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Philippe St-Martin, Elisa Le Roux, Audrey Bergouignan

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## Precision Medicine in Space

### Metabolic adaptations to microgravity

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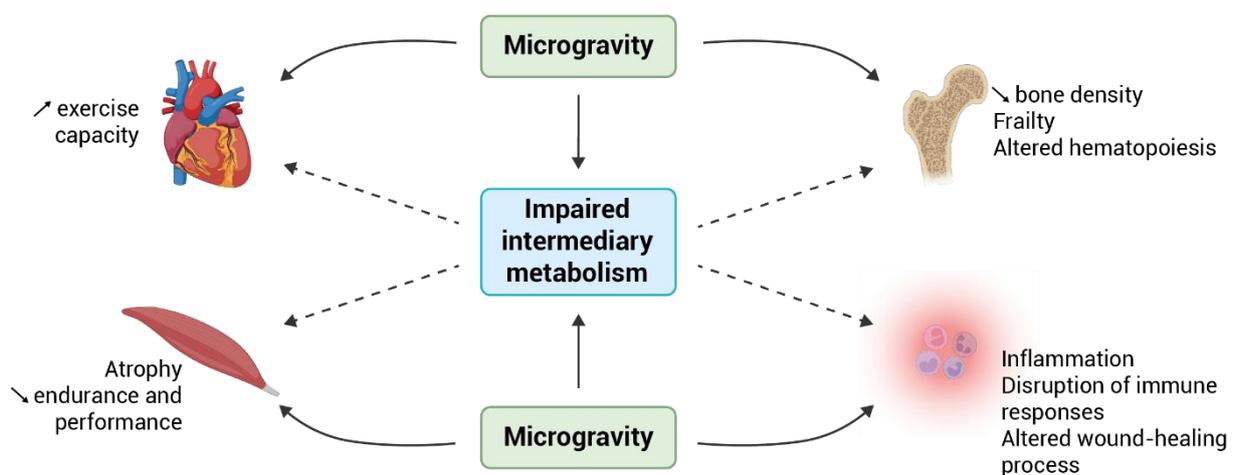
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## 1 Introduction

The study of metabolic adaptations to microgravity is relatively recent in bioastronautics. The cumbersome nature of the clinical tests to be carried out to study endocrinologic and metabolic adaptations to the space environment has been and still is an obstacle to experimental studies. Almost all the results at our disposal have been obtained during simulations of weightlessness using ground-based space analogs including head-down tilt -6° bedrest (HDBR) and dry immersion models in humans, and hind-limb suspension in rodents. These studies demonstrate the development of hormonal and metabolic disorders at the whole-body level as well as at the tissue and cell, protein and gene levels. Intermediary metabolism is at the crossroads of all physiological systems. Thus, changes induced by microgravity can contribute to alterations in other physiological systems. These alterations may manifest as cardiovascular deconditioning, reduced muscular endurance and performance, and muscle atrophy. Furthermore, when examining the impact on the skeletal system, disturbances in intermediary metabolism are not only associated with the well-documented decrease in bone density and increased fragility among astronauts but can also affect another critical bone function – haematopoiesis. This, in turn, influences the immune response and the body's ability to



heal, highlighting the far-reaching consequences of metabolic changes in space (**Figure 1**).

*Figure 1 Contribution of microgravity-induced impairments in intermediary metabolism to dysfunctions of other physiological systems altered by microgravity.*

This chapter reviews how metabolism adapts to both short-term and long-term exposure to microgravity, indicating a series of metabolic events triggered by this condition. With forthcoming missions to the Moon and Mars, it is essential to understand these microgravity-induced metabolic changes to assess and enhance the countermeasure programs. The chapter also summarizes the

metabolic impacts of exercise and nutrition countermeasures tested so far and outlines future directions and major challenges for anticipated human interplanetary exploration.

## 2 Short-term metabolic effects of microgravity

### 2.1 Early hormonal changes associated with stress and the loss of hydrostatic gradient

The first effects of the space environment on the human body occur rapidly likely because of the drastic changes in gravity during take-off and the anticipation surrounding the mission's beginning. For example, cortisol secretion increased on the first day of the Spacelab LifeSciences-1 (SLS1) and SLS2 missions (Stein et al., 1996). Cortisol, a hormone synthesized from cholesterol by the adrenal gland, influences nearly every tissue and organ in the body. It plays several crucial roles, such as managing the body's stress response, increasing blood sugar levels via gluconeogenesis, dampening certain immune system activities, regulating the circadian rhythm, and orchestrating the metabolism of proteins, fats, and carbohydrates, among other functions. This almost instantaneous change indicates that spaceflight is perceived as a stressor by the organism. Interestingly, increases in urinary cortisol are observed during medium-term HDBR and last up to 6 weeks (Blanc et al., 1998). Without the effect of gravity on the body, blood flow to the heart is facilitated, and compensatory hemodynamic and neuroendocrine mechanisms that promote diuresis (excretion of urine) and natriuresis (sodium excretion by urine) activate (Vernikos, 1996). Within 24 to 48 hours, the overall outcome is a decrease in blood plasma (Diedrich et al., 2007) that remains below pre-flight levels until after landing on Earth, with an increase in plasma protein (Leach et al., 1996). Norepinephrine plasma levels and urinary excretion rates decrease following this redistribution. To maintain the homeostasis of blood flow and water metabolism, urinary antidiuretic hormone (ADH) levels and the activity of the renin-angiotensin-aldosterone system (RAAS) decrease after the first hours following launch (Watenpaugh, 2001). ADH, a hormone produced in the brain, causes the kidneys to release less water, resulting in an overall decrease in the volume of urine output. The RAAS is an essential modulator of systemic vascular resistance, blood volume, and electrolyte balance. The combination of these neurohumoral reactions leads to diuresis and natriuresis. Blood volume decreases as a result, enabling suitable central circulation essential to survive in space. However, it is worth noting that there are gender-based differences in short-term responses. In males, urinary cortisol remains high in microgravity-like conditions, while females exhibit higher aldosterone (ALD) levels and plasma renin activity compared to males during HDBR (Vernikos et al., 1993). ALD regulates blood pressure

by controlling sodium and potassium levels and blood volume. Although further studies are needed to better understand sex differences in the response to microgravity exposure, these early studies demonstrate acute hormonal changes in response to stress and the loss of hydrostatic gradient.

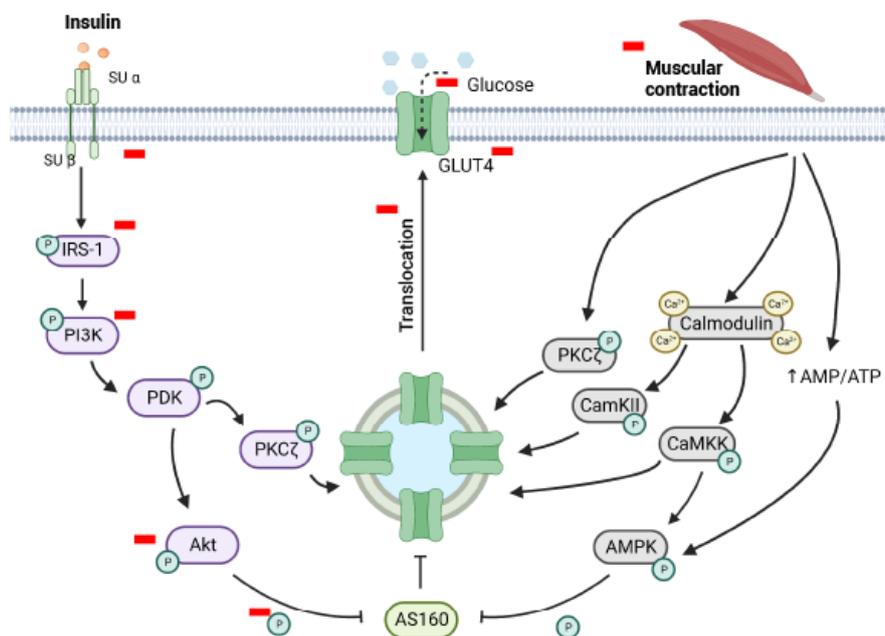
## 2.2 Rapid adaptations of the intermediary metabolism

### 2.2.1 An early decrease in insulin sensitivity

Insulin is a hormone secreted by the pancreas in response to increased blood glucose. Among its many roles, the most important one is the regulation of glucose metabolism by promoting its absorption by fat cells, liver cells and skeletal muscle cells. A simplified cascade of events leading to muscle glucose uptake is represented in **Figure 2**.

At rest, glucose transport is primarily regulated by the insulin metabolic pathway and involves multiple proteins. First, insulin binds to and activates its receptor (IRS-1) resulting in the receptor's phosphorylation. It then interacts with the Akt-regulating PI3K kinase. This facilitates the translocation of the glucose receptor (GLUT-4) from the cytosol to the cell membrane. This pathway is said to be insulin-dependent.

Muscle contractions can modulate a second regulatory pathway. AMPK (AMP-activated protein kinase) is activated and phosphorylated by an increase in the AMP/ATP ratio, which is caused by repeated muscle contractions. AMPK is a kinase that, upon activation, inhibits ATP-consuming pathways and activates ATP-maintaining pathways. During muscle contraction, the increase in cytoplasmic calcium also activates CaMK (Ca<sup>2+</sup>/calmodulin-dependent protein kinase). AMPK and CaMK, in turn, allow GLUT-4 translocation to the membrane, but this time independently of insulin effects. This second insulin-independent signaling pathway enables the muscle to utilize glucose more rapidly, which is necessary for the body to function effectively during sudden and rapid movements.



**Figure 2 Schematic representation of the insulin- and non-insulin-dependent pathways involved in glucose uptake by the skeletal muscle cell and the effects of microgravity.**

The left-hand side represents the insulin-dependent pathway and the right-hand side the non-insulin-dependent pathway. The red minus sign indicates a microgravity-induced decrease in activity. Akt, protein kinase B; AMP, adenosine monophosphate; AS160, Akt substrate of 160 kDa; ATP, adenosine triphosphate; CaMKII, Ca<sup>2+</sup>/calmodulin-dependent protein kinase II; CaMKK, Ca<sup>2+</sup>/calmodulin-dependent protein kinase kinase; IRS-1, insulin receptor substrate-1; PDK, pyruvate dehydrogenase kinase; PI3K, phosphatidylinositol 3 Kinase; PKCζ, protein kinase C, zeta

Clinically, insulin sensitivity is traditionally measured using an oral glucose tolerance test (OGTT) or an euglycemic-hyperinsulinemic clamp. The first is a simple procedure that measures the kinetics of plasma insulin and glucose during the two hours following oral administration of 75g of glucose. This glucose tolerance test can then be used as a surrogate for insulin insensitivity or insulin resistance. The development of insulin resistance necessitates a higher-than-normal concentration of insulin to bring plasma glucose concentration to 1 g/L. Additionally, there is an intravenous glucose administration variant. In the second, more complicated test, a constant flow of exogenous insulin is combined with a variable glucose infusion to maintain constant blood glucose levels. The amount of glucose infused reflects insulin sensitivity: the more glucose infused, the greater the insulin sensitivity.

The sensitivity of tissues to insulin is highly variable, with adipose tissue being the most sensitive organ, followed by the liver and muscle. Consequently, during a meal, the lipolysis of adipose tissue is quickly suppressed, followed by the inhibition of glucose synthesis in the liver and increased

glucose absorption by skeletal muscle. Therefore, the first half hour of an OGTT is ascribed to the liver response, while the remaining time is attributed to the insulin sensitivity of the muscle. It should also be noted that during a clamp, various insulin concentrations can be used to test the liver or muscle-specific sensitivity. Decreased sensitivity to the effects of insulin is an early observation in astronauts.

Several analog studies of short duration (< 7 days) showed a reduction in insulin sensitivity. In fasting state during dry immersion, insulin concentrations increase, glucose levels tend to rise only after the first 24 hours, and concentration of circulating insulin growth factor 1 (IGF1), the major mediator of growth hormone, progressively increases over the following 3 days (Linossier et al., 2017; Robin et al., 2023). A few studies investigated the mechanisms that lead to systemic insulin resistance, focusing on the three main peripheral organs involved in energy homeostasis regulation, i.e., skeletal muscle, the liver and adipose tissue. A decrease in muscle glycogen content and whole-body glucose clearance are observed following 3 days of HDBR, suggesting that decreases in insulin sensitivity occurs at a very early stage (Shur et al., 2022). After 7 days of bedrest, a decrease in insulin action on glucose uptake by muscle and its storage in the form of glycogen can be noted, indicating a reduction in the insulin sensitivity of skeletal muscle (Dirks et al., 2016; Mikines et al., 1989, 1991). After 7 days of HDBR, a decrease in the content of the main muscle glucose transporters, GLUT4 proteins, is observed (Biensø et al., 2012; Tabata et al., 1999). Recent immunofluorescence microscopy experiments also showed reduced GLUT4 localization in muscle cell membranes after 6 days of HDBR (Eggelbusch et al., 2024), highlighting impaired translocation of GLUT4 to the membrane. These alterations are associated with transcriptional changes and with modifications in the activity of proteins involved in glucose uptake and metabolism (GLUT4, hexokinase II, Akt, glycogen synthase). This supports the development of insulin resistance following short-term exposure to microgravity (Alibegovic, Sonne, et al., 2010; Biensø et al., 2012; Kiilerich et al., 2011; Mortensen et al., 2014).

Studies have investigated the impact of real microgravity on glucose tolerance and insulin sensitivity by OGTT or by measuring fasting insulinemia and urine excretion of C-peptide (i.e., the insulin precursor and index of insulin secretion) in astronauts after only 4 days in space. Animal models also help to elucidate some of the mechanisms involved in the microgravity-induced insulin resistance (**Figure 2**). In a rat immobilization protocol, tail suspension led to a reduction in glucose tolerance and glucose uptake (O'Keefe et al., 2004). Although no changes are observed in the IRS-1/PI3K/Akt pathway, increased phosphorylation of p38 MAPK (p38 mitogen-activated protein kinase), known to inhibit IRS-1 (Fujishiro et al., 2003) and to be associated with the development of type 2 diabetes (Henriksen et al., 2011), is noted after 3 and 7 days of suspension (Machida & Booth, 2005; O'Keefe et al., 2004). On the other hand, a decrease in both IRS-1 protein phosphorylation and PI3K protein

activity is observed in rats in which one hind limb is immobilized for 7 days, compared with the other non-immobilized control limb (Hirose et al., 2000). In a protocol of insulin stimulation of rat muscle cells subjected to unilateral hindlimb immobilization for 6 hours, a decrease in phosphorylation of Akt and AS160 is observed (Kawamoto et al., 2016). Stated differently, microgravity induces a decrease in Akt activation, thereby reducing its ability to inhibit AS160, which constitutively inhibits GLUT4 translocation from the cytosol to the muscle membrane. Altogether these ground-based and space studies in humans along with the animal studies demonstrate an early decrease in insulin sensitivity essentially due to alterations of skeletal muscle insulin action. This defect has been associated with major changes in nutrient metabolism.

### 2.2.2 A shift in fuel preference in favor of carbohydrate use

No change in whole-body fuel oxidation is detected after 3 days of HDBR (Shur et al., 2022) and 5 days of dry immersion (Robin et al., 2023). However, significant mRNA abundance changes in proteins involved in the regulation of fuel oxidation and muscle glycogen storage are observed in muscle, suggesting that some early adaptations are already occurring at the skeletal muscle level. Recently, we showed that these metabolic changes are not only transcriptional but also imprinted in the skeletal muscle (Le Roux, 2023). Briefly, harvested skeletal muscle biopsies from active, normal-weight men before and after 5 days of dry immersion present impaired capacity of muscle cells for glycogen synthesis and altered insulin signaling. Following 5 days of dry immersion, skeletal muscle's capacity to inhibit the oxidation of fatty acids is reduced in response to an increase in glucose concentration. This short-term adaptation indicates a decrease in metabolic flexibility i.e., the ability to adjust nutrient oxidation to changes in nutrient availability. A more in-depth examination of this aspect is covered later in the chapter (section 3.2.3). These changes at the skeletal muscle level are associated with reduced *in vivo* insulin sensitivity but not with decreased *in vivo* metabolic flexibility, supporting that muscle cells' metabolic dysfunction may precede whole-body metabolic inflexibility. Indeed, a decrease in metabolic flexibility was noted after 9 days (Damiot, 2018) and 21 days of bed rest (Rudwill et al., 2018).

Regarding lipid metabolism, although no increase in intermuscular adipose tissue (IMAT) is detected after 3 days of dry immersion, a significant increase in IMAT-associated markers is noted (Pagano et al., 2018). Similarly, the expression of markers of fibro-adipogenic progenitors, which are the main source of IMAT, is significantly increased after 5 days of dry immersion, suggesting the onset of disturbances at the skeletal muscle level (Guilhot et al., 2022). The recent 5-day dry immersion study (Le Roux, 2023) found elevated plasma ALT, a liver enzyme linked to steatosis, steatohepatosis, and

NAFLD-related metabolic (Harrison et al., 2008; Zderic & Hamilton, 2006), as well as an increase in triglyceridemia and hepatic fat accumulation as measured by MRI. Additionally, seven days of HDBR do not impact fasting lipogenesis in men but significantly raise it in women (Blanc et al., 2000). Both sexes experience a reduction in fasting lipid oxidation, though men exhibit increased carbohydrate oxidation after bed rest, unlike women. To date, this is the only HDBR study that investigates liver metabolism and showed in men, but not in women, a decrease in endogenous glucose production. This suggests that during short-term simulated microgravity without exercise, there are sex differences in fuel metabolism, with women showing insulin resistance in both muscle and liver, while men show it only in muscle. Although further investigations are needed to better understand the sex differences to microgravity exposure, these observations collectively support the early onset of a deterioration of the liver metabolism and whole-body lipid metabolism.

### 3 Long-term metabolic effects of microgravity

The long-term metabolic effects of microgravity have essentially been investigated during long-term HDBR. Overall, evidence shows that exposure to microgravity progressively leads to a metabolic state similar to the one seen in people with overweight, obesity, or type 2 diabetes on Earth.

#### 3.1.1 The development of hypertriglyceridemia

As described above, reductions in insulin sensitivity and increases in levels of plasma triglycerides are metabolic adaptations developed early in response to simulated microgravity. Both are sustained over the long term (Bergouignan et al., 2006, 2009; Hamburg et al., 2007; Ward et al., 2020) and observed after 6 months in space (Grigor'ev & Noskov, 1997; Hughson et al., 2016; Maass et al., 1997; Macho et al., 2003; Smirnov et al., 1991; Stein et al., 1994; Stein, Schluter, et al., 1999). Elevated blood lipid levels do not seem to result from increased lipolysis (Alibegovic et al., 2009; Alibegovic, Højbjerg, et al., 2010; Højbjerg et al., 2010) but rather from decreased fatty acid clearance. Isotopic tracer studies have revealed that after 60 days of HDBR, there is a significant rise in non-esterified fatty acid spillover from chylomicrons and very low-density lipoproteins (VLDL) hydrolysis (Bergouignan et al., 2009). This suggests a reduced uptake of free fatty acids from peripheral tissues, partially attributed to decreased skeletal muscle gene expression of lipoprotein lipase (LPL) and FAT/CD36 proteins, responsible for lipoprotein breakdown and fatty acid transport into cells, respectively. Furthermore, two 10-day bedrest studies demonstrated elevated LPL expression in subcutaneous adipose tissue, suggesting increased fatty acid uptake in this region (Højbjerg et al., 2010, 2011). Finally, it also appears that while total body fat increases overall, a more pronounced increase in visceral adipose tissue is observed compared to adipose tissue of the limbs and trunk after 60 days of HDBR (Belavy et al., 2014). Collectively, these findings suggest that microgravity redirects plasma lipids from muscle to adipose tissue. This change is probably associated with a shift in lipid metabolism from oxidation to storage.

#### 3.1.2 An impaired capacity to burn fat as fuel in favor of the use of carbohydrates

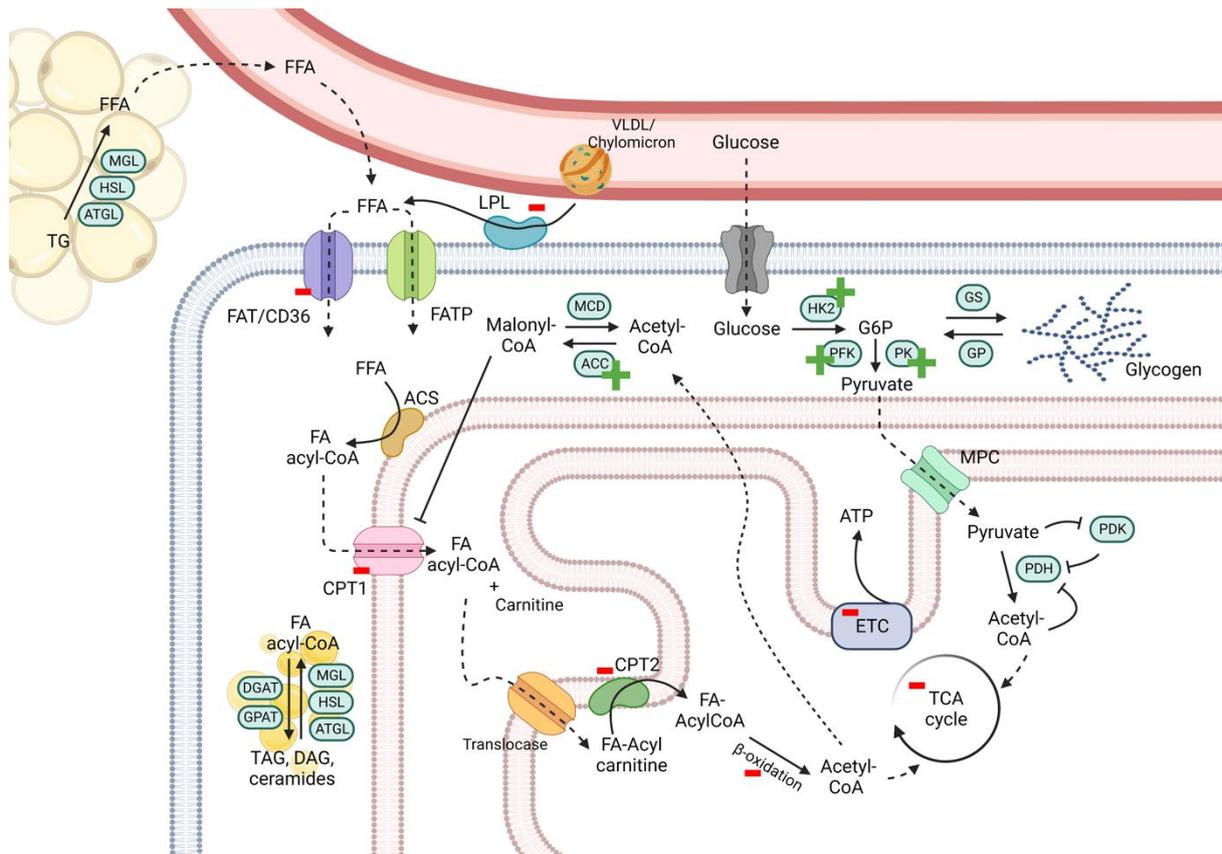
Under physiological state, the oxidation of nutrients depends on their type and availability within the cell. In the fasting state, lipids are mainly oxidized to produce energy. Exposure to microgravity induces a change in the utilization of fasting substrates. A 37% reduction in palmitate oxidation was observed in rats that had spent 9 days aboard the Columbia space rocket (Baldwin et al., 1993). In

humans, a 4 to 14% increase in fasting respiratory quotient (RQ) – calculated as the ratio between  $VCO_2$  and  $VO_2$  measured by respirometry – is noted in men and women after 42, 60, and 90 days of HDBR (Bergouignan et al., 2006, 2009; Blanc et al., 2000), thus indicating a shift in fuel preference in favor of carbohydrates and in detriment to lipids. Indeed, these protocols highlight a marked decrease in fasting lipid utilization, ranging from 33% to 90%, along with an increase in carbohydrate utilization by 20% to 40% compared to baseline. These metabolic changes are also observed in postprandial situations. Importantly, changes in substrate oxidation are all reported independent of detectable changes in body composition and energy balance.

### 3.1.2.1 Potential mechanisms underlying the shift in substrate use in detriment of lipid oxidation

Although the change in substrate utilization is observed independent of fat-free mass loss, it is likely related to a shift in the skeletal muscle fiber types, i.e. an increase in the proportion of fast glycolytic fibers (also known as type II fibers) along with a decrease in slow oxidative fibers (also known as type I fibers). This morphological change has been widely documented in mice and monkeys during stays in space of 11 to 91 days (Gambara et al., 2017a, 2017b; Harrison et al., 2003; Kischel et al., 2001; Sandonà et al., 2012; Tascher et al., 2017; Ulanova et al., 2015) and in men and women during 55 and 84 days of HDBR (Moriggi et al., 2010; Salanova et al., 2008; Trappe et al., 2004, 2007). Glycolytic fibers are known to have lower lipid oxidation rates and insulin sensitivity than oxidative fibers, which, in contrast, have greater mitochondrial density and higher oxidative enzyme activity (Scott et al., 2001).

In addition to changes in muscle fiber typology, transcriptional alterations likely contribute to changes in substrate utilization for energy production. These changes are summarized in **Figure 3**. In parallel with the reduced uptake of circulating fatty acids by muscle cells, a decrease in the expression of genes coding for proteins transporting fatty acids from the cytoplasm to the mitochondria is noted in tail-suspended rats (Stein et al., 2002) and in women during prolonged HDBR (Bergouignan et al., 2009). Gene expression of CPT1 (mitochondrial outer membrane passage) and CPT2 (mitochondrial inner membrane passage) decreases by 36% and 37%, respectively, in rats immobilized for 21 days (Stein et al., 2002). Sixty days of HDBR lead to a 50% decrease in CPT1 gene expression in the *vastus lateralis* muscle of women (Bergouignan et al., 2009). Omic analyses performed on soleus muscle biopsies from mice that spent 30 days in space and 38 days aboard the International Space Station (ISS) show a decrease in the abundance of proteins involved in the transport and  $\beta$ -oxidation of fatty acids (Da Silveira et al., 2020; Tascher et al., 2017) as well as in subjects bedridden for 21 days (Kenny et al., 2020). In parallel, pathways involved in the regulation of glucose metabolism appear to be activated in mice exposed to real microgravity (Da Silveira et al., 2020; Gambara et al., 2017a; Tascher et al., 2017) and in suspended rats, as evidenced by increases in the expression of enzymes involved in glycolysis such as phosphofructokinase (PFK) and pyruvate kinase (PK) (Stein et al., 2002).



**Figure 3 An overview of current knowledge on long-term adaptations of carbohydrate and lipid metabolism to microgravity.**

A decrease and/or increase in gene expression and activity of the involved proteins are indicated by the minus sign in red and the plus sign in green, respectively. ACC, acetyl-CoA carboxylase; ACS, acyl-CoA synthetase; ATGL, adipose tissue triglyceride lipase; ATP, adenosine triphosphate; CPT1, carnitine palmitoyltransferase 1; CPT2, carnitine palmitoyltransferase 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.

The metabolism of the skeletal muscle mitochondria, the site of energy production, is also profoundly altered by real or simulated microgravity. These changes are also shown in **Figure 3**. The Krebs cycle, the step that precedes mitochondrial respiration, appears to be disrupted under microgravity conditions, as shown by the reduced activity of citrate synthase in the mice's muscle after spending 11 days on Endeavour rocket (Harrison et al., 2003) and men's muscle during 21 days of HDBR (Kenny et al., 2020). Altered omic responses of mechanisms involved in the Krebs cycle are also noted in mice boarded on the ISS for 38 days (Da Silveira et al., 2020). A decrease of about 30% in the expression of the cytochrome oxidase gene (also known as COX4, a protein of the respiratory

chain complex) is noted after two months of HDBR (Bergouignan et al., 2009). Astronauts who spend 6 months onboard the ISS present a decrease in cytochrome oxidase activity (Fitts et al., 2013). More widely, omics analyses reveal a decrease in the abundance of proteins involved in the mitochondrial respiratory chain in mice that spent around 30 days in Earth orbit (Da Silveira et al., 2020; Tascher et al., 2017). On Earth, 21 days of HDBR lead to an overall decrease in mitochondrial respiration, not due to altered mitochondrial function but rather to a decrease in mitochondrial density (Kenny et al., 2017). While these changes may be perceived as mitochondrial stress (Da Silveira et al., 2020), they could represent adaptations of the organelle to a new energy state, namely a lower energy requirement due to muscle disuse, reflected notably by a change in mitochondrial function and number. These mitochondrial adaptations may limit the capacity of the mitochondria to cope with these sudden changes induced by either exercise, extra-vehicular activities, or dietary changes.

In addition to transcriptional changes within metabolic pathways in the skeletal cell, animal studies suggest alterations in liver metabolism. Increases in the mRNA of key enzymes involved in gluconeogenesis, i.e., the ability to produce glucose from non-carbohydrate compounds (phosphoenolpyruvate carboxykinase [PEPCK], glucose-6-phosphatase [G6Pase], fructose-1,6-biphosphatase [FDPase], pyruvate carboxylase [PC]) are observed in rats suspended during 21 days (Stein et al., 2005). The activity of PEPCK is also increased in mice suspended for 11 days (Ramirez et al., 2014). Proteomic analyses carried out on rat livers suspended by the tail for 3 and 21 days revealed profound changes in the abundance of proteins involved in glucose and lipid metabolism regulation (Chen et al., 2020).

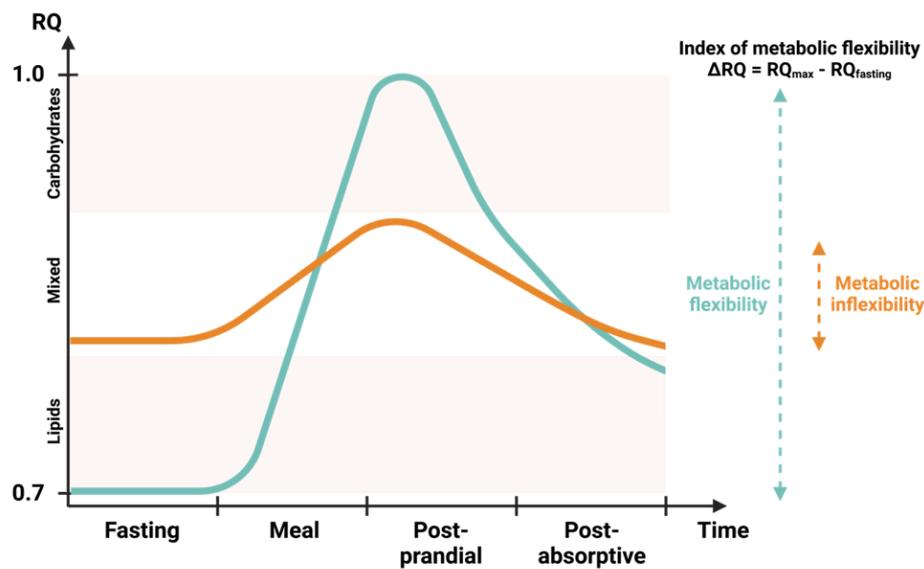
Altogether these metabolic alterations at the adipose tissue, skeletal muscle and liver level promote a change in fuel preference and nutrient partitioning with a reduced trafficking of fatty acids towards mitochondria for oxidation in the oxidative tissues, along with a greater storage of fatty acids in adipose tissue, especially visceral adipose depot. These structural, transcriptomic and molecular changes can alter the ability of the body to adjust substrate use to changes in substrate availability and energy demand, i.e. the so-called metabolic flexibility (Bergouignan et al., 2011).

### 3.1.3 Development of metabolic inflexibility

#### 3.1.3.1 *Data from long-term HDBR studies*

Metabolic flexibility was initially defined in the late 1990s as the body's ability to adjust substrate utilization to changes in substrate availability (Kelley & Mandarino, 2000). The ability to switch substrate utilization can be measured using RQ. In the fasting state, RQ is close to 0.7, indicating

preferential oxidation of lipids, as shown in **Figure 4**. After a meal, insulin secretion activates glucose uptake by peripheral tissues and thus oxidation of carbohydrates to produce energy, increasing RQ close to 1.0. A metabolically flexible individual can alternate between substrates to produce energy, i.e., the use of fatty acids in fast carbohydrates in fed states. In contrast, the ability to alternate substrate oxidation is impaired in inflexible individuals. As an index of metabolic flexibility, the amplitude of change in RQ between fasting and post-meal conditions is used. A decrease in this amplitude indicates metabolic inflexibility (**Figure 5**).



*Figure 4* Changes in respiratory quotient ( $\Delta RQ$ ) to assess metabolic flexibility

(Adapted from Muoio, 2014) The turquoise line represents an individual who is metabolically flexible and characterized by significant daily variations of RQ. The orange line represents an individual with reduced metabolic flexibility/metabolic inflexibility.

It is demonstrated that 21 days of HDBR induces metabolic inflexibility without altering glucose tolerance (Rudwill et al., 2018), thus indicating that metabolic inflexibility is a precursor to glucose intolerance in the pathophysiological context of insulin resistance; glucose intolerance has been observed after long-term exposure to real or simulated microgravity.

### 3.1.3.2 Inflight substrate use measured during the ENERGY protocol

The ENERGY protocol was carried out between 2011 and 2017 in 11 male astronauts onboard the ISS and investigated nutrient metabolism for the very first time inflight (Le Roux et al., 2024). Every astronaut underwent two identical metabolic examinations, one conducted on Earth's surface and

the other while aboard the ISS, at least 3 months after the beginning of the mission. By measuring gas exchange ( $VO_2$  and  $VCO_2$ ) by respirometry before and after a standard meal, fasting and postprandial total substrate oxidation were measured. After 3 to 5 months of actual microgravity, a shift in total substrate use in favor of carbohydrates and in detriment to lipid oxidation was observed in the fasting state. However, this shift seemed to be primarily due to changes in dietary intake marked by an increase in carbohydrate intake in detriment to lipid intake. Specifically, we estimated that the dietary changes explained about 69% of the changes in fasting substrate use observed during the flight. A question that will need to be addressed in the future is whether astronauts shifted their diet to food enriched in carbohydrates because of the food supply on board or because of a change in their food preference due to changes in taste, smell, exercise training, or other reason (Laurens et al., 2019).

Contrary to the large body of evidence accumulated during HDBR studies, these inflight data did not show a shift in postprandial substrate use, which may appear controversial. However, it is important to keep in mind that astronauts exercise, which is not the case for the study participants subjected to strict HDBR; exercise countermeasure was shown to mitigate the shift in substrate use in postprandial conditions (Le Roux et al., 2022). On this note, a large between-astronaut variability in postprandial substrate oxidation was observed during the ENERGY protocol and that was associated with changes in body composition changes and inflight physical activity. As previously suggested by HDBR and clinical studies (Bergouignan et al., 2011), variations in physical activity therefore seem to play a major role in the prevention/development of metabolic alterations under microgravity conditions.

In summary, in response to microgravity exposure without concomitant physical activity and/or exercise, the metabolic alterations at the adipose tissue, skeletal muscle, and liver levels described above promote a metabolically inflexible state and a change in fuel preference and nutrient partitioning, with reduced trafficking of fatty acids towards mitochondria for oxidation in the oxidative tissues. The unoxidized lipids can be stored in adipose tissue, especially visceral adipose depot, but also in non-adipose tissues.

#### 3.1.4 Ectopic fat storage

Ectopic fat storage refers to the deposition of lipids in tissues other than adipose tissue, such as muscle tissue, liver, or bone. Under physiological conditions, non-adipose tissues contain little triglyceride. Fatty acids can be accumulated in muscle cells, mainly in the form of triacylglycerols, diacylglycerols and/or ceramides, which are grouped as intramyocellular lipids (IMCL), but also

between muscle cells, constituting IMAT. Several ground-based studies have described how physical inactivity leads to the formation of lipid accumulations within the muscle. In a 12-day unilateral leg immobilization protocol in humans, using <sup>14</sup>C-labelled palmitate failed to reveal any increase in its incorporation into triacylglycerols and diacylglycerols in the inactive leg (Bilet et al., 2020). In contrast, MRI analyses of the *tibialis anterior* and histological sections of *vastus lateralis* showed an increase in IMCL of around 23% and 53% respectively in the hanging leg (Bilet et al., 2020). Twenty-eight and sixty days of bedrest, respectively, led to a mean increase in IMCL in the soleus muscle of 76% (Cree et al., 2010) and 3% in the lumbar muscles (De Martino et al., 2021). In a 60-day HDBR protocol, intramuscular lipids within the soleus increase by almost 2.7% in women (Bergouignan et al., 2009). This increase in intramuscular lipid content was correlated with the reduction in the oxidation of saturated fatty acids from the meal induced by prolonged HDBR. This suggests that unoxidized dietary lipids are incorporated into IMCL.

The liver was also shown to be an organ susceptible to lipid accumulation following microgravity exposure. Systemic markers suggest the formation of lipid reserves within the liver. Indeed, two indices of hepatic steatosis, aspartate aminotransferase (AST) and alanine aminotransferase (ALT), increase significantly after 60 days of HDBR (Rudwill et al., 2015). In real microgravity, histological analyses showed that the lipid droplet content of the liver increased by a factor of 3.5 in mice that spent 13 days in space, compared with control mice that remained on Earth (Jonscher et al., 2016). The same analyses performed on mouse livers from three different missions show an increase in lipid droplet content after 14, 21, and 37 days in orbit, irrespective of flight duration (although a trend seemed to emerge). The increase in transaminases during HDBR likely reflects the onset of hepatic steatosis in humans.

Bone marrow can also be a site of ectopic fat storage. The lipid fraction in the hematopoietic marrow of the lumbar vertebrae increased by almost 2.5% in women after 60 days of HDBR (Trudel et al., 2009). This increase corresponds to the equivalent of four years of physiological evolution (Meunier et al., 1971; Gurevitch et al., 2007) and was still visible one year after the end of the protocol.

This accumulation of lipids is not without consequences for the tissues concerned. In muscle, while IMCLs are an important energy source, particularly during exercise (Badin et al., 2013), increased IMCL causes lipotoxic intermediate lipids such as ceramides and diacylglycerols to accumulate. These compounds interfere directly with the insulin signaling pathway (Savage et al., 2005), and leads to increased release of adipokines, cytokines, and chemokines, resulting in chronic inflammation in muscles and potentially limiting mitochondrial function (Aldahhan et al., 2022). Altogether, IMAT and hyperinsulinemia resulting from insulin resistance are linked to accelerated muscle degradation and

compromised skeletal muscle protein synthesis (Aldahhan et al., 2022; Masgrau et al., 2012; Tamilarasan et al., 2012). An accumulating body of data suggests that this lipotoxicity may be responsible for the development of anabolic resistance and subsequent muscle atrophy observed during actual and simulated microgravity (Guilhot et al., 2022; Pagano et al., 2015, 2018), but further studies are needed to confirm this hypothesis and delineate the mechanistic underpinnings.

## 4 The cascade of events triggering the development of microgravity-induced metabolic disruptions

Over the past 30 years, in-flight research and HDBR studies have produced a substantial amount of data that can be used to better understand how exposure to microgravity causes metabolic alterations, including muscle atrophy, a shift in muscle fiber characteristic toward fast and glycolytic muscle fibers, insulin resistance, hypertriglyceridemia, a decrease in the ability to use lipids as energy substrates, metabolic inflexibility, and ectopic lipid storage. **Figure 5** summarizes the cascade of events believed to be triggered by microgravity. In brief, microgravity causes a decrease in mechanical load on the muscle, which triggers muscle atrophy and a complete reprogramming of all metabolic regulatory pathways. These lead to a decrease in the insulin signaling pathway, a decrease in glycogen synthesis, and reduced metabolic flexibility to glucose. These changes result in decreased systemic insulin sensitivity and hyperinsulinemia at the whole-body level. The latter participates in the installation of hyperglycemia and probably in the decrease of metabolic flexibility at the whole-body level, which precedes the decrease in glucose tolerance. Regarding lipids, the rapid increase in circulating lipids suggests a lower uptake of dietary lipids by peripheral tissues including muscles and the liver. The increase in intrahepatic lipids further suggests a rapid decrease in the oxidative capacity of the liver. Ectopic storage in the muscle appears to occur later, perhaps related to the secondary and progressive establishment of metabolic inflexibility to lipids. It remains to be confirmed that it translates at the level of the whole body. The decrease in mitochondrial density and oxidative capacity in the skeletal muscle and the change in the typology of muscle fibers contribute to the shift in substrate 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 a decrease in muscle insulin sensitivity

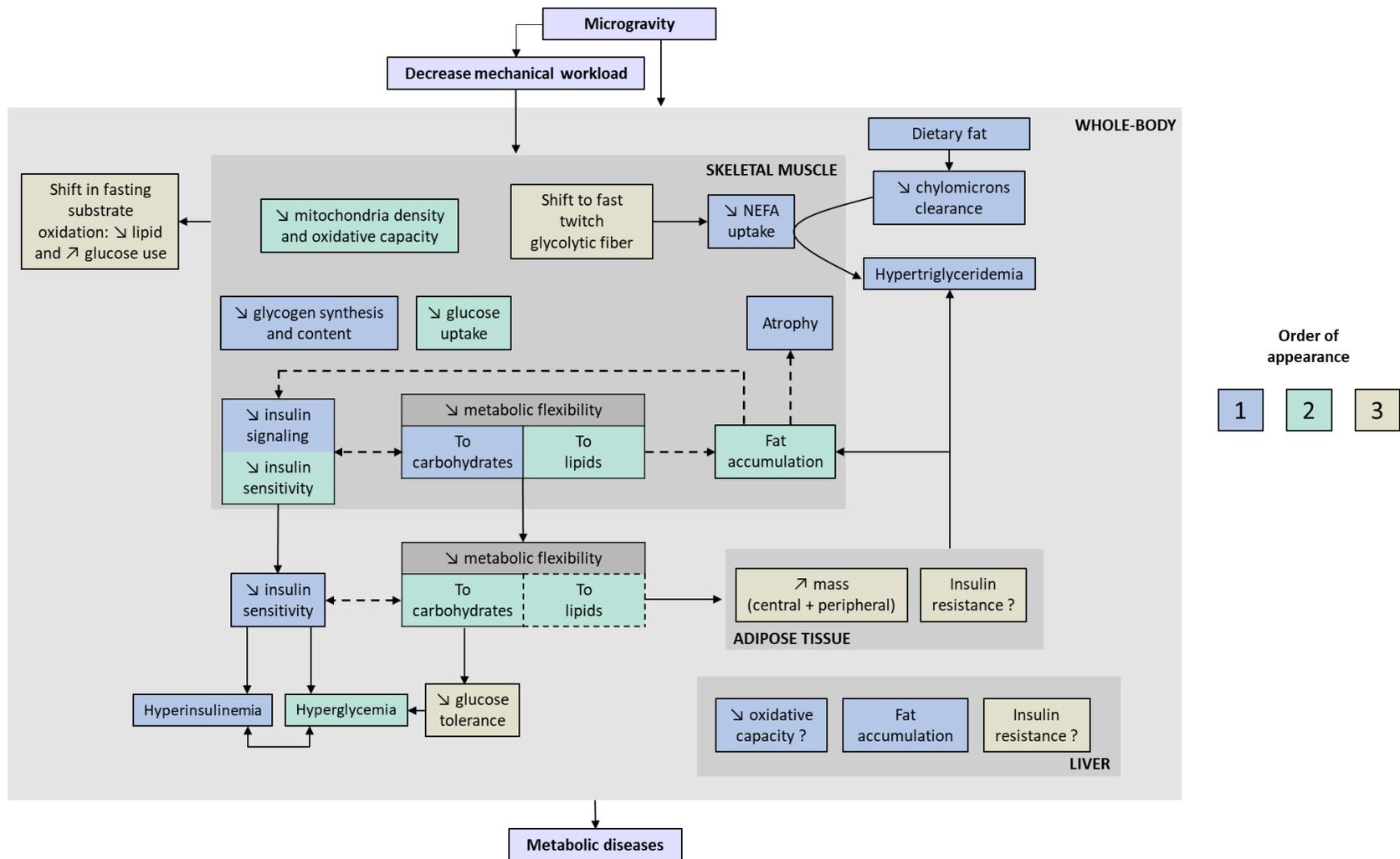


Figure 5 Hypothetical cascade of metabolic adaptations induced by microgravity leading to the development of metabolic diseases, update from Bergouignan et al., 2011. Dotted arrows represent relationships that requires further investigations.

## 5 Energy balance regulation in space

### 5.1 The adverse health consequences of a chronic loss of body mass

Loss of body mass has been a frequent observation during spaceflights. Body mass losses of over 5% have been observed in astronauts who spent 4 months aboard the Mir station (Smith et al., 1999, 2001), after 4 to 19 days aboard the Shuttle rocket (Stein, Leskiw, et al., 1999; Wade et al., 2002) or, more recently, after 4-to-6-month missions aboard the ISS (Matsumoto et al., 2011; Smith et al., 2005) despite the presence of sufficient food. In certain instances, mass loss surpasses 10% of the initial weight, reaching a level of clinical significance. In contrast, body mass was maintained during the SLS1 and SLS2 missions aboard the Spacelab spacecraft in the 1990s (Stein et al., 1996) or, more recently, aboard the ISS (Smith et al., 2005, 2012). The most recent reports show that astronauts onboard the ISS are still losing between 2 and 5% of their initial mass, with a wide inter-individual variability (Matsumoto et al., 2011; Zwart et al., 2014). A mathematical model that extrapolates the body mass loss and uses a large body of data on body mass change during space missions (n=619 missions) shows that the average cumulative body mass loss would be linear and equal to 2.4% every 100 days (Matsumoto et al., 2011). Therefore, a journey to Mars might result in a 15% or more loss in body mass, which would have major adverse health consequences.

Chronic energy imbalance can lead to a cascade of health issues and exacerbate those already triggered by microgravity (Bergouignan et al., 2016), including orthostatic intolerance, loss of muscle mass and strength, bone loss, and aerobic deconditioning (Smith & Zwart, 2008; Stein, 2002). For these reasons, roadmaps establishing research priorities for planetary exploration have raised the significance of energy balance control to a critical level for both medical and operational reasons (Bergouignan et al., 2016; Stein, 2000).

### 5.2 The potential role of exercise in the inflight regulation of energy balance

The reduction in body mass can result from either a decrease in energy intake, an increase in total daily energy expenditure, or a mismatch between both. Total daily energy expenditure, assessed by using the reference method of doubly labeled water (DLW), was measured during short flights (Lane et al., 1997; Stein et al., 1996; Stein, Leskiw, et al., 1999). According to two of these studies, exercising is associated with a negative energy balance. Exercise is the cornerstone of the countermeasure program that has been developed to mitigate microgravity-induced physiological alterations, as detailed in the next section. Besides having a negative energy balance, the astronauts

who exercise display a negative nitrogen balance, which likely contributes to the loss of fat-free mass. *A posteriori* analyses suggest that changes in body mass during flight are primarily linked to daily energy expenditure (Laurens et al., 2019). However, this assumption needed to be verified during extended periods of microgravity.

## 6 Effect of the lifestyle-related countermeasures on metabolic health outcomes

Beyond research to understand the physiological adaptations to microgravity, the international space agencies seek to test and put into practice efficient countermeasures that prevent or mitigate these adaptations. The countermeasure program includes exercise, low body negative pressure, artificial gravity, pharmacology, nutrition, and others. Because the metabolic adaptations to microgravity are essentially due to muscle disuse and physical inactivity, exercise countermeasure has naturally been the focus of attention to prevent microgravity-induced metabolic responses. In more recent years, space agencies have also deployed efforts to investigate the preventative effects of nutritional countermeasures. The primary ways in which these two countermeasures mitigate their effects on metabolic parameters are outlined below.

### 6.1 Exercise

To date, physical exercise remains the fundamental pillar of countermeasure programs. Estimating the effectiveness of exercise countermeasures in space is a challenge, given that all astronauts exercise in flight. It would be unethical to ask astronauts to act as control subjects and not perform exercise when we know the significant physiological repercussions that could result from such an approach. This is why simulated microgravity studies can provide us with this kind of information, by comparing a bedridden group exercising according to different modalities and intensities (resistive exercise alone or combined with aerobic exercise) and a strict bedridden group (no exercise). The effects of exercise countermeasures on microgravity-induced metabolic adaptations have recently been reviewed (Le Roux et al., 2022).

In summary, resistance training on its own can effectively halt the loss of muscle mass and function, including the decrease in fiber diameter that usually happens during prolonged HDBR (Moriggi et al., 2010; Trappe et al., 2004). However, the exact mechanisms responsible for exercise's protective effect against muscle atrophy remain elusive. Previous research has demonstrated that resistive

exercise counteracts HDBR-triggered reductions in muscle protein synthesis (Ferrando et al., 1997) and represses the expression of myostatin (Irimia et al., 2017), a myokine known for its role in promoting muscle loss. Furthermore, a 21-day HDBR resulted in an upregulation of metabolic pathways associated with autophagy and protein degradation, whereas performing resistive exercise on a vibration platform mitigates these effects (Kenny et al., 2020). Despite these beneficial effects on skeletal muscle, resistive exercise only partly prevents the metabolic impairment induced by HDBR. It hinders the elevation of visfatin (Rudwill et al., 2013), an adipokine mimicking insulin effects, yet it does not counter the elevation of pro-inflammatory markers interleukin 6 and C-reactive protein. Moreover, resistive exercise does not halt the decline in adiponectin levels (Brooks et al., 2014), which are associated with inflammation, lipid metabolism disturbances, and obesity. Although performed at high intensity, resistance exercise does not prevent HDL cholesterol decline (Brooks et al., 2014; Guinet et al., 2020), nor the onset of insulin resistance, hyperlipidemia or the shift in substrate oxidation in detriment to lipids (Bergouignan et al., 2006). This finding is intriguing, considering that resistive exercise impedes the change of muscle fiber typology from oxidative to glycolytic (Trappe et al., 2004), compensates for transcriptomic alterations in muscle related to aerobic energy metabolism (electron transport chain, the  $\beta$ -oxidation of fatty acids and the Krebs cycle) (Fernandez-Gonzalo et al., 2020), and partially preserves the activity and gene expression of enzymes controlling oxidative metabolism at mitochondrial level (such as citrate synthase and succinate dehydrogenase) (Irimia et al., 2017). Whilst resistance exercise alone does not restore fatty acid oxidation levels to baseline values (Bergouignan et al., 2006), no lipid accumulation in bone (Trudel et al., 2012) or visceral regions has been documented (Belavy et al., 2014). At present, there is a lack of available data regarding the impact of resistive exercise on ectopic lipid storage in the liver or muscle during HDBR. In all these studies, the exercise session is conducted as a singular, uninterrupted session. However, when exercise is performed in the form of squats intermittently throughout the day, loss of muscle mass is avoided, but not the decrease in whole-body and peripheral insulin sensitivity (Ward et al., 2020). Overall, the low energy expenditure associated with resistance exercise may explain the partial or limited preventive effects on metabolic health indices (Le Roux et al., 2022).

HDBR trials integrating both resistive and aerobic exercise probably yield higher exercise-related energy expenditure than protocols involving only resistive exercise. In addition, this training has the advantage to be closer to the exercise training prescribed onboard the ISS. Combined aerobic and resistance exercise training appears to preserve, or at least attenuate, aspects such as muscle structure and function, leg muscle volume and power, muscle strength and endurance, muscle fiber composition and diameter, as well as mitochondrial content and oxidative capacity (Bergouignan et

al., 2009; Krainski et al., 2014; Lee et al., 2014; Ploutz-Snyder et al., 2018; Salanova et al., 2008; Trappe et al., 2007; Trappe et al., 2007). In those studies, although muscle impairments are prevented by resistance and aerobic exercise protocols irrespective of training type, duration, intensity and frequency, the protective effects on metabolism remain variables. The integration of aerobic and resistive training prevents the development of an inflammatory state (Mutin-Carnino et al., 2013) and mitigates the decrease in insulin sensitivity and the substrate oxidation from lipids to carbohydrates (Bergouignan et al., 2009). However, exercise fails to prevent the rise in fasting triglycerides, the decrease in the rate of dietary fatty acid oxidation – likely due to altered fatty acid transport within myocytes – and the accumulation of lipids in skeletal muscle (Bergouignan et al., 2009) and bones (Trudel et al., 2009). Interestingly, the simulated microgravity-induced liver lipid accumulation, as indicated by liver markers, seems to be compensated (Rudwill et al., 2015).

Exercise energy requirements during spaceflight might have some adverse effects on human physiology and microgravity alterations. While a high volume of exercise countermeasures was of particular importance during early and short spaceflight to maintain a certain level of physical activity, such a high volume may be less important for modern space missions, during which astronauts are called upon to perform all kinds of tasks that require more physical movement. In the context of planning planetary exploration to Mars or the Moon, it seems necessary to update the current exercise countermeasure program. This calls for new countermeasures that successfully limit the body's deconditioning in microgravity while minimally affecting total energy expenditures and, by extension, energy requirements.

#### 6.1.1 The ENERGY protocol: Study of body composition and energy expenditure onboard the ISS

One aim of the ENERGY study was to measure total energy expenditure and activity energy expenditure in relation to body composition using the gold standard DLW method after at least 3 months onboard the ISS (Bourdier et al., 2022). As mentioned before, the study was conducted between 2011 and 2017 in 11 astronauts. The findings support a small drop in body mass on average, i.e. -1.5%, despite no significant modifications in body composition or energy expenditures overall (Bourdier et al., 2022). Contrary to what was expected, averaged total physical activity time and workload, as measured by an activity monitor coupled with an accelerometer, were not reduced while onboard the ISS; instead, non-ambulatory activities compensate for the decrease in ambulatory activity. Upon closer examination of the data, two subgroups emerged: astronauts whose total energy expenditure onboard the ISS was not different from the one measured pre-flight,

and astronauts who present decreased total energy expenditure during the mission. Those who maintained their total energy expenditure maintained their fat-free mass but presented a decrease in fat mass, indicating a negative energy balance. In contrast, those who reduced their total energy expenditure exhibited a loss in fat-free mass but an increase fat mass, thus indicating a positive energy balance. The changes in fat mass reflect unmatched energy intake to energy expenditure during the mission. This large inter-individual variability was associated with ground-based astronaut fitness levels and overall physical activity onboard the ISS, i.e. exercise and any body movement. Regarding energy intake, this study estimated that astronauts consumed approximately 85% of dietary prescriptions. This observation could potentially account for the decrease in fat mass in astronauts who maintained their total energy expenditure despite energy intakes being close to their theoretical energy requirements. Overall, this suggests that inflight energy requirements cannot be derived from general population equations, and inflight changes in body composition, energy intake, and energy expenditure need to be closely tracked to ensure astronaut health and performance, especially in the context of the foreseen planetary exploration.

These findings suggest that microgravity and exercise during spaceflight might affect appetite and eating behaviors, thereby influencing energy intake. Although no published data currently exists on astronauts' eating behaviors and reported appetite, studies using microgravity analogs offer some insights. During long-term HDBR study, there is a rise in the levels of leptin, a hormone secreted by adipose tissue that is known to induce satiety and decrease food intake (Bergouignan et al., 2010; Blanc et al., 2000; Linossier et al., 2017). While ghrelin, the only orexigenic hormone known to date, remains unaltered during HDBR, plasma concentrations of glucagon-like peptide-1 (GLP-1), a satiety hormone secreted by the gut, increase. Throughout 60 days of HDBR in healthy women, fasting plasma leptin concentrations were correlated with energy intake and fat mass (Bergouignan et al., 2010). Further studies are needed to better understand the effect of exercise training on the regulation of appetite, energy intake, and hence energy balance under microgravity conditions.

## 6.2 Nutrition

The main role of the space food system is to provide astronauts with the nutrients they need to meet their requirements. Defining and satisfying the nutritional requirements of astronauts has been an ongoing challenge since the very first spaceflights. For short missions, from a few days to a few weeks, inadequate nutritional intake would have few consequences. For long-duration missions, on the other hand, a nutritional imbalance could have major consequences, such as clinically significant

weight loss, hormonal problems, bone fragility, etc. For long-duration space travel beyond Earth orbit, it will be essential that nutrition not only prevents nutrient deficiencies but also serves as a countermeasure to the many negative effects induced by microgravity on physiological systems. To date, this represents a gap in our understanding of the role of nutrition in spaceflight.

On Earth, amino acid supplementation has been evaluated to counteract the loss of muscle mass and strength observed during spaceflight and prolonged HDBR studies. In their review, Stein and Blanc report that of the six studies that tested this countermeasure, only three show positive effects (Stein & Blanc, 2011). Results depend on basal protein intake, with the efficacy of supplementation decreasing when daily protein intake exceeds 1.2 g/kg/d. Other strategies have been tested, including supplementation with compounds with antioxidant and anti-inflammatory properties. Several studies in humans and rats demonstrate the positive effects of nutrients such as polyphenols, certain vitamins, and essential fatty acids on microgravity-induced adaptations (Momken et al., 2011; Zwart et al., 2009). A cocktail of antioxidant and anti-inflammatory compounds ( $\omega$ -3 fatty acids, vitamin E, polyphenols, selenium) has been tested during 60 days of HDBR in healthy men (Arc-Chagnaud et al., 2020; Blottner et al., 2021). Proteomic and gene expression analyses on muscle biopsies from this study show that the cocktail prevented protein nitrosation (a marker of RNS-induced protein damage), muscle atrophy, and decreased antioxidant defense (Blottner et al., 2021). Simultaneously, a decrease in carbonylated protein levels (an indicator of oxidative stress-induced protein damage) in the supplemented group is highlighted, while no effect on decreased muscle strength, muscle fiber atrophy, or decreased oxidative capacity is noted (Arc-Chagnaud et al., 2020). The findings regarding whole-body metabolic outcomes have not been published yet. However, a preliminary investigation indicates a potential preventative impact on changes in lipid metabolism, though not on insulin sensitivity, among healthy men undergoing sedentarization protocol (i.e. drastic reduction in daily steps) (Damiot et al., 2019).

## 7 Current challenges & Future directions

As demonstrated earlier, an accurate assessment of astronauts' energy requirements requires an understanding of the factors affecting the regulation of the energy balance during flight. On one hand, a chronic negative energy balance (i.e. either insufficient energy intake, excessive energy expenditure, or both) can contribute to the other deleterious consequences induced by simulated or real microgravity such as orthostatic intolerance, decreased muscle mass loss and functionality, bone loss, and aerobic deconditioning (Doucet et al., 1998; Schmitz-Peiffer et al., 1999). On the other hand, positive energy balance (i.e. excessive energy intake, insufficient energy expenditure, or both) is shown to accelerate muscle atrophy and promote chronic inflammation during HDBR studies. Clinical research has also clearly established that energy surplus, especially under physically inactive conditions, adversely impacts metabolic health. The physical exercise regimen and extravehicular activities both need high energy expenditure. These factors cannot be overlooked and must be considered when assessing energy needs. It is important to develop accurate tools to estimate physical activity patterns and the time spent in each type of physical activity (time spent walking, running, cycling, resistance training, and others) and to objectively measure the specific energy cost of the different types of physical activity. Currently, there is limited understanding regarding the regulation of feeding behavior and appetite control in microgravity conditions.

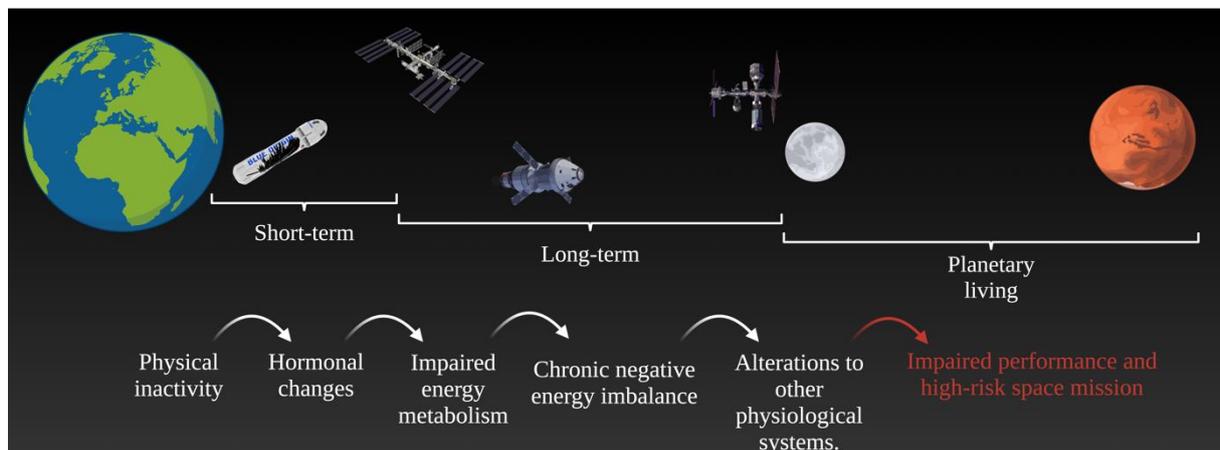


Figure 6 Current Challenges for Interplanetary Spaceflight

After decades of data collection from ground-based studies, we have made significant strides in understanding the mechanisms triggered by microgravity, which result in a metabolic state similar to certain lifestyle-related metabolic pathologies observed on Earth. However, the precise sequence of these mechanisms remains to be further elucidated. Such knowledge is crucial for developing more

effective countermeasures to mitigate the adverse impacts of microgravity on metabolism and, by extension, on other physiological systems.

The emergence of space tourism promises to accentuate the already significant interindividual variations observed in physiological responses to microgravity. These variations, influenced by factors such as age, physical activity level, and sex, contribute to the complexity of understanding the synergistic effects of physiological alterations in space. While space travel was historically confined to astronauts, the ongoing evolution of space tourism is set to democratize access to microgravity, inviting a diverse array of individuals into this unique environment. As a result, there is a growing need to shift our focus from studying groups of subjects to examining individual physiological responses to microgravity, allowing for the development of personalized recommendations. Furthermore, the impact of repetitive space expositions on the human body remains uncertain and warrants investigation, as it could potentially emerge as a new frontier in space health research. This requires combining state-of-the-art and accurate methods to measure biomarkers of nutritional and metabolic states, changes in body mass, energy intake, and energy expenditure along with modeling, artificial intelligence, and the use of numerical twins to thus predict the upcoming changes and anticipate the personalized countermeasure to implement (Nicolou et al., 2023).

## 8 Major takeaways

- By impacting all the tissues involved in the regulation of energy homeostasis, microgravity induces the development of metabolic disturbances including hypertriglyceridemia, insulin resistance, glucose intolerance, impaired capacity to burn as fuel along with a shift in fuel preference for carbohydrates, metabolic inflexibility, and ectopic fat storage, which may contribute to muscle loss. These metabolic alterations are close to the metabolic features observed in people with metabolic diseases on Earth including overweight, obesity, type 2 diabetes, and metabolic syndrome.
- During spaceflight, a wide inter-astronaut variability in changes in body composition, energy expenditure, and nutrient metabolism exists. These variations are probably related to the astronauts' physical activity during the flight and their usual fitness levels.
- Future research needs to better understand the influence of individual factors including sex, race/ethnicity, age, aerobic fitness, body composition, etc. on the metabolic response to microgravity.

- Interplanetary human exploration requires better tracking of changes in the nutritional and metabolic state of space travelers along with changes in food intake, energy expenditure, and physical activity to tailor and implement efficient personalized countermeasure programs.

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