaceflight. This may be because microgravity and/or physical inactivity are not the primary determinants of the shift in nutrients use. Using dietary surveys, we estimated a rough increase in carbohydrate intake from 43% of energy intake in the habitual diet of the astronauts on the ground to

50% of energy intake inflight. A concomitant approximative decrease in fat intake from 34% to 30% of energy intake was estimated. Recently, Péronnet and Haman extensively reviewed the relationship between intrinsic substrate oxidation, energy balance, FQ and fasting RQ (Peronnet & Haman, 2019). They discussed that intra- and inter-subjects' variabilities observed in fasting RQ may be related to various factors not fully identified and difficult to control. However, the principles of biochemistry implies that fasting RQ is necessarily influenced by energy balance and diet macronutrients composition (Goris & Westerterp, 2000; Miles-Chan *et al.*, 2015). In a study that aimed to determine the variability and determinants of resting RQ in trained cyclists, a multivariate analysis showed that 59% of the variance in fasting RQ was explained by type I muscle fibers content, muscle glycogen content, training volume, plasma free fatty acid and lactate concentration, and the percentage of dietary fat intake (Goedecke *et al.*, 2000). We observed that 90% of the variations observed in the changes in fasting RQ induced by the spaceflight were related to inflight dietary intake along with baseline RQ. This observation suggests that individual oxidative capacity along with changes between ground and inflight diet primarily drive the spaceflight-induced shift in substrates use. Muscle biopsies collected before and after the flight would have helped determining whether the structural, functional, and molecular adaptations widely described in studies of prolonged bed rest (Bergouignan *et al.*, 2011) also participate in the variation of inflight fasting substrate oxidation, like what was observed in the trained cyclists (Goedecke *et al.*, 2000). Of note, decreases in type I muscle fibers have been repeatedly observed in animals spending between 11 to 91 days in space (Kischel *et al.*, 2001; Harrison *et al.*, 2003; Ulanova *et al.*, 2015; Gambara *et al.*, 2017a; Gambara *et al.*, 2017b; Tascher *et al.*, 2017) and in men and women bed-rested from 55 to 84 days (Trappe *et al.*, 2004; Trappe *et al.*, 2007a; Salanova *et al.*, 2008; Moriggi *et al.*, 2010). In the absence of blood samples and muscles biopsies, we cannot exclude the role of muscle oxidative capacity alteration in response to microgravity.

In this study, we also assessed metabolic flexibility in response to a standardized moderately high-fat and protein mixed-meal that allowed to reproduce the dynamic process of absorption and digestion of complex foods and the use of mixed substrates. We showed that metabolic flexibility was unchanged after long-term exposition to microgravity. Given that the changes in basal substrate oxidation are largely explained by the dietary changes between the inflight diet and habitual diet, one could question whether those dietary changes may have influenced the assessment of metabolic flexibility and/or the changes in metabolic flexibility. As far as we know, only one clinical research study assessed the impact of diet on metabolic flexibility. By comparing the effect of two 6-weeks eucaloric diets (western vs "healthy" diet) differing in carbohydrate (48% vs 40% of energy intake) and protein (14% vs 20% of energy intake) intakes (Fechner *et al.*, 2020), the authors showed that both fasting RQ and RQ measured in response to a high-fat mixed meal challenge were lower in the group who

consumed the “healthy” diet compared to those who consumed the western diet. However, the difference in postprandial RQ between the two groups did not persist after adjusting for differences in fasting RQ, which suggested no influence of the habitual diet on the postprandial use of substrates as fuel. In line with these data, we observed no relationship between changes in metabolic flexibility and inflight FQ (data not shown). Taken together, these data suggest that inflight diet largely contributes to the shift in fasting substrate oxidation but likely does not affect postprandial substrate use and metabolic flexibility in astronauts, at least in response to a standard-meal enriched in fat and proteins. A high-carbohydrate test meal, like commonly used to test metabolic flexibility, may elicit different results in the ability to adjust the use of carbohydrates over lipids as fuel when transitioning from a fasted to a fed state.

The inter-relationships between metabolic flexibility, body composition and exercise

We showed that flight-induced changes in postprandial substrate use and metabolic flexibility were associated with changes in body composition and exercise. Changes in FM were positively associated with changes in postprandial lipid oxidation, and negatively with postprandial carbohydrate oxidation and metabolic flexibility. A recent systematic review (Glaves *et al.*, 2021) that combined clinical studies on the relationship between adiposity and metabolic flexibility reported inverse correlations between metabolic flexibility to glucose plus insulin stimulation (*i.e.*, measured during a hyperinsulinemic euglycemic clamp) and total FM, visceral adipose tissue and waist circumference. However, the authors did not find any association between adipose tissue characteristics and metabolic flexibility to dietary challenges. This being said only nine studies were included in the systematic review and none with longitudinal measurements of body composition, which may have precluded the detection of relationships with metabolic flexibility measured in response to mixed-meal consumption.

Changes in ΔRQ in response to mixed-meal ingestion, *i.e.*, metabolic flexibility, was also positively associated with flight-induced changes in FFM. Although no relationship was noted between the changes in metabolic flexibility and any physical activity variables, inflight total FFM was previously positively associated with overall physical activity, time spent in vigorous physical activity and T2 relative workload in these same astronauts (Bourdier *et al.*, 2022). This is in line with previous data from our research group showing that on Earth habitual physical activity predicts metabolic flexibility (Bergouignan *et al.*, 2011; Bergouignan *et al.*, 2013a). In addition, while decreases in physical activity concomitant or not of simulated microgravity favor the development of metabolic inflexibility, the

performance of combined aerobic and resistive exercise training prevented, at least partially, bed-rest-induced alterations in muscle outcomes known to participates in metabolic inflexibility (Le Roux *et al.*, 2022), including decreases in mitochondrial oxidative capacity, whole-body oxidative capacity and insulin sensitivity. Like what was observed on Earth, physical activity may therefore modulate metabolic flexibility in space. Taken altogether, these data collected on Earth and in space suggest that the relationship we observed between spaceflight-induced changes in FFM and metabolic flexibility may be partially mediated via the impact of the exercise countermeasure and/or overall physical activity on FFM. Because correlations cannot inform cause-and-effect relationships, we cannot rule out a reciprocal relationship. Some evidence suggests that the changes in metabolic flexibility may play a role in the regulation of muscle mass (Meex *et al.*, 2019). Finally, the negative association previously observed in the ENERGY experiment between inflight overall exercise and inflight FM (Bourdier *et al.*, 2022) further suggests that physical activity/exercise may also modulate the metabolic response to long-term spaceflights via changes in FM.

Limitations and strengths

Strengths and limitations need to be acknowledged. This is the first study to investigate substrate oxidation and metabolic flexibility in astronauts during long-term missions onboard the ISS. We used a standardized mixed-meal as a metabolic challenge to assess changes in metabolic flexibility under physiological conditions, unlike the use of insulin stimulation or an oral glucose tolerance test that create supraphysiological conditions. However, the relatively high proportion of both fat and proteins in the test meal did not allow to test metabolic flexibility to carbohydrate and further infer on its relationship with insulin sensitivity. Other limitations include the relatively small sample size due to the challenges inherent to inflight experiments and the fact that only male astronauts were included. Although the assessment of the macronutrient composition of the inflight diet was reliable, the assessment of the astronauts' habitual diet on the ground was challenging due to their very busy schedule. As a consequence, acceptable data were obtained in seven astronauts only. Finally, the absence of plasma and skeletal muscle samples, for obvious logistical and ethical reasons, precluded the examination of changes in whole-body and skeletal muscle insulin sensitivity and ectopic fat storage in muscle.

Conclusions

We showed that spaceflight is associated with a shift in substrate use in favor of carbohydrate oxidation as observed during bed rest studies. However, it seems to be primarily driven by the macronutrient composition of the inflight diet, rather than by microgravity *per se*. Whereas no change was detected on average, the high between-astronauts variability in both post-prandial substrate use and metabolic flexibility highlighted the influence of body composition and inflight aerobic exercise on these metabolic outcomes. These data support the importance of maintaining stable FM and hence, energy balance during spaceflight. They also confirm the role of physical activity in the regulation of metabolic flexibility in space and suggest that the exercise countermeasure as currently prescribed allows to prevent the development of metabolically inflexibility as long as compliance is observed. Because metabolic flexibility influences metabolic health and likely other physiological functions, future studies will need to optimize and personalize the dietary and exercise countermeasures.

Contributorship: SB, CS, DAS, GGK and AM designed the study. ELR, AB, CS and SB drafted the manuscript. AZ, CT, AM, GGK, CS and SB collected data. ELR, PB, AZ, DAS, IC, MG, LVDB, AB, CS and SB analyzed the data. CS realized the statistical analysis. All the authors mentioned significantly contributed to the realization of this study and approved the final version of the manuscript.

Ethical approval: The study was yearly approved by the NASA Institutional Review Board under NASA 7116301606HR. The ESA Medical Board and the JAXA Institutional Review Board for human experiment also approved the experiment. The study was conducted in conformity with the policy statement regarding the use of human participants as outlined in the Declaration of Helsinki. All astronauts received a detailed presentation of the experiment before enrolling the study and signed a written informed consent.

Acknowledgments: The authors are grateful to the astronauts for their dedication to the study. Special thanks also to the Danish Aerospace Company (former DAMEC, Odense DK) and CADMOS Center (Toulouse, France) for the preparation, operational support during baseline data collection on the different training sites (Houston, USA and Cologne, Germany) but also during the ground support of the inflight sessions. The authors sincerely thank SM Smith and SR Zwart for providing urinary nitrogen excretion data.

Supplemental table 1: Examples of dishes proposed to astronauts for standardized meals.

Recipes	Nutritional values (kcal per 100g)	Macronutrients composition (g per 100g)		
		Proteins	Lipids	Carbohydrates
Salmon with candied Menton lemon	246	35.0	11.7	0.2
Rivieira style swordfish	191	12.0	11.0	0.0
Britany lobster, quinori with seaweed, Menton lemon condiment	194	12.9	5.7	22.8
Scottish salmon, candied tomatoes and grilled eggplant	281	33.2	15.5	2.2
Shredded chicken Parmentier	266	26.6	13.1	10.6
Spiced chicken stir-fried thaï vegetables	276	56.2	4.6	0.0
Duck confit with capers	396	46.5	23.3	0.2
Lamb shoulder confit with sage, pearl barley and candied tomatoes	269	21.7	11.0	22.0
Landes yellow poultry cooked as "Poule au pot"	280	36.4	5.0	12.6
Beef cheeks in Bourguignon style, carrots and mushrooms	197	29.9	7.26	2.64
Carrots tops	29	0.6	0.4	6.5
Caponata	62	1.9	3.2	9.5
Vegetables and tomatoes fondue	36	0.9	0.9	6.8
Omelet cake with tomatoes and herbs	125	11.9	5.9	6.7
Egg cocotte, Basque style condiment	112	6.4	6.7	7.4
Cheese-cake	191	4.0	12.5	15.6
Muesli	282	5.5	14.3	33.9
Chocolate cake	340	5.0	27.9	18.8
Semolina with dried apricots	269	9.0	2.3	52.0
Apple fondant pieces	74	0.2	0.5	18.1
Creamy lemon baked	250	19.1	19.1	17.4

Supplemental table 2: Unadjusted ground and inflight energy expenditure and substrate oxidation data of the astronauts.

	Ground	Inflight	Changes from ground							
	LSmeans (SE)	LSmeans (SE)	LSmeans (95%CI)	P Value	Min to max changes	Ground mean (SD)	Inflight mean (SD)	LSmeans (SE) changes	IQ Range BDC	IQ Range Inflight
Fasting state										
Energy expenditure (KJ/min)	4.73 (0.23)	4.52 (0.24)	-0.22 (-0.54 to 0.11)	0.17	-1.04 to 0.34	4.73 (0.82)	4.43 (0.68)	-0.22 (0.14)	1.65	0.76
Glucose oxidation (g/min)	0.13 (0.02)	0.18 (0.02)	0.06 (0.01 to 0.11)	0.03	-0.05 to 0.16	0.13 (0.06)	0.18 (0.05)	0.06 (0.02)	0.10	0.05
Lipid oxidation (g/min)	0.04 (0.01)	0.01 (0.00)	-0.03 (-0.05 to -0.01)	<0.01	-0.08 to 0.01	0.04 (0.03)	0.01 (0.01)	-0.03 (0.01)	0.04	0.02
Protein oxidation (g/min)	0.06 (0.00)	0.07 (0.00)	0.00 (0.00 to 0.01)	<.001	0.00 to 0.01	0.06 (0.01)	0.07 (0.01)	0.00 (0.00)	0.02	0.02
Respiratory quotient (RQ)	0.85 (0.02)	0.92 (0.02)	0.07 (0.02 to 0.12)	<0.01	-0.04 to 0.17	0.85 (0.06)	0.92 (0.04)	0.07 (0.02)	0.08	0.07
Lipid oxidation (%)	34 (6)	10 (3)	-23 (-37 to -9)	<0.01	-55 to 10	34 (19)	10 (10)	-23 (7)	27	20
Glucose oxidation (%)	44 (5)	65 (5)	21 (6 to 36)	0.01	-17 to 53	44 (20)	65 (13)	21 (7)	26	22
Protein oxidation (%)	22 (1)	24 (1)	2 (0 to 4)	0.02	-1 to 7	22 (3)	24 (5)	2 (1)	5	5
Post breakfast-challenge										
Energy expenditure iAUC (kJ)	302 (31)	447 (55)	145 (9 to 280)	0.04	-105 to 537	302 (103)	447 (174)	145 (63)	164	189
Glucose oxidation iAUC (g)	16.81 (6.13)	21.70 (3.74)	4.89 (-10.30 to 20.09)	0.51	-48.81 to 49.55	16.81 (20.33)	21.70 (11.81)	4.89 (7.18)	38.43	14.35
Lipid oxidation iAUC (g)	0.95 (2.23)	2.91 (0.80)	1.97 (-3.17 to 7.10)	0.42	-6.77 to 22.26	0.95 (7.39)	2.91 (2.52)	1.97 (2.37)	8.20	3.84
ΔRQ	0.06 (0.02)	0.05 (0.02)	-0.02 (-0.07 to 0.04)	0.56	-0.20 to 0.09	0.06 (0.06)	0.05 (0.06)	-0.02 (0.03)	0.09	0.05

Data are estimated Lsmeans (SE), LSmeans (95%CI) and P values from mixed-effects models accounting for repeated measurements.
iAUC, incremental area under the curve over the 260 min period of measurement; ΔRQ, maximal postprandial RQ – fasting RQ.

Discussion

The main goal of this thesis was to better understand the impact of microgravity on metabolic flexibility in humans and characterize some of the underlying physiological, cellular and molecular mechanisms. A secondary aim was to examine the relationships between metabolic flexibility, diet, physical activity and body composition.

In the first study we showed that five days of simulated microgravity induced by dry immersion were sufficient to reduce whole-body insulin sensitivity but not metabolic flexibility.

We further reported that these *in vivo* metabolic changes were related to decreased *in vitro* capacity for glycogen synthesis, metabolic flexibility to glucose and insulin signaling pathways in myocytes cultured from the quadriceps muscle of the study participants. These findings support the concept that alterations in the capacity of skeletal muscle to adjust substrates use to substrate availability and insulin action contribute to the early onset of metabolic disruptions observed in response to microgravity. We further showed that that an epigenetic imprinting of satellite cells rapidly occurs in response to short-term physical inactivity.

In the second study, we showed that the shift in substrate oxidation in detriment of lipid use and in favor of carbohydrates we observed in astronauts who spent more than 3 months onboard the ISS, like it was repeatedly reported on Earth during bed-rest studies, was associated with a change in astronauts' inflight diet. Although long-term exposure to microgravity did not affect postprandial fuel selection and metabolic flexibility at the whole-group level, the variability in the individual responses allowed to highlight that changes in metabolic flexibility and postprandial substrate use were associated with changes in body composition. We believe that exercise may mediate, at least partly, these relationships.

Here the following points will be discussed: 1) the impact of the diet macronutrient composition on substrate oxidation and metabolic flexibility under microgravity environments, 2) methodological considerations for measuring metabolic flexibility, 3) the relationships between metabolic flexibility, body composition and exercise. Finally, the Earth-related benefits of these studies will be presented, especially to help informing the role of physical inactivity in the development of chronic metabolic pathologies.

1 Role of diet on substrate oxidation and metabolic flexibility under microgravity conditions

1.1 Relationships between fuel selection and diet in previous bed-rest studies

In the ENERGY study, we observed that the shift in fasting substrate oxidation was primarily due to changes in macronutrients diet composition. Based on this unexpected observation, the question arose about the changes observed during the previous bed-rest studies. We revisited the data from the five bed-rest studies that had investigated the metabolic effects of simulated microgravity and examined the relationships between RQ and FQ during the baseline ambulatory period of the study and the bed-rest period (**Table 9, Figure 26**).

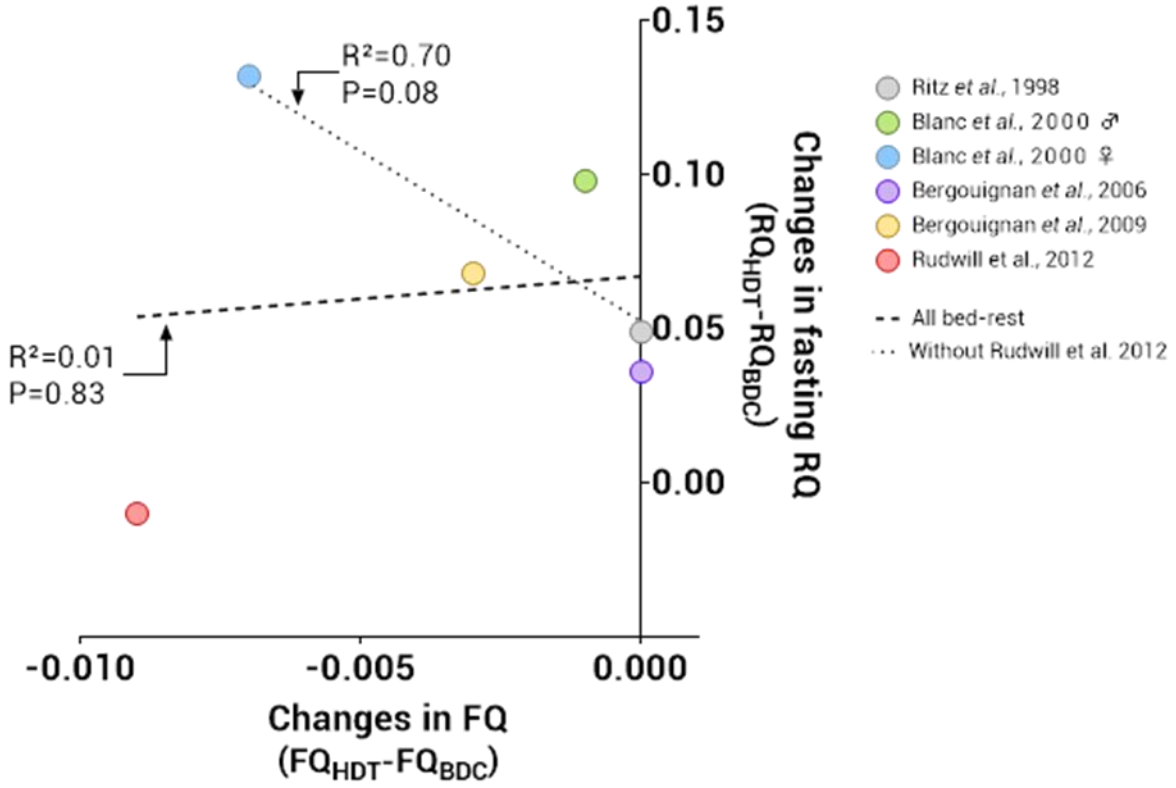


Figure 26: Relationship between changes in food quotient and respiratory quotient during baseline data collection and head-down tilt period following previous bed-rest.

Changes in FQ refers to the difference between FQ during HDT and FQ during BDC. Changes in fasting RQ refers to the difference between fasting RQ during HDT and fasting RQ during BDC. The dashed line represents linear regression including data from all bed-rest. The dotted line represents the linear regression excluding Rudwill et al. 2012.

BDC, baseline data collection period; FQ, food quotient; HDT, head-down tilt test period; RQ, respiratory quotient.

Table 9: Changes in FQ and RQ during previous bed-rest.

Study, country, year and bed-rest duration		Free-living condition				BDC period					HDT period					FQ_{BDC}-FQ_{hab}	FQ_{HDT}-FQ_{hab}	FQ_{HDT} - FQ_{BDC}	RQ_{HDT} - RQ_{BDC}
		Diet macronutrient composition[a]			FQ	Days of diet control	Diet macronutrient composition			FQ	Fasting RQ	Diet macronutrient composition			FQ	Fasting RQ				
		Carb %EI	Fat %EI	Prot %EI			Carb %EI	Fat %EI	Prot %EI			Carb %EI	Fat %EI	Prot %EI						
(Ritz *et al.*, 1998) France, 1994 15 days BDC and 42 days HDT n=7 healthy men		45	42	13	0.854	15	48	40	12	0.861	0.841	48	40	12	0.861	0.890	0.007	0.007	0.000	0.049
(Blanc *et al.*, 2000) France, 1998 4 days BDC and 7 days HDT	n=8 healthy men	45	42	13	0.853	0	51	31	19	0.884	0.906	51	32	18	0.883	1.004	0.031	0.030	-0.001	0.098
	n=8 healthy women	45	42	13	0.853	0	52	32	16	0.877	0.969	49	33	18	0.870	1.101	0.023	0.017	-0.007	0.132
(Bergouignan *et al.*, 2006) France, 2001-2002 15 days BDC and 90 days HDT n=9 healthy male		45	42	13	0.853	10	55	30	15	0.885	0.890	55	30	15	0.885	0.926	0.031	0.031	0.000	0.036
(Bergouignan *et al.*, 2009) France, 2006 20 days BDC and 60 days HDT n=8 healthy women		46	42	13	0.855	5	58	29.5	12.5	0.891	0.791	56.5	30	13.5	0.887	0.859	0.036	0.032	-0.003	0.068
Rudwill et al., unpublished data Germany, 2011-2012 7 days BDC and 21 days HDT n=10 healthy male		51	37	12	0.870	4	56	30	14	0.886	0.840	51	30	19	0.877	0.830	0.016	0.007	-0.009	-0.010

[a]Data from Our World in Data.

BDC, baseline data collection; FQ, food quotient; HDT, head-down tilt; RQ, respiratory quotient.

Of note, this interpretation needs to be considered with caution given the very small sample size. When taking into account four out the five studies, the linear relationship shown in **Figure 27** (dotted line) suggests that the bed-rest-induced changes in fasting RQ were influenced by the changes in the diet of the study participants between baseline data collection (BDC) and head-down tilt bed-rest period (HDT). This supports the observation we made in the astronauts onboard the ISS. However, by adding unpublished data from our group from another bed-rest study (Rudwill et al.), the relationship disappeared (dashed line). Because this last bed-rest study was conducted in Cologne, Germany and not in Toulouse, France like all the others, we wondered whether the habitual diet of the participants may have influenced the fasting RQ measured in BDC and therefore the changes induced by bed-rest between BDC and HDT periods. By using national dietary surveys and taking into account the year the bed-rest study was conducted, we estimated the theoretical FQ of the research participants for each study. The subjects in Germany had likely a greater habitual FQ than the participants in France. This may partly explain why no change (-0.01) was observed in RQ between BDC and HDT.

Because the macronutrient composition of the diet and energy balance status of the days prior to the test are known to influence RQ measurement (Hill *et al.*, 1991; Flatt, 1993; Astrup, 2011; Peronnet & Haman, 2019), the number of days the participants received a standardized diet during the BDC period prior to the experimental study visit may impact the fasting RQ value in BDC and the change between BDC and HDT (**Figure 28**). In support of this, we observed a linear relationship between changes in RQ and number of days of diet control when taking the prior bed-rest studies that measured fasting RQ (dotted line). This indicates that the closer the RQ measurement is made to the onset of HDT, *i.e.*, the longer the period of diet standardization will be, the smaller the change in RQ will be. Of note, the relationship was however no longer significant when including the unpublished data from participants in Cologne, Germany (dashed line).

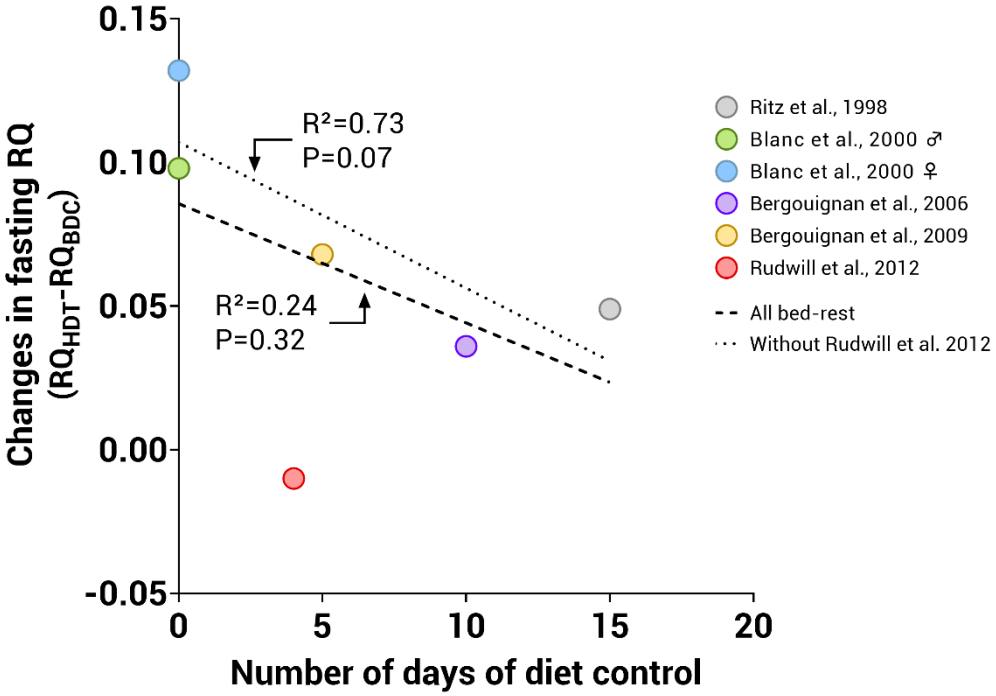


Figure 27: Relationship between changes in respiratory quotient during baseline data collection and head-down tilt period and the number of days of diet standardization following previous bed-rest.

BDC, baseline data collection; HDT, head-down tilt; RQ, respiratory quotient.

Although we could not run rigorous statistical analysis, these prior bed-rest data along with the astronauts data are providing us with important insights for future studies on the effects of microgravity on metabolic outcomes (**Figure 28**): 1) it is crucial to strictly control the diet of the subjects for at least a week prior to the test in BDC to collect baseline data, 2) dietary surveys should be run in

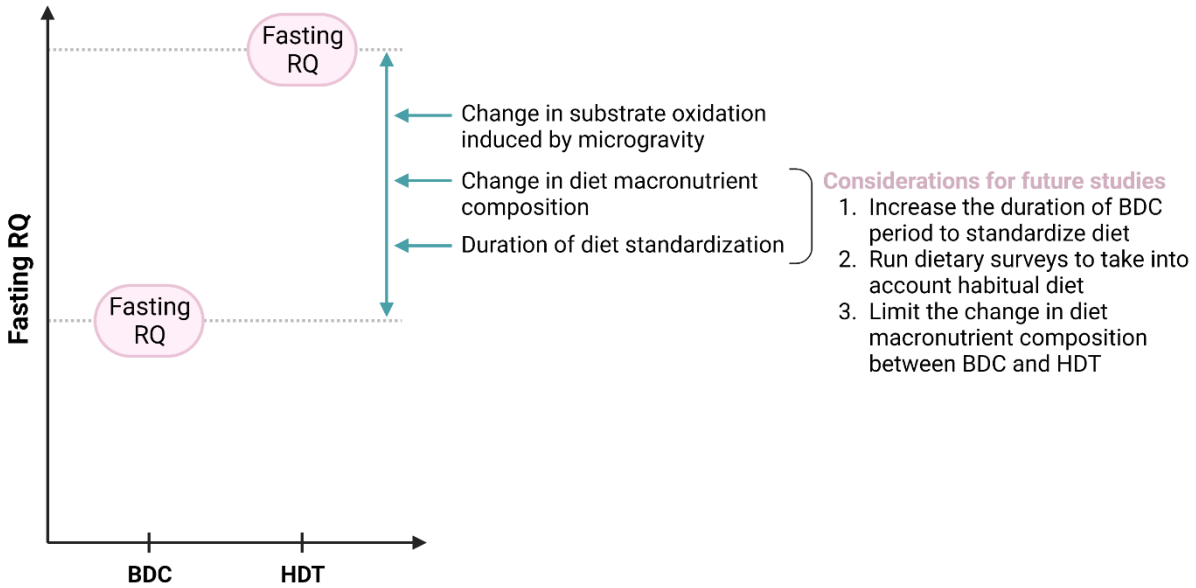


Figure 28: Summary of the impact of change in diet macronutrient composition, the duration of diet standardization and how to decrease their impact on microgravity-induced changes in fasting respiratory quotient.

BDC, baseline data collection; HDT, head-down tilt; RQ, respiratory quotient.

participants to record their habitual diet and take this information into subsequent statistical analyses, like it was done in the astronauts during the ENERGY protocol, and 3) it is important to limit as much as possible changes in diet macronutrient composition between the BDC period and the HDT period, unless it is for testing a specific nutritional countermeasure.

1.2 Do changes in diet macronutrient composition impact metabolic flexibility?

Knowing that changes in diet macronutrient composition influences basal substrate oxidation, one can wonder whether diet composition affects results we obtained on metabolic flexibility. In ENERGY, the astronauts who participated came from 7 different countries and therefore likely had habitual diets with different macronutrient composition. However, as shown in **Figure 29**, no relationship was noted between metabolic flexibility (ΔRQ measured in response to an acute mixed-meal consumption) and FQ both on the ground and inflight, suggesting that astronaut's metabolic flexibility may not have been affected by diet composition.

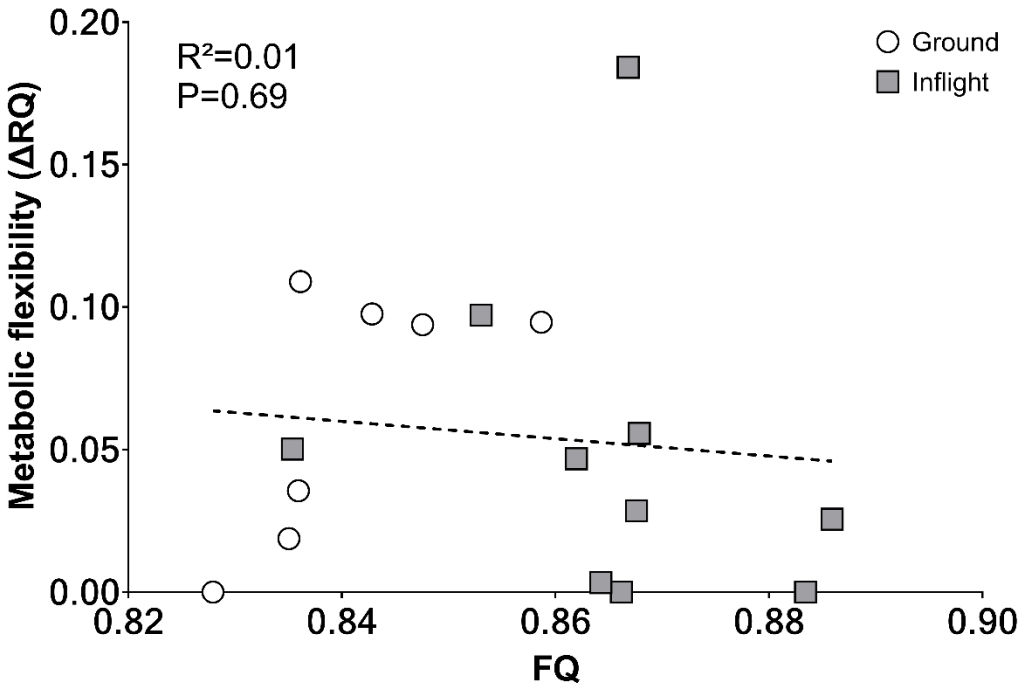


Figure 29: Relationship between metabolic flexibility and food quotient in astronauts before and after at least 3 months onboard the ISS.
Data are from the ENERGY study. White circles refer to ground data and grey squares refer to inflight data. ΔRQ, difference between maximal post-prandial RQ and fasting RQ; FQ, food quotient.

This goes along with results obtained in few ground-based clinical studies. Galgani & Fernandez-Verdejo have recently summarized studies that investigated the effects of various diets on metabolic flexibility and are summarized in **Figure 31** (Galgani & Fernandez-Verdejo, 2021). Three studies tested the effect of healthy diets on metabolic flexibility. In two of these studies, the effect of

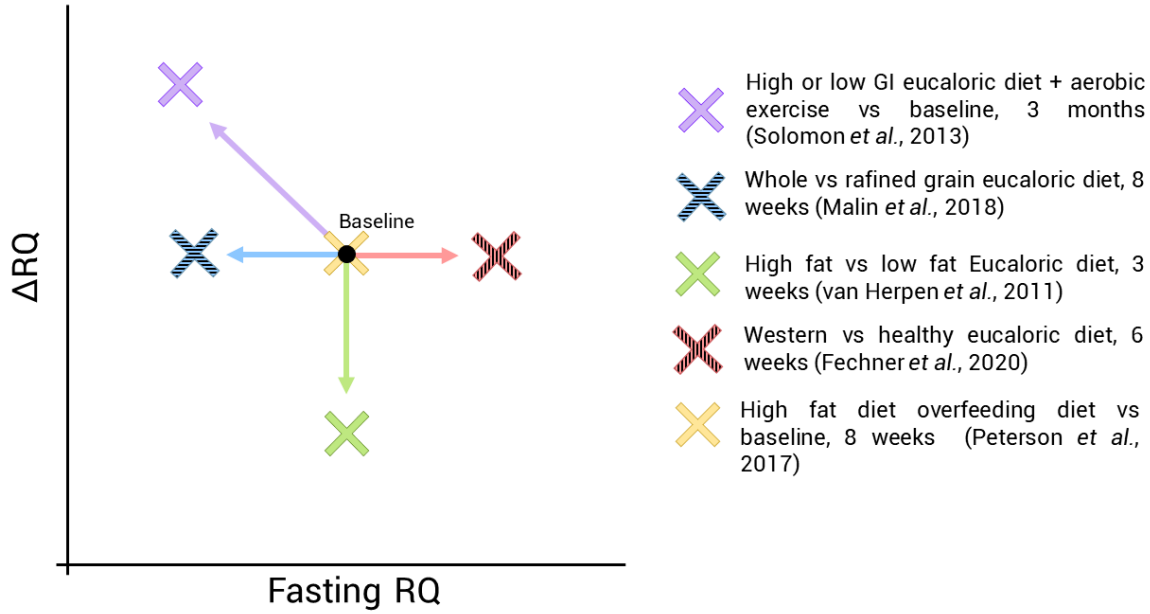


Figure 30: Effects of nutritional interventions on metabolic flexibility.

Full cross: metabolic flexibility assessed during euglycemic-hyperinsulinemic clamp. Cross with vertical hatches: metabolic flexibility assessed during a standardized meal. Cross with horizontal hatches: metabolic flexibility assessed during OGTT.

diet alone on metabolic flexibility was primarily explained by differences in fasting RQ (Malin *et al.*, 2018; Fechner *et al.*, 2020). The first study (in blue in **Figure 30**) compared the effect of an 8-week whole-grain diet to a refined-grain diet (50g of whole or refined carbohydrate per 1000 kcal) on glucose control and metabolic flexibility (difference between postprandial carbohydrate oxidation value at 60 min after an OGTT and fasting carbohydrate oxidation value) (Malin *et al.*, 2018). Although these study participants, adults with obesity and at risk of developing type 2 diabetes, had increased glucose tolerance and peripheral insulin sensitivity after consuming the whole carbohydrate diet, no changes were reported in glucose disposal or oxidation. No improvement in body fat was noted either. Metabolic flexibility did increase but due to a lower rate of carbohydrate oxidation in the fasting state and not because of greater increases in carbohydrate oxidation following acute meal consumption (Malin *et al.*, 2018). The second study (in orange in **Figure 30**) compared the effect of a healthy diet essentially based on the consumption of fruits, vegetables, and fatty fish to a Western diet (Fechner *et al.*, 2020). Macronutrient composition differed with lower carbohydrate percentage in favor of proteins in the healthy diet (on average 40% carbohydrate, 36% fat and 20% protein) compared to the Western diet (48% carbohydrate, 35% fat and 14% protein). The postprandial response to a high-fat meal with a high glycemic index was studied before and after 6 weeks of consuming one of these two diets in a randomized intervention study. Subjects were in energy balance, as indicated by stable weight and body composition. The mean postprandial RQ was lower in the group assigned to the healthy diet compared with the group consuming the Western diet, but the difference did not persist

after adjustment for fasting RQ (Fechner *et al.*, 2020). The authors of the third study (in purple in **Figure 30**) tested the effect of a low versus high glycemic index eucaloric diet (on average 57% carbohydrate, 30% fat and 18% protein) in subjects with obesity. Unlike the two studies mentioned above, this protocol consisted of an aerobic training protocol in addition to a nutritional intervention (Solomon *et al.*, 2013). Independent of diet, insulin sensitivity and ΔRQ during a clamp (difference in RQ between the last 30 minutes of the clamp and fasting RQ) increased after the intervention. It is unclear whether the improvement in insulin sensitivity fully explained the increase in ΔRQ because tissue glucose availability was not taken into account when assessing metabolic flexibility. In addition, a clear decrease in adiposity was noted in both groups. In this study, it is therefore difficult to know whether this improvement in metabolic flexibility was due to diet or aerobic training (Solomon *et al.*, 2013). Together, these data suggest that the effects of healthy eucaloric diets on metabolic flexibility depend on changes in fasting substrate oxidation rather than on changes in the capacity to respond to an acute change in substrate bioavailability induced by a metabolic challenge.

Conversely, two studies tested the effect of high-fat eucaloric and hypercaloric diets on metabolic flexibility (van Herpen *et al.*, 2011; Peterson *et al.*, 2017). In the first study (in green in **Figure 30**) healthy subjects consumed a low fat diet (65% carbohydrate, 20% fat and 15% protein) for 3 weeks and then were randomly assigned to continue low fat diet for another 3 weeks or consumed a high fat diet (30% carbohydrate, 55% fat and 15% protein) for 3 weeks (van Herpen *et al.*, 2011). Metabolic flexibility was measured during a clamp before and after 3 weeks of low or fat diet. The subjects were in stable energy balance. Although the lipid content of the diet did not influence insulin sensitivity and muscle lipid content, liver lipid content increased after the high-fat diet and decreased after the low-fat diet. On the other hand, ΔRQ was decreased after 3 weeks of the high-fat diet compared with 3 weeks of low-fat diet while fasting RQ was unchanged (van Herpen *et al.*, 2011). This decrease in metabolic flexibility could reflect a lower capacity to rely upon carbohydrates in response to insulin stimulation with the eucaloric high fat diet. In response to 8 weeks of a high-fat, hypercaloric diet (1.4*energy intake for body mass maintenance; 41% carbohydrate, 44% fat and 15% protein), Peterson and colleagues (in yellow in **Figure 30**) measured metabolic flexibility in a metabolic chamber based on ΔRQ calculated either as the difference between waking and sleeping periods during a 24h stay in a whole-room calorimeter or as the difference of RQ measured during a low- or high-insulin clamp (Peterson *et al.*, 2017). Although overnutrition reduced insulin sensitivity, it did not affect the indices of metabolic flexibility and fasting blood glucose and triglyceride levels, suggesting that impairments in insulin sensitivity precede alterations in metabolic flexibility (Peterson *et al.*, 2017). Over the five studies discussed here that investigated the effects of changes in diet macronutrient composition on metabolic flexibility: two of them did not show any changes (van Herpen *et al.*, 2011; Fechner *et al.*,

2020), one noted improved metabolic flexibility but this improvement did not persist after taking into account the decrease in fasting RQ (Malin *et al.*, 2018) and one showed increased metabolic flexibility but we do not know whether this change was due to dietary intervention and/or to exercise training and the last one observed decreased metabolic flexibility (Solomon *et al.*, 2013). It is therefore unclear whether change in diet macronutrient composition impact metabolic flexibility and further rigorous research is needed to address this question.

2 Methodological considerations to measure metabolic flexibility

There is a plethora of challenges to test metabolic flexibility, both to carbohydrate and lipid, and at different fuel availability levels such as intake, circulating, tissue/cell and mitochondria (**Figure 32**). This diversity raises several questions. First, what is the influence of the metabolic challenge on the magnitude response of metabolic flexibility? We assessed metabolic flexibility following two test meals that differed in macronutrient composition (*i.e.*, two different metabolic challenges). To address this point, we will discuss how carbohydrate composition of the test meal may influence metabolic flexibility (**Figure 31**). Knowing whether being flexible in one challenge implies being flexible in another condition would allow us to compare the results of different studies, which is to date difficult. Second, while the metabolic flexibility challenges used in the literature essentially tested metabolic flexibility

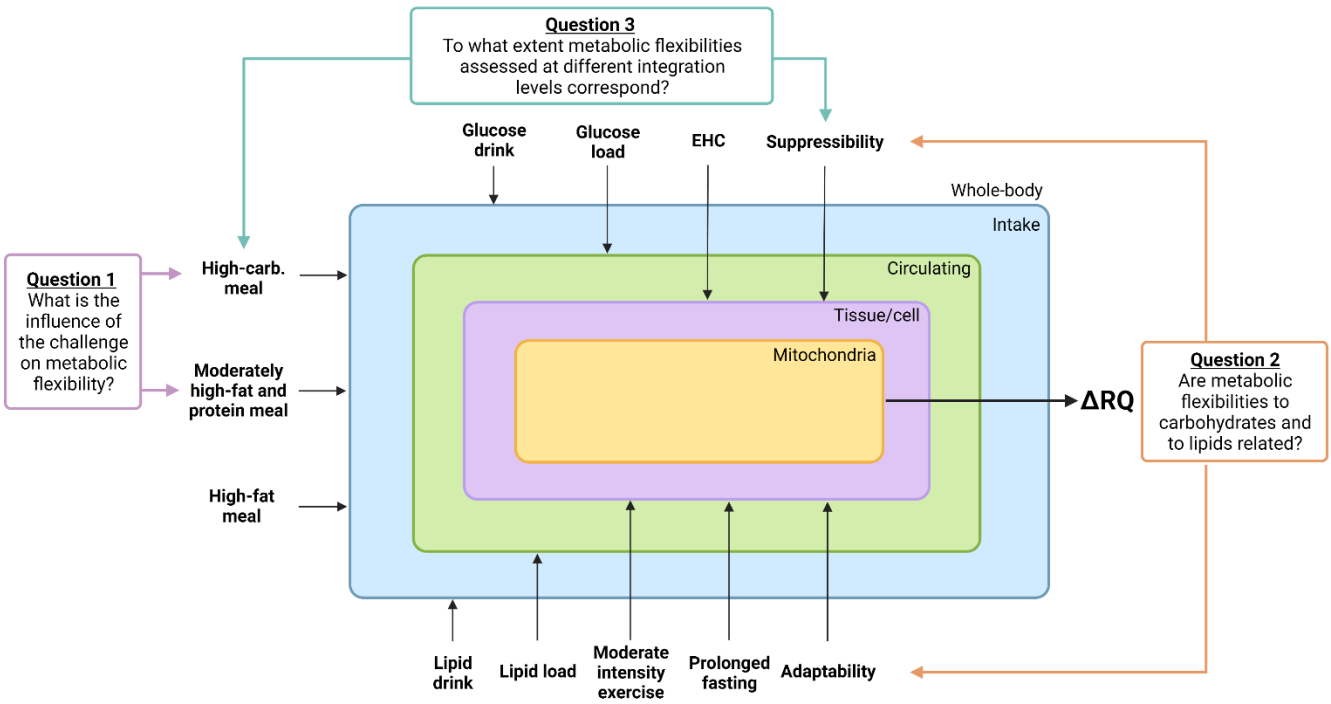


Figure 31: The different challenges to test metabolic flexibility and the questions arising from them.
The different challenges to test metabolic flexibility (ΔRQ) modulate fuel availability at different levels: intake (meals and drinks), circulating (intravenous load), tissue/cell (suppressibility/adaptability) and mitochondria.
ΔRQ, difference between post-challenge RQ and pre-challenge RQ; EHC, euglycemic hyperinsulinemic clamp.

to acute changes in carbohydrate availability, is metabolic flexibility to carbohydrates and to lipids related (**Figure 32**)? Finally, we examined metabolic flexibility at both whole-body and skeletal muscle level. The third question that arises is: to what extent do metabolic flexibility measurements at the tissue, cellular, or organelle levels correspond to metabolic flexibility measurements at the whole-body level (**Figure 32**)?

2.1 Concordance of different challenges to measure metabolic flexibility: influence of carbohydrate proportion of the test mixed meal on metabolic flexibility

Given the diversity of existing metabolic challenges to measure whole-body metabolic flexibility to carbohydrates (euglycemic hyperinsulinemic clamp, intravenous glucose load, glucose drink, high-carbohydrate meal, etc) or to lipids (intravenous lipid load, lipid drink, high-fat meal, prolonged fasting, etc), it would then be important to know whether being flexible in one challenge implies being flexible in another condition. This would allow to compare the results of the studies between them. This aspect of metabolic flexibility has been poorly addressed. As recently underlined, answering this question would be relevant to translate findings from non-physiological (euglycemic hyperinsulinemic clamp) to physiological conditions (high-carbohydrate meal) (Galgani & Fernandez-Verdejo, 2021). Whereas OGTTs and clamps allow to control changes in substrate availability, they create supraphysiological conditions. For example, the clamp utilizes steady-state insulin levels at unphysiological levels, and the steady-state condition does not realistically mimic the dynamic processes that occur after meals consumption. The OGTT has the advantage to be easy to perform but ingesting an acute dose of 75-g of glucose is unpleasant for participants and unlikely to be representative of daily life. Moreover, the process of absorption and digestion of complex foods is not reproduced. In their recent review, Galgani & Fernandez-Verdejo re-examined data from previous studies and more precisely the relationship between metabolic flexibility to glucose measured after 1h of OGTT and during a euglycemic hyperinsulinemic clamp (Galgani & Fernandez-Verdejo, 2021). They showed that ΔRQs adjusted respectively for glucose disposal rate during the clamp and for glucose dose during OGTT were moderately associated with a R^2=0.39 (P=0.04). This suggests that the results of the two challenges may be concordant. In the future studies, it would be interesting to perform several challenges of metabolic flexibility to glucose (OGTT vs high-carbohydrate meal vs euglycemic hyperinsulinemic clamp) or to lipids (high-fat meal vs lipid load vs prolonged fasting) in the same subjects and assess if the measured metabolic flexibilities in response to those challenges are related.

To overcome the limitations of supra-physiological metabolic challenges mentioned above, we chose to assess metabolic flexibility with standard mixed-meals. However, no standardization on the macronutrient composition of this test meal exists to date. In **Figure 32** are compiled the studies during which metabolic flexibility was assessed following a single mixed-meal and where the index of metabolic flexibility was measured as the difference between postprandial and fasting RQ on healthy normal weighted individuals (Heilbronn *et al.*, 2007; Huda *et al.*, 2009; Purtell *et al.*, 2015; Rudwill *et al.*, 2018; Assaad *et al.*, 2019).

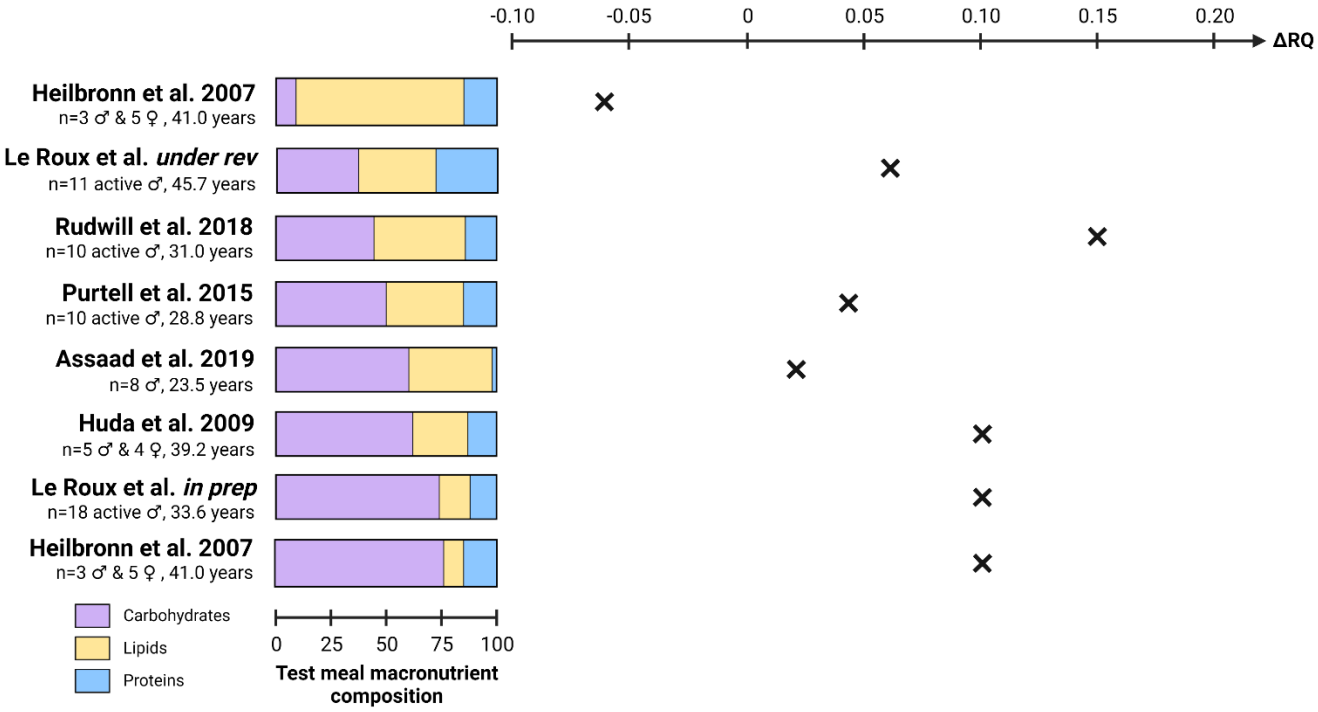


Figure 32: Meal macronutrient composition and metabolic flexibility.

ΔRQ refers to the difference between postprandial RQ and fasting RQ.

In light of these data, it is difficult to rule out the impact of the macronutrient composition of the standard meal on metabolic flexibility. Only one study tested metabolic flexibility (difference between RQ 4 hours after meal ingestion and fasting RQ) with high-carbohydrates meal (76% carbohydrate, 9% fat and 15% protein) and with high-fat meal test (9% carbohydrate, 76% fat and 15% protein) in the same subjects (Heilbronn *et al.*, 2007). Although the data included in this figure are from healthy normal weighted individuals, several factors may contribute to the fact that no clear trend emerges, such as gender, age, and habitual physical activity level. To address this question, we would have to test metabolic flexibility in the same subjects, on different days, using meals with various proportion of nutrients. Now that mixed meals are more commonly used as a metabolic challenge to examine metabolic flexibility, it is important to identify macronutrient composition of the standard diet that allows to discriminate the people with difference levels of metabolic flexibility but also detect small changes in metabolic flexibility in response to interventions. Of course, multiple factors may

influence the absorption and assimilation of the nutrients, including fiber content, glycemic index and types of carbohydrates, fats and proteins of the diet, but also gut microbiota, and others, which would impact the quantity of nutrients being available for the tissues. Further studies are therefore warranted.

2.2 Relationship between metabolic flexibility to carbohydrates and to lipids

If metabolic flexibility has been essentially tested in response to acute changes in glucose availability, a question that remains poorly investigated is whether a similar response would be observed in response to acute changes in lipid availability. To date, only one study in the literature tested both carbohydrates and lipids metabolic flexibility in the same human subjects and assessed if they were associated (Fernandez-Verdejo *et al.*, 2020). In healthy men, they assessed metabolic flexibility to carbohydrates using a hyperinsulinemic euglycemic clamp and metabolic flexibility to lipids in response to prolonged fasting. ΔRQ defined as the difference between post-challenge RQ and pre-challenge RQ was used to assess metabolic flexibility. They showed that metabolic flexibility measured during the clamp was directly related with metabolic flexibility during prolonged fast. In other words, individuals who are able to increase carbohydrate oxidation when carbohydrate availability is increased are also able to increase fat oxidation when fat becomes the predominant substrate. Other studies can bring insights to this question. After 3 days of high fat diet, sleep RQ was shown to be negatively associated with metabolic flexibility assessed during clamp in healthy individuals with or without family history of diabetes (Ukropcova *et al.*, 2007), indicating that high metabolic flexibility to carbohydrate is associated with high lipid oxidation. After an acute high-fat meal, subjects with family history of type 2 diabetes have lower decrease in RQ from fasting compared to subjects without predisposition to type 2 diabetes. In contrast, the increase in RQ following a high carbohydrate meal was similar in the two groups (Heilbronn *et al.*, 2007). Taking these data altogether suggest that metabolic flexibility to carbohydrates and fats are likely related and highlight the need for more studies to investigate this relationship. Another question is to know whether this relationship between metabolic flexibility to glucose and lipids also exists at other levels of integration.

In **Chapter 6** we demonstrated that at skeletal muscle cells level, although suppressibility – the capacity to switch from palmitate to glucose to produce energy – is reduced after 5 days of microgravity (**Figure 22**), the capacity to increase palmitate oxidation following an increase of its availability – adaptability – was not significantly impacted (**Figure 22**). We examined the associations between adaptability and suppressibility measured *in vitro* in the cultured primary myotubes. No relation was observed neither when pooling the data obtained in ambulatory and dry immersion

periods together (**Figure 33, left panel**) nor when looking at the dry immersion-induced changes in adaptability and suppressibility (**Figure 33, right panel**). This suggests that metabolic flexibility to carbohydrates and to lipids are independent that may involve different molecular mechanisms. However, we only had a small number of subjects and while adaptability (*i.e.*, metabolic flexibility to lipids) decreased in five subjects, it increased in one, thus inducing a large heterogeneity in the results. Further studies would be needed to better understand whether metabolic flexibility to glucose and lipids are interrelated or whether alterations in metabolic flexibility to glucose precedes defects in the response to acute changes in lipid availability as our results currently suggest it.

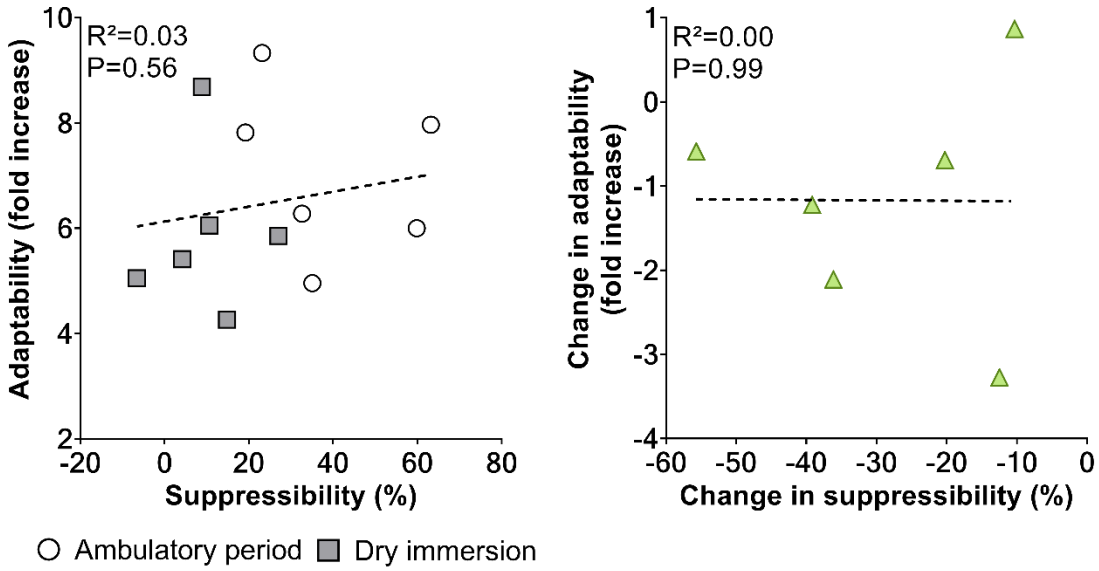


Figure 33 : Relationships between adaptability and suppressibility.

Left: Relationship between adaptability and suppressibility. Adaptability represents the capacity to increase palmitate oxidation following increased levels of palmitate in extracellular medium. Suppressibility represents the capacity to reduce palmitate oxidation following increased levels of glucose in extracellular medium. The dashed line represents the linear regression for all data irrespective of the time of the study. Right: Relationship between change in adaptability (difference between adaptability during dry immersion and ambulatory period and change in suppressibility (difference between suppressibility during dry immersion and ambulatory period). The dashed line represents the linear regression for all the subjects.

One parameter that may need to be considered is the lipids used for the adaptability test. Only palmitate was used like in other *in vitro* studies. However, humans do not eat diet only composed of palmitate but of a mix of lipids. In 2015, palmitate represented only 19% of the French habitual diet and oleate represented 29% of total lipid intake (ANSES, 2015). Therefore, it would be interesting to test the adaptability with a mixture of these two fatty acids to be more representative of what happens in real life.

2.3 Integration of metabolic flexibility assessed at different levels

In **Chapter 6** we tested metabolic flexibility to carbohydrates both at whole-body and skeletal muscle levels and microgravity reduced only at muscle level. This could suggest that impairments in metabolic flexibility to glucose develop first at skeletal muscle level and then extend to whole-body level. This hypothesis would be in line with the first study conducted in the early 2000s that investigated whether the characteristics of the skeletal muscle mirrored those observed in the whole body (Ukropcova *et al.*, 2005). In this study suppressibility and adaptability were measured under the same conditions as in our study and whole-body metabolic flexibility was assessed during a clamp in healthy men and women. Significant relationships were observed between ΔRQ (difference between RQ during the clamp and fasting RQ) and suppressibility and adaptability. In our study, no relationship exists between the index of adaptability, i.e. an index of metabolic flexibility to lipid, and *in vivo* metabolic flexibility. Although no significant correlation is observed, a trend for a linear relationship between in vitro suppressibility, metabolic flexibility to glucose, and in vivo metabolic flexibility tested in response to high carbohydrate mixed meal can be noted (**Figure 34**). Unfortunately, we may not have the power to investigate such relationship.

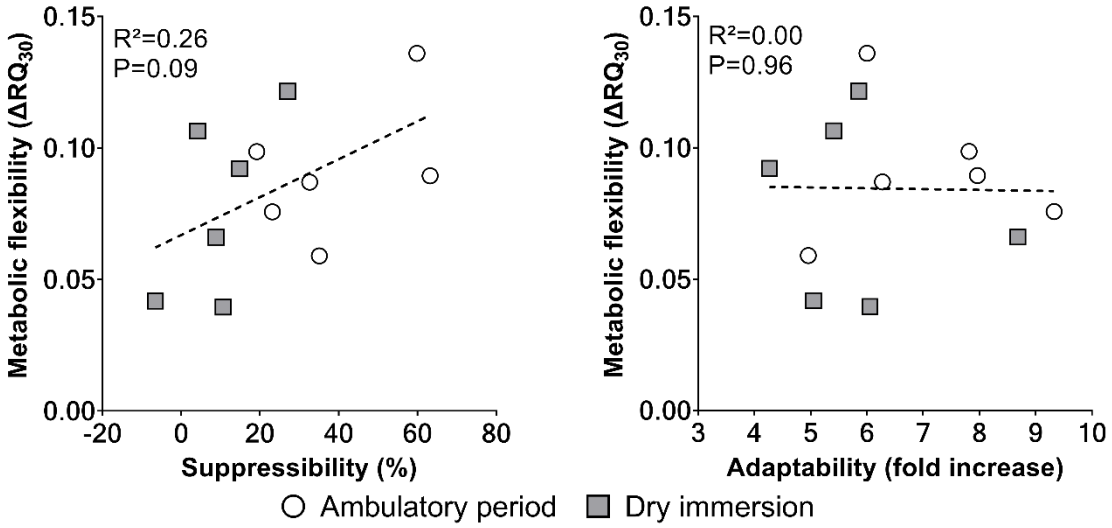


Figure 34: Relationships between whole-body and skeletal muscle metabolic flexibility.

Left: Relationship between whole-body metabolic flexibility and suppressibility. ΔRQ_{30} is the difference between RQ 30 min after high-carbohydrate meal ingestion and fasting RQ. Suppressibility represents the capacity to reduce palmitate oxidation following increased levels of glucose in extracellular medium Right: relationship between whole-body metabolic flexibility and adaptability. Adaptability represents the capacity to increase palmitate oxidation following increased levels of palmitate in extracellular medium. The dashed line represents the linear regression for all data irrespective of the time of the study.

The use of this *in vitro* metabolic flexibility technique allows to investigate metabolic flexibility at the mitochondrial level. Knowing that metabolic inflexibility was a characteristic of individuals with type 2 diabetes or obesity at both the whole body and muscle tissue levels (Kelley *et al.*, 1999; Kelley & Mandarino, 2000; Corpeleijn *et al.*, 2010), Boyle and colleagues investigated whether metabolic

flexibility of the mitochondria could also be a characteristic of this population (Boyle *et al.*, 2012). By incubating permeabilized myotubes with palmitoyl carnitine (thereby overriding the roles of the fatty acid transporters CPT1 and CPT2 within the mitochondria), mitochondrial respiration was increased twofold in the cells from lean subjects but not from subjects with obesity. This suggests that the whole-body metabolic inflexibility phenotype is also noticed at the mitochondrial level. It would have been interested to test the changes in mitochondrial respiration in presence of carbohydrate-like or fatty acid-like substrates in our muscle cell cultures as well. Collectively, these data shows that metabolic flexibilities assessed at different integration levels are consistent, and further studies are needed to understand the impact of physical inactivity on each of them.

3 Relationships between metabolic flexibility, body composition and exercise

The high variability of astronauts in age, body composition and physical activity allowed to examine interesting relationships between metabolic flexibility, body composition and exercise. **Figure 35** shows the relationships between changes in metabolic flexibility and body composition when combining data collected in healthy male adults from both studies, *i.e.*, ENERGY and dry immersion. Changes in ΔRQ, index of metabolic flexibility, were negatively associated with changes in fat mass and positively with changes in fat-free mass.

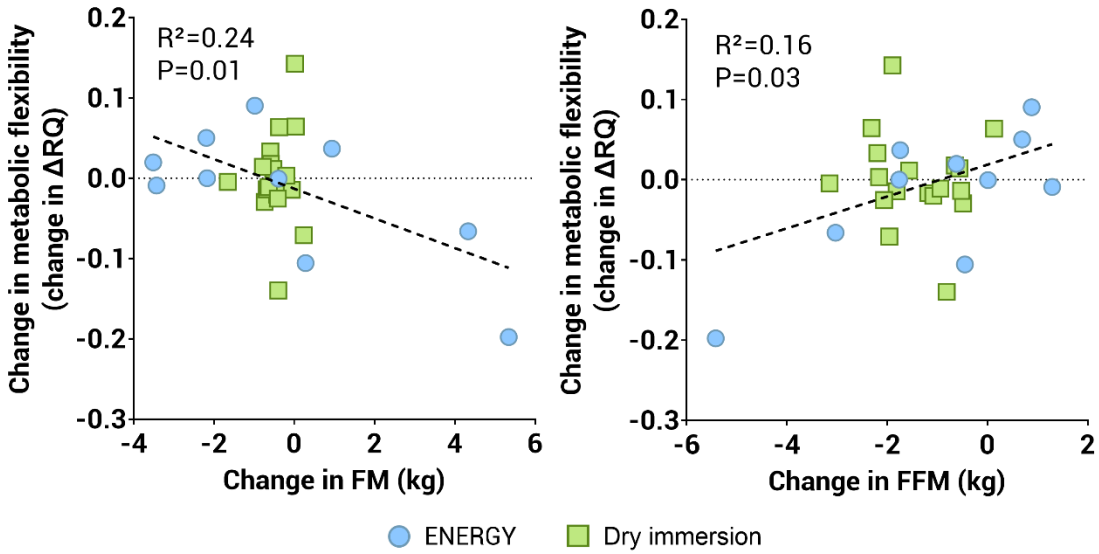


Figure 35: Relationships between changes in metabolic flexibility and body composition.

Changes in ΔRQ refers to inflight/dry immersion ΔRQ – baseline ΔRQ. ΔRQ was calculated as maximal postprandial RQ – fasting RQ. Changes in FM and FFM refers to inflight/dry immersion FM and FFM – baseline FM and FFM. The dotted lines represent linear regression between changes in ΔRQ and changes in FM (left panel) and changes in FFM (right panel) including data from both studies.

3.1 Role of adipose tissue in the regulation of metabolic flexibility

Adipose tissue is a major organ involved in energy homeostasis and has two major functions: NEFA release in the bloodstream to provide energy to the body in fasting state and triglycerides uptake and storage in the postprandial phase. On the other hand, adipose tissue is an exocrine organ that secretes adipokines that regulate the oxidation of substrates in other tissues (Deng & Scherer, 2010). For example, adiponectin stimulates lipid oxidation and glucose uptake by peripheral tissues (Yamauchi *et al.*, 2002). Secreted adipokines and the level and profile of fatty acids released differ according to the adipose tissue deposits (subcutaneous, visceral, femoral, etc.) (Tchkonia *et al.*, 2013; Chait & den Hartigh, 2020). Knowing that adipose tissue function is impaired in insulin-resistant states (Frayn, 2002) and that adiponectin levels are low in obesity and type 2 diabetes (Balsan *et al.*, 2015), adipose tissue can modulate (positively or negatively) metabolic flexibility.

In **Chapter 7**, we showed that changes in metabolic flexibility and fat mass of astronauts in response to long exposure to microgravity were negatively associated. Although no relationship was detected when considering data from the dry immersion only, combining data from both ENERGY and the dry immersion study showed negative associations between changes metabolic flexibility and fat mass thus suggesting that characteristics from adipose tissue may influence metabolic flexibility (**Figure 36**). Although fat distribution could not be measured in astronauts during spaceflights nor in the dry immersion study, enhanced fat accumulation in the visceral adipose depot was previously observed in response to simulated microgravity in healthy adults (Belavy *et al.*, 2014). Adiponectin levels were also not measured, but a decrease in its circulating concentration was noted during long-term bed-rest (Trim *et al.*, 2022). Because abdominal obesity (*i.e.*, high waist circumference and visceral adipose tissue) is known to be directly related to insulin resistance (Westphal, 2008), and insulin sensitivity is directly associated with metabolic flexibility (Ukropcova *et al.*, 2007; Galgani *et al.*, 2008), we may speculate that metabolic flexibility is influenced by both changes in adipose tissue mass and distribution. By assessing metabolic flexibility during euglycemic hyperinsulinemic clamp in healthy men, Sparks and collaborators showed that total body fat, fat cell size and insulin suppression of fatty acids are negatively related to metabolic flexibility. Moreover, adiponectin levels are positively associated with metabolic flexibility (Sparks *et al.*, 2009b). They further showed that no difference was detected between men and women in the relationship between visceral adipose tissue mass and metabolic flexibility and that fat cell size was similar. However, women are more metabolically flexible and have greater capacity to suppress fatty acid release in the insulin-stimulated state, higher adiponectin levels and higher expression levels of gene involved in adipogenesis, fat storage and fat

oxidation in adipose tissue than men (Sparks *et al.*, 2009a). In a recent systematic review, Glaves and colleagues sought to identify whether certain characteristics of adipose tissue were associated with metabolic flexibility measured in response to different energy challenges in humans (Glaves *et al.*, 2021). The 37 cross-sectional and interventional studies included in this review were classified by metabolic flexibility measurement technique, *i.e.*, euglycemic-hyperinsulinemic clamp, in response to a nutritional challenge, and other challenges (sleep, acute physical activity episode, epinephrine infusion). The authors concluded that metabolic flexibility was negatively associated with some adiposity parameters (total fat mass, waist circumference and visceral adipose tissue) but only when metabolic flexibility was measured by euglycemic-hyperinsulinemic clamp. The major difference between a clamp and a dietary challenge is that the availability of substrates is at the level of intake. Metabolic flexibility therefore depends on all levels, from the digestive system to oxidation at the mitochondrial level as well as hormonal responses (mainly insulin). Moreover, the relative influence of each tissue on the oxidation of substrates is still unknown (Galgani & Fernandez-Verdejo, 2021). The authors suggested the lack of association between adiposity characteristics and metabolic flexibility measured after a meal is due to the fact that the meal represents a more physiological situation than the clamp and that the adaptation of substrate oxidation in the face of this challenge is the result of a more complex interaction between several systems (Glaves *et al.*, 2021). As indicated in section 2.1 of the discussion, the macronutrient composition of the test meal may also have an impact on the metabolic outcomes. From the graph on the left of F**igure 35**, we cannot exclude the reciprocal relationship, *i.e.*, that metabolic flexibility could influence adipose tissue. This would be consistent with the fact that metabolic inflexibility to lipids has been identified as a factor that may participate to ectopic fat storage (Galgani *et al.*, 2008).

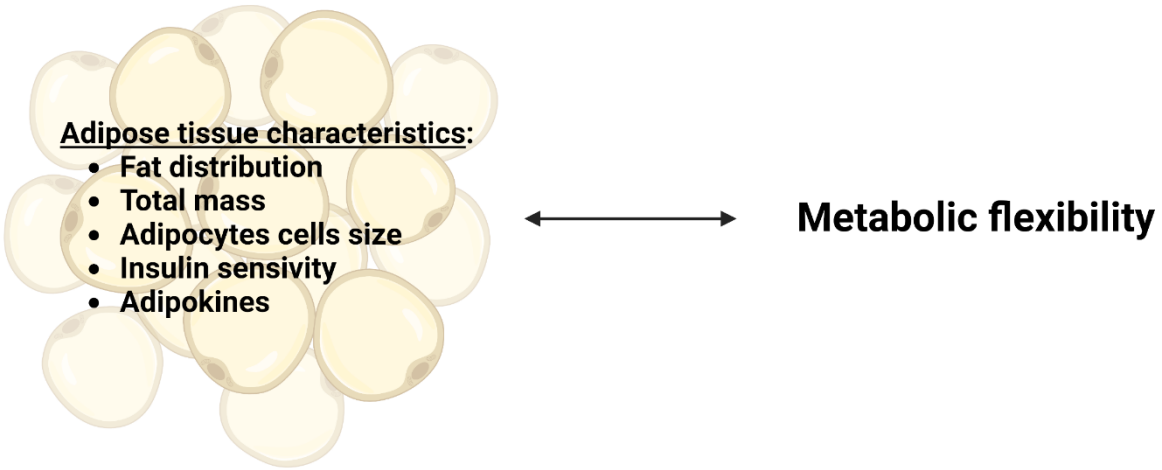


Figure 36: Adipose tissue characteristics and metabolic flexibility.
Reciprocal relationship between adipose tissue characteristics and metabolic flexibility.

3.2 Influence of metabolic flexibility on skeletal muscle

Although the relationship we observed between changes in metabolic flexibility and changes in muscle mass indicates a link in the role of skeletal tissue in metabolic flexibility, a reciprocal role of metabolic flexibility in the regulation of fat-free mass may also exist (Meex *et al.*, 2019). Metabolic inflexibility to lipids has been deemed to be one of the main factors triggering the accumulation of lipids in non-lipid-storing organs such as skeletal muscle (Galgani *et al.*, 2008). Of note, accumulation of intramuscular lipids following two months of bed-rest was previously reported (Bergouignan *et al.*, 2009). By altering the insulin (protein kinase C and Akt) (Petersen & Shulman, 2018) and anabolic (Akt and mTOR) (Léger *et al.*, 2006; Zoncu *et al.*, 2011) and activating catabolic signaling pathways (**Figure 5**), this lipotoxicity likely contributes to the development of muscle insulin resistance (Galgani *et al.*, 2008; Moro *et al.*, 2008) and anabolic resistance (Ferrando *et al.*, 1996; Biolo *et al.*, 2004; Moro & Bourlier, 2015), *i.e.* resistance to the stimuli known to activate protein synthesis such as essential amino acids, insulin or exercise. Altogether these studies suggest that metabolic flexibility and insulin resistance may be interrelated and precede anabolic resistance and loss of muscle mass observed during spaceflights, and likely under physical inactive conditions. Further studies are however warranted to confirm or not this hypothesis.

3.3 Exercise, a potential mediator of the relationships between metabolic flexibility, skeletal muscle and adipose tissue

We previously published that fat-free mass and fat mass are respectively positively and negatively associated with overall physical activity, vigorous intensity activity as well as reported treadmill load (Bourdier *et al.*, 2022), see **Appendix 2**. Although no relationship between metabolic flexibility and exercise was noticed, we previously reported on Earth that the level of physical activity predicts metabolic flexibility (Bergouignan *et al.*, 2011; Bergouignan *et al.*, 2013a) and the practice of resistive and aerobic exercise partially prevents alterations known to participate in the decrease of metabolic flexibility (Le Roux *et al.*, 2022). Based on this, we can therefore hypothesize that the relationships between metabolic flexibility, skeletal muscle and adipose tissue may be mediated by physical exercise (**Figure 37**).

Exercise training increases adipose tissue mitochondrial activity, increases glucose and improves the profile of adipokines released from adipose tissue that may be beneficial for health (Mika *et al.*, 2019). As detailed in section 3.5.3 of the introduction, exercise increases muscle outcomes known to be linked to metabolic flexibility such as fatty acid uptake and oxidation, mitochondrial

function and insulin sensitivity. These effects could be mediated by adipokines and myokines which by their autocrine, paracrine and endocrine roles are known to contribute to an inter-organ cross-talk that participates in the regulation of energy homeostasis (Oh *et al.*, 2016; Laurens *et al.*, 2020).

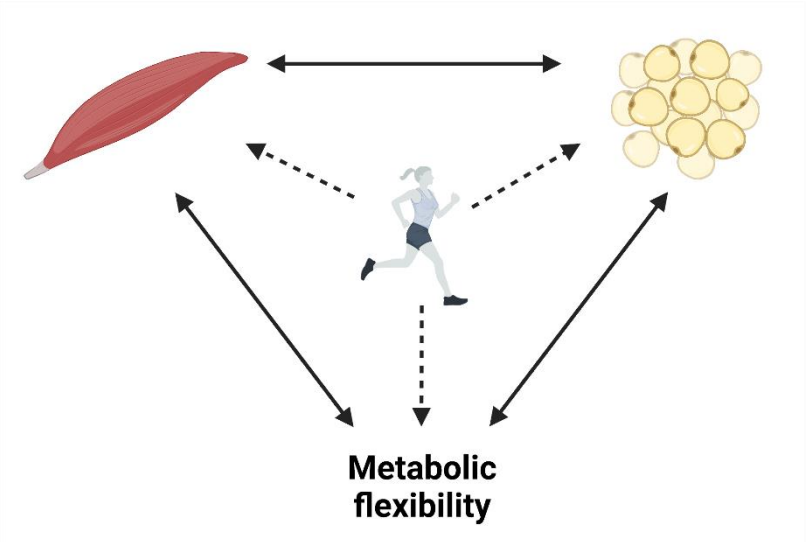


Figure 37: Exercise as mediator in the relationships between skeletal muscle, adipose tissue and metabolic flexibility.

By improving some parameters involved in metabolic flexibility and through the intermediary of myokines and adipokines, exercise would play an integral role in the triad between skeletal muscle, adipose tissue, and metabolic flexibility.

In the ENERGY study, having muscle biopsies and blood samples would have allowed us to investigate how long exposure to microgravity impacts the other health outcomes participating in metabolic flexibility and their relationship to exercise. Although metabolic flexibility was not affected at the whole-astronauts group level, data from bed-rest studies suggest that while we observe a dose-effect relationship (**Figure 15**), exercise practice may not be sufficient to prevent or attenuate the adaptations participating to metabolic flexibility induced by microgravity (Le Roux *et al.*, 2022). We estimated that an average of 45 min/day combined resistive and aerobic exercise during bed-rest may counteract only 70% of the metabolic adaptations. Onboard the ISS, astronauts performed 24 min/day of aerobic exercise (sum of reported daily time spent on T2 and CEVIS) and about 29 min/day of resistive exercise (ARED) (Bourdier *et al.*, 2022). Of note, the latter is an approximation based on the 1481 repetitions/week from NASA log. A question then arises on how to prevent all the adaptations induced by microgravity on metabolic health. Could increasing the time or intensity of exercise onboard the ISS be effective? For two reasons, this would not be the solution. Firstly, the astronauts' schedule onboard the ISS is very tight and increasing exercise duration would create a conflict with the other tasks they have to complete (scientific experiments, maintenance of the station etc.). Second, we estimated, although inter-individual variability is important, that astronauts' energy intakes (estimated by changes in body composition) were on average lower than the dietary requirement intake (85 [SD 14] % of DRI) (Bourdier *et al.*, 2022). Therefore, increasing exercise time and/or intensity

would induce, if energy intake is not spontaneously increased to compensate, an energy imbalance leading to energy deficit. This latter would have consequences on body composition, favor cardiovascular deconditioning and impair other physiological function that could ultimately affect astronauts' performance and health (Bergouignan *et al.*, 2016). Research must then continue to optimize exercise protocols to preserve maximum metabolic outcomes without adversely impacting others. This also highlights the value of developing and testing countermeasures that could potentially be combined with exercise.

4 Role of physical activity in the onset of metabolic diseases: insights from space science

4.1 Metabolic consequences of physical inactivity

This thesis was conducted in the context of space science. However, it allows to address some question of public health issues and bring new insights to better understand the role of physical inactivity in the pathophysiology of metabolic diseases.

Since the first study led by Jerry Morris in 1953 showing differences between occupations requiring different levels of physical activity on the risk of developing cardiovascular pathologies (Morris *et al.*, 1953), a plethora of studies underline the benefits of physical activity on health (Hawley *et al.*, 2014; Ekelund *et al.*, 2020; Thyfault & Bergouignan, 2020). Although there is now ample evidence of the deleterious effects of physical inactivity on health (Booth *et al.*, 2012; Booth *et al.*, 2017), studying physical inactivity directly with a rigorous experimental approach can be challenging. Space science models such as bed-rest and dry immersion in humans and hindlimb suspension in animals are particularly relevant as they induce enforced physical inactivity under tightly controlled lab conditions. In 2011, our research group gathered data from these models and proposed a sequence of events to explain the development of metabolic adaptations induced by physical inactivity leading to the development of metabolic flexibility (Bergouignan *et al.*, 2011). This cascade is represented in **Figure 8**.

By coupling this former cascade with the data obtained over the past 30 years along with data from this thesis, we propose to update this cascade triggered by physical inactivity and provide a more precise timeline as shown in **Figure 38**. First, physical inactivity leads to a decrease in mechanical load on the muscle, which triggers muscle atrophy and a reprogramming of all metabolic regulatory pathways. These lead to a decrease in the insulin signaling pathway, a decrease in glycogen synthesis and metabolic inflexibility to glucose. These changes result in decreased systemic insulin sensitivity

and hyperinsulinemia at the whole-body level. The latter participate in the installation of hyperglycemia and probably in the decrease of the metabolic flexibility of the whole body which itself precedes the decrease of glucose tolerance. Concerning lipids, the increase in triglyceridemia after 5 days of immersion suggests a lower uptake of dietary lipids by peripheral tissues including muscle. The increase in intrahepatic lipids suggests a rapid decrease in the oxidative capacity of the liver. Ectopic storage in the muscle seems to come later, perhaps related to the secondary and progressive establishment of metabolic inflexibility to lipids. It remains to be confirmed that it is translated at the level of the whole body. The decrease in the density and oxidative capacity of the mitochondria, the change in the typology of muscle fibers as well as all the changes in the muscle tissue and peripheral tissues contribute to the shift in substrates oxidation, with a decrease in the use of lipids in favor of carbohydrates. By becoming resistant to the effects of insulin, adipose tissue also participates in the accumulation of lipids within the muscle, which could contribute to muscle atrophy and the decrease in muscle insulin sensitivity.

Although the space models are extreme models, these alterations have been observed in individuals from the general population (Bergouignan *et al.*, 2013b; Lefai *et al.*, 2017; Damiot *et al.*, 2019). Collectively, the data accumulated over the 30 past years support the idea that physical inactivity itself is a major factor contributing to the development of chronic diseases. Of note, research over the past decade has enable to gain a deeper understanding of the mechanistic underpinnings of the physical inactivity pathophysiology, but the sequence of events triggered by the transition from an active to inactive lifestyle is still unclear. Understanding this sequence of events would be important to know what are the key parameters to prevent in priority.

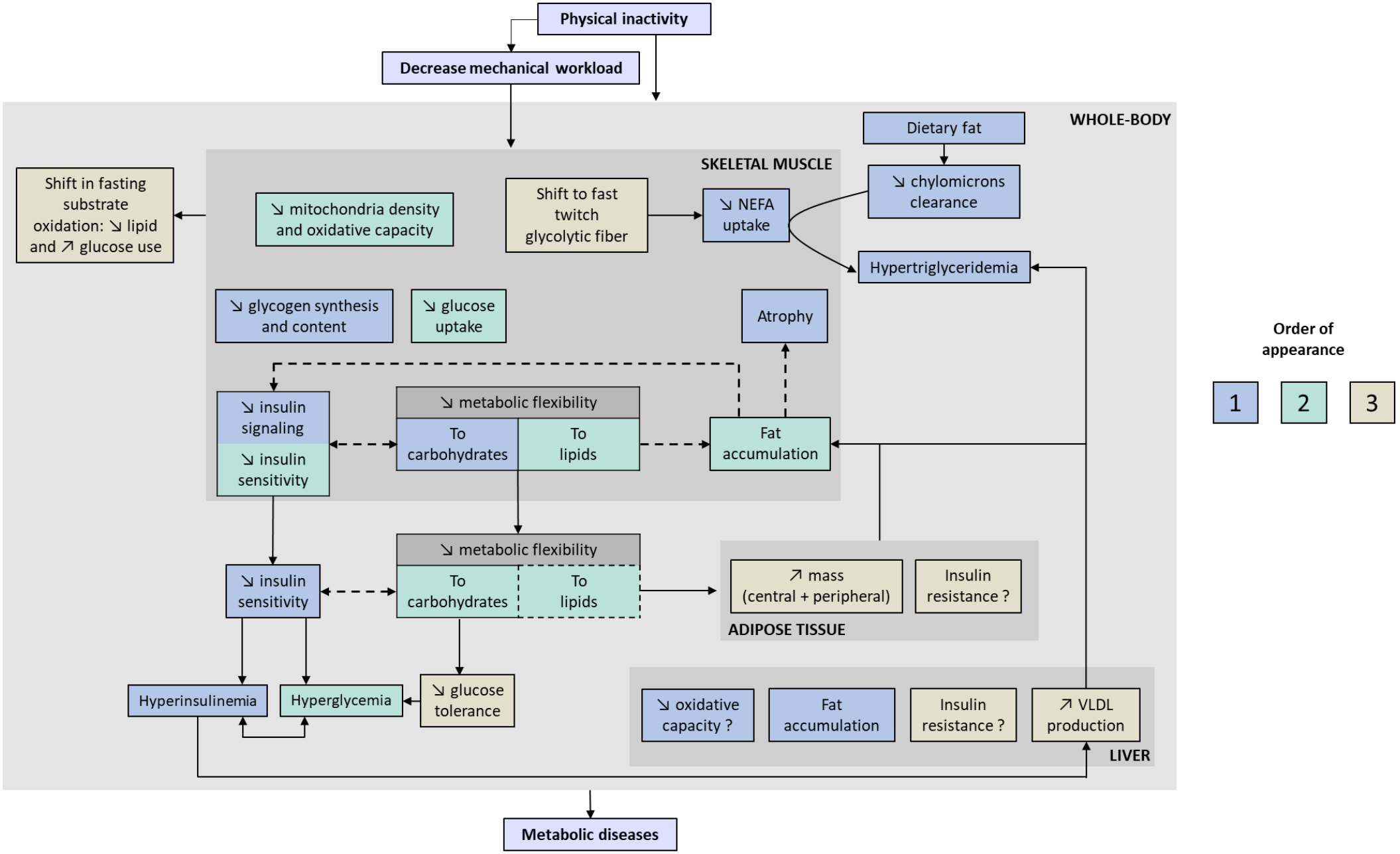


Figure 38: Hypothetical metabolic alterations induced by physical inactivity-induced bed-rest and dry-immersion that can explain how physical inactivity contributes to the development of metabolic diseases, updated from (Bergouignan *et al.*, 2011).
Dotted arrows represent relationships that requires further investigations.

4.2 Role of sedentary behaviours and non-exercise activity on the regulation of metabolic health

Adapted from the following reviews *Physiology of physical inactivity, sedentary behaviours and non-exercice activity: insights from the bed-rest model* J Physiol 600(5): 1037-1051 (**Appendix 1**) and *Inactivité physique et sédentarité : impact sur la santé métabolique, de quoi parle-t-on ?* Nutritions & Endocrinologie Vol. 19 n°98 (**Appendix 3**)

Through research efforts to develop strategies to combat physical inactivity, scientists have identified another health risk behaviour: sedentary behaviours (SB). SB are distinct from physical inactivity. Although physical inactivity is defined as engaging in less physical activity than is necessary to meet the current guidelines (<150 min/week moderate or <75min/week vigorous physical activity (moderate/vigorous physical activity, MVPA), with energy expenditure above 3.0 metabolic equivalent or METs), SBs correspond to "any waking behaviour characterized by an energy expenditure <1.5METs, while in a sitting or reclining posture" (Tremblay *et al.*, 2017). Although the recommendations encourage reducing periods of SB, no specific strategy has been proposed to combat the effects of sedentary lifestyles (Bull *et al.*, 2020).

SB are found in every domain of modern daily life: transportation, occupational (*e.g.* desk-bound work) and leisure time activities (*e.g.* video gaming and internet). Adults in Westernized societies spend between 7.7 and 9.7 h/day sitting, which corresponds to up to 60% of adult wake time (Ekelund *et al.*, 2019). Several epidemiological studies have reported associations between sedentary time and health outcomes including early mortality and risk of type 2 diabetes, metabolic syndrome and cardiovascular disease (Dunstan *et al.*, 2012). These associations were observed in both sexes and all ages and ethnicities and were independent of adiposity. They were also found in individuals who reach the recommended levels of MVPA. Those inactive and sedentary lifestyles were particularly increased in the last few years with pandemic crisis of COVID-19. An international study highlights that during confinements, physical activity duration (all intensities) decreased by 33.5% while time spent sitting increased by 28.6% (Ammar *et al.*, 2020). In France, a study from the Observatoire national de l'activité physique et de la sédentarité (ONAPS) reported that 45% of adults physically active reduced physical activity time and 74% of adults initially spending less than 6h/day sitting increased their sedentary time during confinement (ONAPS & sédentarité, 2020). These data suggest that SB is a stand-alone factor in the relationship between physical activity and health. In other words, spending too much time sitting may have different health effects from not exercising enough. While a plethora of epidemiological data have been published, experimental evidence supporting the adverse health

effects of SB independent of time spent physically active is lacking. This is mainly due to challenges in isolating the effects of SB from those of PA.

The bed-rest model can provide unique insights into the independent health effects of SB. During these studies, physically active healthy participants free from any predisposition for chronic diseases stay in bed 24 h/7 days. They are both physically inactive and highly sedentary (**Figure 39**). In some bed-rest studies, the efficacy of exercise training protocols to protect the body against the harmful effects of microgravity have also been tested. Participants in these studies perform exercise training (**Figure 39**) while in bed-rest. They are both sedentary and physically active and represent an extreme but unique model of "sedentary exercisers". Another distinctive characteristic of these bed-rest exercisers is that they have very low levels of non-exercise activities of daily living, which correspond to light physical activity (LPA) with an energy expenditure between 1.6 and 2.9 METs (walking, taking the stairs, standing, etc.) (**Figure 39**). The physiological effects of strict bed-rest (*i.e.*, or physical inactivity coupled with sedentariness) are presented in **sections 2 and 3** of the introduction and summarized in **Figure 15**. All these metabolic features are commonly observed in individuals with obesity, type 2 diabetes or metabolic syndrome. These observations therefore support a key role of physical inactivity in the onset and progression of metabolic diseases. Although the health enhancing effects of exercise (or MVPA) on these metabolic outcomes are well established, it is unclear whether they are sufficient to reverse the adverse health effects of sedentariness.

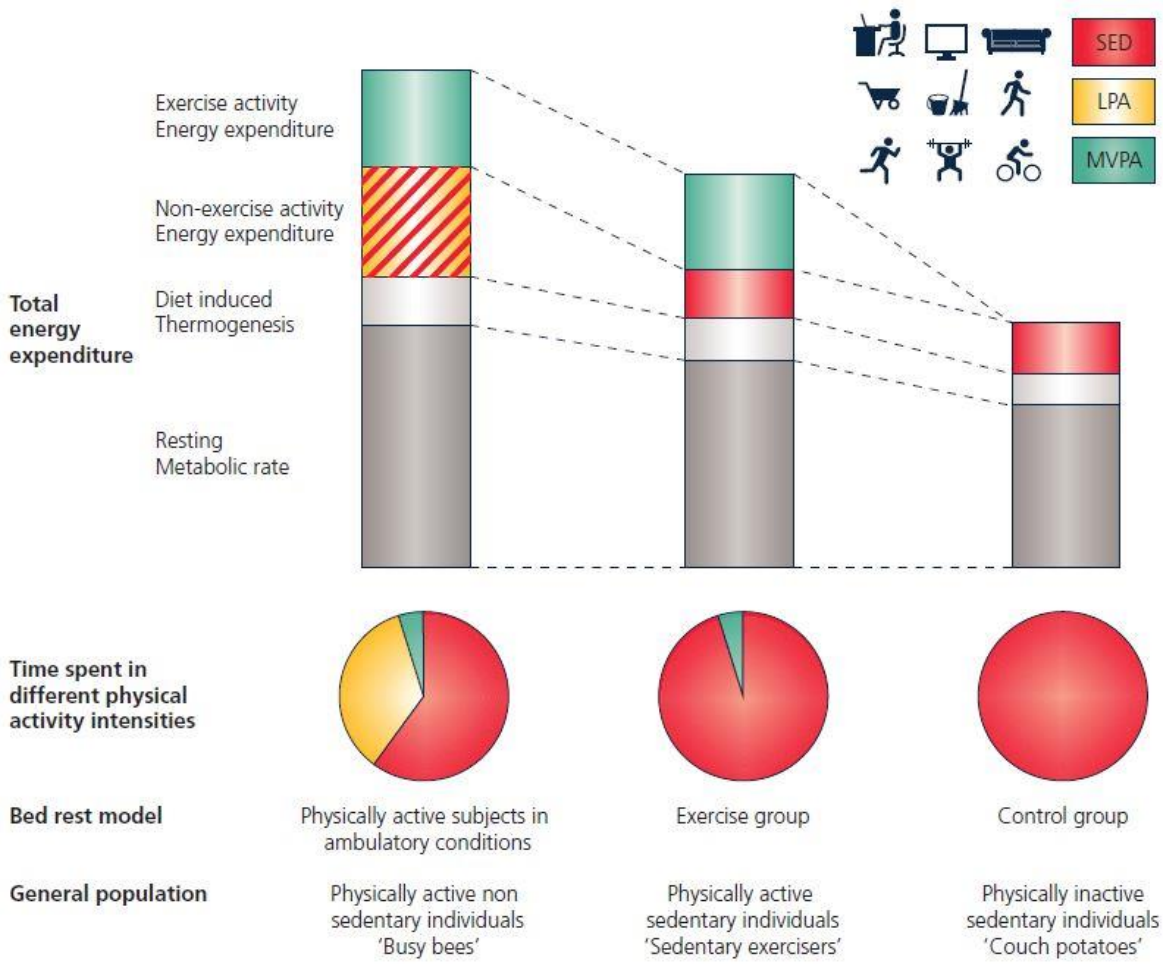


Figure 39: Schematic representation of the components of total energy expenditure during bed-rest conducted with or without exercise training (Le Roux *et al.*, 2022).

Based on total energy expenditure, participants enrolled in bed-rest protocols can be compared to the general population. Strict bed-rest suppresses both components of physical activity energy expenditure: exercise activity energy expenditure and non-exercise activity energy expenditure. Exercise activity energy expenditure refers to the energy spent in MVPA and/or structured exercise. Non-exercise activity energy expenditure corresponds to any activity of daily life, which is essentially LPA. Participants who are subjected to moderate to vigorous exercise training along with bed-rest maintain high exercise activity energy expenditure mainly due to MVPA. However, they are sedentary with very low levels of non-exercise activity energy expenditure and are lacking LPA. These individuals represent an extreme but unique model of 'sedentary exercisers', *i.e.* physically active yet sedentary people. Strict bed-rest leads to a decrease of both MVPA and LPA while increasing SB. These bed-rest individuals represent a model of the modern physically inactive sedentary individuals. Abbreviations: SED, sedentary activities; LPA, light physical activity; MVPA, moderate-to-vigorous physical activity. Adapted from Bergouignan et al. (2010), figure 1 of (Le Roux *et al.*, 2022). Use authorized by John Wiley and Sons, licence 5486670832494.

4.2.1 Can exercise offset the adverse health effects of physical inactivity and SB?

The physiological effects of resistive combined or not with aerobic exercise on the adaptations induced by strict bed-rest are presented in **section 5.3.2** and represented in **Figure 15**. To summarize, based on the metabolic adaptations to strict bed-rest presented in **Figure 15**: 1) an average of 14 min/day of MVPA (mean estimated daily MVPA time from resistive exercise training during bed-rest) can partially/fully counteract about 50% (7 among 14) of the adaptations induced by sedentariness

(**Figure 40**, blue line); 2) an average of 45 min/day of MVPA (mean estimated daily MVPA time from combined resistive and aerobic exercise training during bed-rest) – which is superior to the WHO recommendations about daily practice of MVPA (Bull *et al.*, 2020) – may counteract about 70% of the metabolic adaptations (**Figure 40**, orange line). Based on the existence of a linear relationship – which we know to be oversimplistic, 94 min/day of MVPA would be required to totally prevent the sedentary-induced adaptations on metabolic health (**Figure 40**, dashed line). Of note, Ekelund and colleagues, who estimated that 60 to 75 min/day of MVPA is required to fully counteract the effects induced by at least 9 h/day of SB on mortality (Ekelund *et al.*, 2016). Taken together these studies show that exercise (or MVPA) protects skeletal muscle mass and function, and cardiorespiratory function against large volumes of SB induced by bed-rest. However, even if a dose–response relationship exists (**Figure 15, 40** and **Table 2**), very high levels of exercise do not fully prevent the manifestation of metabolic dysfunction, *i.e.*, whole-body insulin resistance, glucose intolerance, alterations of lipid metabolism and systemic inflammation. These observations support the role of organs other than muscle in the health-enhancing effects of physical activity (Thyfault & Bergouignan, 2020), and the existence of health effects of SB independent of those from MVPA. It further highlights the importance of non-exercise activity (*i.e.*, LPA), which mainly corresponds to daily living activities performed throughout the day.

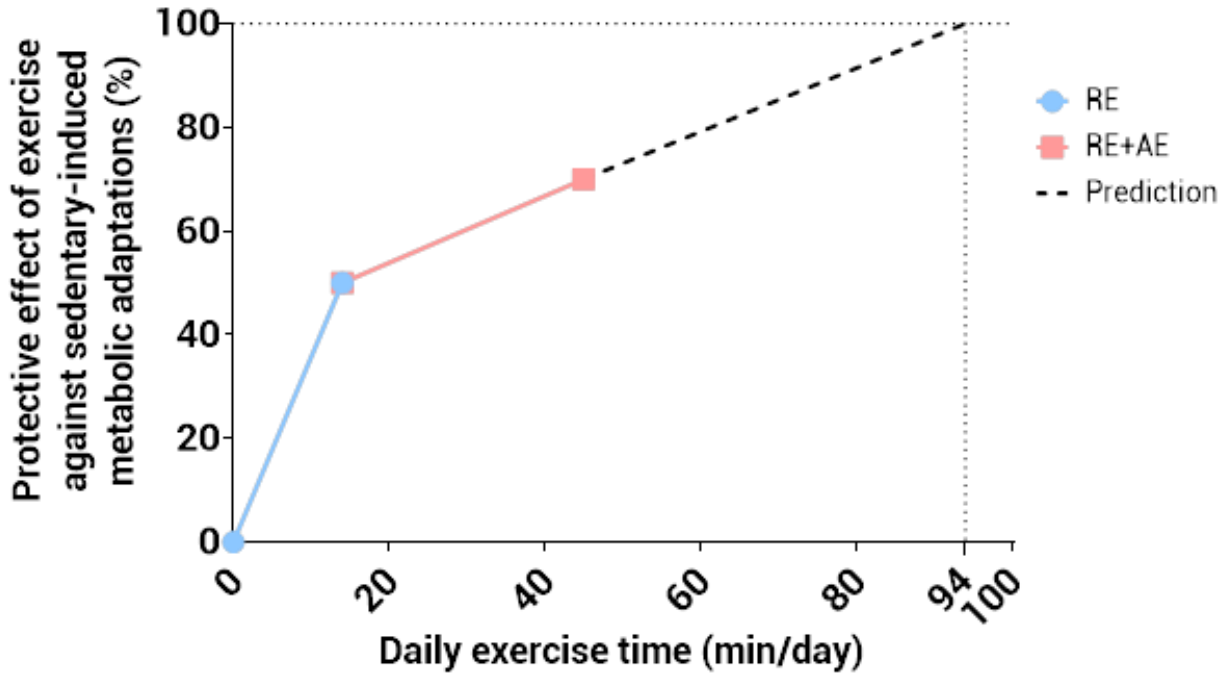


Figure 40: Dose-effect relationship between daily exercise time and their protective effect against sedentary-induced metabolic adaptations.

Based on bed-rest studies, 12 min/day of resistive exercise (blue line) and 45 min/day of combined resistive and aerobic exercise (orange line) may fully/partially counteract 50% and 70% of the adaptations induced by SB, respectively. To offset the entire SB-induced metabolic adaptations 94 min/day of combined resistive and aerobic exercise would be necessary.

4.2.2 Health benefits of daily living activities

Evidence from epidemiological studies indicates that LPA has a potential role in reducing the risk of early mortality. In cross-sectional studies, LPA is favorably associated with waist circumference, BMI, plasma triglyceride, insulin, HDL-cholesterol concentrations (Amagasa *et al.*, 2018) and 2h plasma glucose (Healy *et al.*, 2007), independent of time spent in MVPA. Iso-temporal substitution modelling suggests that replacing 30 min of SB per day with 30 min of LPA (but not MVPA) is associated with lower waist circumference and BMI (Healy *et al.*, 2015). A growing number of experimental studies have also examined the effects of LPA prescribed as short bouts spread throughout the day on metabolic health (**Table 10**).

As previously reviewed (Dempsey *et al.*, 2016a), LPA bouts (15–40 min) acutely decrease postprandial glycaemia and insulinemia. Even brief intermittent bouts (≤5 min) of walking spread throughout the day reduce glucose and insulin concentrations following meal ingestion, with more potent effects observed in adults with overweight to obesity and type 2 diabetes (Chastin *et al.*, 2019), and those with lower cardiorespiratory fitness compared to healthy lean individuals (McCarthy *et al.*, 2017). Importantly, acute exposure to bouts of LPA elicits similar responses to those observed with short, frequent bouts of MVPA. With regards to standing, although some studies did not show a reduction in postprandial glycemic response (Bailey & Locke, 2015; Pulsford *et al.*, 2017) (Bailey & Locke, 2015; Pulsford *et al*. 2017), others did (Benatti *et al.*, 2017). Benatti and colleagues even reported that intermittent standing, but not a single continuous bout of MVPA, lowers postprandial glycemia in healthy adults. The difference in the observed effects may be explained by the duration of the standing bouts (2 min *vs* 15 min) and the total active duration (30 min MVPA *vs* 15 min standing every 30 min for 8.5 h). Although these acute studies suggest that LPA of higher energy expenditure or longer duration decreases glycemia and insulinemia in a dose-dependent manner, none of these studies controlled for energy expenditure across the interventions. In an elegant series of studies, Duvivier and colleagues compared the metabolic effects of replacing SB with LPA walking and standing to those of 1 h/day of MVPA. Both interventions lasted 4 days and were matched for energy expenditure. Replacing SB with high volumes of LPA without any increase in MVPA decreased postprandial insulin, fasting triglycerides and non-HDL cholesterol in healthy adults (Duvivier *et al.*, 2013). In adults with type 2 diabetes, increasing time spent standing and walking improved glucose control and insulin sensitivity. It further reduced diastolic blood pressure, blood triglycerides and non-HDL cholesterol while increasing HDL cholesterol (Duvivier *et al.*, 2017a; Duvivier *et al.*, 2017b). The MVPA intervention tended to improve these metabolic parameters, but the effects were less pronounced.

Table 10: Summary of the acute metabolic health effects of light-intensity physical activity from experimental laboratory-controlled studies.

Publication	Sample size and characteristics	Study design Study conditions	Primary results
Studies investigating light-physical activity compared to sitting			
Bailey et al. 2015	n=10 (3♀/7♂) BMI: 26.5 ± 4.3 kg/m^2 Non-insulin resistant	Cross-over (5h/condition) 1) Uninterrupted Sitting 2) Sit + 2min stand every 20min 3) Sit + 2min LIW every 20min	Plasma glucose AUC: LIW < sitting and standing; standing vs. sitting: NS Blood pressure: No between-conditions difference Plasma lipids: No between-conditions difference
Benatti et al. 2017	n=14 ♂ BMI kg/m^2: 24.9 ± 4.3 kg/m^2 Non-insulin resistant	Cross-over (27h/condition) 1) Uninterrupted Sitting 2) Sit + 15min stand every 30min 3) Sit + 30min MIW 4) Sit + 30min MIW and 15min stand every 30-min	Postprandial plasma glucose iAUC: standing < sitting; MIW vs sitting: NS
Dempsey et al. 2016b	n=24 (10♀/14♂) BMI: 33.0 ± 3.4 kg/m^2 Type 2 diabetes	Cross-over (8h/condition) 1) Uninterrupted sitting 2) Sitting + 3min LIW every 30min 3) Sitting + 3min resistance activities every 30min	Blood pressure: LIW < sitting Noradrenaline concentration: LIW < sitting
Duvivier et al. 2017a	n=24 (11♀/13♂) BMI: 29.0 ± 2.0 kg/m^2 Non-insulin resistant	Cross-over (4 days/condition) 1) Sit 13.5h/d, stand 1.4h/d, LIW 0.7 h/d 2) Sit 7.6h/d, stand 4.0h/d, LIW 4.3h/d	OGTT insulin AUC: LIW < sitting Insulin sensitivity (Matsuda index): LIW < sitting Fasted lipids: LIW < sitting Fasted lipoproteins: LIW < sitting Diastolic blood pressure: LIW < sitting
McCarthy et al. 2017	n=34 (18♀/16♂) BMI: 23.8 ± 6.1 kg/m^2 Non-insulin resistant	Cross-over (7.5h/condition) 1) Uninterrupted Sitting 2) Sit + 5min LIW every 30min	Plasma glucose iAUC: LIW < sitting Plasma insulin iAUC: LIW < sitting
Pulsford et al. 2017	n=25 ♂ BMI: 24.9 ± 4.3 kg/m^2	Cross-over (7h/condition) 1) Uninterrupted sitting	Postprandial plasma glucose AUC: LIW < sitting Postprandial plasma insulin AUC: LIW < sitting

	Non-insulin resistant	2) Sit + 2min stand every 20min 3) Sit + 2min LIW every 20min	Insulin sensitivity (Matsuda index): LIW < standing and sitting
Thosar et al. 2015	n=12 ♂ BMI: 23.7 ± 3.4 kg/m^2 Non-insulin resistant	Cross-over (3h/condition) 1) Uninterrupted sitting 2) Sit + 5min LIW every hour	Flow-mediated dilation: LIW > sitting Shear rate: LIW > sitting
Studies comparing the health effects of light-intensity and moderate-vigorous physical activity to sitting			
Duvivier et al. 2013	n=18 (16♀/2♂) BMI: 22.6 ± 2.6 kg/m^2 Non-insulin resistant	Cross-over (4d/condition) 1) Sit 14h/d 2) Sit 13h/d and 1h EX 3) Sit 8h/d, 4h LPA, 2h stand	OGTT plasma insulin AUC: LPA < sitting and exercise Fasting lipids: LPA < sitting; LPA vs exercise: NS Fasting lipoproteins: LPA < sitting; LPA vs exercise: NS
Duvivier et al. 2017b	n=19 (6♀/13♂) BMI: 30 ± 2.0 kg/m^2 Type 2 diabetes	Cross-over (4d/condition) 1) Sit 14h/d with 4415 steps/d 2) Sit + 1.1h/d EX with 4823 steps/d 3) Sit + stand 2.5h/d and LIW 2.2h/d with 17,502 steps/d	Glycemia (CGMs): LIW < exercise Insulin sensitivity index (HOMA2-IR): LIW < exercise
Duvivier et al. 2018	n= 61 (33♀/28♂) BMI: 27.8 ± 4.3 kg/m^2 Non-insulin resistant and type 2 diabetics	Pooled analysis Cross-over (4d/condition) (1) Sit 14h/d (2) Sit + 1h/d MIW (3) Sit + 5–6h/d LIW and standing	Endothelial function: LIW < MIW Insulin sensitivity index (HOMA2-IR): LIW < MIW Plasma lipids: LIW < MIW

BMI: body mass index (kg/m2); h: hours; d: day; EX: exercise; body mass index; AUC: area under the curve; iAUC: incremental area under the curve; LIW: light-intensity walking; MIW: moderate-intensity walking; OGTT: oral glucose tolerance test; LPA: light-intensity physical activity; CGMs: continuous glucose monitoring system.

These studies show that when energy expenditure is matched, replacing SB with high volumes of LPA (*i.e.*, non-exercise activity) is more beneficial than performing MVPA as a single continuous bout (*i.e.*, structured exercise), at least for glucose control, insulin sensitivity and circulating lipids. On the contrary, if frequent LPA bouts (>2 min) improve endothelial function (Thosar *et al.*, 2015; Dempsey *et al.*, 2016b), a single bout of MVPA may be more beneficial for microvascular function than increasing LPA throughout the day (Duvivier *et al.*, 2018). These findings suggest that MVPA (*i.e.*, exercise) and LPA (*i.e.*, non-exercise activity) might elicit differential cardiometabolic effects. Future studies will need to further compare the effects of LPA versus MVPA on cardiometabolic health outcomes, including maximal aerobic capacity, muscle strength, substrate metabolism, glucose control and insulin sensitivity in different populations and investigate the mechanistic underpinnings.

4.2.3 Where do we go from here?

Although MVPA produces myriad health benefits, it does not reduce time spent sedentary. Indeed, physically active people, even those who exceed the current guidelines, can be as sedentary as their inactive counterparts (Rantalainen *et al.*, 2018). Increasing MVPA can even trigger spontaneous behavioral compensations in sedentary adults leading to a decrease in non-exercise activities (*i.e.*, LPA) in favor of sedentary time (Lefai *et al.*, 2017). Furthermore, MVPA does not fully offset the adverse health effects of large volumes of SB, as shown by the bed-rest studies with concomitant exercise training. The remaining question is how much MVPA is needed to offset the effects of a certain amount of SB. It was shown that independent of physical activity, every hour spent sitting increases the risk of mortality by 5.9%, of type 2 diabetes by 22% and of obesity by 23% (Hu *et al.*, 2003; Wilmot *et al.*, 2012; Chau *et al.*, 2013). A meta-analysis including more than 1 million individuals further showed that 60–75 min/day of MVPA is needed to prevent the risk of premature death associated with 9 h/day or more of sitting time (Ekelund *et al.*, 2016), 9 h/day being close to the average sitting time observed in modern societies. When most of the population does not reach the recommended guidelines (*i.e.*, 30 min/day of MVPA, 5 days/week.), adding 30–45 more minutes per day of MVPA is unrealistic. Therefore, other pragmatic and efficient strategies are needed.

To conclude (**Figure 41**), activities of daily living (*i.e.*, LPA) are inversely associated with SB; increases in LPA are associated with reductions in sedentary time (Pate *et al.*, 2008). In addition, large volumes of LPA, here considered as any body movements associated with activities of daily living, have been shown to confer health benefits. Knowing that lack of time is a major barrier to the practice of exercise/MVPA, reintroducing LPA into daily life could be an effective strategy to reduce sedentary time and prevent its effects on metabolic health. Along these lines, the latest guidelines from the World Health Organization (Bull *et al.*, 2020) promote the practice of physical activity of any intensity

to reduce sedentary activities. In other words, moving is better than sitting. Future mechanistic studies will need to establish the physiology of SB and LPA to better understand the respective negative and positive health effects, and thus better define the dose–response relationship between the components of PA behavior and key health outcomes. Experimental research examining these relationships will foster the development of more specific and pragmatic public health guidelines.

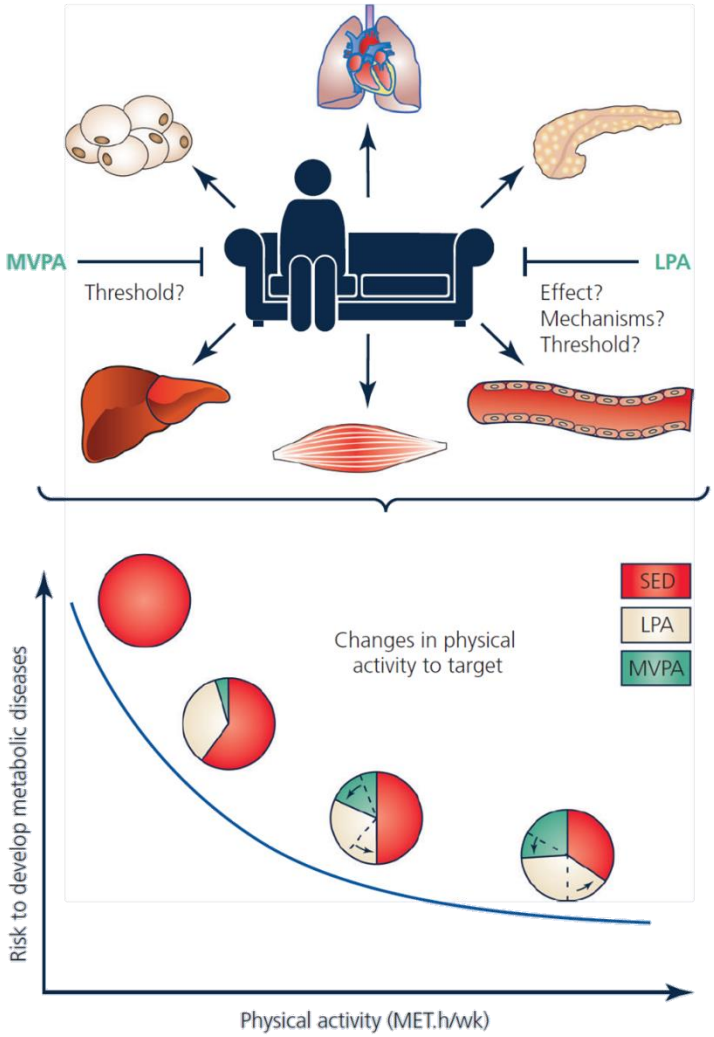


Figure 41: The goals of the future studies investigating the effect of moderate-to-vigorous and light physical activity on metabolic health.

Perspectives

All the topics covered in this thesis allow to bring significant perspectives on both astronauts' health and performance and the understanding of the development of chronic metabolic diseases.

The impact of nutrition on microgravity-induced metabolism adaptations deserves to be further investigated. We showed that the basal shift in substrate oxidation in astronauts observed after long-term spaceflight was driven by increased FQ. However, the reasons behind this greater carbohydrate intake inflight compared to on the ground are unclear. Is it because of changes in food preference, due to potential modifications of taste, smell or other factors involved in the regulation of food intake, or because the food available on board the ISS is richer in carbohydrates? Although to date dietary recommendations for astronauts are set to avoid deficiencies, the next step would be to optimize these recommendations so that diet may also serve as a countermeasure. Beyond the higher amount of carbohydrates ingested (which does not seem to be a problem from the metabolic viewpoint), the impact of dietary fiber would be interesting to investigate. A review of the astronauts' dietary intake on board ISS showed that neither the crewmembers' choices of food nor the standard menu had enough fiber to satisfy the daily recommendations. Knowing that fiber intake is associated with better glucose control and insulin sensitivity, increasing fibers intake may have health benefits. The type of fat present in the diet is another important parameter to consider. The "standard menu" on the ISS and the food selected by the crew are typically higher in saturated fats and cholesterol than recommended. Knowing that dietary saturated fat is known to promote atherosclerosis and inflammation and that in conditions of microgravity monounsaturated lipid oxidation is reduced and redirected to ectopic fat depot in muscle, it would be therefore interesting to investigate whether this could interact with the impaired lipid metabolism widely documented in ground-based models.

In this thesis, we showed that metabolic flexibility of skeletal muscle is an important element in the development of metabolic inflexibility at the whole-body level in response to inactivity. We also highlighted that adipose tissue and the liver, the other organs involved in the homeostatic regulation of metabolism, may have an important role in the regulation of metabolic flexibility. However, the underpinning mechanisms are still unknown and needs to be investigated. Stable isotopes could be used to analyze the effects of microgravity on the two primary functions of adipose tissue, *i.e.*, triglyceride storage and lipid mobilization, to better understand its role. The intrinsic adaptability and suppressibility of adipose tissue may also be assessed *in vitro* using adipocytes obtained from biopsies. For obvious ethical reasons, liver biopsies are not possible, making it challenging to assess hepatocytes' metabolic flexibility the same way we did for skeletal muscle cells. It could be possible to simulate *in*

vitro the effect of microgravity from pre-established cell cultures of healthy hepatocytes using three-dimensional centrifugation techniques. This technique allows by centrifugation in the three planes in a random way to recreate the effects of microgravity at the cell level. Thus, we could compare the metabolic flexibility and oxidative capacity of hepatocytes before and after the microgravity exposure. Alternatively, it would then be interesting to develop indirect markers of liver metabolic flexibility which could include hepatokines (FGF21, Fetuin-A) whose synthesis may vary according to the metabolic demand or using stable tracer isotopes.

It is beyond doubt how important endocrine regulation of metabolic flexibility is, particularly in coordinating the complex interorgan regulation of energy homeostasis. The circulating factors such as myokines, adipokines and hepatokines, respectively secreated by muscle, adipose tissue and liver, would be the main actors of the organs crosstalk and therefore may be involved in the regulation of metabolic flexibility. However, their respective function remains to be elucidated. It could be possible to measure in vitro metabolic flexibility of adipocytes and hepatocytes that have been incubated or not with cell culture medium of muscle cells that have been previously stimulated by electric pulse stimulation, thus reproducing the muscle contractions of exercise. If metabolic flexibility is indeed impacted between the two conditions, this would suggest that a molecule synthesized by the electrically stimulated skeletal muscle cells has an effect on adipocytes and hepatocytes metabolic flexibility. Performing proteomic analyses on the muscle cell culture medium would help identifying potential targets of myokines involved in the regulation of metabolic flexibility in adipose tissue and the liver. Conversely, by analyzing the differences in the gene and proteomic profiles of adipose tissue and liver samples collected before and after exercise in humans or animals (only in animals for the liver), it would be possible to identify certain peptides that could modulate the metabolic flexibility of other tissues.

Although metabolic flexibility and insulin resistance are known to be interrelated, the cause-and-effect relationship is still the object of controversies. Inconsistent findings have been reported so far and further investigations are needed to know which one comes first. This would help designing more efficient preventative and therapeutic strategies to prevent metabolic diseases, including obesity and type 2 diabetes.

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Annexe 1 : Physiology of physical inactivity, sedentary behaviors and non-exercise activity: insights from the space bed-rest model

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TOPICAL REVIEW

Physiology of physical inactivity, sedentary behaviours and non-exercise activity: insights from the space bedrest model

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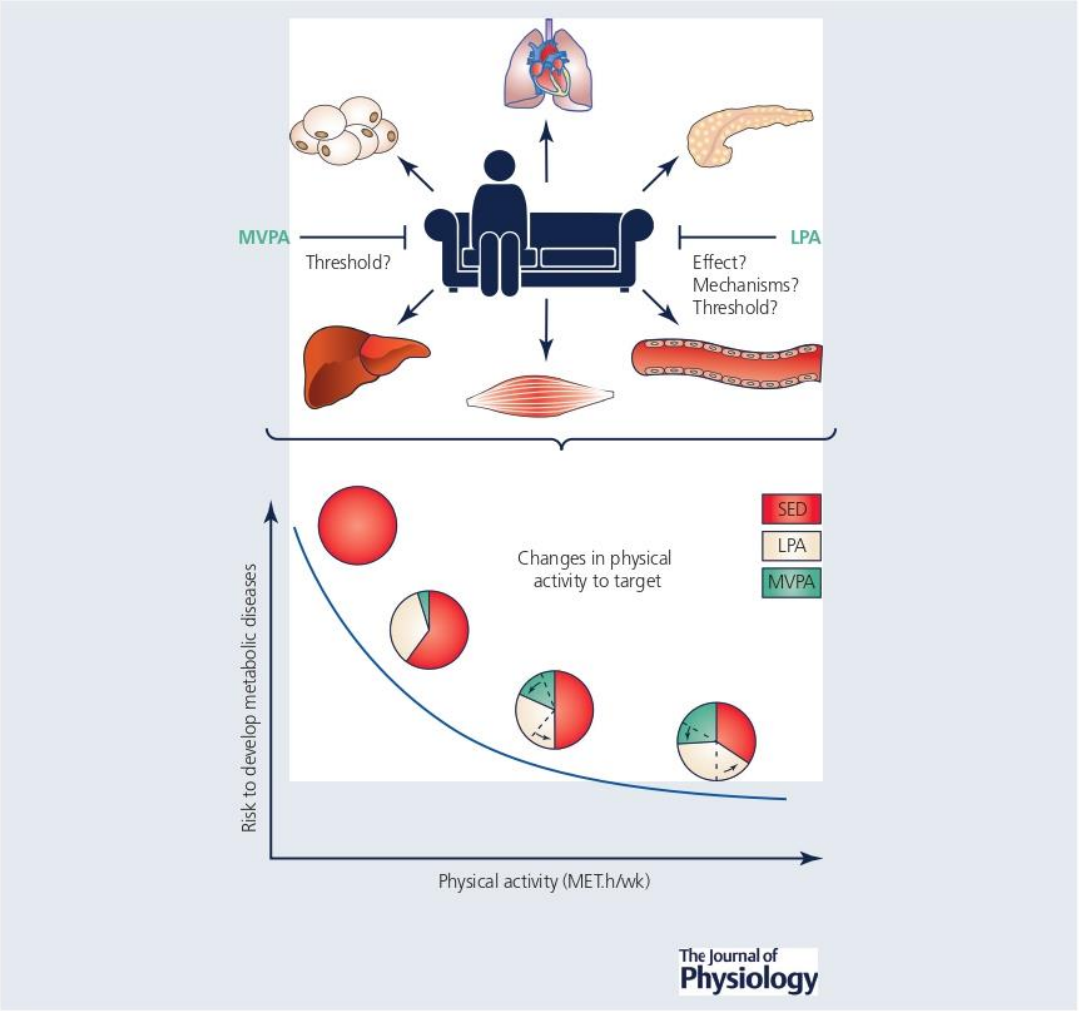

E. Le Roux and N. De Jong contributed equally to this work.

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Abstract Physical inactivity, i.e. not reaching the recommended level of physical activity (PA), and sedentary behaviours (SB), i.e. sitting time, have been associated with increased risk for common metabolic diseases. Recent epidemiological data suggest that high volumes of SB are detrimental to metabolic health, even in the presence of regular exercise, i.e. moderate/vigorous PA. This suggests that the health effects of SB are independent from those of exercise. However, experimentally testing this hypothesis is complicated because of the difficulty in disassociating SB from PA. Bedrest studies, a traditional space science model, can offer new insights. In some bedrest studies, an exercise training protocol has been used to counteract the harmful effects of inactivity. While bedrest induces an inactive and sedentary state, exercise with bedrest represents a unique model of sedentary yet physically active people. Here, we review bedrest studies with and without exercise training. Although exercise training prevents the loss of muscle mass and function, even large volumes of exercise are not sufficient to fully counteract the negative metabolic adaptations triggered by inactivity. This observation supports the existence of independent adverse health effects of SB, but also the potential benefits of non-exercise activity, i.e. daily living light PA. We gathered available data to examine the complex relationships between exercise, non-exercise activity, SB and health outcomes. Given the large amount of SB in modern societies, the sole promotion of exercise, i.e. moderate/vigorous PA may be insufficient, and promotion of light PA may be a complimentary approach to improve health.

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Abstract figure legend Although the exact physiological mechanisms remain unclear, sedentariness has been shown to negatively affect metabolic health and the main organs involved in its regulation. The 2020 World Health Organization (Bull *et al*. 2020) guidelines on physical activity recommend practicing a weekly volume of 150–300 min of moderate intensity or 75–150 min of vigorous intensity or an equivalent combination of moderate/vigorous physical activity (MVPA) to limit the appearance of certain maladaptations. Recently, some large-scale studies have revealed the importance of light physical activity (LPA) to prevent the effects of sedentary lifestyles. Since then, limiting periods of sedentary time have become part of the recommendations for the first time, raising the question of how to reduce periods of sedentary living. This review aims to highlight the potential role of LPA in effectively reducing sitting time and preventing its associated harmful health effects. Intervention studies specifically targeting LPA and sedentary behaviour, in addition to MVPA, are necessary to develop specific recommendations and limit the risk of developing metabolic diseases.

The authors are a group of researchers from the University of Colorado in the USA, the French National Centre for Scientific Research (CNRS) and the University of Lyon who are experts in metabolism, exercise and physical inactivity physiology. **Elisa Le Roux** and **Nathan De Jong** are graduate students working under the mentorship of Audrey Bergouignan. **Stéphane Blanc**, PhD, is studying the role of environment on the regulation of energy balance. **Chantal Simon**, MD, PhD, is professor of Nutrition and expert in metabolism, physical activity and sedentary behaviours. **Daniel Bessesen**, MD, is an endocrinologist who studies the regulation of body weight at the University of Colorado. **Audrey Bergouignan**, PhD, is an expert in metabolism, sedentary behaviours and physical inactivity physiology. The group, led by Dr Bergouignan, is building an international lab including members from the two institutions to address the role of sedentary behaviours in the onset and progression of metabolic diseases.

Introduction

Insufficient physical activity (PA) is a public health concern and a major risk factor for early mortality and common chronic diseases including obesity, metabolic syndrome, insulin resistance, type 2 diabetes (T2D), certain type of cancers, mental health disorders and others (Lee *et al.* 2012). Through research efforts to develop strategies to combat physical inactivity, scientists have identified another health risk behaviour: sedentary behaviours (SBs). SBs are distinct from physical inactivity. Although physical inactivity is defined as engaging in less PA than is necessary to meet the current guidelines (<150 min/week moderate or <75min/week vigorous physical activity (moderate/vigorous PA, MVPA), with energy expenditure above 3.0 metabolic equivalent or METs), SBs correspond to 'any waking behaviour characterized by an energy expenditure <1.5 METs, while in a sitting or reclining posture' (Tremblay *et al.* 2017). Although the recommendations encourage reducing periods of SB, no specific strategy has been proposed to combat the effects of sedentary lifestyles (Bull *et al.* 2020).

SBs are found in every domain of modern daily life: transportation, occupational (e.g. desk-bound work) and leisure time activities (e.g. video gaming and internet). Adults in Westernized societies spend between 7.7 and 9.7 h/day sitting, which corresponds to up to 60% of adult wake time (Ekelund *et al.* 2019). Several epidemiological studies have reported associations between sedentary time and health outcomes including early mortality and risk of T2D, metabolic syndrome and cardiovascular disease (Dunstan *et al.* 2012). These associations were observed in both sexes and all ages and ethnicities, and were independent of adiposity. They were also found in individuals who reach the recommended levels of MVPA, which suggests that SB is a stand-alone factor in the relationship between PA and health. In other words, spending too much time sitting may have different health effects from not exercising enough. While a plethora of epidemiological data have been published, experimental evidence supporting the adverse health effects of SB independent of time spent physically active is lacking. This is mainly due to challenges in isolating the effects of SB from those of PA.

The bedrest model can provide unique insights into the independent health effects of SB. Bedrest studies have traditionally been used by the international space agencies to understand the physiological effects of microgravity. During these studies, physically active healthy participants free from any predisposition for chronic diseases stay in bed 24 h/7 days. They are both physically inactive and highly sedentary (Fig. 1). In some bedrest studies, the efficacy of exercise training protocols to protect the body against the harmful effects of microgravity have also been tested. Participants in these studies perform exercise training (Fig. 1) while in bedrest. They are both sedentary and physically active, and represent an extreme but unique model of 'sedentary exercisers'. Another distinctive characteristic of these bedrest exercisers is that they have very low levels of non-exercise activities of daily living, which correspond to light physical activity (LPA) with an energy expenditure between 1.6 and 2.9 METs (walking, taking the stairs, standing, etc.) (Fig. 1).

The objective of this review is to present experimental evidence from bedrest studies with or without concomitant exercise training to provide new information on the metabolic health effects of SB independent from those of MVPA/exercise. To better understand the complex relationship between SB, LPA, MVPA and metabolic health outcomes, we will also review briefly the health benefits of LPA.

The physiology of physical inactivity: insights from strict bedrest investigations

To understand the respective effects of SB and PA, it is important to first briefly summarize the physiological effects of combined sedentariness and physical inactivity induced by strict bedrest (Fig. 2). Bedrest induces hypokinesia (loss of body movements) and hypodynamia (loss of strength and power), which leads to modifications in all physiological systems (Bergouignan *et al.* 2011). Among other changes, bedrest reduces muscle function and mass as demonstrated by muscle atrophy and a shift from slow oxidative to fast glycolytic muscle fibres (Trappe *et al.* 2007*a*, 2008; Salanova *et al.* 2008), reduced mitochondrial volume and oxidative capacity (Kenny *et al.* 2017), and an impaired expression of genes involved in mitochondrial function (Alibegovic *et al.* 2010*b*). Bedrest also rapidly decreases insulin sensitivity in muscle (Alibegovic *et al.* 2009) in association with lower content and activity of key proteins involved in glucose transport, phosphorylation and storage (Biensø *et al.* 2012). This results in hyperinsulinaemia to maintain normal glucose disposal. This development of glucose intolerance seems to be preceded by a metabolically inflexible state, i.e. an inability of the body to adjust substrate use to changes in substrate availability (Rudwill *et al.* 2018). Gene expression and activity of enzymes coupled with oxidative metabolism are decreased (Bergouignan *et al.* 2009; Alibegovic *et al.* 2010*b*; Fernandez-Gonzalo *et al.* 2020) in association with a reduction in lipid oxidation in favour of carbohydrate oxidation (Bergouignan *et al.* 2006, 2009). These changes are particularly relevant following meal ingestion since they lead to decreased clearance of dietary lipids, which contributes to hyperlipidaemia. Despite reduced adipose tissue lipolysis (Alibegovic *et al.* 2009, 2010*a*), excess of plasma lipids enhances fat accumulation in the visceral

adipose depot (Belavý *et al.* 2014) and ectopic fat storage in muscle, liver and bone (Bergouignan *et al.* 2009; Trudel *et al.* 2009, 2012; Rudwill *et al.* 2015). This in turn exacerbates the development of insulin resistance. Fat accumulation in liver likely stimulates *de novo* lipogenesis and an increased synthesis of atherogenic lipid particles (very-low-density lipoprotein; VLDL), as suggested by a recent study in free-living individuals who reduced their PA levels (Damiot *et al.* 2019). This increased secretion of VLDL further facilitates hyperlipidaemia and ectopic fat storage. A decrease in high-density lipoprotein (HDL) cholesterol, known to be associated with a reduction in cardiometabolic risk, has also been observed (Alibegovic *et al.* 2009). Concomitantly, the liver is less able to suppress hepatic glucose production, which results in increased gluconeogenesis, thus worsening hyperinsulinaemia. These changes are finally associated with the development of low-grade inflammation as indicated by an increase in plasma pro-inflammatory markers (Rudwill *et al.* 2013; Mutin-Carnino *et al.* 2014).

All these metabolic features are commonly observed in individuals with obesity, T2D or metabolic syndrome. These observations therefore support a key role of physical inactivity in the onset and progression of metabolic diseases. Although the health enhancing effects of exercise (or MVPA) on these metabolic outcomes are well established, it is unclear whether they are sufficient to reverse the adverse health effects of sedentariness.

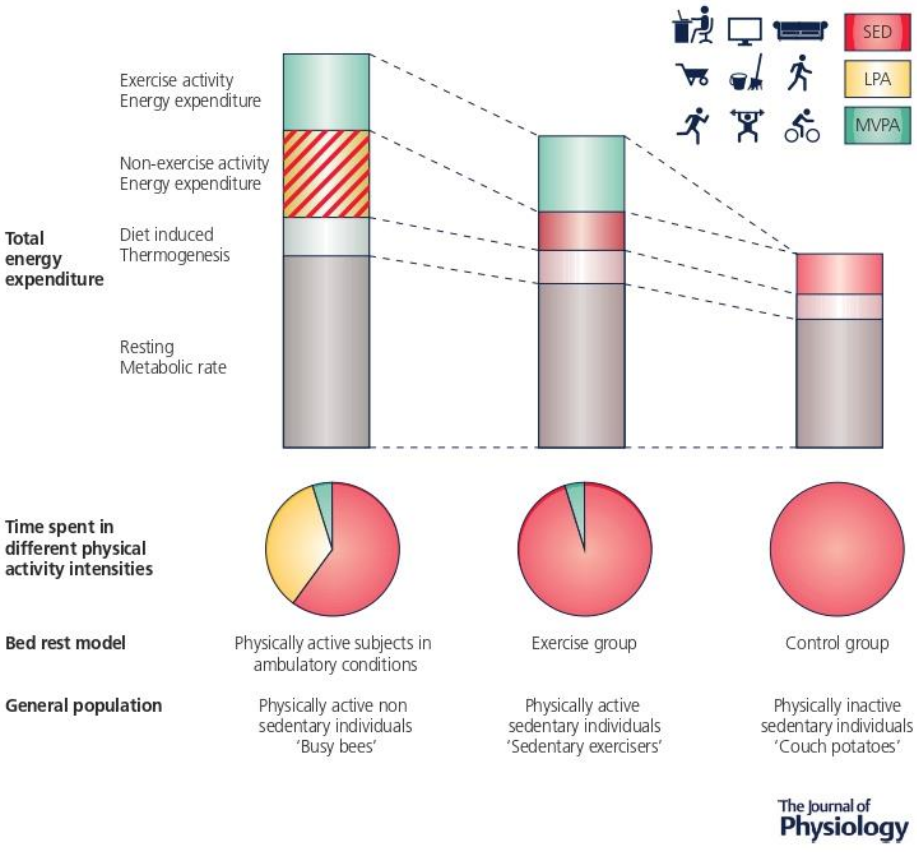

Figure 1. Schematic representation of the components of total energy expenditure during bedrest, conducted with or without exercise training
Based on total energy expenditure, participants enrolled in bedrest protocols can be compared to the general population. Strict bedrest suppresses both components of physical activity energy expenditure: exercise activity energy expenditure and non-exercise activity energy expenditure. Exercise activity energy expenditure refers to the energy spent in MVPA and/or structured exercise. Non-exercise activity energy expenditure corresponds to any activity of daily life, which is essentially LPA. Participants who are subjected to moderate to vigorous exercise training along with bedrest maintain high exercise activity energy expenditure mainly due to MVPA. However, they are sedentary with very low levels of non-exercise activity energy expenditure and are lacking LPA. These individuals represent an extreme but unique model of 'sedentary exercisers', i.e. physically active yet sedentary people. Strict bedrest leads to a decrease of both MVPA and LPA while increasing SB. These bedrest individuals represent a model of the modern physically inactive sedentary individuals. Abbreviations: SED, sedentary activities; LPA, light physical activity; MVPA, moderate-to-vigorous physical activity. Adapted from Bergouignan *et al.* (2010).

Can MVPA reverse the adverse health effects of physical inactivity and SB? Insights from bedrest studies with concomitant exercise training

To the best of our knowledge, 10 bedrest studies have tested the protective effects of an exercise training programme against the metabolic alterations induced by bedrest. These studies span from 14 to 90 days and the exercise prescriptions varied in the type (resistance or aerobic exercise), duration, frequency and intensity (Table 1). Some training protocols were below the current recommendations for PA while others were above. Results have been reported in 26 published articles and are summarized in Fig. 2.

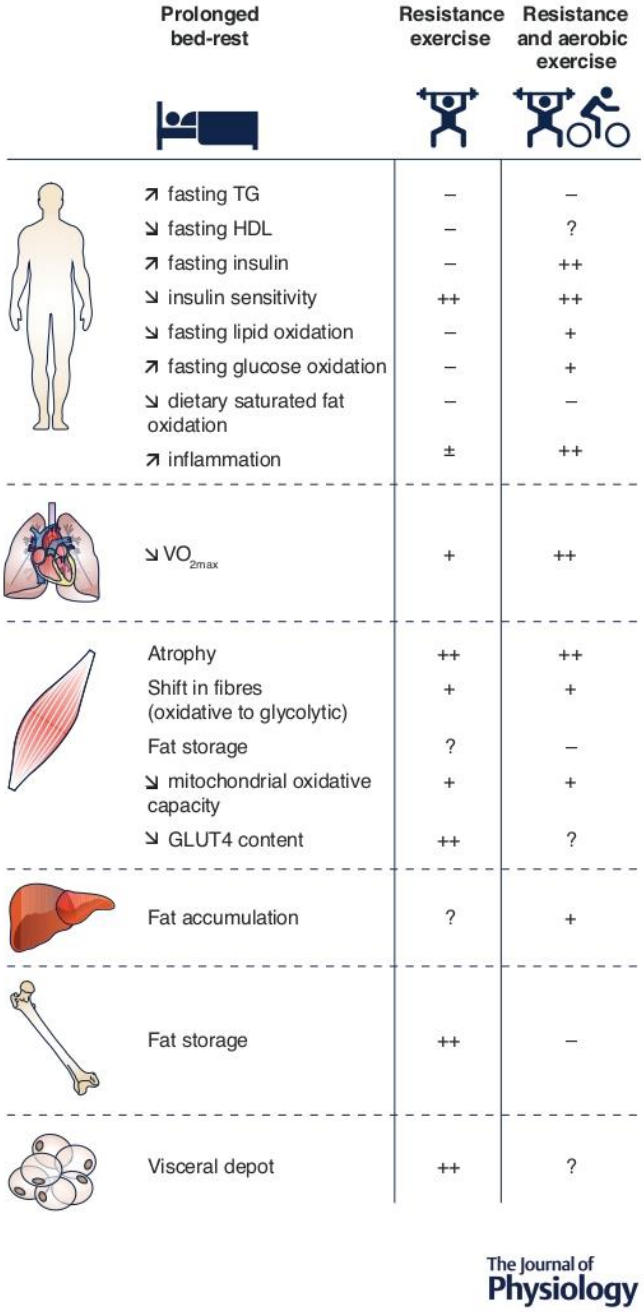

Figure 2. Preventive effect of exercise (resistance exercise or resistance plus aerobic exercise) against bedrest-induced physiological and metabolic alterations
–: no effect; +: partially protected; ++: fully protected; ?: no data available; ±: no consensus.

Effect of resistance exercise alone. Resistance exercise has been shown to mitigate the decrease in cardiorespiratory fitness (Guinet *et al.* 2020; Kenny *et al.* 2020) and prevent the loss of muscle function and mass including the reduction in fibre diameter during bedrest (Trappe *et al.* 2004; Moriggi *et al.* 2010). However, the mechanisms underlying the protective effects of resistance exercise against unloading-muscle atrophy are not fully clear. Resistance exercise was shown to prevent the bedrest-induced decrease in muscle protein synthesis (Ferrando *et al.* 1997) and downregulate the gene expression of myostatin (Irimia *et al.* 2017), a myokine known to contribute to muscle wasting. The effect of resistance exercise on muscle protein balance, i.e. muscle protein synthesis and breakdown, during bedrest is, however, still unknown. Despite these positive effects on skeletal muscle, resistance exercise only partially prevents the whole-body metabolic alterations induced by bedrest. It protects against the rise in visfatin (Rudwill *et al.* 2013), an adipocytokine that mimics the effects of insulin, but does not prevent the increase in interleukin-6 and C-reactive protein, two pro-inflammatory markers, or the decrease in adiponectin (Brooks *et al.* 2014), a change associated with inflammation, lipid abnormalities and insulin resistance. Even when performed at high intensity, resistance exercise does not mitigate the decrease in HDL (Brooks *et al.* 2014; Guinet *et al.* 2020), the development of insulin resistance, hyperlipidaemia, or the shift towards the use of carbohydrate as fuel (Bergouignan *et al.* 2006). This latter observation is surprising knowing that resistance exercise prevents the shift of muscle fibres from oxidative to glycolytic types (Trappe *et al.* 2004), offsets the transcriptomic alterations in muscle related to aerobic energy metabolism (e.g. electron transport chain, fatty acid β-oxidation and tricarboxylic cycle pathways) (Fernandez-Gonzalo *et al.* 2020), and partially maintains the activity and gene expression of enzymes controlling oxidative metabolism (e.g. citrate synthase, succinate dehydrogenase) at the mitochondrial level (Irimia *et al.* 2017). Although resistance exercise alone does not restore the levels of fatty acid oxidation to baseline values (Bergouignan *et al.* 2006), no accumulation of fat in bone (Trudel *et al.* 2012) or visceral depots (Belavý *et al.* 2014) was reported. There are no data on the effects of resistance exercise on ectopic fat storage in liver or muscle during bedrest. In all these studies, the resistance exercise session

Table 1. Summary of the exercise training protocols tested during bedrest studies

Publication	Study name, duration of BR, sample size	Exercise modalities	Estimated duration of MVPA	Estimated energy cost
		Resistance exercise		
Fernandez-Gonzalo *et al*. (2020) Irimia *et al*. (2017) Bergouignan *et al*. (2006) Rudwill *et al*. (2013) Trappe *et al*. (2004)	LTBR 2001–2002 90 d HDT-BR $n = 18$ ♂	35 min every 3 d during BR on flywheel ergometer. Progressive warm-up + 4 × 7 max concentric/eccentric squat + 4 × 14 in calf press. 2 min rest between sets and 5 min between EX	82 min MVPA/wk 12 min MVPA/d	8 MET h/wk 1.2 MET h/d
Brooks *et al*. (2014)	28 d HDT-BR $n = 31$ ♂	1 h/d, 6 d/wkTarget intensity: 70%–80% of 1RM as estimated by the OMNI rating of perceived EX 10 category scale 7–8 REX targeting major muscle groups during each session. Lower body (squats, single leg squats, diagonal jump, calf raise, single-leg hip extension, leg curl, single-leg hip abduction) and upper body (pull-ups, pull-over, triceps press, chest fly, shoulder press, biceps curl, upright row, lateral arm raise) EX were performed on alternating days	360 min MVPA/wk 51 min MVPA/d	36 MET h/wk 5.2 MET h/d
Ferrando *et al*. (1997)	14 d HDT-BR $n = 6$ ♂	Squat on horizontal leg-training device every 2 d. 3 × 12 squats, training volume progressively increased to reach 5 × 8 squat at session 3 till the end of BR	?	?
Kenny *et al*. (2017) Guinet *et al*. (2020) Kenny *et al*. (2020)	MNX 21 d HDT-BR $n = 12$ ♂	5 sessions of EX, on leg press machine with a vibration platform (8 mm peak-to-peak, 25 Hz): bilateral squats (10 rep, 75% 1RM, 8 s/rep), single heel raises (×1.3 body weight, contractions performed as fast as possible until fatigue) and bilateral heel raises (×1.8 body weight, contractions performed as fast as possible until fatigue). A 5% load adjustment was made based on the ability of volunteers to complete the set of EX	5–15 min MVPA/wk 0.7–2.1 min MVPA/d	0.5–1.5 MET h/wk 0.01–0.2 MET h/d
Moriggi *et al*. (2010)	BBR1 55 d HDT-BR $n = 12$ ♂	2 bouts/d of EX (6 min each) of RVE at preset frequencies ranging from 19 at the beginning to 25Hz. Total of 89 sessions (1) Squatting EX: knees were extended from 90° to almost full extension in cycles of 6 s for each squat (knee extensors) (2) Heel raises: with knees almost extended, heels were raised to fatigue. Only then, brief rests (<5 s) were allowed with the entire foot on the vibration platform in order to recover, and subjects started to raise their heels again (foot plantar flexors) (3) Toe raises: similar to 2, but toes were raised instead of heels (foot dorsi-flexors) (4) 'Kicks': with the same loading as in 1−3, knees were extended as quickly and forcefully as possible. The platform was struck with the balls of the feet, and legs rested on the Galileo Space framework in between the kicks. This was done 10 times with 10 s of rest inserted	36 min MVPA/wk5.1 min MVPA/d	3.6 MET h/wk 0.5 MET h/d

(*Continued*)

Table 1. (Continued)

Publication	Study name, duration of BR, sample size	Exercise modalities	Estimated duration of MVPA	Estimated energy cost
Belavý *et al*. (2014) Trudel *et al*. (2012)	BBR2-2 60 d HDT-BR $n = 24$ ♂	3 d/wk:(1) Bilateral leg press (∼75–80% of pre-bed-rest max voluntary contraction) (2) Dingle-leg heel raises (∼1.3 times body weight) (3) Double leg heel raises (∼1.8 times body weight) (4) Back and forefoot raise (performing hip and lumbar spine extension against gravity with ankle dorsiflexion, but with ∼1.5 times body weight applied at the shoulders) The RVE group performed the same exercises as the REX group, except that whole body vibration was applied. The corresponding vibration parameters were as follows: (1) Frequency 24 Hz, amplitude 3.5–4 mm and peak acceleration ∼8.7 *g*, where *g* is 9.81 m s^{-2} (2) Frequency 26 Hz, amplitude 3.5–4 mm and peak acceleration ∼10.2 *g* (3) Frequency 26 Hz, amplitude 3.5–4 mm and peak acceleration ∼10.2 *g* (4) Frequency 16 Hz, amplitude 3.5–4 mm and acceleration ∼3.9 *g*	15.8 min MVPA/wk 2.3 min MVPA/d	1.6 MET h/wk 0.2 MET h/d
		Combined resistance and aerobic exercise		
Bergouignan *et al*. (2009) Bergouignan *et al*. (2010) Lee *et al*. (2014) Mutin-Carnino *et al*. (2014) Salanova *et al*. (2008) Rudwill *et al*. (2015) Trappe *et al*. (2007*b*) Trappe *et al*. (2007*a*) Trappe *et al*. (2008) Trudel *et al*. (2009)	WISE 60 d HDT-BR $n = 16$ ♀	REX: 35 min every 3 d, 4 × 7 max concentric/eccentric squat + 4 × 14 in calf press AEX: 50 min every 2 d, 50 min in lower body negative pressure vertical treadmill at 40%–80% pre-bedrest $\dot{V}_{O_2\,max}$	247 min MVPA/wk 35.3 min MVPA/d	33.1 MET h/wk 4.7 MET h/d

(*Continued*)

Table 1. (Continued)

Publication	Study name, duration of BR, sample size	Exercise modalities	Estimated duration of MVPA	Estimated energy cost
Krainski *et al*. (2014)	35 d HDT-BR $n = 27$ ♂/♀	REX: 25–30 min 2 d/wk. 2 × 8−12 of lower body exercises (leg press, plantar flexion, knee flexion, hip flexion and hip abduction) and 1 × 8−12 of upper body EX (shoulder press, elbow flexion and extension, chest press, pullovers and abdominal crunches) were performed in the supine position, loads were adjusted weekly to reach muscle fatigue during each set of EX. After 5 wk of BR, 2 × 20 plantar flexion exercises on each leg 2/d (6–8 min) against an elastic band were added for all remaining subjects in EX group AEX: 6 d/wk. During each week of BR, subjects completed 1 recovery (low intensity, typically <70% max HR), 2 base (moderate intensity, between 70%–80% max HR), 1 MSS (vigorous intensity, 80−90% maximal HR) and 2 interval sessions (high intensity, 90−95% max HR or above), each lasting a total of 30–46 min and separate warm-up/cool-down phases lasting 5 min each. Intervals consisted of 6 cycles of 3 min at 90−95% of max HR, followed by 3 min at recovery pace	381 min MVPA/wk 54.4 min MVPA/d	49.5 MET h/wk 7.1 MET h/d
Ploutz-Snyder *et al*. (2018)	70 d HDT-BR $n = 26$ ♂	REX: 3 d/wk. 3 × 4 supine lifts (squat, leg press, unilateral leg curl and heel raise); squats and leg press were each performed using a standard shoulder-width stance, single-leg stance, or wide-leg stance on a rotating basis. Training followed a non-linear periodized model in which load and repetitions were varied on a daily basis to optimize adaptations AEX: 6 d/wk. Alternating days of continuous cycle EX for 30 min at 75% of $\dot{V}_{O_2peak}$ (3 d/w) with interval treadmill sessions of 30 s, 2 min, or 4 min intervals (3 d/wk) at nearly max intensity	314.5 min MVPA/wk 45 min MVPA/d	40 MET h/wk 5.7 MET h/d
Ward *et al*. (2020)	RSL 60 d HDT-BR $n = 23$ ♂	48 sessions including 4 types of training sessions based on varying CMJ and repetitive hops between 80–90% of BW during 1.5–3 min preceded by a warm-up and 3 max CMJ at 80% of BW	17.5 min MVPA/wk 2.5 min MVPA/d	2.6 MET h/wk 0.4 MET h/d

Abbreviations: AEX, aerobic exercise; BW, body weight; CMJ, countermovement jump; d, days; EX, exercise; HDT-BR, 6° head-down tilt bedrest; max, maximal; REX, resistive exercise; wk, week.

was performed as a single continuous bout; however, novel data suggest that spreading activity throughout the day as multiple short active bouts may have more potent health-enhancing effects (Loh *et al.* 2019). Nevertheless, when exercise was performed as intermittent, frequent jumping squats spread throughout the day, muscle mass loss was prevented, but not the reduction in whole-body and peripheral insulin sensitivity (Ward *et al.* 2020). Taken together, the low energy expenditure associated with resistance exercise (Table 1) may be responsible for partial or limited protective effects on metabolic health outcomes.

Effect of combined resistance and aerobic exercise. The majority of bedrest studies have combined aerobic exercise with resistance exercise, which likely induced a greater energy expenditure compared to resistance exercise alone. This training approach preserves or at least attenuates cardiorespiratory fitness, muscle structure and function, leg muscle size and power, muscle strength and endurance, muscle fibre composition and diameter, and mitochondrial content and oxidative capacity (Trappe *et al.* 2007*a*, 2008; Trappe *et al.* 2007*b*; Salanova *et al.* 2008; Bergouignan *et al.* 2009; Krainski *et al.* 2014; Lee *et al.* 2014; Ploutz-Snyder *et al.* 2018). Although muscle alterations were prevented by all the tested resistance and aerobic exercise protocols regardless of the type, duration, intensity and frequency of the training, the protective effects on metabolic outcomes were variable. Combined resistance and aerobic exercise training prevents the development of a pro-inflammatory state (Mutin-Carnino *et al.* 2014), insulin resistance and the shift in substrate use from total fat oxidation to carbohydrate oxidation (Bergouignan *et al.* 2009). However, it does not counteract the increase in fasting triglycerides, the reduced oxidative rate of dietary fatty acids likely due to an impaired transport of fatty acids into the myocyte, and fat accumulation in skeletal muscle (Bergouignan *et al.* 2009) and bone (Trudel *et al.* 2009). Hepatic fat accumulation is, however, likely offset (Rudwill *et al.* 2015). Surprisingly, these alterations were observed despite levels of MVPA mostly above recommended levels (Table 1), and total daily energy expenditure maintained at pre-bedrest levels in the exercising participants (Bergouignan *et al.* 2010).

Taken together these studies show that exercise (or MVPA) protects skeletal muscle mass and function, and cardiorespiratory function against large volumes of SB induced by bedrest. However, even if a dose–response relationship exists (Fig. 2 and Table 1), very high levels of exercise do not fully prevent the manifestation of metabolic dysfunction, i.e. whole-body insulin resistance, glucose intolerance, alterations of lipid metabolism and systemic inflammation. These observations support the role of organs other than muscle in the health-enhancing effects of physical activity (Thyfault & Bergouignan, 2020), and the existence of health effects of SB independent of those from MVPA. It further highlights the importance of non-exercise activity (i.e. LPA), which mainly corresponds to daily living activities performed throughout the day.

Health benefits of daily living activities

Evidence from epidemiological studies indicates that LPA has a potential role in reducing the risk of early mortality. In cross-sectional studies, LPA is favourably associated with waist circumference, body mass index (BMI), plasma triglyceride, insulin, HDL-cholesterol concentrations (Amagasa *et al.* 2018) and 2 h plasma glucose (Healy *et al.* 2007), independent of MVPA. Iso-temporal substitution modelling suggests that replacing 30 min of SB per day with 30 min of LPA (but not MVPA) is associated with lower waist circumference and BMI (Healy *et al.* 2015). A growing number of experimental studies have also examined the effects of LPA prescribed as short bouts spread throughout the day on metabolic health (Table 2). As previously reviewed (Dempsey *et al.* 2016*a*), LPA bouts (15–40 min) acutely decrease postprandial glycaemia and insulinaemia. Even brief intermittent bouts ($\leq$5 min) of walking spread throughout the day reduce glucose and insulin concentrations following meal ingestion, with more potent effects observed in adults with overweight to obesity and T2D (Chastin *et al.* 2019), and those with lower cardiorespiratory fitness compared to healthy lean individuals (McCarthy *et al.* 2017). Importantly, acute exposure to bouts of LPA elicits similar responses to those observed with short, frequent bouts of MVPA. With regards to standing, although some studies did not show a reduction in postprandial glycaemic response (Bailey & Locke, 2015; Pulsford *et al.* 2017), others did (Benatti *et al.* 2017). Benatti and colleagues even reported that intermittent standing, but not a single continuous bout of MVPA, lowers postprandial glycaemia in healthy adults. The difference in the observed effects may be explained by the duration of the standing bouts (2 min *vs* 15 min) and the total active duration (30 min MVPA *vs* 15 min standing every 30 min for 8.5 h). Although these acute studies suggest that LPA of higher energy expenditure or longer duration decreases glycaemia and insulinemia in a dose-dependent manner, none of these studies controlled for energy expenditure across the interventions. In an elegant series of studies, Duvivier and colleagues compared the metabolic effects of replacing SB with LPA walking and standing to those of 1 h/day of MVPA. Both interventions lasted 4 days and were matched for energy expenditure. Replacing SB with high volumes of LPA without any increase in MVPA decreased postprandial insulin, fasting triglycerides and non-HDL cholesterol in healthy adults (Duvivier *et al.* 2013). In adults with

Table 2. Summary of the acute metabolic health effects of light-intensity physical activity from experimental laboratory-controlled studies

Publication	Sample size and characteristics	Study design, study conditions	Primary results
Studies investigating light physical activity compared to sitting			
Bailey *et al.* (2015)	$n = 10$ (3♀/7♂) BMI: 26.5 ± 4.3 kg/m^2 Non-insulin resistant	Cross-over (5 h/condition) (1) Uninterrupted sitting (2) Sit + 2 min stand every 20 min (3) Sit + 2 min LIW every 20 min	Plasma glucose AUC: LIW < sitting and standing; standing *vs.* sitting: NS Blood pressure: no between-conditions difference Plasma lipids: No between-conditions difference
Benatti *et al.* (2017)	$n = 14$ ♂ BMI: 24.9 ± 4.3 kg/m^2 Non-insulin resistant	Cross-over (27 h/condition) (1) Uninterrupted sitting (2) Sit + 15 min stand every 30 min (3) Sit + 30 min MIW (4) Sit + 30 min MIW and 15 min stand every 30 min	Postprandial plasma glucose iAUC: standing < sitting; MIW *vs* sitting: NS
Dempsey *et al.* (2016*b*)	$n = 24$ (10♀/14♂) BMI: 33.0 ± 3.4 kg/m^2 Type 2 diabetes	Cross-over (8 h/condition) (1) Uninterrupted sitting (2) Sitting + 3 min LIW every 30 min (3) Sitting + 3 min resistance activities every 30 min	Blood pressure: LIW < sitting Noradrenaline concentration: LIW < sitting
Duvivier *et al.* (2017*a*)	$n = 24$ (11♀/13♂) BMI: 29.0 ± 2.0 kg/m^2 Non-insulin resistant	Cross-over (4 d/condition) (1) Sit 13.5 h/d, stand 1.4 h/d, LIW 0.7 h/d (2) Sit 7.6 h/d, stand 4.0 h/d, LIW 4.3 h/d	OGTT insulin AUC: LIW < sitting Insulin sensitivity (Matsuda index): LIW < sitting Fasted lipids: LIW < sitting Fasted lipoproteins: LIW < sitting Diastolic blood pressure: LIW < sitting
McCarthy *et al.* (2017)	$n = 34$ (18♀/16♂) BMI: 23.8 ± 6.1 kg/m^2 Non-insulin resistant	Cross-over (7.5 h/condition) (1) Uninterrupted sitting (2) Sit + 5 min LIW every 30 min	Plasma glucose iAUC: LIW < sitting Plasma insulin iAUC: LIW < sitting
Pulsford *et al.* (2017)	$n = 25$ ♂ BMI: 24.9 ± 4.3 kg/m^2 Non-insulin resistant	Cross-over (7 h/condition) (1) Uninterrupted sitting (2) Sit + 2 min stand every 20 min (3) Sit + 2 min LIW every 20 min	Postprandial plasma glucose AUC: LIW < sitting Postprandial plasma insulin AUC: LIW < sitting Insulin sensitivity (Matsuda index): LIW < standing and sitting
Thosar *et al.* (2015)	$n = 12$ ♂ BMI: 23.7 ± 3.4 kg/m^2 Non-insulin resistant	Cross-over (3 h/condition) (1) Uninterrupted sitting (2) Sit + 5 min LIW every hour	Flow-mediated dilation: LIW > sittingShear rate: LIW > sitting
Studies comparing the health effects of light-intensity and moderate-vigorous physical activity to sitting			
Duvivier *et al.* (2013)	$n = 18$ (16♀/2♂) BMI: 22.6 ± 2.6 kg/m^2 Non-insulin resistant	Cross-over (4 d/condition) (1) Sit 14 h/d (2) Sit 13 h/d and 1 h EX(3) Sit 8 h/d, 4 h LPA, 2 h stand	OGTT plasma insulin AUC: LPA < sitting and exercise Fasting lipids: LPA < sitting; LPA *vs* exercise: NS Fasting lipoproteins: LPA < sitting; LPA *vs* exercise: NS

(*Continued*)

Table 2. (Continued)

Publication	Sample size and characteristics	Study design, study conditions	Primary results
Duvivier *et al*. (2017*b*)	$n = 19$ (6♀/13♂) BMI: 30 ± 2.0 kg/m^2 Type 2 diabetes	Cross-over (4 d/condition) (1) Sit 14 h/d with 4415 steps/d (2) Sit + 1.1 h/d EX with 4823 steps/d (3) Sit + stand 2.5 h/d and LIW 2.2 h/d with 17,502 steps/d	Glycaemia (CGMs): LIW < exercise Insulin sensitivity index (HOMA2-IR): LIW < exercise
Duvivier *et al*. (2018)	$n = 61$ (33♀/28♂) BMI: 27.8 ± 4.3 kg/m^2 Non-insulin resistant and type 2 diabetics	Pooled analysis Cross-over (4 d/condition) (1) Sit 14 h/d (2) Sit + 1 h/d MIW (3) Sit + 5–6 h/d LIW and standing	Endothelial function: LIW < MIW Insulin sensitivity index (HOMA2-IR): LIW < MIWPlasma lipids: LIW < MIW

Abbreviations: AUC, area under the curve; BMI, body mass index (kg/m^2); CGMs, continuous glucose monitoring system; d, day; EX, exercise; h, hours; iAUC, incremental area under the curve; LIW, light-intensity walking; LPA, light physical activity; MIW, moderate-intensity walking; OGTT, oral glucose tolerance test.

T2D, increasing time spent standing and walking improved glucose control and insulin sensitivity. It further reduced diastolic blood pressure, blood triglycerides and non-HDL cholesterol while increasing HDL cholesterol (Duvivier *et al*. 2017*a*,*b*). The MVPA intervention tended to improve these metabolic parameters, but the effects were less pronounced. These studies show that when energy expenditure is matched, replacing SB with high volumes of LPA (i.e. non-exercise activity) is more beneficial than performing MVPA as a single continuous bout (i.e. structured exercise), at least for glucose control, insulin sensitivity and circulating lipids. On the contrary, if frequent LPA bouts (>2 min) improve endothelial function (Thosar *et al*. 2015; Dempsey *et al*. 2016*b*), a single bout of MVPA may be more beneficial for microvascular function than increasing LPA throughout the day (Duvivier *et al*. 2018). These findings suggest that MVPA (i.e. exercise) and LPA (i.e. non-exercise activity) might elicit differential cardiometabolic effects. Future studies will need to further compare the effects of LPA *versus* MVPA on cardiometabolic health outcomes, including maximal aerobic capacity, muscle strength, substrate metabolism, glucose control and insulin sensitivity in different populations and investigate the mechanistic underpinnings.

Where do we go from here?

Although MVPA produces myriad health benefits, it does not reduce time spent sedentary. Indeed, physically active people, even those who exceed the current guidelines, can be as sedentary as their inactive counterparts (Rantalainen *et al*. 2018). Increasing MVPA can even trigger spontaneous behavioural compensations in sedentary adults leading to a decrease in non-exercise activities (i.e. LPA) in favour of sedentary time (Lefai *et al*. 2017). Furthermore, MVPA does not fully offset the adverse health effects of large volumes of SB, as shown by the bedrest studies with concomitant exercise training. The remaining question is how much MVPA is needed to offset the effects of a certain amount of SB. It was shown that independent of physical activity, every hour spent sitting increases the risk of mortality by 5.9%, of T2D by 22% and of obesity by 23% (Hu *et al*. 2003; Wilmot *et al*. 2012; Chau *et al*. 2013). A meta-analysis including more than 1 million individuals further showed that 60–75 min/day of MVPA is needed to prevent the risk of premature death associated with 9 h/day or more of sitting time (Ekelund *et al*. 2016), 9 h/day being close to the average sitting time observed in modern societies. When most of the population does not reach the recommended guidelines (i.e. 30 min/day of MVPA, 5 days/week.), adding 30–45 more minutes per day of MVPA is unrealistic. Therefore, other pragmatic and efficient strategies are needed.

Activities of daily living (i.e. LPA) are inversely associated with SB; increases in LPA are associated with reductions in sedentary time (Pate *et al*. 2008). In addition, large volumes of LPA, here considered as any body movements associated with activities of daily living, have been shown to confer health benefits. Knowing that lack of time is a major barrier to the practice of exercise/MVPA, reintroducing LPA into daily life could be an effective strategy to reduce sedentary time and prevent its effects on metabolic health. Along these lines, the latest guidelines from the World Health Organization (Bull *et al*. 2020) promote the practice of physical activity of any intensity to reduce sedentary activities. In other

words, moving is better than sitting. Future mechanistic studies will need to establish the physiology of SB and LPA to better understand the respective negative and positive health effects, and thus better define the dose–response relationship between the components of PA behaviour and key health outcomes. Experimental research examining these relationships will foster the development of more specific and pragmatic public health guidelines.

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Additional information

Competing interests

No conflict of interest to declare.

Author contributions

E.L.R., N.D.J. and A.B. wrote the first draft of the review, and all authors contributed to revising and editing the paper. All authors have approved the final version of the manuscript and agree to be accountable for all aspects of the work. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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Keywords

bedrest, exercise, light physical activity, metabolism, moderate to vigorous physical activity, non-exercise activity, physical inactivity

Annexe 2 : Effect of Exercise on Energy Expenditure and Body Composition in Astronauts Onboard the International Space Station: Consideration for Interplanetary Travel

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ORIGINAL RESEARCH ARTICLE

Effect of Exercise on Energy Expenditure and Body Composition in Astronauts Onboard the International Space Station: Considerations for Interplanetary Travel

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Abstract

Objective Body mass (BM) loss and body composition (BC) changes threaten astronauts' health and mission success. However, the energetic contribution of the exercise countermeasure to these changes has never been investigated during long-term missions. We studied energy balance and BC in astronauts during 6-month missions onboard the International Space Station.

Methods Before and after at least 3 months in space, BM, BC, total and activity energy expenditure (TEE and AEE) were measured using the doubly labeled water method in 11 astronauts (2011–2017). Physical activity (PA) was assessed by the SensewearPro® activity-device.

Results Three-month spaceflight decreased BM (− 1.20 kg [SE 0.5]; $P=0.04$), mainly due to non-significant fat-free mass loss (FFM; − 0.94 kg [0.59]). The decrease in walking time (− 63.2 min/day [11.5]; $P<0.001$) from preflight was compensated by increases in non-ambulatory activities (+64.8 min/day [18.8]; $P<0.01$). Average TEE was unaffected but a large interindividual variability was noted. Astronauts were stratified into those who maintained (stable_TEE; $n=6$) and those who decreased (decreased_TEE; $n=5$) TEE and AEE compared to preflight data. Although both groups lost similar BM, FFM was maintained and FM reduced in stable_TEE astronauts, while FFM decreased and FM increased in decreased_TEE astronauts (estimated between-group-difference (EGD) in ΔFFMindex [FFMI] 0.87 kg/m^2, 95% CI+0.32 to+1.41; $P=0.01$, ΔFMindex [FMI] − 1.09 kg/m^2, 95% CI − 2.06 to − 0.11 kg/m^2; $P=0.03$). The stable_TEE group had higher baseline FFMI, and greater baseline and inflight vigorous PA than the decreased_TEE group ($P<0.05$ for all). ΔFMI and ΔFFMI were respectively negatively and positively associated with both ΔTEE and ΔAEE.

Conclusion Both ground fitness and inflight overall PA are associated with spaceflight-induced TEE and BC changes and thus energy requirements. New instruments are needed to measure real-time individual changes in inflight energy balance components.

Audrey Bergouignan, Stéphane Blanc and Chantal Simon are co-last authors.

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Key Points

Space agencies ranked energy balance control to critical level for human space exploration. Although exercise is a cornerstone of countermeasure programs to prevent microgravity-induced physiological alterations, the energy cost of physical activity during long-term spaceflights has never been studied.

Six-month missions on the International Space Station led to large inter-individual variability in body composition changes. Astronauts maintaining pre-flight total and activity energy expenditures (possibly due to both physical training and unexpected non-exercise activity) maintained fat-free mass but lost fat mass. Conversely those who decreased energy expenditures lost fat-free mass but gained fat mass. Fat mass changes reflects unmatched energy intake adaptation to expenditures during flight.

On average, astronauts who maintained energy expenditures during flight were also fitter on the ground.

The large between-astronauts variability suggests that energy requirements cannot be derived from general population equations. Methods to track inflight changes in body composition, energy intake and energy expenditure are needed to determine individual energy requirements to ensure astronaut's performance and mission success.

1 Introduction

The human spaceflight program has entered a new phase of space exploration directed towards the Moon and Mars. Space agencies have developed roadmaps that define research priorities enabling planetary exploration. Among those, understanding inflight energy balance regulation has been placed as a top priority, as energy homeostasis is vital for both medical and operational reasons. Any energy deficit will not only impact fat mass (FM), but it can also increase loss of bone and of muscle mass and strength, favor cardiovascular deconditioning, and impair numerous other physiological functions. Overall it conditions astronaut's performance and health, and ultimately may affect mission success [1].

A loss of body mass (BM) is a hallmark of spaceflights. On Mir (4 months) [2, 3], Shuttle (4–19 days) [4, 5] and early International Space Station (ISS) missions (128–195 days) [6, 7], astronauts lost more than 5% of their preflight BM despite sufficient food onboard with no clear relationship with mission duration [8]. In some cases, this loss even exceeded 10% of preflight BM, which is clinically significant. Conversely, BM was successfully maintained in few missions, such as SpaceLab Life Sciences Space Shuttle missions SLS1 and SLS2 in the 1990s [9] or, more recently, on the ISS [7, 10]. Yet, recent reports from the ISS show that astronauts still lose from 2 to 5% of their initial BM with a large between-subject variability [6, 11]. We recently hypothesized that an energy balance dysregulation, i.e., an uncoupling between energy intake (EI) and expenditure, occurs in space [8]. Very little data, however, exist to support this hypothesis.

The regulation of energy balance was measured with objective doubly-labeled water (DLW) methods in three studies during short-term spaceflights only [4, 9, 12]. The results of two of these studies indicate that energy balance is not optimal in space when exercise is performed, i.e., compensatory changes in EI are insufficient to match increases in energy expenditure [13]. During the 17-day Life and Microgravity Science (LMS) Space Shuttle mission, Stein et al. [4] observed in four astronauts an energy deficit as large as 5.7 MJ/day that was associated with the loss of up to 2 kg of fat mass (FM). Concomitantly, even though heavy exercise training was specifically prescribed during this mission to prevent loss of muscle mass and bone, astronauts presented a negative nitrogen balance indicating protein loss related to a maladjustment of energy and protein intakes to high requirements. By contrast, during the SLS1 and SLS2 missions, during which no exercise was prescribed, the astronauts maintained stable energy balance with only a moderate negative nitrogen balance likely thanks to the maintenance of protein intakes despite an adapted reduction in energy intakes [9]. Because exercise is the cornerstone of the countermeasure program to prevent unloading-induced loss of skeletal muscle mass and strength, bone, aerobic capacity and other health-related outcomes [14, 15], these observations suggest that understanding its role in energy balance regulation is critical, notably for long-duration missions.

This exploratory study was therefore designed to measure total energy expenditure (TEE) and activity energy expenditure (AEE), using the gold standard DLW method, in relation to body composition in 11 astronauts after at least 3 months onboard the ISS. Inferences to evaluate energy requirements in space were derived. We hypothesized that astronauts' individual BM and body composition changes are explained by inflight AEE.

2 Materials and Methods

Protocols, methodological validation and related assumptions of the calculations used are fully described in the Online Supplementary Material (OSM).

2.1 Participants

Eighteen astronauts (16 men and two women) voluntarily took part in the study between 2011 and 2017. All were subjected to extensive physical and medical examination prior to the flight. None had a history of chronic disease, and all of them were healthy throughout the mission. Out of the 18 astronauts, three (two men and one woman) performed preflight measurements only, due to rescheduling priorities during the flight, and four (three men and one woman) served as controls to correct for background isotopic changes on the ISS during an experimental session (see OSM Methods and OSM Figs. 1–3). Data presented here were therefore collected in 11 men.

The study was yearly approved by the NASA Institutional Review Board (IRB) under NASA 7116301606HR. European Space Agency (ESA) Medical Board and Japanese Space Agency (JAXA) IRB for human experiments also approved the protocol. Written informed consent was obtained from all astronauts.

2.2 Protocol

For the 11 remaining astronauts, each completed two research sessions of 10 days, one on Earth (ground) and one onboard the ISS (flight). The ground session was conducted within the year before flying, while astronauts were at the European Astronaut Center (EAC) in Cologne, Germany. On average, the mean delay between ground measurements and the flight was 99 days (standard deviation (SD) 78). The astronauts were deemed in energy balance the year before launch (see Fig. 1a and OSM Methods). The flight session was conducted after at least 3 months in space and before the last month onboard the ISS. This 3- to 5-month window was selected to provide data for long-term spaceflights while avoiding stress associated with the preparation for return to Earth. On average, flight sessions were conducted after 108 days (SD 19) in space.

Ground and flight experiments, strictly similar, were realized under the supervision of ESA and French Space Agency (CNES) science officers in charge of the experiment and the investigators from Toulouse Space Center (CADMOS, France), and were preceded by dry runs of the experiments conducted at EAC. BM and body composition, TEE and its components (resting metabolic rate (RMR) and AEE) and physical activity (PA) were measured. Additionally, postflight BM and composition were measured on the ground after landing.

2.3 Total Energy Expenditure and its Components

TEE was measured over 10 days using the doubly-labeled water (DLW) method as previously described [16]. The DLW is the gold standard to assess TEE in free living conditions. The technique is fully described in OSM Methods. Briefly, it is based on the exponential elimination of the stable isotopes ^{2}H and ^{18}O after a bolus dose of water labeled with both isotopes. The ^{2}H are lost as water, whereas the ^{18}O are lost both as water and as CO_2. Thus, the excess disappearance rate of ^{18}O relative to ^{2}H is a measure of the CO_2 production rate. This latter is converted to TEE using the food quotient or the respiratory quotient and the classic indirect calorimetry equations. On the DLW dosing day, RMR was measured in fasting state, at rest for 45 min using the Pulmonary Function System (PFS, manufactured by the Danish Aerospace Company, former DAMEC) [17]. AEE was calculated from TEE and RMR assuming a diet-induced thermogenesis of 10% of TEE. PA level (PAL) was calculated as the ratio between TEE and RMR.

2.4 Body Composition

Pre- and post-flight ground BM measures were obtained during NASA medical operations with a calibrated scale at EAC, and fat-free mass (FFM) and FM by dual X-ray energy absorptiometry (DXA, Hologic, Marlborough, MA, USA; software version 12.7.3.1 in 2011 to 15.5.3 in 2016). Inflight BM was measured using the SLAMMD (Space Linear Acceleration Mass Measurement Device) within 1 day of the DLW dosing day, and FFM and FM were obtained from the DLW method. BM, FFM and FM indexes (BMI, FFMI, FMI), were calculated by dividing BM, FFM and FM (in kg) by squared height (in squared m).

2.5 Energy Intake

Inflight mean daily EI, expressed in MJ/day, was calculated from the changes in body composition and TEE between the preflight and inflight DLW sessions deeming astronauts were in stable energy balance on ground measurements [18], and compared with theoretical EI calculated from the 2001 Dietary Reference Intakes (DRI) [19]. Details are provided in the OSM.

2.6 Physical Exercise and Activities

Physical training sessions were composed of both aerobic and resistive exercises and prescribed 6 days/week, as detailed in OSM Methods. Briefly, 30–45 min of aerobic exercise per session were prescribed on the cycle ergometer (CEVIS) or on a treadmill (T2) with vibration isolation system (OSM Fig. 4) [20, 21]. During treadmill exercise, an external vertical load (60–100% of BM) was applied to the astronauts to partially compensate for the absence of body weight in microgravity environment. The resistance exercise program consisted of three to three workouts per session that

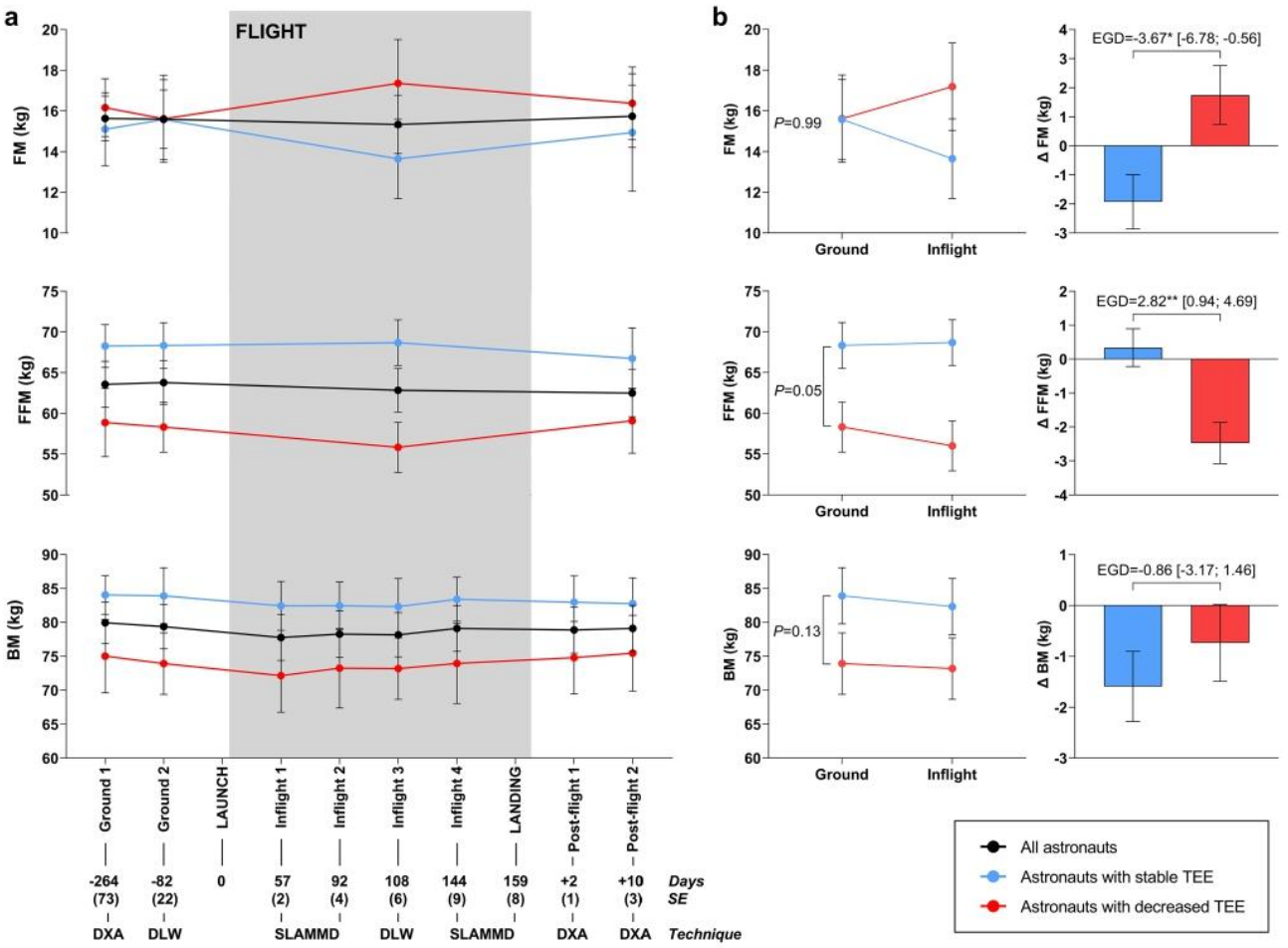


Fig. 1 Body mass and composition changes during flight. Changes in body mass and composition throughout the whole mission (**a**) and during the experimental sessions (**b**). Values are means (SE) (**a**), and lsmeans (SE) (**b**) from mixed-effects models for repeated measurements with estimated group differences (EGD) presented with their 95% confidence interval; $*P<0.05$; $**P<0.01$. *BM* body mass, *FFM* fat-free mass, *FM* fat mass, *DXA* dual X-ray absorptiometry, *SLAMMD* Space Linear Acceleration Mass Measurement Device, *DLW* doubly-labeled water

were performed on the advanced resistive exercise device (ARED) (OSM Fig. 4). Each workout was composed of five to seven exercises primarily focused on the lower body using 8–15 repetitions for 3–4 sets. Loads were individually adapted by the personal exercise trainers throughout the flight. Parameters of the exercise training actually performed during the spaceflight were obtained from the exercise dairy logs. Overall PA was further evaluated from a Sensewear Pro (SWP) activity monitor (Body media Inc®, Pittsburgh, PA, USA).

Self-reported exercise logs were provided by NASA. These logs included date and duration of each aerobic exercise session, the average speed and external load for T2, and average power and revolution per minute for CEVIS. For ARED, they included date and detailed exercise workouts of each resistive exercise session, with number of sets and repetitions, and loads. Mean daily and weekly exercise parameters were calculated over a period beginning 2 months before the experimental flight session (details in OSM files). An estimation of T2 power was derived from speed and external load from the American College of Sports Medicine (ACSM) formulas [22], and aerobic exercise workloads were calculated as power*duration. Resistive exercise workloads were calculated as sets*repetitions*loads [23]. Because the duration for the resistive exercise sessions was not provided, muscular time under tension was estimated from the number of repetitions, assuming a 2- to 5-s count for each repetition (i.e., including both concentric and eccentric phases), according to movement complexity from online video featuring this kind of exercise [24]. An additional 1-min resting time by set was added to approximate time involved with exercise on ARED. The goal of this calculation was to allow a comparison with the SWP-derived data.

The SWP, an activity multi-sensor armband that includes a bi-axial accelerometer, was worn on the non-dominant arm throughout the two 10-day DLW sessions. Its companion software (professional version 8.0) incorporates a proprietary machine-learning activity classification algorithm based on heat flux, galvanic skin response, skin and near-body ambient temperature and accelerometry measures patterns [25]. It was used to identify non-wear periods and, after exclusion of non-valid days, to obtain steps and categorize each minute in four classes: inactivity, walking, running and all other detected activities groups as non-ambulatory PA. Inflight valid data were obtained for nine astronauts only. The norm of the 1-min acceleration signal mean amplitude deviation (MAD) was used as an additional proxy of PA workload and intensity [26]. MAD removes the static component due to gravity from the acceleration signal to keep only the dynamic component due to body movements and changes in velocity. It is therefore poorly influenced by microgravity. Aggregation of 1-min MAD during activities, a composite variable that corresponds to the product of duration by the intensity of a given effort/exercise bout or of overall daily PA, are presented in milli-g (i.e., 0.001 g). Vigorous PA (VPA) was estimated using a MAD-cutpoint of 16 milli-g/min. Details are provided in OSM Methods.

2.7 Statistical Analyses

Data were first analyzed for the entire group of astronauts ($n = 11$). To understand the inter-individual variability, astronauts were then stratified according to inflight TEE, i.e., either maintained close to preflight values (stable_TEE; $n = 6$) or decreased (decreased_TEE; $n = 5$).

Differences between the two groups stratified for inflight TEE changes at baseline and for inflight physical training were examined using unpaired Student's t tests. Linear mixed-effects models accounting for repeated measurements, with subjects as random effect and indicator for flight as fixed factor, were used to test the overall effect of spaceflight on the anthropometric, energetic and PA outcomes. The group effect (stable_TEE vs. decreased_TEE) on the flight-induced changes was tested by adding a group × flight interaction term. Our statistical inference was respectively on the overall outcome net changes and their between-group differences estimated (EGD) with their 95% confidence interval (CI). Effect size was estimated using Hedges'g, calculated as the EGD divided by the estimation of the groups' weighted pooled standard deviation, with a Hedges'g greater than 0.2, 0.5 and 0.8 indicating small, medium and large effect size, respectively [27]. TEE, AEE, EI and EB were further adjusted for mean BM, and RMR for both mean FM and mean FFM (whole group mean baseline values were used as reference values).

General linear models were used to examine the associations of (1) FFMI and FMI changes (inflight-preflight) with TEE and AEE changes (inflight-preflight), and (2) inflight FFMI and FMI with different inflight PA variables.

Baseline values and inflight physical training variables are presented as means (SD). Unless otherwise noted, results of the linear mixed-models are presented as least square means (standard error, SE). Statistical analyses were performed using SAS 9.4 (SAS Institute, Cary, NC, USA) with a significance level at 5%. Figures were realized with Prism 9 (GraphPad, San Diego, CA, USA).

3 Results

3.1 Few Spaceflight Changes for the Whole Group of Astronauts

At baseline (Table 1) astronauts had a mean age of 45.7 years (SD 7.7), a BMI of 24.3 kg/m^2 (2.1) with a normal-to-high FFMI of 19.6 kg/m^2 (1.9). They were quite active, as indicated by a TEE of 13.2 MJ/day (1.9), an AEE of 4.9 MJ/day (1.1) and a PAL of 1.90 (0.20). SWP-derived daily overall PA time was 161.8 min/day (56.3). This included 66.8 min/day (34.1) walking and 8.1 min/day (10.5) running, corresponding to a total of 10,077 steps/day (2834) and 7.4 min/day (9.2) of SWP-derived VPA.

Figure 1a presents the changes in BM and composition throughout the whole mission and Table 2 details the effect of at least 3 months in space on the main outcomes. Astronauts were in energy balance the year before launch and spaceflight had a modest impact on BM, FM and FFM. Compared to preflight values, BM decreased by 1.20 kg (SE 0.50; $P = 0.04$) mainly due to a non-significant reduction in FFM of 0.94 kg (0.59; $P = 0.14$).

No significant overall changes in TEE, RMR and AEE were noted between preflight and inflight (Table 2). However, inflight SWP-derived ambulatory activities dramatically dropped with daily steps decreasing by 6,583 steps/day (SE 730; $P < 0.001$) and walking time by 63.2 min/day (SE 11.5; $P < 0.001$). While SWP-derived running time remained stable at 14.4 min/day (SD 6.4), in agreement with the 13.3 min/day (SD 4.6) of self-reported time spent exercising on the T2, this reduction in ambulatory activities was almost fully compensated by an increase in SWP-derived non-ambulatory activities of 64.8 min/day (SE 18.8; $P < 0.01$). This explained the absence of significant net changes in SWP-derived overall active time and accelerometry MAD, a proxy of overall PA workload. Daily SWP-derived VPA was not significantly different from ground values. The 1-h increase in non-ambulatory activities, leading to a total of 151.8 min/day (SE 18.9; range 119.7–194.5), was only partially explained by the

Table 1 Astronauts' baseline characteristics for the whole group and by inflight-total energy expenditure (TEE)-changes groups

	All astronauts ($n = 11$)	Astronauts with stable TEE ($n = 6$)	Astronauts with decreased TEE ($n = 5$)	Group difference *P* value[b]
Age and anthropometry				
Age (years)	45.7 (7.7)	43.5 (8.7)	48.4 (6.0)	0.32
Height (cm)	180.3 (6.7)	182.0 (5.7)	178.3 (7.9)	0.39
BM (kg)	79.4 (10.6)	83.9 (8.0)	73.9 (11.6)	0.13
BMI (kg/m^2)	24.3 (2.1)	25.3 (1.4)	23.2 (2.4)	0.10
FFM (kg)	63.8 (8.8)	68.3 (6.0)	58.3 (8.9)	0.05
FFMI (kg/m^2)	19.6 (1.9)	20.6 (1.5)	18.3 (1.6)	0.03
FM (kg)	15.6 (3.9)	15.57 (4.5)	15.6 (3.5)	0.99
FMI (kg/m^2)	4.8 (1.0)	4.7 (1.2)	4.9 (1.0)	0.71
Energetics				
TEE (MJ/day)	13.2 (1.9)	14.0 (1.4)	12.2 (2.1)	0.13
TEE_{BM} (MJ/day)[a]	13.2 (1.0)	13.3 (1.2)	13.0 (1.3)	0.70
RMR (MJ/day)	7.0 (1.2)	7.4 (1.4)	6.6 (0.8)	0.32
$RMR_{FFM\ \&\ FM}$ (MJ/day)[a]	7.0 (1.0)	7.0 (1.3)	7.0 (1.4)	0.93
AEE (MJ/day)	4.9 (1.1)	5.2 (1.0)	4.4 (1.1)	0.24
AEE_{BM} (MJ/day)[a]	4.9 (0.9)	5.0 (1.1)	4.7 (1.1)	0.71
PAL	1.90 (0.20)	1.94 (0.26)	1.85 (0.09)	0.50
Energy intake (MJ/day)	13.2 (1.9)	14.0 (1.4)	12.2 (2.1)	0.13
Energy $intake_{BM}$ (MJ/day)[a]	13.2 (1.1)	13.3 (1.2)	13.0 (1.3)	0.70
SWP-derived physical activities				
Steps (number/day)	10,077 (2834)	1046 (2816)	9614 (3111)	0.65
Overall activity and exercise (min/day)	161.8 (56.3)	164.8 (67.5)	158.3 (47.0)	0.86
Walking (min/day)	66.8 (34.1)	60.2 (27.1)	74.6 (43.1)	0.53
Running (min/day)	8.1 (10.5)	13.7 (11.5)	1.5 (2.8)	0.04
Non-ambulatory activity and exercise (min/day)	87.9 (63.4)	90.9 (61.7)	82.3 (72.4)	0.84
Vigorous physical activity (MAD > 16 milli-g; min/day)	7.4 (9.2)	12.4 (9.8)	1.3 (2.6)	0.04
Accelerometry MAD				
Daily total (milli-g/day)	1353 (278)	1487 (309)	1193 (124)	0.08
Overall activity and exercise (milli-g/day)	692 (313)	814 (368)	546 (164)	0.17
Walking (milli-g/day)	286 (171)	250 (104)	329 (235)	0.47
Running (milli-g/day)	188 (242)	322 (259)	27 (54)	0.04
Non-ambulatory activity and exercise (milli-g/day)	218 (141)	241 (151)	190 (140)	0.58
Overall activity and exercise mean intensity (milli-g/min)	4.3 (1.1)	4.9 (0.9)	3.5 (0.9)	0.03

Values are means (SD)

Physical activities were derived from a combination of different physiological signals with an in-built machine-learning activity classification algorithm

BMI body mass index, *FM* fat mass, *FFM* fat-free mass, *FMI* fat mass index, *FFMI* fat-free mass index, *TEE* total energy expenditure, *RMR* resting metabolism rate, *AEE* activity energy expenditure, *EI* energy intake, *MAD* acceleration mean absolute deviation, *SWP* Sensewear Pro activity-device

[a]BM (TEE, AEE, EI) or FFM and FM (RMR) adjusted lsmeans (SD) (whole group mean baseline values were used as reference values). All astronauts were male

[b]Statistical analyses used Student's *t* tests

10.7 min/day (SD 4.4) spent on the CEVIS and the about 29.0 min/day (SD 14.3) on the ARED as reported by the astronauts (OSM Table 1). This suggested an increase in PA not related to physical training. Overall, the astronauts reported 6.2 aerobic exercise sessions/week (SD 1.4; range 3.6–8.6) corresponding to a total duration of 167.8 min/week (SD 43.9; range 86.9–257.8). They reported 4.5 resistance training sessions/week (SD 1.8; range 1.8–6.6), consisting of 5.9–10.2 exercises using 8.8–15.1 repetitions for 2.2–3.2 sets. This led to a total of 1481 repetitions/week (SD 834;

Table 2 Astronauts' anthropometric, energetic and physical activity changes between preflight and flight for the whole group and by inflight-total energy expenditure (TEE)-changes groups

	All astronauts ($n=11$)		Astronauts with stable TEE ($n=6$)	Astronauts with decreased TEE ($n=5$)	Linear mixed model analyses		
	Changes from preflight	*P* value	Changes from preflight	Changes from preflight	EGD in changes from preflight	95% CI	*P* value
Age and anthropometry							
BM (kg)	− 1.20 (0.50)	0.04	− 1.59 (0.69)	− 0.74 (0.75)	− 0.86 (1.02)	− 3.17 to 1.46	0.42
BMI (kg/m^2)	− 0.39 (0.17)	0.04	− 0.49 (0.24)	− 0.28 (0.26)	− 0.22 (0.35)	− 1.01 to 0.57	0.55
FFM (kg)	− 0.94 (0.59)	0.14	0.34 (0.56)	− 2.48 (0.61)	2.82 (0.83)	0.94 to 4.69	< 0.01
FFM index (kg/m^2)	− 0.29 (0.18)	0.13	0.10 (0.16)	− 0.76 (0.18)	0.87 (0.24)	0.32 to 1.41	< 0.01
FM (kg)	− 0.26 (0.87)	0.77	− 1.93 (0.93)	1.74 (1.02)	− 3.67 (1.38)	− 6.78 to − 0.56	0.03
FM index (kg/m^2)	− 0.11 (0.27)	0.70	− 0.60 (0.29)	0.49 (0.32)	− 1.09 (0.43)	− 2.06 to − 0.11	0.03
Energetics							
TEE_{BM} (MJ/day)[a]	− 0.39 (0.73)	0.60	0.90 (0.37)	− 2.08 (0.40)	2.98 (0.54)	1.75 to 4.22	< 0.001
$RMR_{FFM\ \&\ FM}$ (MJ/day)[a]	− 0.15 (0.19)	0.43	− 0.35 (0.28)	0.11 (0.33)	− 0.46 (0.48)	− 1.51 to 0.59	0.36
AEE_{BM} (MJ/day)[a]	− 0.19 (0.70)	0.79	1.12 (0.36)	− 1.90 (0.39)	3.02 (0.53)	1.81 to 4.23	< 0.001
PAL	− 0.02 (0.10)	0.86	0.23 (0.08)	− 0.31 (0.09)	0.54 (0.12)	0.28 to 0.80	0.001
Energy intake (MJ/day)	− 0.82 (0.38)	0.06	0.06 (0.34)	− 1.86 (0.37)	1.92 (0.50)	0.78 to 3.05	< 0.01
Energy $intake_{BM}$ (MJ/day)[a]	− 0.61 (0.40)	0.16	0.29 (0.33)	− 1.75 (0.36)	2.04 (0.48)	0.96 to 3.13	< 0.01
Energy balance (MJ/day)	− 0.15 (0.29)	0.61	− 0.68 (0.33)	0.48 (0.36)	− 1.15 (0.49)	− 2.19 to − 0.12	0.03
Energy $balance_{BM}$ (MJ/day)[a]	− 0.14 (0.30)	0.65	− 0.63 (0.30)	0.50 (0.33)	− 1.13 (0.44)	− 2.06 to − 0.20	0.02
SWP-derived physical activities							
Steps (number/day)	− 6583 (730)	< 0.001	− 6277 (1402)	− 7231 (1554)	954 (2093)	− 3483 to 5391	0.66
Overall activity and exercise (min/day)	13.2 (23.8)	0.59	24.4 (33.2)	− 0.9 (36.8)	25.2 (49.6)	− 79.9 to 130.3	0.62
Walking (min/day)	− 63.2 (11.5)	< 0.001	− 57.3 (16.0)	− 70.1 (17.7)	12.8 (23.9)	− 37.8 to 63.4	0.60
Running (min/day)	6.3 (4.0)	0.13	4.4 (4.5)	8.5 (5.0)	− 4.1 (6.7)	− 18.3 to 10.1	0.55
Non-ambulatory activity and exercise (min/day)	64.8 (18.8)	< 0.01	77.3 (36.3)	60.8 (40.2)	16.5 (54.2)	− 98.3 to 131.4	0.76
Vigorous physical activity (MAD > 16 milli-g; min/day)	2.8 (2.1)	0.23	4.3 (4.2)	3.3 (4.6)	1.0 (6.2)	− 12.2 to 14.1	0.88
Accelerometry MAD							
Daily total (milli-g/day)	− 40.8 (74.7)	0.60	12.6 (147.3)	− 67.9 (163.1)	80.5 (219.8)	− 385.4 to 546.4	0.72
Overall activity and exercise (milli-g/day)	11.1 (93.2)	0.91	70.2 (169.0)	− 40.4 (187.2)	110.7 (252.2)	− 424.0 to 645.4	0.67
Walking (milli-g/day)	− 264.6 (57.3)	< 0.001	− 235.0 (79.4)	− 299.9 (87.9)	64.9 (118.5)	− 186.2 to 316.1	0.59
Running (milli-g/day)	113.2 (73.8)	0.16	138.4 (118.3)	127.5 (131.1)	10.9 (176.6)	− 363.4 to 385.3	0.95
Non-ambulatory activity and exercise (milli-g/day)	151.8 (60.9)	0.02	166.8 (83.8)	132.0 (92.9)	34.8 (125.1)	− 230.4 to 300.0	0.78

Table 2 (continued)

	All astronauts ($n=11$)		Astronauts with stable TEE ($n=6$)	Astronauts with decreased TEE ($n=5$)	Linear mixed model analyses		
	Changes from preflight	*P* value	Changes from preflight	Changes from preflight	EGD in changes from preflight	95% CI	*P* value
Overall activity and exercise mean intensity (milli-g/min)	− 0.23 (0.35)	0.53	− 0.32 (0.55)	− 0.16 (0.61)	− 0.16 (0.82)	− 1.90 to 1.58	0.84
Activity NASA logs[c]							
T2 (min/day)	13.3 (1.4)		15.7 (1.6)	10.4 (1.7)	− 5.3 (2.3)	− 10.5 to − 0.1	0.04
CEVIS (min/day)	10.7 (1.3)		10.0 (1.9)	11.4 (2.1)	1.4 (2.8)	− 4.9 to 7.7	0.63
ARED (min/day)[d]	29.0 (4.3)		30.9 (6.1)	26.8 (6.7)	− 4.1 (9.0)	− 24.5 to 16.4	0.66

Physical activities were derived from a combination of different physiological signals with an in-built machine-learning activity classification algorithm

BMI body mass index, *FM* fat mass, *FFM* fat-free mass, *FMI* fat mass index, *FFMI* fat-free mass index, *TEE* total energy expenditure, *RMR* resting metabolism rate, *AEE* activity energy expenditure, *EI* energy intake, *EB* energy balance, *MAD* acceleration mean absolute deviation, *T2* treadmill device onboard the ISS, *CEVIS* Cycle Ergometer with Vibration Isolation and Stabilization system, *ARED* Advanced Resistive Exercise Device, *SWP* Sensewear Pro device

Values are estimated Lsmeans (SE) from a mixed-effects models for repeated measurements

[a]BM (TEE, AEE, EI, EB) or FFM and FM (RMR) adjusted models (whole group mean baseline values were used as reference values)

[c]Inflight values

[d]ARED duration was approximate from number of repetitions and sets (2-to5 sec/repetition, according to movement complexity, with addition of 1-min recuperation time by set)

range 468–3,044) and a muscular time under tension of 81.8 min/week (SD 43.5; range 28.8–161.2). These numbers exhibit a great inter-individual variability in the adherence to the exercise prescriptions. They are to be compared to the 180–270 min/week aerobic, and six resistive exercise sessions/week (corresponding to about 1500 repetitions/week) recommendations [21].

The astronauts were stratified into two groups, according to their inflight TEE changes, i.e., no change in TEE or decrease in TEE compared to preflight values. Fortuitously, the two groups ended up having an almost equal number of subjects.

3.2 Baseline Characteristics of Inflight-Total Energy Expenditure (TEE)-Changes Groups

At baseline (Table 1), both groups were of the same age and had similar FMI but, compared to the decreased_TEE group, the stable_TEE astronauts had higher FFMI (20.6 kg/m^2 [SD 1.5] vs. 18.3 kg/m^2 [1.6]; $P=0.03$) and presented a more active profile. TEE, AEE and daily SWP-derived overall active time did not differ between the two groups. However, the stable_TEE astronauts spent more time on ground in SWP-derived VPA (12.4 min/day [9.8] vs. 1.3 min/day [2.6]; $P=0.04$) and running ($P=0.04$), and had higher running-related MAD ($P=0.04$) resulting in greater SWP-derived mean activity intensity (4.9 milli-g/min [0.9] vs. 3.5 milli-g/min [0.9]; $P=0.03$) than the decreased_TEE astronauts.

3.3 Anthropometric Changes by Inflight-TEE-Changes Groups

Compared to preflight, both groups lost equivalent BM, but contrasted changes in body composition were noted (Table 2 and Fig. 1a, b). While the stable_TEE group maintained FFMI and displayed a slight FMI loss, their counterparts lost FFMI and gained FMI. The EGD for FFMI changes (stable_TEE compared to decreased_TEE group) was + 0.87 kg/m^2 (95% CI 0.32–1.41; effect size = 1.7; $P<0.01$); and the EGD for FMI changes − 1.09 kg/m^2 (95% CI -2.06 – -0.11; effect size = 1.2; $P=0.03$).

3.4 Energy Expenditure Changes by Inflight-TEE-Changes Groups

The EGD for TEE changes adjusted for BM was + 2.98 MJ/day (95% CI 1.75–4.22; effect size = 2.6; $P<0.001$), mainly related to differences in AEE changes (BM-adjusted EGD + 3.02 MJ/day, 95% CI 1.81–4.23; effect size = 2.7; $P<0.001$) while RMR remained stable in both groups (Table 2 and Fig. 2a). As illustrated in Fig. 2b, the individual FMI net changes were negatively associated with net

changes in TEE ($R^2 = 0.58$; $P < 0.01$) and AEE ($R^2 = 0.54$; $P = 0.01$) while net changes in FFMI were positively associated with net changes in TEE ($R^2 = 0.64$; $P < 0.01$) and, to a lesser degree, net changes in AEE ($R^2 = 0.47$; $P = 0.02$).

3.5 Physical Activity Changes by Inflight-TEE-Changes Groups

Net changes in SWP and accelerometry-derived activity parameters (Table 2 and Fig. 3a) were globally similar across groups. However, as what was observed on the ground, the stable_TEE astronauts still spent more time inflight in SWP-derived VPA (16.7 min/day [SE 3.1] vs. 4.6 min/day [3.4]; $P = 0.02$), had higher overall-activity accelerometry MAD (884 milli-g/day [125] vs. 505 milli-g/day [140]; $P = 0.06$), and SWP-derived mean activity intensity (4.60 milli-g/min [0.41] vs. 3.35 milli-g/min [0.45]; $P = 0.06$) than the decreased_TEE group. Reported inflight resistance training characteristics and time spent performing CEVIS were not significantly different between groups (OSM Table 1). The stable_TEE group reported non-significant higher inflight aerobic workloads than the decreased_TEE group ($P = 0.10$), due to more time spent on T2 (15.7 min/day [SD 3.0] vs. 10.4 min/day [4.6]; $P = 0.05$) but also to higher average speeds (11.2 [0.9] vs. 8.5 [2.1] km/h; $P = 0.02$). This was in good agreement with higher SWP-derived running-times (18.0 min/day [SE 3.3] vs. 9.9 min/day [3.7]; $P = 0.12$) and running-related accelerometry MAD (461 milli-g/day [SE 87] vs. 154 milli-g/day [98]; $P = 0.03$).

As illustrated in Fig. 3b, after at least 3 months on the ISS, individuals' inflight FMI were inversely associated with SWP-derived overall activity-related accelerometry MAD ($R^2 = 0.73$; $P < 0.01$), time spent in SWP-derived VPA ($R^2 = 0.56$; $P = 0.02$), and self-reported T2 relative workload ($R^2 = 0.71$; $P = 0.001$). Conversely FFMI values were positively associated with SWP-derived overall activity-related accelerometry MAD ($R^2 = 0.48$; $P = 0.04$), time spent in SWP-derived VPA ($R^2 = 0.62$; $P = 0.01$) and self-reported T2 relative workload ($R^2 = 0.40$; $P = 0.04$). Practice of ARED and CEVIS was not associated with any body composition parameter.

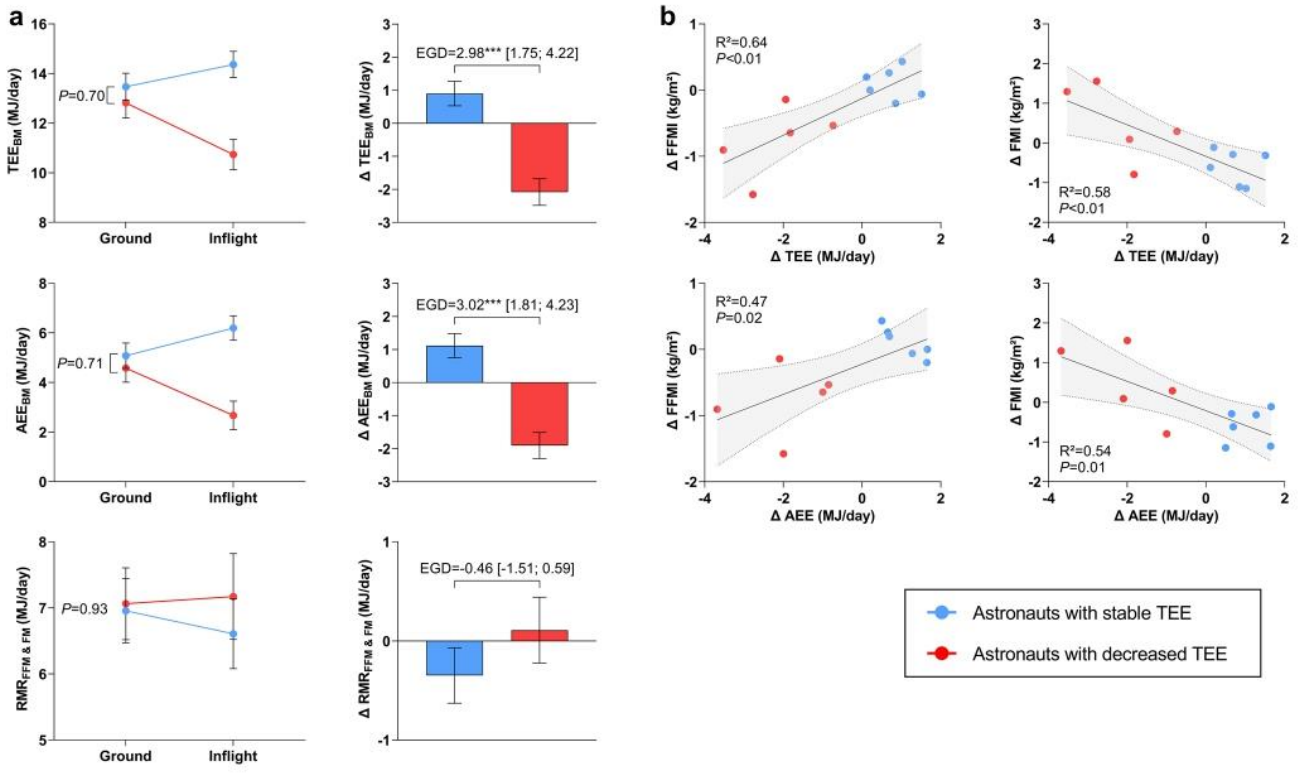
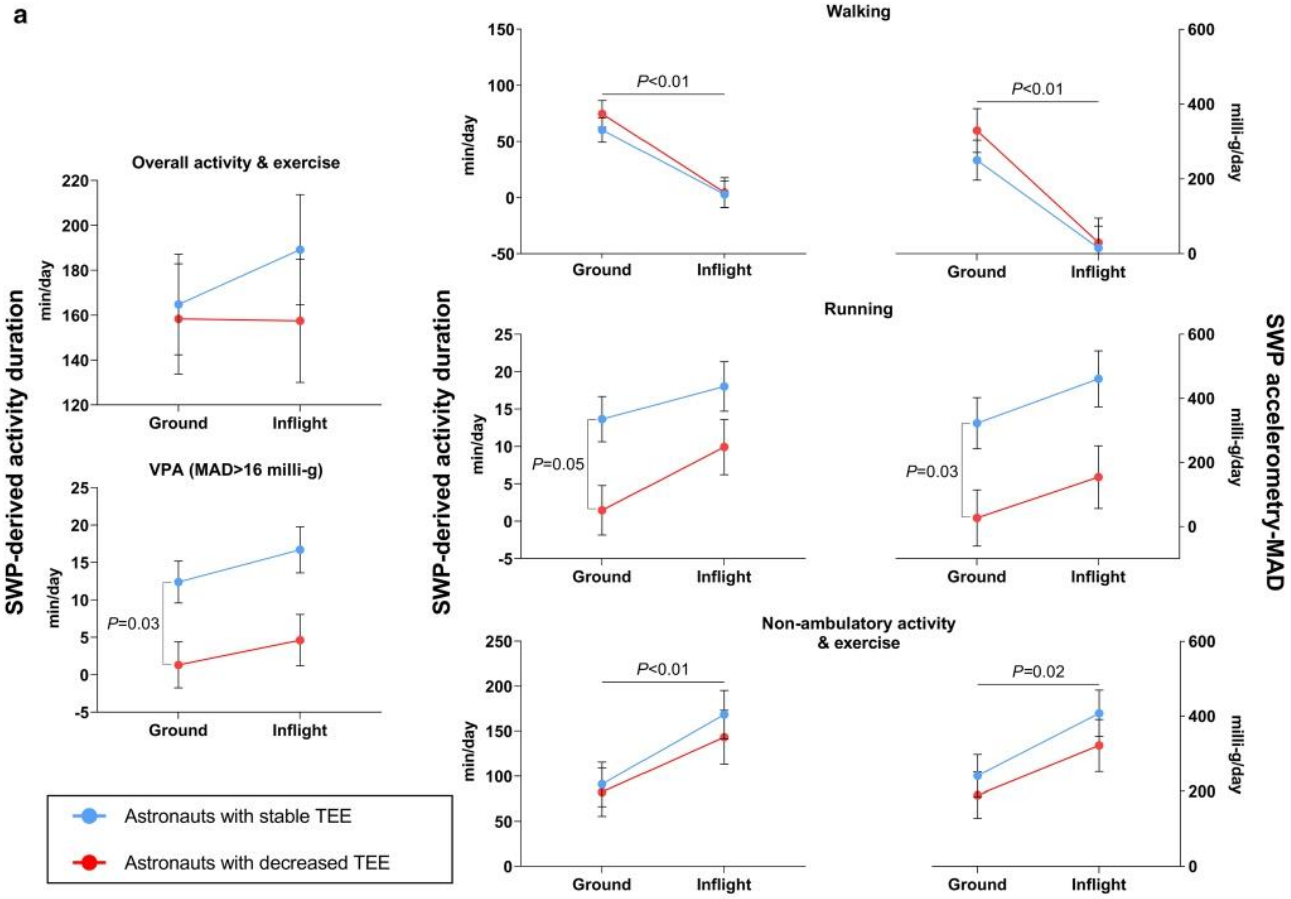
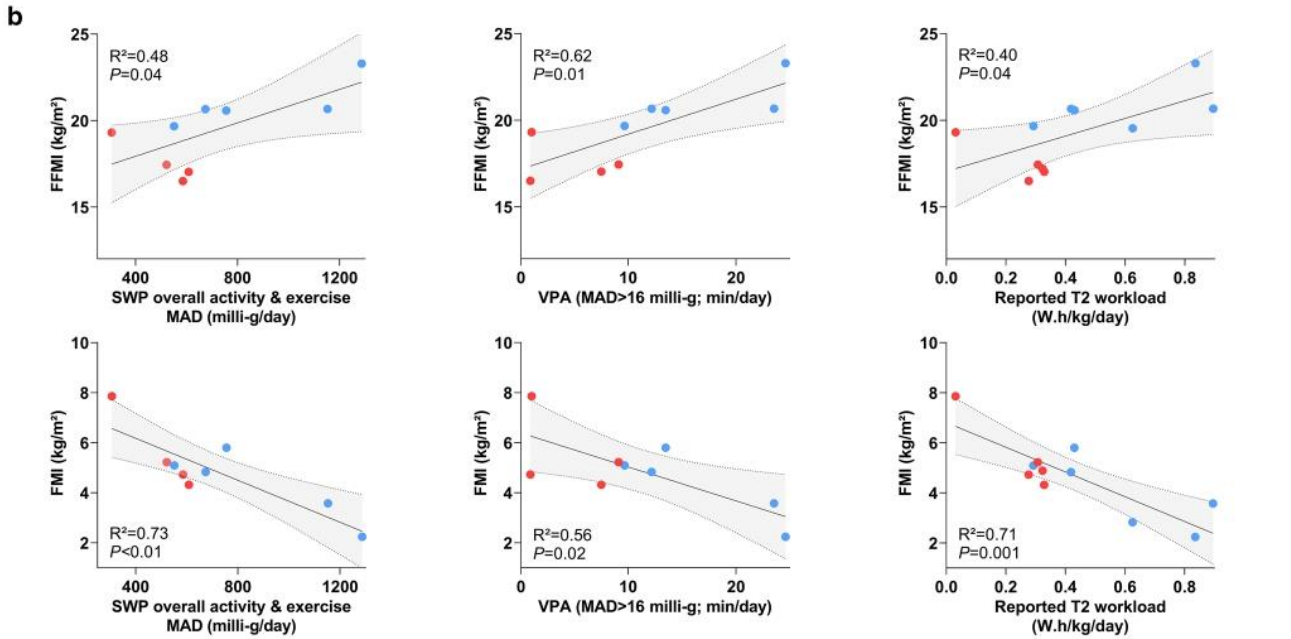


Fig. 2 Energy expenditure changes during flight. Changes in total energy expenditure and its components during the experimental sessions (**a**) and scatterplots for the relationship between body composition changes and energy expenditure components changes (**b**). Values are lsmeans (SE) from mixed-effects models for repeated measurements with estimated group differences (EGD) presented with their 95% confidence interval; ***$P < 0.001$ (**a**). Least square regression lines are plotted with their 95% confidence interval in shaded areas (**b**). TEE_{BM} total energy expenditure adjusted for body mass, AEE_{BM} activity-related energy expenditure adjusted for body mass, $RMR_{FFM\ \&\ FM}$ resting metabolic rate adjusted for fat-free mass and fat mass, *FFMI* fat-free mass index, *FMI* fat mass index


a
SWP-derived activity duration
SWP accelerometry-MAD
Overall activity & exercise
min/day
Ground
Inflight
VPA (MAD>16 milli-g)
P=0.03
Walking
P<0.01
milli-g/day
Running
P=0.05
P=0.03
Non-ambulatory activity & exercise
P<0.01
P=0.02
Astronauts with stable TEE
Astronauts with decreased TEE


b
FFMI (kg/m²)
FMI (kg/m²)
R²=0.48
P=0.04
R²=0.62
P=0.01
R²=0.40
P=0.04
R²=0.73
P<0.01
R²=0.56
P=0.02
R²=0.71
P=0.001
SWP overall activity & exercise MAD (milli-g/day)
VPA (MAD>16 milli-g; min/day)
Reported T2 workload (W.h/kg/day)

◂**Fig. 3** Physical activity changes during flight. Changes in SWP-derived activity duration and acceleration-MAD (as a proxy of activity workload) during the experimental sessions (**a**) and scatterplots for the relationship between inflight body composition and inflight SWP-derived or reported physical activity (**b**). Values are lsmeans (SE) from mixed-effects models for repeated measurements (**a**). Least square regression lines are plotted with their 95% confidence interval in shaded areas; individual values are available for nine astronauts only (**b**). *FFMI* fat-free mass index, *FMI* fat mass index, *T2* treadmill device onboard the International Space Station, *SWP* Sensewear Pro activity monitor, *MAD* acceleration mean amplitude deviation, *VPA* vigorous physical activity (MAD > 16 milli-g)

3.6 Energy Intakes by Inflight-TEE-Changes Groups

EI (BM-adjusted) calculated from changes in body composition from the time of beginning of spaceflight to the time of starting the inflight TEE measurement was 13.6 MJ/day (SE 0.5) in the stable_TEE group and 11.3 MJ/day (0.6) in the decreased_TEE group, representing 93% and 75% of DRIs, respectively (Fig. 4).

4 Discussion

The aim of the ENERGY study was to assess the regulation of energy balance and body composition during 6- month missions onboard the ISS by specifically considering the energy cost of PA using the gold standard DLW method. Despite no significant overall changes in body composition and energy expenditures, a slight decrease in BM (-1.5%) was observed in the whole group of astronauts. A large inter-individual variability was, however, noticed. Astronauts who maintained pre-flight total and activity energy expenditures kept fat-free mass at baseline levels but lost fat mass. Conversely, those who expended less energy during the flight than on the ground lost fat-free mass and gained fat mass. Astronauts who maintained stable TEE during the flight spent more time inflight running and engaged in VPA, and were also the ones with the best fitness on the ground. These results suggest that (1) inflight AEE, possibly due to both physical training and non-exercise PA, drives inflight body composition regulation; (2) inflight energy requirements should be individually evaluated during the whole duration of the spaceflight; (3) baseline participants' characteristics need to be considered for the prescription of both preflight and inflight exercise training; and (4) fat mass changes result from unmatched spontaneous EI adaptation to changes in AEE in space that require further investigation.

4.1 Ground Fitness and Physical Acdtivity (PA) Performance Influence Changes in Body Composition During Spaceflight

After at least 3 months onboard the ISS, the 11 astronauts presented a slight BM loss, mainly due to a reduction in FFM, despite no significant changes in TEE. The modest effect of spaceflight on BM and body composition in the whole group masked a large interindividual variability with half of the group of astronauts who maintained a stable FFM favoring a slight BM loss (stable_TEE group), while the other half displayed a FFM loss but an increase in FM (decreased_TEE group). This between-subject variability seemed to be associated with heterogeneity in astronauts' ground body composition and engagement in exercise of high intensity [15]. Although all the astronauts were quite fit and active on the ground, those who maintained their FFM during the flight had higher FFMI and spent more time running or in VPA on Earth. These results are in line with the study of Matsumoto et al. [6] that showed habitual PA on Earth was a better predictor of inflight BM loss than PA performed during spaceflight. Whether habitual ground activity levels affect inflight body mass and composition directly or are due to greater engagement of fitter astronauts in exercise during the mission requires further investigations. Of interest, ground differences in SWP-derived VPA and running persisted during the flight with a significant reduction in inflight energy expenditures in the astronauts who were the least active on Earth, but not in the others. We also observed significant associations of inflight self-reported running or SWP and accelerometry proxies of PA workload and intensity with body composition. While improvement of exercise hardware capabilities and prescription have led to better post-flight performances, predicting appropriate inflight exercise loads remains difficult because of the difference in training environment (gravity vs. microgravity) and hardware [20, 21]. Beyond their energetics implications, our findings indicate that gaining a better understanding of the associations between ground and inflight exercise characteristics may help designing preflight and inflight prescriptions that favor adherence and enhance inflight performance including for the occupation-related astronaut tasks. Altogether these data suggest that pre-flight astronauts' individual anthropometric and fitness characteristics require more attention for onboard exercise prescriptions and EI recommendations.

4.2 Does the Exercise Countermeasure Play a Role in the Regulation of Body Composition?

The strong associations of TEE and AEE changes with FFMI and FMI changes suggest that activity-related energy expenditure, in interaction with microgravity exposure, plays a role in inflight body composition changes. In the absence of gravity, TEE is driven by the exercise countermeasure and intra- and extra-vehicular activities while energy expenditure related to standing posture and weight-bearing muscle activity is suppressed. For the first time, SWP and accelerometry used in this study gave a more detailed insight into onboard overall PA patterns. It clearly highlighted the

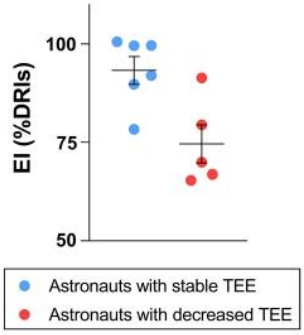


Fig. 4 Estimated energy intakes during flight. Percentage of energy intake (EI) estimated from the changes in body composition and total energy expenditure between the preflight and inflight experimental sessions from the 2001 Dietary Reference Intakes (DRIs) [19] for each groups stratified for inflight TEE changes. Values are individual data and means (SE)

expected decrease in ambulatory activities, except for running activity performed on the T2 treadmill. Contrary to our expectations, averaged overall PA time and workload, as estimated by SWP and accelerometry MAD, were not reduced on the ISS; the decrease in ambulatory activities was compensated by non-ambulatory activities. Of interest, accelerometry MAD removes from accelerometry signal any static gravitation component [26] and SWP utilizes the signature of several physiological systems (temperature, sweat, and heat flux and dissipation) in addition to accelerometry, which allows for the disambiguation of activities and contexts (e.g., microgravity) that may confuse a single sensor [25]. Inflight non-ambulatory activities (almost 2.5 h/day) can only be partially explained by the CEVIS and ARED exercises, which represent about 40 min/day altogether. This suggests that routine and off-nominal mission tasks may be another important component of overall PA. Unfortunately, the identification of specific onboard activities based on the SWP other than walking and running, was not possible due to the arm-placement of the device and the absence of activity learning studies in microgravitational conditions.

Mean self-reported training exercises were slightly lower than the recommendations. This was in agreement with previous observations made on the ISS [28]. The lower levels of aerobic exercise reflect a recent tendency to favor resistive exercise for its effect on muscle and bone mass and strength [29, 30]. More importantly, a large inter-individual variability in exercise adherence was observed with some astronauts reporting no more than 87 min/week of aerobic exercise and less than 500 repetitions/week in resistive exercise with a muscular time under tension lower than 30 min/week.

Even if efficiency is lower in space than on the ground, AEE does not seem to be fully explained by exercise training inflight. Treadmill exercise, characterized by higher weekly duration and speed in the astronauts who maintained their TEE, was undoubtedly a contributor of higher AEE and was inversely associated with astronauts' FM changes. Conversely both inflight SWP-derived VPA and T2 workload were positively associated with FFM confirming the protective role of exercise intensity on muscle mass [30]. Of note, time spent on the treadmill alone did not seem to explain the between-group differences in SWP-derived VPA. This suggests that VPA may also reflect an engagement in energy-demanding mission-related activities. However, any extrapolation from SWP sensors about the exact energy cost of both non-training and specific training activities in the context of spaceflights was not possible. Although resistive exercise is considered important to prevent muscle mass and bone loss, we surprisingly did not find any relationship between ARED practice and FFM.

4.3 Energy Intakes During Long-Term Spaceflight

Even if, by reviewing data from past space missions, no association was found between inflight unadjusted EI and BM loss [6], insufficient EI has often been reported during long-term missions [7, 13]. Astronauts in this study consumed about 85% of dietary prescriptions [19], which is similar to self-reported values observed by Smith et al.[7] during past missions onboard the ISS. Hyporexia due to lower food attractivity, altered smell or taste, sickness, or other microgravity-induced disorders [8] are expected to impair the spontaneous adjustment of EI to the changes in energy needs [7, 31]. This may explain why the astronauts who maintained their TEE to preflight values lost FM despite EI close to their theoretical energy requirements. Conversely, the reduction in EI observed in the less active astronauts was expected as their TEE was reduced by about 20%, due to a decrease in AEE. However, despite EI only representing 75% of the dietary recommendations, the astronauts who displayed a decrease in TEE during spaceflight were still in positive energy and fat balance.

4.4 How to Maintain Energy Balance During Long-Term Spaceflight?

Exercise countermeasure is the cornerstone of countermeasure programs during human spaceflights. Exercise training is known to benefit several physiological systems, including muscle strength, bone density and aerobic capacity, but effects of the exercise on these health outcomes were beyond the scope of this study. Here we showed that FFM was maintained in the stable_TEE astronauts despite some of them completing less than the prescribed exercise sessions. Our results further suggest that the non-ambulatory non-training activities play a role in the regulation of energy balance. The extent to which these activities, likely related to the occupational tasks on the ISS, influence energy expenditures and

body composition remains to be addressed. For example, it is not known how it contributes to the preservation of muscle mass or other critical key performance outcomes. This finding has potential implications for future spaceflights as it calls for considering non-training activities and their associated energy cost when developing the exercise countermeasure [15], especially for long-term space missions.

This does not preclude the need for improving strategies to help astronauts to comply with the exercise prescriptions. Space agencies must find the best combination between modalities, i.e., duration and intensity for the aerobic exercise, and load and repetitions for the resistive exercise, to benefit most of the physiological systems without affecting others. For example, using high intermittent interval training (HIIT) that efficiently stimulates musculoskeletal and cardiovascular systems with a relatively low impact on energy requirements may be a promising alternative [8, 15, 23, 32]. Other countermeasure such as nutrition or artificial gravity may also be used to optimize the benefits of exercise and PA. In parallel, changes in the agenda of the astronauts could be considered to improve time dedicated to meals for the whole crew together, and food could be further improved to favor a better adjustment to actual energy needs [33, 34].

Overall, the results of this study show that both the measurement and regulation of energy balance inflight are challenging. One of the key findings is the large between-subject variability in changes in TEE, AEE and body composition. This variability was previously reported for EI. This has challenging consequences for both exercise and EI prescriptions as a complex tradeoff needs to be assessed. Such a tradeoff would need to consider both the positive impact on health and various physiological functions of high AEE along with adequate EI; but also the negative impact of too low AEE and excessive FM gain. This is why research on both exercise countermeasures and the development of loggers accurately assessing on real-time exercise, energy expenditure and body composition at the individual level is a top priority. Clearly current available devices (along with their algorithms) do not allow to do so and further interdisciplinary studies are needed.

4.5 Limitations and Strengths of the Study

Spaceflight imposes some limitations and strengths that need to be acknowledged. The sample size was relatively low, due to the limited number of inflight experiments. This study was conducted in male astronauts only, and results may not apply to women. Because spaceflights are limited, this study is one of the only three studies that determined energy requirements and body composition changes inflight using gold standard objective methods such as DLW and activity monitors, but it is the first one to focus on long-duration missions. Utilization of a unique combination of sensors for continuous physiological monitoring related to PA is a strength of the SWP device. As already underlined, the activity classification algorithms were, however, developed for detecting activities on Earth, limiting their current recognition capabilities and energy extrapolations in the context of spaceflights. Unfortunately, the R&D service of the SenseWearPro company no longer exists and the SWP software is no longer updated. The current version of the software only provides one-min aggregate measures rather than raw signals, which preludes any additional data treatment and validation. Also, heart rate monitoring, an additional valuable physiological signal in the context of exercise and energy evaluation, was available for few astronauts and during the training sessions only. On the other side, making assumptions on exercise performance based on crewmember exercise logs was challenging and not possible for non-training activities. Many of the findings are correlations, which suggests the need for future studies to prove causation.

5 Conclusion

The between-astronauts variability in body composition and energy expenditure changes observed during spaceflight was related to ground fitness, and inflight practice of activities of high intensity. Importantly, a high inter-individual variability in inflight training was noted with some crewmembers reporting values far below the recommendations, but there was an unexpected engagement in non-ambulatory activities related to the nature of the missions onboard the ISS. These results suggest that energy requirements in space must be individually derived based on real-time measurements of AEE and changes in body composition rather than on current general recommendations and exercise prescriptions. This requires validating in space the use of tri-axial accelerometry on different body parts, along with other sensors including heart rate monitors, and the development of specific algorithms to detect and quantify all physical activities and derive activity-specific energy expenditures during the space missions. Despite some spontaneous adjustments, we further observed an uncoupling between energy intakes and expenditures that led to energy imbalance. Methodological developments are therefore vital for the control of both sides of the energy balance, i.e., energy intakes and expenditures, or at least body composition evolution during long-duration space missions, which are considered a top priority for exploration by the international space agencies.

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Declarations

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Conflicts of interest PB, AZ, DSA, IC, ELR, CT, AM, MG, GGK, AB, CS and SB declare that they have no potential conflicts of interest that might be relevant to the contents of this article.

Availability of data and material Data are accessible upon reasonable request to the authors after validations by space agencies.

Ethics approval The study was approved yearly by the NASA Institutional Review Board under NASA 7116301606HR. The ESA Medical Board and the JAXA Institutional Review Board for human experiment also approved the experiment.

Consent to participate The study was conducted in conformity with the policy statement regarding the use of human participants as outlined in the Declaration of Helsinki of 1964. All astronauts received a detailed presentation of the experiment before enrolling the study and signed a written informed consent.

Consent for publication A global authorization from the European Space Agency was obtained to use and publish the photos (ESA copyright).

Author contributions SB, DAS, GGK and AM designed the study. PB, AB, CS and SB drafted the manuscript. AZ, CT, AM, GGK, CS and SB collected data. PB, ELR, AZ, DAS, IC, MG, AB, CS and SB analyzed the data. CS and PB realized the statistical analysis. All the authors read and approved the final version of the manuscript.

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Annexe 3 : Inactivité physique et sédentarité : impact sur la santé métabolique, de quoi parle-t-on ?

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Inactivité physique et sédentarité : impact sur la santé métabolique, de quoi parle-t-on ?

La pratique d'activité physique (AP) est reconnue pour ses effets bénéfiques sur l'ensemble des systèmes physiologiques. La sédentarité est un nouveau comportement qui suscite l'intérêt des chercheurs depuis une dizaine d'années et semble avoir son importance dans l'équation entre AP et santé métabolique. Réintroduire de l'AP de toute intensité tout au long de la journée serait une stratégie prometteuse.

Mots-clés :
Activité physique, Sédentarité, Inactivité physique, Diabète de type 2, Obésité, Physiologie

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Lien d'intérêt : Les auteurs déclarent ne pas avoir de lien d'intérêt en relation avec le sujet de l'article.

Introduction

Les bénéfices de l'activité physique (AP), définie comme "tout mouvement corporel produit par la contraction des muscles squelettiques entraînant une augmentation de la dépense énergétique par rapport à la dépense énergétique de repos" (1), sur la santé ne sont plus à prouver. Dès le Vème siècle avant JC, Hippocrate déclare que "manger seul ne gardera pas un homme en bonne santé ; il doit également faire de l'exercice". C'est en 1953 que la première étude révélant un lien entre le manque d'AP et le risque de développer des maladies coronariennes a été publiée (2). Être actif améliore entre autres les capacités cardiorespiratoires, l'endurance et la force musculaire, la santé cardiométabolique, les capacités cognitives, la santé mentale et le bien-être. Malgré ce constat indéniable, les révolutions industrielles, technologiques et numériques ont conduit depuis la fin du XIXème siècle à une diminution progressive mais drastique de l'AP dans toutes les sphères du quotidien: activités professionnelles (secteur tertiaire, ordinateur, machines), domestiques (machine à laver, lave-vaisselle), de loisirs (jeux vidéo, Internet, télévision) et lors de déplacements (véhicules motorisés). L'inactivité physique est quant à elle définie comme le fait de ne pas atteindre les recommandations actuelles pour la pratique de l'AP établies par l'Organisation Mondiale de la Santé (OMS) à savoir au minimum 150 min/semaine d'AP modérée c'est-à-dire entraînant une dépense énergétique comprise entre 3 et 5,9 METs (Metabolic Equivalent of Task) (ex : marche rapide, jogging, gymnastique, randonnée, etc.) ou 75 min/semaine d'AP d'intensité vigoureuse définie comme une activité associée à une dépense énergétique supérieure à 6,0 METs (ex: course à pied, natation, vélo sur route à vitesse soutenue, etc.) ou une combinaison des deux (3). Il est maintenant établi que l'inactivité physique augmente le risque de développer de nombreuses maladies chroniques. En parallèle à la diminution du niveau d'AP, un nouveau comportement à risque pour la santé a été identifié par les scientifiques depuis une dizaine d'année : les comportements sédentaires qui sont définis comme "tout comportement éveillé caractérisé par une dépense énergétique <1,5 MET, comme la position assise ou allongée" (4). La sédentarité a aussi été reconnue plus récemment comme un facteur de risque majeur de nombreuses maladies chroniques.

Ces modes de vie inactifs et sédentaires ont été particulièrement favorisés ces derniers mois avec la crise sanitaire liée au virus SARS-CoV-2. Une étude internationale révèle que pendant les confinements, le nombre de minutes d'AP (toute intensité confondue) par semaine a diminué de 33,5% alors que le temps passé assis a augmenté de 28,6% (5). En France, une enquête de l'Observatoire national de l'AP et de la sédentarité (ONAPS) indique que 45,1% des adultes initialement actifs ont réduit leur niveau d'AP et 74,2% des adultes passant initialement moins de 6h par jour en position assise ont augmenté leur niveau de sédentarité pendant le confinement (6).

Depuis la loi de santé de 2016 (décret 2016-1990), il est désormais possible pour les médecins traitants de prescrire une AP adaptée aux patients atteints d'une affection de longue durée (ALD). En réalité, bien que les réseaux AP et santé se développent, des enquêtes révèlent que seulement 10% des médecins prescrivent de l'AP sur ordonnance (7). Parmi les freins à la prescription, le manque de formation et de connaissances fait partie des motifs récurrents. Bien que les bonnes pratiques alimentaires pour rester en bonne santé soient bien identifiées et largement diffusées auprès de la population, les messages ne sont pas toujours clairs concernant l'AP et la sédentarité.

Après avoir brièvement résumé les bienfaits de l'AP sur la santé métabolique, cette revue a pour objectif de présenter nos connaissances actuelles sur la physiopathologie de

l'inactivité physique et de la sédentarité. Une perspective sur les stratégies de lutte contre les effets néfastes de la sédentarité et de l'inactivité physique sur la santé métabolique sera enfin abordée.

Les bienfaits de l'AP chronique sur la santé métabolique

La pratique régulière d'AP aérobie et résistif, permet de diminuer le risque de développer de nombreuses pathologies métaboliques chroniques selon une relation dose-réponse. Par exemple, 35 min/jour de marche rapide diminue la mortalité liée aux maladies cardiovasculaires de 30%, la mortalité toutes causes confondues de 25%, le risque de développer un cancer du sein de 40%, et un diabète de type 2 (DT2) de 40% (8). Il a été montré que pratiquer 150min/semaine d'AP modérée permet de mieux prévenir le développement d'un DT2 chez des sujets pré-diabétiques qu'un traitement par Metformine après 3 ans de suivi (9). Les bénéfices de l'AP régulière sur l'organisme sont le résultat d'adaptations au niveau du tissu musculaire, mais aussi d'une coopération orchestrée entre les principaux tissus impliqués dans la régulation de l'homéostasie énergétique à savoir le muscle squelettique, le foie, le pancréas, le tissu adipeux et le système circulatoire. Ces effets récemment discutés dans une revue complète sur le sujet (10) sont résumés dans la Figure 1.

Les conséquences néfastes de l'inactivité physique sur la santé métabolique

L'inactivité physique est la 2ème cause de décès aux États-Unis et la 4ème dans le monde (11). En Europe, elle provoque le décès prématuré de 600000 personnes par an, soit 10% de la mortalité totale (12). Plus précisément, l'inactivité physique serait une cause majeure et indépendante dans le développement de 35 pathologies (13). Elle serait responsable de 4% des DT2, 4% des maladies coronariennes, 2,2% des cancers du sein et de 2,5% des cancer du côlon mais serait aussi la cause de dépression et de maladies mentales (14).

Les effets de l'inactivité physique sur le corps touchent l'ensemble des systèmes physiologiques. Etudier expérimentalement ces effets chez l'homme est un défi car cela nécessite de supprimer entièrement l'activité physique chez des personnes en bonne santé et physiquement actives. Les modèles d'alitement prolongé (modèle d'étude issu des sciences spatiales pour étudier les effets de la microgravité, voir Figure 2) répondent à cette problématique. Ces protocoles ayant rendus complètement inactifs et sédentaires des hommes et des femmes sains et physiquement actifs, ont montré que les participants développent des profils métaboliques proches de ceux de la personne atteinte d'obésité ou de DT2 en réponse à une inactivité sévère (15). Plus spécifiquement, l'inactivité physique entraîne une perte de la masse et de

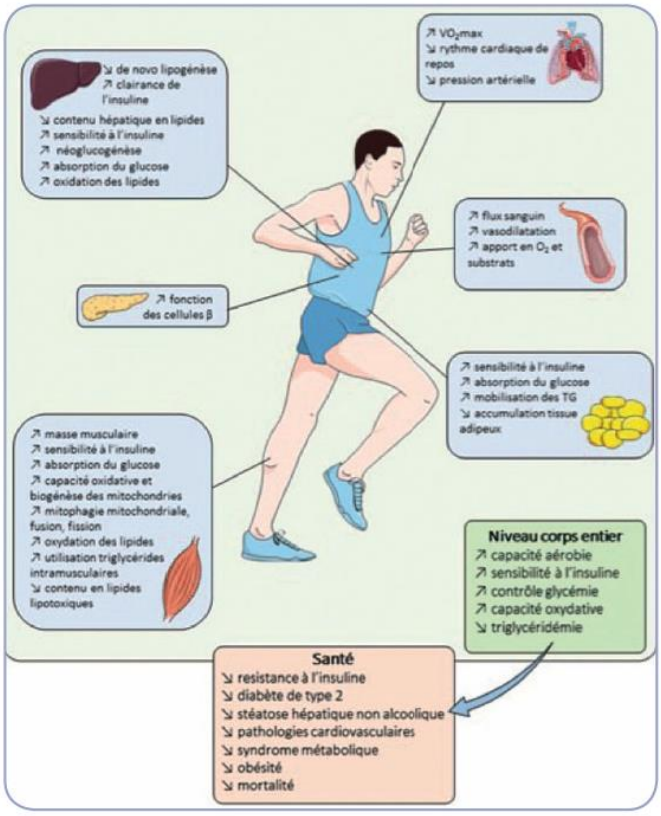

Figure 1 - Effets chroniques de l'exercice sur les organes clés impliqués dans la régulation du métabolisme et effets métaboliques associés.

Au niveau du système cardiovasculaire l'AP chronique, souvent effectuée sous forme d'un entraînement physique, diminue la fréquence cardiaque de repos et la pression artérielle, et augmente la capacité aérobie maximale (VO2max) ainsi que la masse musculaire. Le réseau microvasculaire se développe et la réponse vasodilatatrice est améliorée. La fonction des cellules bêta du pancréas (synthèse et sécrétion d'insuline) augmente ainsi que la sensibilité à l'insuline des tissus périphériques. Ainsi l'absorption du glucose sanguin par les muscles, le tissu adipeux et le foie est améliorée. La capacité de mobilisation des acides gras depuis le tissu adipeux est meilleure de même que la capacité du foie à produire du glucose menant à une diminution de la de novo lipogénèse (synthèse d'acides gras à partir de précurseurs glucidiques et protéiques). La capacité à utiliser les lipides du foie et du muscle ainsi que la capacité oxydative des mitochondries (production d'ATP) et leur biogénèse sont également améliorées. Ces effets entraînent en parallèle une diminution de la masse du tissu adipeux viscéral et du stockage ectopique des graisses (accumulation de graisses dans des tissus autres que le tissu adipeux) dans le foie, le muscle et les os. L'AP chronique, par ces adaptations structurales, fonctionnelles et métaboliques, jouerait ainsi un rôle clé dans la régulation de l'homéostasie énergétique et participe à augmenter la capacité aérobie (indice le plus fiable de mortalité précoce), la sensibilité à l'insuline, le contrôle de la glycémie, la capacité oxydative et à réduire la triglycéridémie. Elle permet également de diminuer l'inflammation chronique qui est impliquée dans le développement de nombreuses pathologies non-transmissibles (11) et d'améliorer le couplage entre énergie ingérée et dépensée par un meilleur contrôle des comportements alimentaires (12). Ces changements réduisent les risques de développer une résistance à l'insuline, un DT2, une stéatose hépatique non alcoolique, un syndrome métabolique, des pathologies cardiovasculaires, un surpoids et une obésité et in fine une mortalité précoce.

Figure 2 - L'alitement prolongé.

la fonction musculaire, un remodelage du muscle avec un changement des fibres musculaires lentes et oxydatives au profit de fibres musculaires rapides et glycolytiques et une refonte complète des voies métaboliques cellulaires caractérisée par une activation des voies impliquées dans la régulation du métabolisme des glucides, et une inhibition des voies de la régulation du métabolisme des lipides et de la fonction mitochondriale. Ces modifications impactent l'utilisation des substrats et entraînent une réduction de l'utilisation des lipides au profit de celle des glucides pour fournir de l'énergie au corps. Ces lipides non utilisés, notamment ceux provenant de l'alimentation, participent au développement d'une hyperlipidémie et participent au stockage de graisses ectopiques, c-à-d dans des organes non prévus pour cette fonction, comme les muscles, le foie ou les os. Ces changements métaboliques contribuent au développement d'une résistance aux effets de l'insuline mais stimulent également la synthèse de nouveaux lipides (i.e. de novo lipogenèse) comme celle des VLDL ("very low density lipoprotein") qui sont des particules lipidiques athérogènes. Cette sécrétion accrue de VLDL renforce à son tour l'hyperlipidémie et le stockage ectopique des lipides. Ces changements sont finalement associés au développement d'une inflammation chronique comme l'indique l'augmentation de marqueurs pro-inflammatoires plasmatiques, laquelle est connue pour contribuer au développement de l'insulino-résistance. Il est important de noter que ces troubles métaboliques se sont développés malgré une alimentation saine équilibrée et ajustée aux besoins des participants pendant toute la durée des études (16). Cela veut dire que ces dérèglements sont bien le résultat de l'inactivité physique seule, et non ceux d'une surnutrition. De plus, ces résultats obtenus dans des conditions d'inactivité physique extrême ont été confirmées à des niveaux d'inactivité observés dans la population générale (17). Ces recherches ont apporté des données fondamentales clé démontrant le rôle de l'inactivité physique dans l'étiologie des maladies métaboliques.

La sédentarité : composante indépendante de la relation entre activité physique et santé ?

Bien que la prévalence de l'inactivité physique soit globalement stable depuis une vingtaine d'années (18), la sédentarité se généralise. Par exemple, le temps consacré aux comportements sédentaires a augmenté d'une heure entre 2007 à 2017 aux États-Unis (19). En France, la proportion des adultes passant au moins 7h/j en position assise est comprise entre 18 et 40 % (20, 21). Toutefois, ayant été obtenues à l'aide de questionnaires, ces valeurs sont probablement imprécises et fortement sous-estimées. L'utilisation de moniteurs placés sur la cuisse pour mesurer le temps passé assis montre que les adultes des sociétés occidentales peuvent passer entre 7,7 et 9,7 heures par jour en position assise, représentant alors jusqu'à 60% du temps d'éveil (22, 23). Or, la mortalité précoce semble augmenter graduellement à partir de 9h de sédentarité par jour, avec un risque de mortalité globale augmenté de 48% pour 10h/j assis et multiplié par 3 pour 12h/j assis (22). De plus, indépendamment de la pratique d'AP, chaque heure passée en position assise augmente le risque de mortalité de 5,9 %, et celui de développer un DT2 et une obésité de 22% et 23% respectivement (24-26). Ces chiffres suggèrent ainsi que les effets de l'AP et de la sédentarité sont indépendants. Alors qu'une pléthore de données épidémiologiques ont été publiées, les preuves expérimentales des effets néfastes de la sédentarité sur la santé métabolique indépendamment de l'AP manquent. Ce constat est principalement dû aux difficultés à isoler les effets de la sédentarité de ceux de l'AP. Certaines études d'alitement prolongé incluent des protocoles d'AP ce qui en revanche peut nous apporter un aperçu unique des effets de la sédentarité indépendamment de l'exercice. Par définition, les activités de légère intensité (dépense énergétique comprise entre 1,6 et 2,9 MET), ici les activités de la vie quotidienne (marcher, monter les escaliers, faire le ménage…), sont supprimées lors de ces protocoles. Ces sujets sont donc très sédentaires mais pratiquent un volume élevé d'AP d'intensité modérée à vigoureuse qui vont souvent bien au-delà des niveaux recommandés d'AP. Ces études récemment résumées dans une revue (27) suggèrent que bien qu'une relation effet-dose semble exister, l'AP ne suffit pas à totalement lutter contre le développement de dysfonctions métaboliques induites par de hauts niveaux de sédentarité comme une résistance à l'insuline, une intolérance au glucose, une altération du métabolisme lipidique et une inflammation chronique. Ces études soutiennent l'existence d'effets indépendants de la sédentarité de ceux de l'AP d'intensité modérée-vigoureuse. En d'autres termes passer trop de temps assis peut avoir des effets différents sur la santé que de ne pas faire assez d'exercice.

Remettre le mouvement au centre de notre quotidien

Au-delà du temps total passé sédentaire, la durée de chaque épisode en position assise semble être un paramètre important pour la santé. Des études de population ont en effet observé des profils cardiométaboliques moins sains chez les personnes qui restent assis pendant de longues périodes que chez ceux qui fragmentent fréquemment leur position assise. A contrario, les adultes dont le temps de sédentarité est le plus souvent interrompu par de brefs épisodes d'AP,

présentent une diminution de l'insulinémie, de la glycémie et de la triglycéridémie à jeun ainsi que du tour de taille, par rapport à ceux qui ne font pas de pauses dans leurs activités sédentaires (28, 29). Il est important de noter que ces observations ont été réalisées indépendamment du temps total passé sédentaire total ou à pratiquer de l'AP modérée-vigoureuse, mais aussi de l'âge, du sexe et de l'origine ethnique. À court terme, il a été testé que pour un même volume et une même intensité d'AP et des dépenses énergétiques égales, réaliser de l'AP d'intensité légère ou modérée en sessions courtes et réparties tout au long de la journée diminue la glycémie et l'insulinémie postprandiale (30-34), augmente l'absorption du glucose (35) et l'oxydation des glucides (36) chez des adultes en surpoids ou atteints de DT2 par rapport à une session unique d'AP effectuée en continu. À plus long terme, des sessions d'AP légère à modérée de 3 min dispensées toutes les 30 min pendant trois semaines diminuent la glycémie à jeun et la variabilité de la glycémie chez des personnes atteintes d'obésité (37).

Des études épidémiologiques mettent en avant le rôle bénéfique de l'AP d'intensité légère et son potentiel pour lutter contre la sédentarité. Effectivement, il a été noté que l'AP légère est fortement et négativement corrélée au temps de sédentarité (29). Au risque de paraître trivial : plus nous bougeons et moins nous sommes sédentaires. De plus, les AP d'intensité légère sont favorablement associées à certains indices de santé métabolique comme le tour de taille, l'IMC (indice de masse corporelle), l'insulinémie, le profil lipidique et la glycémie postprandiale (38, 39), indépendamment de l'AP modérée-vigoureuse. Dans une série d'études, une équipe de chercheurs a comparé les effets du remplacement du temps de sédentarité par de l'AP légère (marche et position debout) à ceux d'une heure par jour d'AP modérée-vigoureuse en veillant à contrôler la dépense énergétique. Remplacer le temps sédentaire par des volumes élevés d'AP légère sans augmenter l'AP modérée-vigoureuse a permis de réduire l'insuline postprandiale, la triglycéridémie à jeun et le cholestérol non-HDL chez des adultes sains (40). Chez des adultes atteints de DT2, l'augmentation du temps passé debout et à marcher a amélioré le contrôle du glucose et la sensibilité à l'insuline. De plus, une réduction de la pression artérielle diastolique et une amélioration du profil lipidique ont également été notés (41, 42). Remplacer la sédentarité par de l'AP modérée-vigoureuse a également amélioré ces paramètres métaboliques mais de façon moins prononcée. Ces études montrent que lorsque les dépenses énergétiques sont équivalentes, remplacer des activités sédentaires par des volumes élevés d'AP légère est plus bénéfique que de réaliser une session unique et continue d'AP modérée-vigoureuse, du moins pour le contrôle du glucose, la sensibilité à l'insuline et la lipidémie. Au contraire, bien que des sessions fréquentes d'AP d'intensité légères améliorent la fonction endothéliale (43, 44), un seul épisode d'AP modérée-vigoureuse permettrait d'apporter des bénéfices d'amplitude supérieure sur la fonction microvasculaire (45). Les études à venir devront donc comparer davantage les effets sur le long terme de l'AP de légère intensité par rapport à ceux de l'AP modérée-vigoureuse sur les facteurs clés de la santé cardiométabolique.

Comment traduire ces recherches en pratique ?

Bien que les effets bénéfiques de l'AP modérée-vigoureuse sur la santé ne soient plus à prouver (8), elle ne tend pas à réduire le temps de sédentarité. En effet, les personnes physiquement actives, même celles qui dépassent les recommandations, peuvent être aussi sédentaires que les personnes physiquement inactives (46). Augmenter le volume d'AP modérée-vigoureuse peut même mener à des compensations comportementales spontanées chez les adultes sédentaires, entraînant ainsi une diminution des AP d'intensité légère (47). De plus, l'AP modérée-vigoureuse ne suffirait pas à compenser entièrement les effets néfastes induits par de grands volumes de sédentarité (27). Nous pouvons alors nous demander quelle est la quantité adéquate d'AP modérée-vigoureuse nécessaire pour compenser les effets de la sédentarité. Une méta-analyse incluant plus d'un million d'individus a montré que 60-75 min/jour d'AP modérée-vigoureuse sont nécessaires pour prévenir le risque de décès prématuré associé à 9 heures ou plus passées assis par jour (48). Alors que la majorité de la population n'atteint pas les recommandations pour la pratique de l'AP (c'est-à-dire

Figure 3 - Impact de l'activité physique suivant l'intensité.

Moins s'asseoir et bouger plus : cette approche implique des étapes de transitions simples en commençant dans un premier temps à se concentrer sur la réduction et la fragmentation du temps passé assis en privilégiant la position debout pour progressivement réintroduire la pratique d'activités légères. Cette augmentation progressive du mouvement dans la vie quotidienne serait alors une préparation vers la pratique d'activités d'intensité plus élevée sur le long terme.

30 min/jour d'AP modérée-vigoureuse, 5 jours/semaine), ajouter 30 à 45 minutes d'AP supplémentaires par jour n'est pas réaliste. Par conséquent, bouger régulièrement de façon à réintroduire de l'AP de toute intensité tout au long de la journée semble être une stratégie plus pragmatique et prometteuse.

Conclusion

Jusqu'à récemment, les recommandations en pratique d'AP se basaient essentiellement sur la pratique d'AP modérée à vigoureuse. Depuis quelques années, grâce à l'apparition de moyens de mesures objectifs et précis des comportements de mouvement, les activités sédentaires ont révélé être des éléments ayant une place à part entière dans la relation entre AP et santé métabolique. De plus, il a été démontré que des volumes importants d'AP légère, considérée ici comme tous les mouvements corporels associés aux activités de la vie quotidienne, sont bénéfiques pour la santé. Sachant que le manque de temps est un obstacle majeur à la pratique d'exercice/AP modérée-vigoureuse, réintroduire de l'AP légère tout au long de la journée pourrait être une stratégie efficace pour réduire le temps sédentaire et ainsi prévenir ses effets négatifs sur la santé métabolique. Dans cette optique, les dernières directives de l'OMS (3) publiées en 2020 encouragent la pratique d'une activité physique de toute intensité pour réduire les comportements sédentaires. En pratique, il est nécessaire d'encourager à changer les habitudes progressivement (Figure 3) en s'asseyant moins et en bougeant plus. Réduire le temps passé assis et incorporer des épisodes d'AP tout le long de la journée pourrait constituer une première étape importante dans la modification durable des habitudes d'AP au profit de la santé cardiométabolique, favorisant ainsi la transition progressive vers la pratique d'AP modérée à vigoureuse. En résumé, être en bonne santé nécessite deester moins assis, bouger plus et bouger le plus souvent possible. ■

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Annexe 4 : Metabolic profile in women differs between high versus low energy spenders during a low intensity exercise on a cycle-desk

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Metabolic profile in women differs between high versus low energy spenders during a low intensity exercise on a cycle-desk

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Active-desks are emerging strategies aiming at reducing sedentary time while working. A large inter-individual variability in energy expenditure (EE) profile has been identified and has to be explored to better optimize and individualize those strategies. Thus the present study aimed at comparing the metabolic and physical profile of individuals characterized as high spenders (H-Spenders) *versus* low spenders (L-Spenders) based on EE during a cycle-desk low intensity exercise. 28 healthy women working in administrative positions were enrolled. Anthropometric, body composition and fasting metabolic profile parameters were assessed. EE was determined by indirect calorimetry, at rest and during a 30-min cycle-desk use. Participants were categorized as H-Spenders and L-Spenders using the median of the difference between EE at rest and during the 30-min exercise. H-Spenders had higher mean EE ($p < 0.001$) and carbohydrate oxidation ($p = 0.009$) during exercise. H-Spenders displayed higher values for fasting plasma insulin ($p = 0.002$) and HOMA-IR ($p = 0.002$) and lower values for HDL-cholesterol ($p = 0.014$) than L-Spenders. The percentage of body fat mass was significantly higher in H-Spenders ($p = 0.034$). Individuals expending more energy during a low intensity cycling exercise presented a less healthy metabolic profile compared with L-Spenders. Future studies will have to explore whether the chronic use of cycle-desks during work time can improve energy profile regarding metabolic parameters.

Over the last century, the technological revolution (i.e. work automation, increase in transports use) led to tremendous changes in human behaviors favoring a global reduction in physical activities (PA) and an increase in sedentary behaviors (SB)[1], particularly in high-income countries[2,3]. The independent and joint effects of those more recently adopted behaviors raise the risks of cardio-metabolic morbidity and all-cause mortality[2,4]. With the growth of desk-bound activities in the work environment, SB have taken an important part in individuals' daily time[5] resulting in a reduction in PA and total energy expenditure (EE)[6]. Active workstations (sit-to-stand, treadmill or cycle-desks) have been suggested as potential solutions to counterbalance the excessive amount of time spent seated at work[7,8]. Standing desks have been suggested to increase slightly but significantly EE at work ($\approx$1.2 kcal.min^{-1})[9] compared with sitting position. However, this strategy may not benefit everyone to the same extent; inter-individual variability has been previously reported in energy during a sit-to-stand protocol[10,11]. Some individuals displayed a significant increase in EE during a steady-state standing position compared to a sitting position, while only a small increase in EE was detected in others in response to this postural change. These previous results from Miles-Chan and al.[10,11] raised questions regarding standing as an effective strategy to increase EE in the overall population. The variability in EE adaptation has been associated with some health parameters such as body fat mass that is positively correlated with the energy cost of standing posture in healthy

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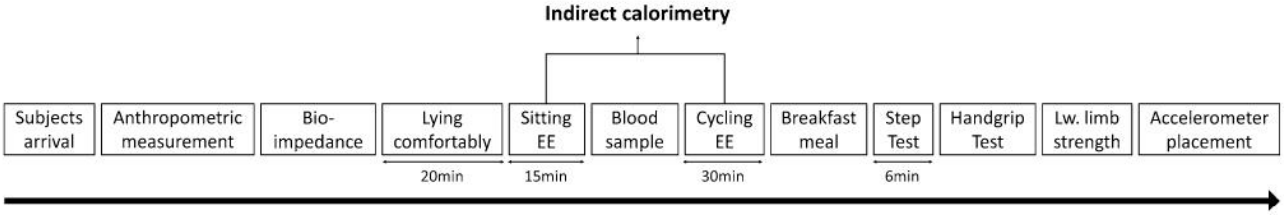

Figure 1. Schematic representation of the experimental design. EE, energy expenditure; Lw., lower.

inactive individuals[12]. Several studies have questioned the energetic cost of other different dynamic workstations such as walking on a treadmill or cycling desk[9,13]. While these studies obviously reported a substantial increase in EE (≈2–4 kcal.min^{-1}) compared to seating position[9,14,15], cycling desks have been suggested to be the best active workstation in terms of work and psychobiological performances[13]. Nevertheless, none of the studies investigating EE during cycle-desk utilization have identified the parameters that could explain these different energy profiles. Several authors have noticed that training status can influence cycling gross efficiency[16,17], with higher trained subjects being more efficient (i.e. more thrifty). However, the exercise intensities used in these studies are moderate to high and might not be representative of EE adaptations during low intensity exercise on a cycle-desk. Hence, it remains unknown whether the energetic profile of individuals during low intensity activities such as cycle-desk can be explained by specific anthropometric, body composition, cardiometabolic parameters or physical fitness. Indeed, understanding the characteristics of individuals' energetic profiles will enable a better optimization and individualization of active-desks strategies. In this context, the present study is the first to aim at comparing body composition, the cardiometabolic and physical fitness profile of individuals characterized as spenders *versus* non-spenders during a low intensity cycle-desk exercise, based on EE measurement. We hypothesized that participants with a more efficient energy profile will present healthier body composition, metabolic health and physical fitness.

Methods

Participants. Twenty-eight healthy women, administrative employees, with a body mass index (BMI) ranging from 18.5 to 29.9 kg/m^2 and aged between 18 to 60 years, participated in the present study. To be included in the study participants had to: i) be engaged in less than 150 min of moderate-to-vigorous physical activity per week based on self-reported data; ii) declare having regular menstrual cycles; iii) not be pregnant or lactating; iv) be free of any cardiovascular or metabolic disorders; v) not be dieting; vi) be free of any medication (excepted oral contraceptive); and vii) have a stable body weight (< 3 kg change during the 6 months prior to screening). This study was approved by the French ethical committee (Comité de Protection Personne Ile De France VIII 19 09 66) and all methods were performed in accordance with the relevant guidelines and regulations. Written informed consent was obtained for all participants in the present study.

Experimental design. After a full medical examination to assess eligibility, all subjects were asked to join the laboratory (laboratory AME2P, Aubière, France) for an experimental visit between January 6th and January 24th, 2020. Subjects were asked to keep their habitual daily activities, avoid any stressful situations and not consume caffeine for the 24 h prior to the test day. All participants completed this experimental session (Fig. 1) during the follicular phase of their menstrual cycle. Subjects reported to the laboratory at ~ 08.00 am, after a 12-h overnight fast. Evaluation started with body composition assessment and EE at rest was then investigated. Blood sample was obtained before a light intensity cycling exercise during which EE was measured. Participants' physical fitness was evaluated on the same day after a standardized breakfast meal. Finally, before leaving the laboratory, participants received an accelerometer to be worn for the following 7 days in order to assess their daily physically active and sedentary time.

Anthropometric measurement and body composition. Height was measured with a stadiometer at the nearest 0.1 cm, waist circumference (WC) was measured with a tape measure at the nearest 0.5 cm and WC to height ratio (WHtR) was calculated. Body weight was assessed using a calibrated scale (SECA, Les Mureaux, France) and fat mass percentage (%FM) and fat-free mass (FFM-kg) were evaluated by bioelectrical impedance (Tanita MC-780, USA, Arlington Heights), following the manufacturer's instructions.

Energy expenditure and substrate oxidation. After calibration of the device, indirect calorimetry with a facemask (MetaMax 3b, Cortex Biophysik, Leipzig, Germany) was used to measure VO_2 and VCO_2 for EE and substrate oxidation assessment. A heart rate monitor (Polar A300, Polar, Kempele, Finland) was used for the length of the experiment. Prior to resting condition subjects were sitting quietly for 15 min. For resting condition, subjects were lying comfortably in a deckchair in a thermoneutral environment for at least 20 min. After this period, subjects were asked to stay calm, not speak and avoid any movement. Gas exchanges were recorded for 15 min minimum and only the last 5 min were analyzed as previously suggested[18] and were defined as "Rest" time measure.

During the exercise condition, subjects were submitted to a 30-min light exercise using a cycle-desk (DeskCycle, 3D Innovations LLC., Greeley, CO, USA) with a resistance set at 2 out of 8 per design of the ergometer and

a revolution per minute (RPM) at 50 during the whole test, representing a power of ~ 16 Watts. An investigator supervised that participants respected the speed during the cycling test and reported at the end of the exercise the distance covered to ensure the test condition was similar between subjects. After the 30-min exercise testing, subjects had 1-min of recovery. Gas exchanges were measured during the entire exercise test and recovery period. EE, using Weir's equation[19], respiratory quotient (RQ; VCO_2/VO_2) and substrate utilization, using Péronnet & Massicotte equations[20] were calculated for the whole 30-min exercise session and also at rest, and after 5 (Start), 10, 20, 30 min of exercise. Mean values of the last 2 min of each period were considered for analysis as done in previous studies[21]. The first minute of recovery was also considered for analysis.

Cardiometabolic outcomes. Systolic and diastolic blood pressures were measured in a seated position using an auditory stethoscope with a blood pressure cuff adapted to the arm circumference. Subjects remained comfortably installed on a deckchair to collect a fasting blood sample. Plasma glucose, triglycerides, light-density lipoprotein cholesterol (LDL-cholesterol), high-density lipoprotein cholesterol (HDL-cholesterol) and total cholesterol were measured by enzymatic commercial assays. Insulin was assessed by chemiluminescent enzyme immunoassays. The enzymatic kits can be found in Supplemental file 1. All blood samples were centrifuged and plasma was kept frozen in aliquots at − 80 °C prior to analyses. The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated by the following formula: fasting blood insulin (mU/L) x fasting blood glucose (mmol/L) / 22.5[22].

Physical fitness. *Aerobic fitness.* Participants performed a 6 min step test as described before[23]. Participants wore a heart rate monitor (Polar A300, Polar, Kempele, Finland) to continuously record heart rate from the start to the end of the test, 30 s and 1 min in recovery.

Upper and lower limb strength. Participants performed a handgrip test as described in previous studies[24]. Then, participants were seated with a hip joint at 105° of flexion and were attached on the trunk, the hip and the left leg to the dynamometer chair (Biodex System 2, Biodex, Shirley, USA) with Velcro straps. Torque was measured on isometric 3 s-Maximum Voluntary Contraction (MVC) and on concentric MVC at a velocity of 60°/sec and 120°/sec.

Daily physical activity and sedentary time. From the day after the experiment, every subject was asked to wear triaxial accelerometers (ActiGraph wGT3X-BT, ActiGraph, Inc., Pensacola, FL) during 7 days with at least one weekend day. Participants wore the device on the right hip[25] on an elastic belt. Data were collected at a frequency of 60 Hz and converted to counts per 1 s epoch using the manufacturer's software (ActiLife version 6.13.4). Non wear time was defined as 90 min of 0 count per minute (cpm) with an allowance of 2 min of activity when it is placed between two 30-min windows of 0 cpm[26]. To be accepted in the analysis, accelerometer data had to be at least 4 days (including 1 weekend day) of wear with a monitor wear time of ≥ 10 h/day (600 min/day)[27]. SB was calculated with the vertical axis and PA with vector magnitude. SB was defined as < 150 counts min^{-1}[28], light intensity PA (LIPA) was obtained by subtracting SB and data below 2689 counts min^{-1}, MVPA was defined as 2,690–6166 counts min^{-1}, vigorous PA (VPA) was defined as < 6467 counts min^{-1}[29].

Statistical analyzes. The sample size was estimated in order to compare the metabolic and physical profile of individuals characterized as high spenders (H-Spenders) versus low spenders (L-Spenders) based on EE during a cycle-desk low intensity exercise. To highlight significant differences greater than 1 point effect-size, 14 participants by group (H-Spenders vs. L-Spenders) were needed for 80% satisfactory statistical power and a two-sided type I error at 5%.

Statistical analysis was performed using Stata software (version 15, StataCorp, College Station, Texas, USA). Data were presented as mean and standard deviation. The Shapiro–Wilk test was used to test the assumption of distribution normality for quantitative parameters. Energy profile was determined by categorizing difference between EE at rest and 27 min of exercise (3-27 min) (Delta Exo-Rest) according to statistical distribution, i.e. to median of the sample[30,31]. This categorization enabled to have two different groups: High Spenders (H-Spenders) and Low Spenders (L-Spenders). The comparisons between groups (above versus below the median value), were performed by repeated-measures ANOVA and post-hoc Bonferroni test was used for multiple comparisons with significance levels set at $p < 0.05$. The statistical tests were two-sided, with type I error at 0.05. Then, a sensitivity analysis was conducted to guaranty that these analyzes realized according to median value were robust and that conclusions can be supported by the results. Delta Exo-Rest was categorized according to values ranged between interquartile ranges. The comparisons were performed as aforementioned. More precisely, for each value of Delta Exo-Rest between first and third quartile, continuous variables were compared among < or ≥ of each value of Delta Exo-Rest. The results were expressed as Hedges' effect size (ES) and 95% confidence intervals, and were interpreted according to Cohen's rules of thumb, which defines effect-size bounds as: small (ES: 0.2), medium (ES: 0.5) and large (ES: 0.8: grossly perceptible and therefore large). Multivariate analysis was conducted using multiple linear regression to adjust results on weight of participants. The assumption of residuals normality was analyzed as aforementioned. When appropriate, a logarithmic transformation was applied. As these analyzes could be considered as exploratory, individual p-values have been reported without applying any mathematical correction but with specific attention to the magnitude of differences (i.e. ES), according to several works reported in the literature like those discussed by Bender and Lange[32]. Furthermore, principal component analysis was also performed to investigate relationships between quantitative variables using R software (R Foundation for Statistical Computing, Vienna, Austria). This statistical method was useful for analyzing assets as elements of

Variables	Low spenders	High spenders
N	14	14
Age (years)	41.9 (10.9)	37.7 (7.6)
Height (cm)	164.4 (4.7)	163.8 (7.3)
Body weight (kg)	58.4 (4.6)	64.5 (11.8)
BMI (kg/m^2)	21.6 (1.7)	23.9 (3.8)
Body fat mass (%)	25.9 (5.9)	31.5 (6.6)*
Body fat-free mass (kg)	40.9 (2.4)	41.4 (4.8)
Waist circumference (cm)	73.2 (6.0)	82.0 (11.3)
Waist circumference/height	0.44 (0.04)	0.50 (0.07)*
Systolic blood pressure (mmHg)	112.1 (5.8)	121.1 (14.9)
Diastolic blood pressure (mmHg)	70.0 (6.0)	76.1 (8.4)
Glucose (mmol/L)	4.79 (0.32)	5.12 (0.90)
Insulin (mIU/L)	4.04 (1.72)	9.25 (6.46)**
HOMA-IR	0.86 (0.36)	2.30 (2.35)**
Total cholesterol (g/L)	1.73 (0.45)	1.68 (0.25)
HDL-Cholesterol (g/L)	0.66 (0.09)	0.54 (0.13)*
LDL-Cholesterol (g/L)	0.99 (0.24)	1.00 (0.27)
Triglycerides (g/L)	0.83 (0.38)	0.72 (0.28)

Table 1. Characteristics of the study population. BMI, body mass index; HOMA-IR, homeostasic model assessment of insulin resistance; HDL, high-density lipoprotein cholesterol; LDL, light-density lipoprotein cholesterol. Values are presented as mean score (standard deviation) or percentage. Boldface indicates statistical significance (*$p < 0.05$, **$p < 0.01$), respectively with Mann–Whitney test.

quantitative variables in order to i) uncover the underlying relationships and structures of the measured variables (latent constructs) and ii) to aggregate subjects into clusters such that each cluster represents a topic.

Results

Anthropometric, body composition and cardiometabolic outcomes. H-Spenders and L-Spenders were aged 37.7 ± 7.6 and 41.9 ± 10.9 y.o., respectively, with a mean BMI of 23.9 ± 3.8 and 21.6 ± 1.7 kg/m^2. H-Spenders had a higher percentage of body fat mass ($p = 0.034$) and WHtR ($p = 0.025$) and lower fasting plasma concentration of HDL-C ($p = 0.014$) compared to L-Spenders (Table 1). A lower insulin sensitivity was observed for H-Spenders compared to L-Spenders, as indicated by greater plasma insulin concentrations ($p = 0.002$) and HOMA-IR ($p = 0.002$) values (Table 1). No other between-group significant difference was reported in body composition and cardiometabolic outcomes (Table 1).

Daily physical activity, sedentary time and physical fitness. As displayed in Table 2, no significant difference was observed between the two groups for aerobic fitness, upper and lower limb strength, total and segmented (by intensities) physical activity levels and sedentary time (Table 2). Based on the recorded physically active and sedentary time, our population can be considered sedentary and physically inactive[1].

Energy expenditure, heart rate and substrate oxidation. Overall, Delta Exo-Rest for EE showed large variability (0.5 to 1.8 kcal.min) (Fig. 2). Mean EE during the 30-min exercise increased significantly compared to mean resting EE in both H-Spenders (2.26 ± 0.2 *vs* 0.98 ± 0.12 kcal/min, $p < 0.001$) and L-Spenders (1.91 ± 0.15 *vs* 0.93 ± 0.11 kcal/min, $p < 0.001$). There was no between-group difference in EE at rest (0.98 ± 0.12 *vs* 0.93 ± 0.12 kcal/min, respectively). However, H-Spenders had higher EE than L-Spenders at every time point of the exercise test: start (2.38 ± 0.19 *vs* 1.93 ± 0.16 kcal/min, $p < 0.001$, respectively), 10 min (2.26 ± 0.18 *vs* 1.92 ± 0.13 kcal/min, $p < 0.001$, respectively), 20 min (2.21 ± 0.22 *vs* 1.88 ± 0.15 kcal/min, $p < 0.001$, respectively), and 30 min (2.18 ± 0.17 *vs* 1.92 ± 0.15 kcal/min, $p < 0.001$, respectively) (Fig. 3A). At 1 min-recovery, EE was not significantly different between the groups (1.56 ± 0.30 *vs* 1.39 ± 0.20 kcal/min, H-Spenders *vs* L-Spenders, respectively).

The light cycling exercise significantly increased heart rate compared to resting position in both H-Spenders (86 ± 11 *vs* 70 ± 12 beats/min, $p < 0.001$) and L-Spenders (81 ± 10 *vs* 68 ± 9 beats/min, $p < 0.001$) with no differences between the two groups. This increase was consistent across the entire duration of cycling for both groups (Fig. 3B).

RQ was similar between H-Spenders and L-Spenders at rest (0.84 ± 0.05 *vs* 0.83 ± 0.04, respectively) but was significantly higher in H-Spenders during the whole duration of exercise compared to L-Spenders ($p = 0.021$) (Fig. 4A). Taking all data together, there was a time effect ($p = 0.009$) for carbohydrates (CHO) oxidation, which was significantly higher during exercise compared to rest ($p = 0.008$) and recovery ($p = 0.006$). Resting CHO oxidation was significantly higher in H-Spenders compared to L-Spenders (3.33 ± 1.2 *vs* 2.82 ± 0.94 mg/min/kgFFM, $p = 0.017$, respectively). No significant difference was observed between groups at start, while H-Spenders oxidized significantly more CHO than L-Spenders during cycling at 10 min (6.52 ± 2.28 *vs* 4.53 ± 1.48 mg/min/

Variables	Low spenders	High spenders
N	14	14
Valid days of accelerometer wear	5.7 (0.4)	6.0 (0)
Weekdays	3.9 (0.3)	4.0 (0)
Weekend days	1.8 (0.4)	2.0 (0)
Number of minutes of accelerometer data (min/day)	352.9 (64.3)	368.9 (55.4)
Sedentary time (%/daily waking hours)	87.8 (2.9)	87.9 (3.1)
Total physical activity (%/daily waking hours)	12.2 (2.9)	12.0 (3.1)
LPA (%)	3.1 (1.1)	3.4 (1.1)
MVPA (%)	7.9 (1.9)	7.7 (2.7)
VPA (%)	1.2 (0.5)	0.9 (0.4)
Handgrip dominant hand (kg)	29.1 (4.7)	29.3 (4.7)
Handgrip non-dominant hand (kg)	28.0 (4.8)	26.5 (5.0)
Rest heart rate step test (bpm)	65.8 (6.8)	74.8 (12.7)
Heart rate step test (bpm)	147.9 (18.0)	160.3 (16.7)
Heart rate step test +30 (bpm)	124.5 (19.9)	136.0 (19.3)
Heart rate step test +60 (bpm)	106.2 (20.1)	118.1 (17.6)
Isometric strength (nm)	136.1 (31.7)	131.1 (36.6)
Isokinetic power 60°/sec (w)	142.7 (33.2)	140.1 (34.0)
Isokinetic power 120°/sec (w)	236.5 (55.7)	215.1 (60.5)

Table 2. Physical activity level, sedentary time and physical fitness of the study population. LPA, light intensity physical activity; MVPA, moderate-to-vigorous physical activity; VPA, vigorous physical activity. Values are presented as mean score (standard deviation) or percentage.

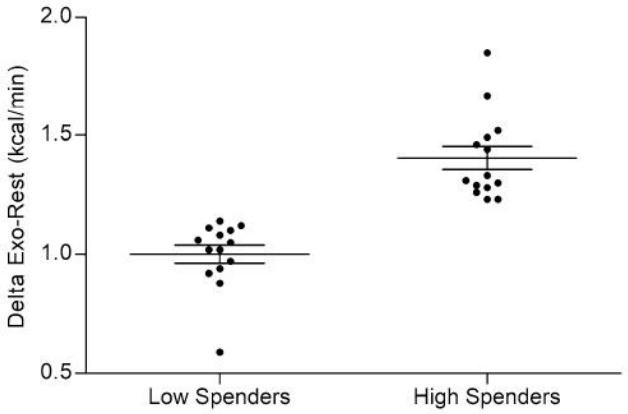

Figure 2. Characterization of Delta Exo-Rest between H-Spenders and L-Spenders. Data are presented as mean ± SEM.

FFM, p = 0.009, respectively), 20 min (6.52 ± 2.34 *vs* 4.79 ± 1.47 mg/min/kgFFM, p = 0.049, respectively) and 30 min (6.47 ± 2.15 *vs* 4.14 ± 1.38 mg/min/kgFFM, p = 0.008, respectively). No significant difference was reported at recovery between groups and compared to rest.

There was also a time effect for lipid oxidation, which was higher during exercise compared to rest for both H-Spenders (3.88 ± 1.55 *vs* 1.28 ± 0.33 mg/min/kgFFM, p < 0.001) and L-Spenders (3.85 ± 1.48 *vs* 1.27 ± 0.34 mg/min/kgFFM, p < 0.001). A group effect was noticed at the start of exercise and during recovery, with H-Spenders oxidizing more lipid than L-Spenders at start (5.94 ± 1.69 *vs* 4.76 ± 1.03 mg/min/kgFFM, p = 0.030, respectively) and oxidizing less lipid than L-Spenders during recovery (2.1 ± 0.44 *vs* 4.84 ± 2.34 mg/min/kgFFM, p = 0.002, respectively). No significant difference was reported for any other time of the exercise test.

Relative to total EE at rest, there was no significant difference in CHO oxidation in percentage between H-Spenders and L-Spenders (53.5 ± 17.8% *vs* 49.5 ± 14.9%, p = 0.26) or for lipid oxidation (46.5 ± 15.5% *vs* 50.4 ± 12.4%, p = 0.46) (Fig. 4B). During exercise, CHO oxidation was representing a greater percentage of total EE (44 ± 10.9% vs 35.7 ± 8.6%, p = 0.050) and lipid oxidation a lower percentage (56 ± 8.9% vs 64.3 ± 7.2%, p = 0.045) in H-Spenders compared to L-Spenders (Fig. 4C). No specific correlation were found between EE or substrate oxidation parameters and body composition, anthropometric data or blood parameters.

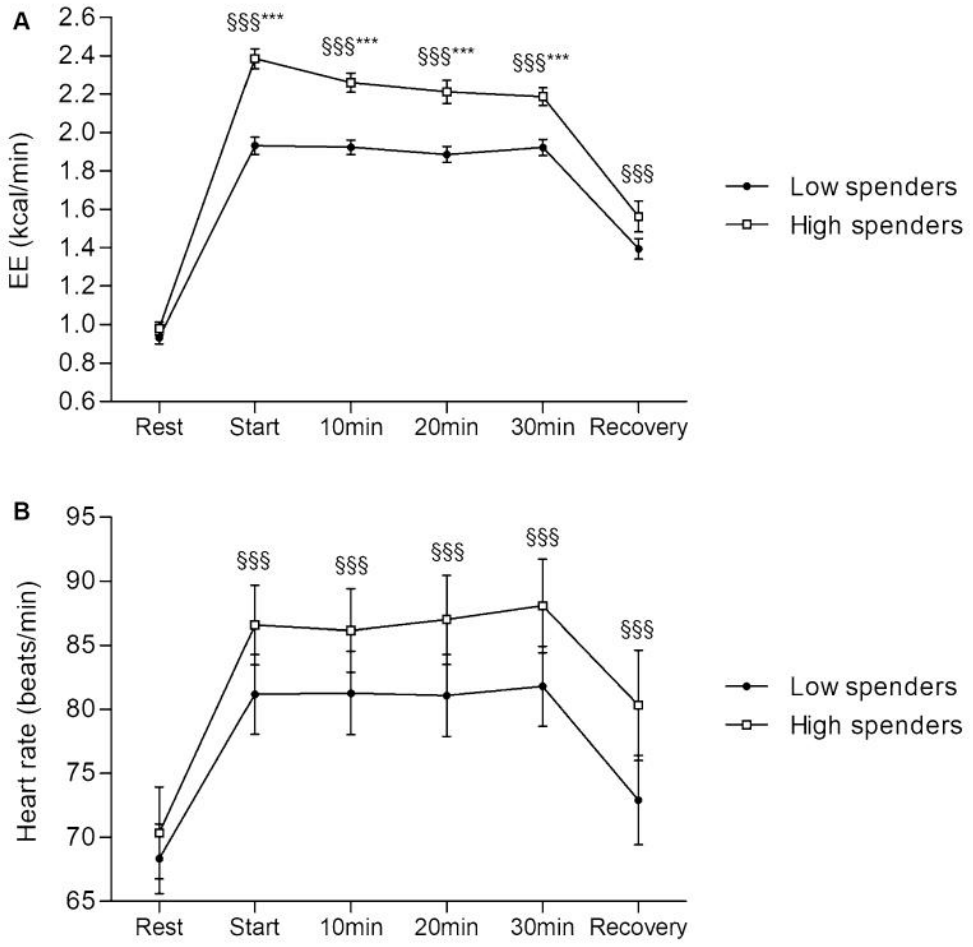

Figure 3. Comparison of EE (**A**), heart rate (**B**), from resting condition to light cycling exercise and recovery in each EE response group: H-Spenders and L-Spenders. Data are presented as mean ± SEM. §§§, time effect at $p < 0.001$; ***, significantly different between low spenders and high spenders at $p < 0.001$.

Principal component analysis. Lastly, the associations between the different parameters studied were illustrated by a principal component analysis (Fig. 5). Our data has shown a strong correlation between Delta Exo-Rest and some cardiometabolic parameters, such as inulin, HOMA-IR, LDL-cholesterol, glucose and triglycerides (Fig. 5). Also, the variability of energy expenditure between rest and low intensity cycling was strongly associated with higher values of body composition and anthropometric parameters (fat mass, fat-free mass, BMI, WC and WC/height) (Fig. 5).

Discussion

Active workstations are currently promoted to decrease office-related sedentary time and increase PA in a public health perspective. The aim of the present study was to examine associations between energy expenditure during a low intensity exercise on a cycle-desk device and body composition, cardiometabolic parameters and physical fitness of tertiary employees. Our data shows that two energetic profiles (H-Spenders or L-Spenders) can be identified in premenopausal women. More importantly, those two profiles show significant differences in anthropometric data, body composition (fat mass and WHtR) and metabolic outcomes (insulin, HOMA-IR, and HDL-Cholesterol), with H-Spenders presenting a less healthy metabolic profile.

Our results show that a light intensity cycle-desk exercise can significantly increase EE between 1.9 and 2.4 METs compared to resting. This result is in line with previous studies[14,33] and demonstrates that light intensity cycling allows to increase EE above EE associated with sedentary activities (i.e. 1.5 METs). A number of studies have questioned the effect of cycle-desk use on EE[14,21,34] but, none of them has looked for the potential factors that could explain this EE variability. Heterogeneity in energy responses has been reported in other studies from a sitting position to a steady-state standing position[10,11] with individuals characterized as "energy-savers" or "energy-spenders". While studies of Miles-Chan et al.[10] reported only 18% of their subject having a significant increase in EE compared to sitting (increase > 5% resting EE), all subjects of our study significantly increased their EE during the low-intensity cycling session. Differences in the magnitude of responses between the two studies are likely explained by the higher energetic demand induced by cycle-desk used in the present study compared to the standing position alone (1.9 to 2.3 METs vs ~ 1.2 METs)[9].

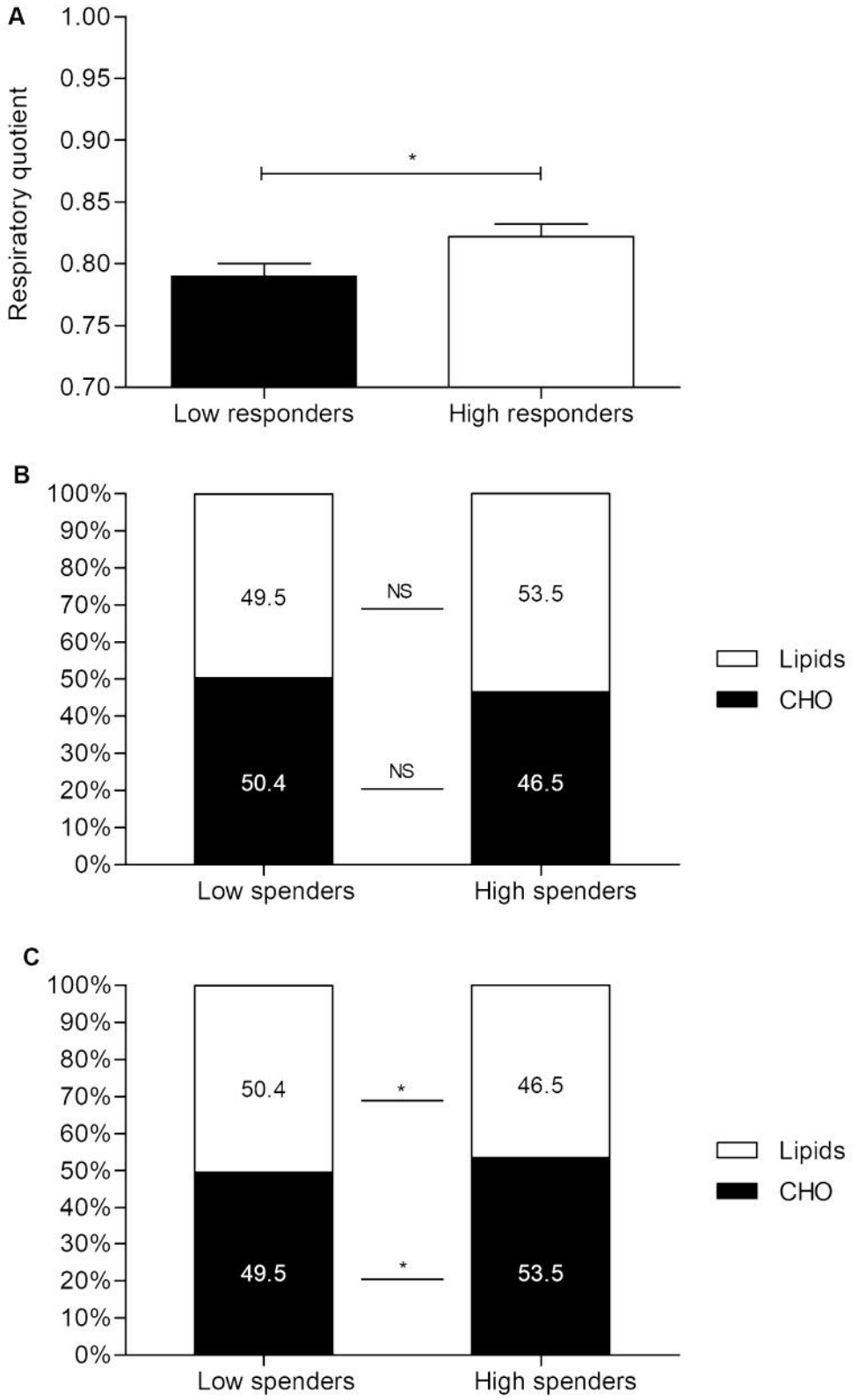

Figure 4. Respiratory quotient (**A**) during light cycling exercise. Substrate oxidation during Rest (**B**) and light cycling exercise (**C**). (**A**) data are presented as mean ± SEM. (**B**) and (**C**) data are expressed as mean percentage of CHO and lipids consumption relating to total energy expenditure. NS, not statistically significant; *, significantly different between low spenders and high spenders at $p < 0.05$.

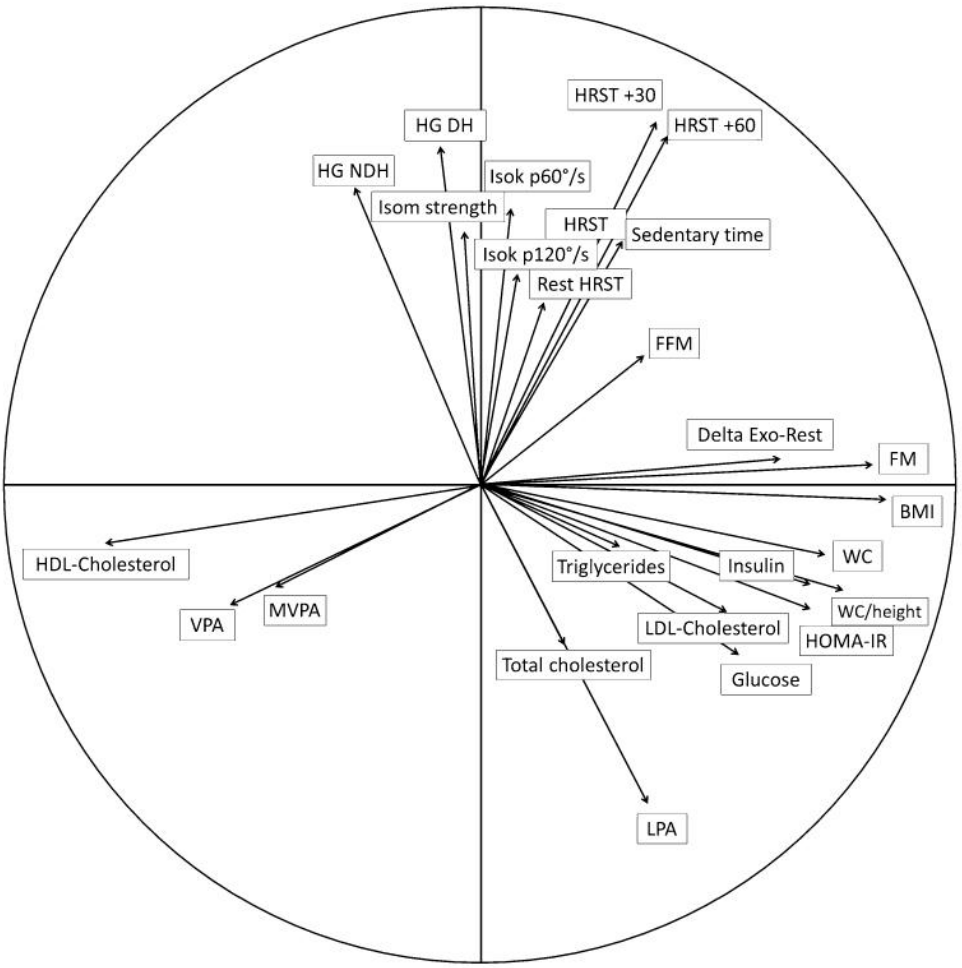

Figure 5. Principal component analysis of the study parameters. BMI, body mass index; DH, dominant hand; FFM, fat-free mass; FM, fat mass; HG, handgrip; HOMA-IR, homeostasic model assessment of insulin resistance; HDL, high-density lipoprotein cholesterol; HRST, heart rate step test; Isok, isokinetic; Isom, isometric; LDL, light-density lipoprotein cholesterol; LPA, light intensity physical activity; MVPA, moderate-to-vigorous physical activity; NDH, non-dominant hand; VPA, vigorous physical activity; WC, waist circumference.

Light-intensity cycling was more demanding for H-Spenders who were eliciting higher EE at each period of exercise than L-Spenders. During exercise, H-Spenders oxidized more CHO, both in total amounts and relatively to EE, but a lower percentage of lipids compared to the L-Spenders, while H-Spenders had significantly more fat mass than L-Spenders. Relationships between fat mass percentage, body weight and substrate oxidation during exercise have been investigated in several studies with no clear association between these parameters[35,36]. Studies comparing substrate oxidation during exercise in women with normal weight and overweight did not show clear differences[35,36]. It suggests that excess of fat mass does not necessarily result in a decrease in the ability to oxidize lipids. However, fat mass localization in normal or overweighed subjects seems to be more associated with substrate oxidation during exercise[37,38] than percentage of fat mass per se, with lower body fat mass profile being associated with better ability to oxidize lipids. In this line, we found that H-Spenders displayed higher %FM and WHtR, suggesting higher abdominal repartition of fat mass in individuals with this energy profile. The ability to rely predominantly on lipids or carbohydrates during submaximal exercise has been associated with the concept of metabolic flexibility, which is defined as the capacity to adjust fuel utilization to changes in fuel availability[39]. Metabolic state associated with glucose intolerance or insulin resistance has been shown to favor CHO oxidation during low intensity exercise compared to control subjects[40] and has been associated with metabolic inflexibility[41]. The metabolic challenge induced in our study by a 30-min low intensity cycling exercise suggests that H-Spenders are less metabolically flexible than L-Spenders as they are less able to rely on lipids during a low intensity exercise[42]. Physical fitness and training status are also known to influence the ability to preferentially rely on lipids during low and moderate intensity exercise[43]. Thus, the H-Spenders and L-Spenders profiles could have been explained by differences in physical capacities of the subjects. This appears however unlikely here since heart rate during step test and higher and lower limb strength did not differ significantly between the two groups.

The potential mechanisms explaining heterogeneity in energy profile have been poorly investigated in previous studies questioning strategies to decrease SB during work time. Miles-Chan et al.[10] did not find any association between body weight or height and EE when comparing energy cost in sitting *vs* standing positions. In a second study of the same research group, the energy cost of standing posture maintenance was positively correlated with body weight and WC[12]. Recently, Amaro-Gahete et al.[44] showed that FFM could partly explain differences in EE profiles in sitting *vs.* standing position. Although H-Spenders had a higher percentage of fat mass, no difference in FFM was observed. These results are also concordant with the study of Chen et al.[45], in which were reported relationships between energy efficiency and fat mass during walking, with subjects with obesity having decreased work efficiency compared to individuals with normal-weight during normal-speed walking. We further examined the cardiometabolic parameters of the two energy profiles. Our results suggest that H-Spenders showed a less healthy cardiometabolic profile as indicated by higher levels of fasting insulin, HOMA-IR and lower level of HDL-Cholesterol than L-Spenders. Metabolic profile and substrate oxidation during exercise of H-Spenders further feature similarities with those of subjects with obesity and/or type 2 diabetes[40].

Individualization of exercise programs is a cornerstone of health management. Our results suggest that physical activity level and fitness capacities are not sufficient to discriminate people and that an energy evaluation at rest and during exercise should be assessed to personalize prescription. In light of our results, we can assume the H-Spenders benefit more from the same cycle-desk program than L-Spenders. Depending on the energy profile, it could be expected that cycle-desk use recommendations may need to differ in terms of time and/or intensity of pedaling. Given the increased demand and/or necessity in the utilization of active desks, this could have important implications for metabolic health management.

One potential limitation needs to be considered. We only studied women, thus those results are only applicable to the female population. However, Miles-Chan et al.[10] reported different energetic profiles among male individuals during an activity at a lower intensity suggesting that the existence of different energy profiles might not be sex dependent. Nevertheless, the relation with body composition or metabolic profile could depend on this factor as shown by Chen et al.[45]. It is well known that hormonal status affects EE and two of our participants were taking oral contraceptives. Currently, there is no clear scientific evidence that oral contraceptives could induce modification of EE at rest or during exercise.

Conclusion

This study confirms that light cycling exercise enables to increase EE compared to resting but, inter-individual heterogeneity exists in the magnitude of energetic response. Differences in physical fitness, habitual time spent active or sedentary are not explaining this inter-individual variability. However, female individuals who spend less energy during a low intensity cycling activity present a healthier metabolic profile than those who displayed higher EE. Identification of energy profile could represent a strategy to better individualize the use of dynamic workstations to optimize EE during workdays. Future studies will need to investigate whether long-term utilization of light-intensity cycling desk at work can improve metabolic health outcomes of sedentary office workers, especially those with less healthy metabolic profiles.

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Author contributions

T.G., L.M., M.D. and D.T. designed the study; T.G., P.B., E.L.R. and A.B. performed the experiments; T.G., L.M., D.T., L.I., B.P., A.B. and M.D. contributed significantly to the writing and revision of the manuscript. All the authors read and approved the final manuscript.

Competing interests

The authors declare no competing interests.

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Elisa LE ROUX

Effet de la microgravité réelle et simulée sur la flexibilité métabolique chez l'homme

Résumé en français

Les études de simulation de microgravité ont mis en évidence des adaptations du métabolisme intermédiaire et une diminution de la flexibilité métabolique (c-à-d la capacité à ajuster l'utilisation des substrats en fonction des changements de leur disponibilité), pouvant entrainer des conséquences néfastes sur la performance et la santé des astronautes. Dans le contexte de la nouvelle phase exploratoire spatiale, ce travail de thèse a cherché à combler les lacunes actuelles pour mieux comprendre l'impact de la microgravité sur la flexibilité métabolique et les mécanismes cellulaires et moléculaires sous-jacents. Nous avons montré chez des hommes sains que seuls 5 jours d'exposition à la microgravité par immersion sèche induisent une diminution de la sensibilité à l'insuline, une hypertriglycéridémie et une accumulation de lipides hépatiques. Au niveau du muscle squelettique, une altération de la voie de signalisation de l'insuline ainsi qu'une réduction de la flexibilité métabolique au glucose ont été notées. Cette dernière semble précéder l'altération de la flexibilité métabolique au niveau du corps entier. Chez des astronautes ayant séjourné au moins 3 mois à bord de la station spatiale internationale, la flexibilité métabolique et l'oxydation postprandiale des substrats n'ont pas été affectées en moyenne. Toutefois les variabilités inter-individuelles ont mis en évidence des relations entre la flexibilité métabolique, l'exercice, le régime alimentaire et les changements de composition corporelle. Ces résultats apportent de nouveaux éléments pour aider à la préparation des futures missions de longue durée. Enfin, ces travaux ont des retombées sur terre en aidant à mieux comprendre les effets de l'inactivité physique sur le développement de pathologies métaboliques.

Mots clés : microgravité, flexibilité métabolique, oxydation des substrats, santé métabolique, exercice

Résumé en anglais

Simulated microgravity studies have shown adaptations of intermediate metabolism and a decrease in metabolic flexibility (i.e., the ability to adjust substrate utilization according to changes in their availability), which can have adverse consequences on astronaut performance and health. In the context of the new space exploratory phase, this thesis work aimed at filling the current gaps to better understand the impact of microgravity on metabolic flexibility and the underlying cellular and molecular mechanisms. We have shown in healthy men that only 5 days of exposure to simulated microgravity by dry immersion induce a decrease in insulin sensitivity, hypertriglyceridemia, and an accumulation of hepatic lipids. At the level of skeletal muscle, an alteration of the insulin signaling pathway as well as a reduction of the metabolic flexibility to glucose have been noted. The latter seems to precede the alteration of metabolic flexibility at the level of the whole body. In astronauts who spent at least 3 months on the International Space Station, metabolic flexibility and postprandial oxidation of substrates were not affected on average. However, inter-individual variabilities revealed relationships between metabolic flexibility, exercise, diet, and body composition changes. These results provide new insights to assist in the preparation of future long-duration missions. Finally, this work has implications on earth by providing a better understanding of the effects of physical inactivity on the development of metabolic pathologies.

Key words: microgravity, metabolic flexibility, substrate oxidation, metabolic health, exercise