Chapter 9

INTERACTIONS BETWEEN ARTIFICIAL GRAVITY, THE AFFECTED PHYSIOLOGICAL SYSTEMS, AND NUTRITION

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1 INTRODUCTION

Malnutrition, either by insufficient supply of some nutrients or by overfeeding, has a profound effect on the health of an organism. Therefore, optimal nutrition is a necessity in normal gravity on Earth, in microgravity, and when applying artificial gravity to the human system.

Reduced physical activity, such as observed in microgravity or bed rest, has an effect on many physiological systems, such as the cardiovascular, musculoskeletal, immune, and body fluids regulation systems. There is currently no countermeasure that is effective to counteract both the cardiovascular and musculoskeletal deconditioning when applied for a short duration (see Chapter 1). Artificial gravity therefore seems the simplest physiological approach to keep these systems intact. The application of intermittent daily dose of artificial gravity by means of centrifugation has often been proposed as a potential countermeasure against the physiological deconditioning induced by spaceflight.

However, neither the optimal gravity level, nor its optimal duration of exposure have been enough studied to recommend a validated, effective, and efficient artificial gravity application. As discussed in previous chapters, artificial gravity has a very high potential to counteract any changes caused by reduced physical activity. The nutrient supply, which ideally should match the actual needs, will interact with these changes and therefore has also to be taken into account. This chapter reviews the potential interactions between these nutrients (energy intake, vitamins, minerals) and the other physiological systems affected by artificial gravity generated by an on-board short-radius centrifuge.

2 ENERGY INTAKE AND MACRONUTRIENT SUPPLY

It is well known that astronauts, except perhaps during the Skylab missions, were and are still not optimally nourished during their stay in space (Bourland *et al.* 2000, Heer *et al.* 1995, Heer *et al.* 2000b, Smith *et al.* 1997, Smith and Lane 1999, Smith *et al.* 2001, Smith *et al.* 2005). It has also been described anecdotally that astronauts have lower appetites during space missions. One possible explanation is that taste and smell sensations are altered during spaceflight. Although some early observations suggest that this is not the case (Heidelbaugh *et al.* 1968, Watt *et al.* 1985), data from recent head-down bed rest studies showed significant decrease in smell sensation (Enck *et al.*, unpublished data). This finding suggests that fluid shifts might have an impact in the decrease in smell sensation. If this finding is confirmed during spaceflight, a decrease in smell could be responsible for lowered food intake, causing insufficient energy intake and subsequently insufficient supply of most of the macro- and micronutrients to the organism.

On the other hand, other nutrients are taken in excess, as it is the case for sodium. It is well known (especially from the company that manufacture packaged food) that food with high salt content seems to be more palatable than food with low salt content. Salt also functions as a preservative, which is very important taking into account the food system limitations during spaceflight, such as the limited amount of refrigerator and freezer space. The preference for food with high salt intake by astronauts might therefore very likely be caused by altered smell and taste sensations in microgravity.

2.1 Energy Intake

During most past space missions, astronauts have had an insufficient energy intake. On average their energy intake was about 25% less than their expenditure, thus leading to a loss in body mass (Bourland *et al.* 2000), including in muscle and fat tissue. Although caloric intake in the recent ISS missions has been slightly improved, it is still not optimal (Smith *et al.* 2005).

Energy expenditure consists of the *resting energy expenditure* (REE) plus the energy requirements for any activity (e.g., exercise, walking), plus the thermogenesis derived from the metabolism of protein, fat, and carbohydrates. As mentioned previously, voluntarily chosen energy intake by the astronauts in microgravity does usually not match the energy needs (Bourland *et al.* 2000, Smith *et al.* 2005). Experience from head-down tilt bed rest studies also shows that volunteers are rather reluctant to consume all the food prescribed to meet their energy expenditure.

Animals exposed to centrifugation increase their energy expenditure substantially. Wade *et al.* (2002) have shown that 2-week centrifugation (24 hour per day; 2.3 g or 4 g) led to a 40% increase of REE in rats, independently from the gravity level. In another experiment where rats were continuously exposed to 1.25, 1.5, and 2 g for 14 days, the mean body mass was significantly lower than non-centrifuged controls, but no differences were found in food intake (expressed in 'g per day per 100 g of body mass') between the hypergravity group and the controls. Epididymal fat mass was 14 to 21% lower than controls in the centrifuged group. Plasma insulin was also significantly lower (about 35%) in the hypergravity groups than controls, suggesting an improved sensitivity to insulin (Warren *et al.* 2000, Warren *et al.* 2001, Moran *et al.* 2001).

Lowered energy intake has a profound effect on the cardiovascular system (Mattson and Wan 2005). This has mainly been shown in obese people during semistarvation (Hafidh *et al.* 2005, Brook 2006, Sharma 2006, Poirier *et al.* 2006), in pilots during Ramadan (Bigard *et al.* 1998), and in a metabolic ward study in normal weight subjects during head-down bed rest (Florian *et al.* 2004). Hence, in the latter, moderate energy restriction of 25% of energy intake led to profound decrease in orthostatic tolerance, which was even higher than the effect of bed rest. Taking into account that centrifugation will lead to a fluid shift towards the lower legs, insufficient caloric intake and concomitant cardiovascular reactions might jeopardize the compensating effect of artificial gravity because symptoms of presyncope might on one hand lead to an early stop of the centrifugation protocol and on the other hand might interact with any countermeasure effect to the cardiovascular system.

When total energy intake is less than total energy expenditure, endogenous energy stores (e.g., glycogen, protein, fat) have to be mobilized. In order to provide sufficient energy for the body, these endocrine energy stores are used. After the glycogen stores are used up, muscle protein is used as an amino acid/energy source, thus leading to a decrease in muscle mass in addition to the muscle mass loss caused by disuse. In microgravity or during bed rest, protein synthesis is reduced while protein breakdown stays the same, thus resulting in a loss of muscle mass (Biolo *et al.* 2004, Ferrando *et al.* 1996). In these conditions, hypocaloric nutrition, even at moderate levels, will exacerbate muscle loss, since muscle protein functions as an energy delivering nutrient (Lorenzon *et al.* 2005). A severe decrease in energy intake increases bone resorption, as shown in patients suffering from *anorexia nervosa* (Heer *et al.* 2002, Heer *et al.* 2004c) and in exercising women (Ihle and Loucks 2004). Moderate restriction in energy intake, however, seems to have no effect in bed rested male test subjects (Heer *et al.* 2004b).

The application of artificial gravity may have an anabolic effect on bone, and lead to an increase in bone modeling (see Chapter 7). Severe low caloric intake will lead to a suppression of osteoblast activity, if this activity is not stimulated by the mechanical loading induced by passive centrifugation (Heer *et al.* 2002, Heer *et al.* 2004c). Sufficient energy supply is therefore a prerequisite for using artificial gravity as a countermeasure to bone loss in immobilized subjects.

If increased REE during centrifugation occurs in humans as well, and if a combination of artificial gravity and exercise countermeasures is more effective for compensating cardiovascular deconditioning, maintaining muscle mass and strength, and bone mass, then assuring optimal energy intake will be a critical co-factor for the success of artificial gravity as a countermeasure.

2.2 Protein Supplementation

Protein intake during spaceflight is about 102 ± 29 g per day (Smith *et al.* 2005) or 1.4 ± 0.4 g per kilogram of body weight per day. So, protein intake in microgravity is a concern because of too much intake rather than not enough intake. As mentioned above, reduced physical activity leads to a decrease in protein synthesis, constant protein breakdown, and concomitant loss in muscle mass. Paddon-Jones *et al.* (2004) have shown that increasing protein intake to about 1.5 g per kilogram of body weight per day by using branched-chain amino acid together with carbohydrate supplementation preserves not only muscle mass but also muscle strength. In addition, Biolo *et al.* (1995b, 1997) have shown that increased protein intake combined with resistive exercise lead to an increased amount of muscle protein. Centrifugation of a passive subject lying on a short-radius device is equivalent to isometric resistive exercise. Therefore, supplementing protein during passive centrifugation might be a potential measure to keep up muscle mass and strength. However, the timing of this protein supplementation is very important. According to Biolo *et al.* (1997) protein has to be supplemented shortly before or after the resistive exercise training in order to induce an increase in muscle protein synthesis.

An increase in protein supplementation, however, has some disadvantages for bone metabolism. As discussed in Chapter 7, immobilization *per se* leads to decrease in bone mass and strength in the lower legs. Increase in protein intake, however, might also have a bone resorption effect, which is highly dependent on the nutrients provided with the higher protein intake (Massey 2003). In this context, the intake of potassium seems to be very important. The effect of increase in bone resorption during rather low potassium intake together with high protein intake is even more important during immobilization where bone turnover is already increased. Our group has observed an increased relationship of animal protein intake to potassium intake during immobilization in bed rested healthy test subjects, that exacerbated the effect of mere bed rest (Zwart *et al.* 2004). As previously suggested by others authors, this effect seems to be mediated by changes in the acid-base balance. High animal protein intake, together with low potassium intake, leads to a rather high potential of renal acid load. This might lead to mild metabolic acidosis. Mild metabolic acidosis has shown to be a strong cause for increasing bone resorption (Meghji *et al.* 2001, Riond 2001, Bushinsky 1994, Bushinsky *et al.* 1999). Therefore, applying high protein intake plus artificial gravity might have a positive effect on muscle mass and strength. However, mild metabolic acidosis, which potentially increases bone resorption, must be counteracted by other countermeasures.

2.3 Insulin Resistance

The sensitivity to insulin has been shown to decrease in many bed rest studies (Mikines *et al.* 1989, Mikines *et al.* 1991, Shangraw *et al.* 1988, Smorawinski *et al.* 1996, Stuart *et al.* 1990, Yanagibori *et al.* 1994, Yanagibori *et al.* 1997, Blanc *et al.* 2000, Smorawinski *et al.* 2000, Stuart *et al.* 1988). Physical fitness and training status of the subjects might have an impact on insulin sensitivity, according to studies carried out in trained and untrained test subjects (Wegmann *et al.* 1984, Smorawinski *et al.* 1996, Smorawinski *et al.* 2000). Furthermore, studies in trained and untrained test subjects have demonstrated that insulin resistance in untrained volunteers is due to a reduced sensitivity to insulin of their inactive muscles (Mikines *et al.* 1991, Stuart *et al.* 1988, Blanc *et al.* 2000). The effects of isometric, resistance exercise training on insulin sensitivity were tested in a prospective study by Tabata *et al.* (1999). Their data showed an improved glucose uptake of the muscles, indicating that resistance exercise training during bed rest could overcome the effect of inactivity (Tabata *et al.* 1999).

Besides its effects on glucose metabolism, insulin is also a regulator of protein metabolism. The synthesis of myofibrillar protein requires physiological levels of insulin. Hyperinsulinemia caused by insulin infusion, while holding blood amino acid concentrations normal, leads to increased rates of protein synthesis without changing protein breakdown in muscle in ambulatory healthy volunteers (Biolo *et al.* 1995a, Biolo *et al.* 1999). However, in the case of decreased insulin sensitivity, such an increased protein synthesis may not take place. Like in patients with type II-diabetes (Tessari *et al.* 1986), the insulin resistance in bed rest subjects might be responsible for a decreased muscle protein synthesis during immobilization.

Artificial gravity generated by a short-radius centrifuge in some way mimics isometric, resistance exercise, and one might speculate that artificial gravity might have a positive effect on insulin sensitivity. Thereby increased insulin sensitivity might also have a positive effect on muscle mass and strength. In order to distinguish between the potential effects of changed insulin sensitivity and resistive exercise on muscle mass and strength, further studies are mandatory to validate the effect of resistive exercise as well as artificial gravity.

3 VITAMINS AND ARTIFICIAL GRAVITY

3.1 Vitamin A

Vitamin A is a general term that refers to a family of fat-soluble compounds that are structurally similar to retinol and share its biological activity. Among these are retinol, β-carotene, and retinyl palmitate. Trans-retinol is the primary biologically active form of vitamin A. Many carotenoids, such as β-carotene, can be converted to trans-retinol and thus contribute to vitamin A activity. Collectively, these carotenoids are termed provitamin A carotenoids and are measured in retinol equivalents.

Vitamin A plays a role, albeit sometimes indirectly, in the function of almost all of the body's organs (Ross 1999). Vitamin A is directly involved in vision, bone growth, cell division, reproduction, and immunity. Vitamin A and β-carotene serve as biological antioxidants and have been shown in multiple studies to reduce the risk of cancer and coronary heart disease (Kohlmeier and Hastings 1995, van Poppel and Goldbohm 1995).

Deficiency of vitamin A leads to xerophthalmia, loss of appetite, drying and keratinization of membranes, or infection. Likewise, ingestion of large amounts of vitamin A are commonly associated with adverse skeletal effects (Dickson and Walls 1985, Hough *et al.* 1988, Scheven and Hamilton 1990). The mechanisms are thought to include suppressed osteoblast activity, stimulated osteoclast formation, and impaired function of vitamin D (Jackson and Sheehan 2005).

Serum levels of retinol and retinol-binding protein are decreased after long-duration spaceflight. One supporting animal study found that both serum retinol and retinol binding protein were decreased after prolonged immobilization (Takase *et al.* 1992), and the changes were thought to be related to a stress response.

Artificial gravity may induce changes in stress hormones (see Chapter 10), which may in turn affect vitamin A metabolism. Furthermore, care must be taken to avoid ingestion of large supplemental amounts of vitamin A during bed rest or artificial gravity studies due to its known toxic effects on the skeletal system.

3.2 Vitamin K

Vitamin K plays a role as a cofactor in the carboxylation of a limited number of proteins. The vitamin K-dependent carboxylase is an enzyme responsible for the posttranslational conversation of specific glutamate to *gamma-carboxyglutamate* (Gla) residues. Three carboxylated proteins, osteocalcin, matrix Gla protein, and protein-S, have been identified in bone (Hauschka *et al.* 1989, Vermeer *et al.* 1995). Osteocalcin is a protein synthesized by osteoblasts, and in its carboxylated form, osteocalcin exhibits strong calcium binding properties and is related to the bone mineralization process (Shearer 1995). In case of vitamin K deficiency undercarboxylated osteocalcin, which lacks some or all of the Gla residues, is synthesized. Therefore blood concentration of undercarboxylated osteocalcin is a sensitive marker for vitamin K nutritional status (Knapen *et al.* 1989, Sokoll *et al.* 1997, Vermeer and Hamulyak

1991). The discovery of these vitamin K-dependent proteins in bone has led to research on the role of vitamin K in maintaining bone health. Epidemiological studies provide evidence for an association between low vitamin K intake and an enhanced osteoporotic fracture risk (Hart *et al.* 1985, Booth *et al.* 2000). A higher incidence of femoral neck (Vergnaud *et al.* 1997) and hip (Szulc *et al.* 1996) fractures has been observed in patients with high levels of undercarboxylated osteocalcin. Moreover, as a result of the vitamin K supplementation, the urinary calcium excretion was decreased by 30% in the fast losers (Knapen *et al.* 1989, Knapen *et al.* 1993).

While bone resorption can be counteracted (e.g., by bisphosphonates), there is no proven countermeasure for the decrease in bone formation. Vermeer *et al.* (1998) and Caillot-Augusseau *et al.* (2000) observed a profound effect of Vitamin K on bone formation in microgravity. During the 179-day Euromir 95 mission, one astronaut received vitamin K supplementation of 10 mg Vitamin K1 (Konakion®) for 6 weeks during the second part of the mission, as a countermeasure for spaceflight induced bone loss. This astronaut showed a very promising effect: while bone formation markers, PICP and serum *bone alkaline phosphatase* (bAP) had decreased in the first part of the mission (without Vitamin K supplementation), their concentration levels were comparable to preflight with vitamin K supplementation (Vermeer *et al.* 1998). In two other astronauts, undercarboxylated osteocalcin increased from preflight levels of 12-15% to 25% within the first 5 days in-flight. In one of these astronauts, a supplementation with 10 mg vitamin K 1 was able to decrease the levels of undercarboxylated osteocalcin into the preflight range. Moreover, Vermeer and Ulrich (1986) showed that the amount of Gla-residues is reduced by more than 50% in the postflight samples.

With regard to artificial gravity, the vitamin K status of the astronauts would need to be adequate to optimize the counteractive potential of artificial gravity. Resistive exercise leads to an increase in bone formation markers (Shackelford *et al.* 2004, Maimoun *et al.* 2005) and therewith to an increase in osteocalcin. If there is a lack of substrate, such as vitamin K, for carboxylation of osteocalcin, this undercarboxylated osteocalcin can not bind to hydroxyapatite and therefore might not play its role in the mineralization process. A supplementation with vitamin K seems to have a very high potential to reduce the amount of undercarboxylated osteocalcin and, moreover, counteracts the decreased bone formation.

3.3 Vitamin B6

Vitamin B6 comprises a group of three compounds and their 5’-phosphates: *pyridoxal* (PL) and PLP, *pyridoxine* (PN) and PNP, and *pyridoxamine* (PM) and PMP. These vitamers of B6 serve as coenzymes in many transamination, decarboxylation, and trans- and desulfydration reactions involved in immune function and synthesis of several neurotransmitters (Institute of Medicine 1998, McCormick 2001).

Approximately 70% of vitamin B6 is stored in muscle tissue associated with glycogen phosphorylase (Coburn *et al.* 1988): 10% is stored in the liver, and 60% is stored in the plasma pool (Institute of Medicine 1998). Since vitamin B6 is mainly stored in muscle tissue, a decrease in muscle mass could reduce the amount of the vitamin that is stored, or even influence vitamin B6 metabolism. Supportive of this, urinary excretion of 4-pyridoxic acid is indeed elevated after long-duration (17 weeks) bed rest when muscle mass is known to decrease (Coburn *et al*. 1995). Based on data from 4-6 month spaceflights, there is no change in red blood cell transaminase activation (Smith *et al.* 2005), However, plasma PLP has not been determined after long-duration spaceflight.

Vitamin B6 may also be involved with oxidative stress due to its role in homocysteine, cysteine, and glutathione metabolism (Kannan and Jain 2004, Mahfouz and Kummerow 2004). Vitamin B6 deficiency increases oxidative stress and decreases antioxidant defense systems (Taysi 2005, Voziyan and Hudson 2005). Furthermore, pyridoxamine supplementation can reduce oxidative damage in both animal and human studies (Anand 2005, Voziyan and Hudson 2005).

Because both oxidative stress and decreased muscle mass are observed during spaceflight and during head-down-tilt bed rest (Ferrando *et al.* 2006, LeBlanc *et al.* 2000, Zwart and Oliver 2006, Smith *et al.* 2005), vitamin B6 metabolism should be monitored during these instances. With respect to artificial gravity, we expect muscle mass may be maintained, and therefore artificial gravity may maintain vitamin B6 status.

4 MINERALS AND ARTIFICIAL GRAVITY

4.1 Calcium and Vitamin D

During most of the space missions, calcium intake and vitamin D supply were below the recommended intake values (Bourland *et al*. 2000). For example, although calcium intake has been improved recently, during the first 8 increments on board ISS calcium intake was about 1000 mg per day (Smith *et al.* 2005, Heer *et al.* 1999, Smith and Heer 2002). Adequate calcium intake is a prerequisite to mineralize bone during life. Convincing evidence has emerged with respect to the effects of dietary calcium intake on bone health in all age groups. A number of reports led to a consensus view on the effectiveness of calcium together with vitamin D supplementation in postmenopausal osteoporosis (Chee *et al.* 2003, Lau and Woo 1998, Cumming and Nevitt 1997, Ilich and Kerstetter 2000, Prentice 2004). High calcium intake cannot prevent bone loss but can reduce the rate of bone loss in older women. Dawson-Hughes *et al.* (1997) showed that combined supplementation with calcium and vitamin D for three years significantly reduced non-vertebral fracture rates in men and women (mean age 71 years).

Astronauts in space have high serum calcium levels because of increased bone resorption (Smith *et al.* 2001). High serum calcium concentration and low 25-hydroxyvitamin D levels are also observed during bed rest (van der Wiel *et al.* 1991). One might argue that increasing calcium intake above the recommended levels, together with vitamin D supplementation, might counteract the microgravity-related and bed rest-induced bone losses. However, data from the Mir-97 mission and bed rest studies show that calcium absorption is reduced (Smith *et al.* 1999, Zittermann *et al.* 2000) and calcitriol concentrations are decreased (Heer *et al.* 1999, Rettberg *et al.* 1999), so that increased calcium intake above the recommended level is not absorbed.

In short-term (6-14 day) head-down bed rest studies it was shown that bone turnover was unchanged by increasing calcium intake from 1000 mg per day to 2000 mg per day (Heer *et al.* 2004a). Increasing calcium and vitamin D intake above the recommended levels appear to be ineffective as a nutritional countermeasure to maintain bone mass in bed rest without any mechanical loading. If artificial gravity acts as a form of isometric exercise, it might activate bone-forming cells. When bone formation is increased and bone built, all mandatory nutrients including calcium and vitamin D should be supplied in a sufficient amount in order not to limit bone formation because of malnutrition. In case of bed rest combined with centrifugation, the questions remains if calcium intake above the recommended level is necessary to maintain bone mass and strength.

In addition to its effect on calcium homeostasis, vitamin D also affects skeletal muscle (Bischoff-Ferrari *et al.* 2006). Vitamin D binds to specific receptors on skeletal muscle for 1,25-dihydroxyvitamin D (Bischoff-Ferrari *et al.* 2006). Investigations in the elderly showed that muscle strength is related to vitamin D status. Low serum 25-hydroxyvitamin D levels are related to lower muscle strength (Bischoff *et al.* 1999, Zamboni *et al.* 2002) and to a loss of muscle mass and muscle strength (Visser *et al.* 2003). Snijder *et al.* (2006) showed that low physical performance is associated with low serum 25-hydroxyvitamin D levels. With regard to artificial gravity, the supply of vitamin D in a sufficient amount might be preventive to achieve muscle strength as well.

4.2 Phosphorus and Magnesium

Phosphorus and magnesium are critical minerals for human health. Phosphorus is a critical element of many enzymes, cellular messengers, and carbohydrate fuels. Osteomalacia, a defect in bone mineralization, often occurs as a result of long-term phosphorus deficiency. Inadequate intake of phosphorus can cause the release of calcium from bone, impaired granulocyte function, and cardiomyopathy (Knochel 1999).

Magnesium is required as a cofactor for over 300 enzyme systems and serves as a substrate for phosphate transfer reactions in all cells. Adequate intake of magnesium is necessary to prevent hypocalcemia, resistance to vitamin D, and resistance to parathyroid hormone (Shils 2006). Magnesium is also critical for cardiovascular health.

There is evidence that magnesium and phosphorus are altered after long-duration spaceflight. Urinary magnesium and phosphorus were about 45% less after landing than before launch in 11 ISS crewmembers (Smith *et al.* 2005). Results of previous spaceflight studies are consistent with a significant decrease in urinary magnesium (Leach and Rambaut 1977, Leach 1992), possibly owing to a decrease in magnesium intake. Decreased urinary magnesium could be a point of concern for long-duration flights because of the role of magnesium in inhibiting calcium oxalate renal stones (Su *et al.* 1991, Grases *et al.* 1992).

The cause, extent, and impact of alterations in magnesium and phosphorus homeostasis during spaceflight are not well defined. However, it is quite possible that artificial gravity effects on musculoskeletal health may help to reverse these changes. This too, remains to be proven.

4.3 Sodium

Sodium is the major cation of the extracellular volume and plays a major role in keeping up the membrane potential, nutrient absorption, as well as the maintenance of blood volume and blood pressure. However, as for the majority of people in the western world, sodium intake of astronauts in spaceflight is far above the recommended levels. We have shown that during the recent ISS missions (increment 1-8) the average sodium intake was 4556 ± 1492 mg per day (Smith *et al.* 2005).

High *sodium chloride* (NaCl) intake affects most of the physiological systems, like body fluid regulation, cardiovascular as well as the musculoskeletal system. We have recently shown that in space sodium intake mainly as NaCl leads to sodium retention without fluid retention (Drummer *et al.* 2000). In some metabolic balance studies we demonstrated that on Earth high NaCl intake also leads to sodium retention without fluid retention (Heer *et al.* 2000a) and may induce mild metabolic acidosis (Frings *et al.* 2005). Now, mild metabolic acidosis has a significant effect on release and function of several hormones including defects in growth hormone, IGF-1, insulin, glucocorticoids, thyroid hormone, parathyroid hormone and vitamin D (Mitch 2006). It also affects the musculoskeletal system as described in the section on protein metabolism. For muscle, decrease in pH may inhibit protein synthesis, may lead to insulin resistance (which, as described above, is a risk because of immobilization already) and concomitantly may activate proteolytic mechanisms leading to protein breakdown. Application of artificial gravity by centrifugation as described above may act as a resistive exercise and if so might lead to anaerobic processes and consequently reduce pH by increasing lactate acid production (McCartney *et al.* 1983, Kowalchuk *et al.* 1984, Putman *et al.* 2003, Lindinger *et al.* 1995). The anabolic effect aimed at with applying artificial gravity might be at risk, in case of high salt intake because of induced mild metabolic acidosis. The prescription of artificial gravity should therefore be developed in such a way that all the impacting metabolic changes are taken into account.

It has been shown in studies in pre- and postmenopausal women (Nordin *et al.* 1993) and calcium stone-forming patients (Martini *et al.* 2000) that increasing sodium intake has also a profound effect on bone metabolism like increase in calcium excretion (Nordin *et al.* 1993) associated with lower area bone mass density (Martini *et al.* 2000). Nordin et al (Nordin *et al.* 1993) postulated that the rise in urinary calcium excretion is sodium driven. Increasing sodium intake by each 100 mmol (2300 mg) raises urinary calcium excretion by 1 mmol (40 mg). Taking into account that the average calcium excretion is around 120 to 160 mg per day, the rise in calcium excretion by higher salt intake is substantial. These findings were supported by Arnaud *et al.* (2000) in a 7-day bed rest study. The mechanism by which high sodium intake exacerbates urinary calcium excretion is not fully understood. As mentioned above we have shown that high salt intake decreases blood pH bicarbonate and base excess levels (Frings *et al.* 2005). Concurrently, bone resorption markers were significantly increas. This supports the notion of Arnett (2003) who stated that even mild metabolic acidosis (pH-changes of <0.05) may activate osteoclasts and may cause appreciable bone loss over time in ambulatory conditions, and may exacerbate bone loss in bed rest. Application of exercise on top of high salt intake though has to be applied with caution. As mentioned above, exercise may increase blood lactate levels and reduce thereby blood pH. When applying artificial gravity as a resistive exercise training blood lactate levels should not lead to a strong metabolic acidosis in order to not jeopardize and bone forming process initiated by the mechanical loading.

4.4 Potassium

As the major intracellular cation, potassium has a significant role in many physiological processes (Preuss 2001). Potassium is critical to regulation of acid-base balance, energy metabolism, blood pressure, membrane transport, and fluid distribution within the body. It is also involved in the transmission of nerve impulses and cardiac function (Kleinman and Lorenz 1984). Disordered potassium metabolism because of excess or deficient circulating levels has negative consequences for cardiac, muscle, and neurological function.

Potassium levels cannot be maintained at intakes under 10–20 mmol per day (Perez and Delargy 1988). Moderate depletion of potassium in humans is associated with clinically significant cardiovascular risks (Srivastava and Young 1995). During long-duration spaceflight, serum potassium is decreased and potassium balance is negative, suggesting potassium loss from the body (Johnston and Dietlein 1975, 1977, Leach-Huntoon and Schneider 1987). One of the main concerns for decreased potassium status during spaceflight is related to the increased cardiovascular risks.

Potassium metabolism and status may also contribute to an individual’s predisposition to orthostatic intolerance after exposure to microgravity or even tolerance to artificial gravity. In one study, subjects who failed a 60-min centrifugation on a short-radius centrifuge had higher salivary potassium than subjects who successfully withstood 60-min of centrifugation (Igarashi *et al.* 1994). The authors suggest that the potassium response may be due to the changes in autonomic nervous system function and stress response induced by centrifugation. Others show that orthostatic intolerant individuals during bed rest have higher baseline urinary potassium excretion (Grenon *et al.* 2004). Whether the differences in potassium metabolism are causes or effects in these instances are unknown.

While it is important to keep potassium intake at recommended levels for appropriate age groups (Institute of Medicine 2004), it is also important to monitor potassium status during artificial gravity experiments to minimize cardiovascular risks that may accompany changes in potassium status induced by stress responses. While potassium depletion is a concern during spaceflight, and this may in part be related to loss of muscle mass, artificial gravity may help to mitigate some of this concern.

4.5 Iron

Iron, while having multiple functions in the body, is critical for *red blood cell* (RBC) production and function. Maintenance of blood volume and RBCs has been of interest from the initial days of spaceflight, with concerns over a “spaceflight anemia”. The mass of RBCs in the body is decreased during flight, and the rate of loss is slightly greater than 1% per day, and reaching a net loss of 10 to 15% of RBC volume after 10 to 14 days of launch. Further decreases do not occur with longer flight durations.

Experiments performed on the Space Shuttle showed that the release of new RBCs is halted upon entry into weightlessness, and furthermore that newly released RBCs are selectively removed from the circulation (Alfrey *et al.* 1996b, Alfrey *et al.* 1996a, Udden *et al.* 1995). These changes in RBC mass seem to be adaptive, and reach a new plateau after the first weeks of flight, as evidenced by long-term flight data (Alfrey *et al.* 1996a, Leach and Rambaut 1975).

One consequence of the change in RBC mass is the associated increase in iron storage. Serum ferritin, an index of iron storage, is increased after short- and long-term flights. All other indices also suggest increased iron storage and availability during and after spaceflight. Serum iron concentrations are normal to elevated during and after flight. The concentrations of circulating transferrin receptors, which are lower during conditions of iron overload, are decreased on landing day. The implications of this increased iron storage not known, but concern exists about iron overload during extended-duration spaceflight (Smith 2002).

Artificial gravity may have an impact on iron metabolism and red blood cell metabolism. The decreased RBC mass during flight is believed to be in part related to the loss of pooling of RBCs in the lower extremities related to gravity. When entering weightlessness, these cells become part of the circulating population of RBCs, and the body senses an excess of available oxygen carrying capacity. Artificial gravity might cause a transient (depending on the duration of artificial gravity application) restoration of the pooling effect, which in turn might stimulate erythropoietin and RBC synthesis. Whether this would be beneficial (or detrimental) requires further study. On the positive side, this might help to alleviate the iron storage issues associated with flight, it might also increase plasma and red blood cell volumes, which might improve muscle cardiovascular function. On the negative side, this might stimulate erythropoiesis during the application of artificial gravity, followed by a re-adaptation to microgravity afterwards.

5 IMPACT OF ARTIFICIAL GRAVITY ON GI-TRACT

Gastrointestinal (GI) function may be altered during weightlessness. However, this has not been systematically studied, but has been discussed in several reviews (Da Silva *et al.* 2002, Lane *et al.* 1993, Smirnov and Ugolev 1996). Fluid shifts, inadequate fluid intake, altered blood flow would be expected to decrease gastrointestinal motility. Bed rest studies have confirmed this, where it was noted that the mouth-to-cecum transit time is increased during head-down-tilt when compared to ambulatory periods. As discussed above (Section 3.2), vitamin K is a concern for space travelers, and might be part of the mechanism of spaceflight-induced bone loss. While difficult to study, it is possible that the production and absorption of vitamin K by the gastrointestinal microflora is impaired during weightlessness due to changes in gastrointestinal function.

Artificial gravity may help with gastrointestinal function, and the intermittent application may physically stimulate motility. This would help with anecdotal reports of constipation. What effect this would have on nutrient and drug absorption is yet to be determined, but depending on the frequency and duration of exposure, it might provide an effective countermeasure. It might also be possible (or necessary) to coordinate the timing of application of artificial gravity with either meal times or ingestion of medication, to ensure optimal absorption.

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