# Interactions between Artificial Gravity, Affected Physiological Systems, and Nutrition

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## *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 mandatory on Earth (1 g), in microgravity and also when applying artificial gravity to the human system.

Immobilization like in microgravity or bed rest also has a profound effect on different physiological systems, like body fluid regulation, the cardiovascular, the musculoskeletal, the immunological system and others. Up to now there is no countermeasure available which is effective to counteract cardiovascular deconditioning (rf. Chapter 5) together with maintenance of the musculoskeletal system in a rather short period of time. Gravity seems therefore to be one of the main stimuli to keep these systems and application of certain duration of artificial gravity per day by centrifugation has often been proposed as a very potential countermeasure against the weakening of the physiological systems. Up to now, neither optimal intensity nor optimal length of application of artificial gravity has been studied sufficiently to recommend a certain, effective and efficient protocol. However, as shown in chapter 5 on cardiovascular system, in chapter 6 on the neuromuscular system and chapter 7 (bone and connective system) artificial gravity has a very high potential to counteract any degradation caused by immobilization. But, nutrient supply -which ideally should match the actual needs- will interact with these changes and therefore has also to be taken into account.

It is well known that astronauts –beside 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 & Lane 1999;Smith *et al.* 2001;Smith *et al.* 2005). It has also been described anecdotally that astronauts have lower appetites. One possible explanation could be altered taste and smell sensations during space flight, although in some early space flights no significant changes were found (Heidelbaugh *et al.* 1968;Watt *et al.* 1985).

However, data from a recent head-down bed rest study showed significant decrease in smell sensation (Enck et al. unpublished data) suggesting that fluid shifts might have an impact. If this holds true and which has to be validated in further studies, this seems to play an important role for lowered food intake causing insufficient energy intake and subsequently insufficient supply of most of the macro- and micronutrients. Other nutrients are taken in excess, for example sodium. As it is very well known from daily food consumption especially premanufactured food with high salt content seems to be more palatable than that with low salt content. Salt also functions as preservation which is very important taking into account the space food system limitations (i.e., lack of refrigerators and freezers). The preference for food with high salt intake by astronauts might therefore very likely be caused by altered smell and taste sensations in microgravity.

### *1. Energy intake and macronutrient supply: its effects during immobilization and implication of artificial gravity*

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

Energy expenditure consists of REE plus the energy requirements for any activity (exercise, walking etc.) plus 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 needs (Bourland *et al.* 2000;Smith *et al.* 2005). Experience from head-down tilt bed rest studies, a model to simulate the physiological effects of microgravity, also shows that volunteers are rather reluctant to consume all the food prescribed to meet energy expenditure.

Application of artificial gravity in animal studies (with 24 hour centrifugation) increases energy expenditure substantially. Wade et al. (Wade *et al.* 2002) have shown in rat experiments that 2-week centrifugation (24 hours a day) at 2.3 g or 4.0 g led to a 40% increase of resting energy expenditure (REE) independent from the g-load. In another animal experiment where rats were exposed to 1.25, 1.5 and 2.0 g loads for 14 days, 24 hours a day, the mean body mass of the hyper-G groups were significantly ($P < 0.05$) lower than controls, and no differences were found in food intake (g/day/100 g body mass) between the hypergravity groups and controls. Epididymal fat mass was 14 to 21% lower than controls in the centrifuged groups. Plasma insulin was significantly lower (about 35%) in the hypergravity groups than controls suggesting improved insulin sensitivity (Warren *et al.* 2000;Warren *et al.* 2001;Moran *et al.* 2001).

Lowered energy intake has a profound effect on the cardiovascular system (Mattson & 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 with and without 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 stores) have to be mobilized. In order to provide sufficient energy for the body endocrine energy stores are used, which also effects muscle protein. After the glycogen stores are used up, muscle protein will be used as an amino acid/energy source leading to a decrease in muscle mass in addition to the muscle mass loss caused by disuse. Now, in microgravity or bed rest protein synthesis is reduced while protein breakdown stay the same resulting in a loss of muscle mass (Biolo *et al.* 2004;Ferrando *et al.* 1996). Furthermore, hypocaloric nutrition will exacerbate the muscle loss even at moderate levels of hypocaloric nutrition- since muscle protein will function 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) or in exercising women (Ihle & Loucks 2004). Moderate restriction in energy intake, however, seems to have no effect in bed rested male test subjects (Heer *et al.* 2004b). However, as described in the bone chapter, application of artificial gravity may have an anabolic effect on bone and lead to an increase in bone modeling. In severe cases of low caloric intake, this leads to a suppression of osteoblast activity which might not be compensated by mechanical loading induced by 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 immobilization.

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

- **Protein (supplementation, interaction acid-base balance)**

Protein intake during spaceflight is about 102 ± 29g/d (Smith *et al.* 2005) or 1.4 ± 0.4 g/kg body weight/day. So, protein intake in microgravity is rather of concern because of high rather than low intake. However, as mentioned above immobilization leads to a decrease in protein synthesis, constant protein breakdown and concomitant loss in muscle mass. Paddon-Jones et al. (Paddon-Jones *et al.* 2004) have shown that increasing protein intake to about 1.5 g per kilogram body weight per day by applying 33% as branched-chain amino acid together with carbohydrate supplementation keeps not only muscle mass but also muscle force. Biolo et al. (Biolo *et al.* 1995b;Biolo *et al.* 1997) have shown additionally that combining increased protein intake with resistive exercise leads to an increased muscle protein. Now, applying centrifugation of certain g-loads leads to isometric resistive exercise. Supplementing protein might therefore be a potential measure to keep up muscle mass and force. However, timing of a respective protein supplementation is also very important. According to Biolo et al. (Biolo *et al.* 1997) protein has to be supplemented shortly before or after the respective resistive exercise training in order to induce an increase in muscle protein synthesis.

Increase in protein supplementation, however, has some disadvantages for bone metabolism. As already shown in the bone chapter, 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 very much dependent on the nutrients provided with the high protein intake (Massey 2003). In this context the intake of potassium seems to be extremely 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. We have shown in an immobilization study in bed rested healthy test subjects, that an increased relation of animal protein intake to potassium intake during immobilization exacerbates the effect of mere bed rest (Zwart *et al.* 2004). As suggested by others already, this effect seems to be mediated by changes in acid-base balance. High animal protein intake together with low potassium intake leads to a rather high potential of renal acid load, might lead to an at least 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 on one hand have a strong effect on muscle mass and force. However, mild metabolic acidosis which potentially increases bone resorption should be counteracted by other measures.

- **Insulin resistance**

In numerous bed rest studies it has been shown that the sensitivity to insulin is decreased (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/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). Further studies in trained and untrained test subjects have demonstrated that insulin resistance in untrained volunteers is due to reduced sensitivity of inactive muscles to insulin (Mikines *et al.* 1991;Stuart *et al.* 1988;Blanc *et al.* 2000). In a prospective study the effects of isometric, resistance exercise training on insulin sensitivity was tested (Tabata *et al.* 1999). For glucose metabolism the data of this study show that resistance exercise training during bed rest could overcome the effect of inactivity during bed rest, indicating an improved glucose uptake of the muscles (Tabata *et al.* 1999).

Apart from the effects of insulin 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 this increased protein synthesis may not take place. As in patients with Type II-Diabetes (Tessari *et al.* 1986) bed rest induced insulin resistance might therefore be an added cause for the decreased muscle protein synthesis during immobilization.

Artificial gravity 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 force. In order to distinguish between the potential effects of changed insulin sensitivity and resistive exercise on muscle mass and forth further studies are mandatory to validate the effect of resistive exercise as well as artificial gravity.

## *2. Vitamins and artificial gravity*

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 & Hastings 1995;van Poppel & 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 & Walls 1985;Hough *et al.* 1988;Jackson & Sheehan 2005;Scheven & Hamilton 1990). The mechanisms are thought to include suppressed osteoblast activity, stimulated osteoclast formation, and impaired function of vitamin D (Jackson & Sheehan 2005).

Serum levels of retinol and retinol-binding protein are decreased after long-duration space flight. 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, 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.

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 and therefore blood concentration of undercarboxylated osteocalcin is a sensitive marker for vitamin K nutritional status (Knapen *et al.* 1989;Sokoll *et al.* 1997;Vermeer & 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 -for example- by bisphosphonates, there is no proven countermeasure for the decrease in bone formation. Vermeer et al. (Vermeer *et al.* 1998) and Caillot-Augusseau et al. (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 space flight induced bone loss and showed a very promising effect: While bone formation markers, PICP and bAP, were decreased without Vitamin K supplementation in the first part of the mission, serum bone alkaline phosphatase (bAP) concentration revealed levels comparable to preflight with vitamin K supplementation (Vermeer *et al.* 1998). Caillot-Augusseau et al. (Caillot-Augusseau *et al.* 2000) found in two inflight-studies (Euromir 95 mission and MIR 97 mission) in two cosmonauts, that undercarboxylated osteocalcin increased from pre-flight levels of 12-15% to 25% within the first 5 days inflight. In one astronaut a supplementation with 10 mg vitamin K 1 was able to decrease the levels of undercarboxylated osteocalcin into the pre-flight range. Moreover Vermeer et al (Vermeer & Ulrich 1986) showed that the amount of Gla-residues is reduced by more than 50% in the post-flight samples.

With regard to artificial gravity vitamin K status of the subjects or astronauts needs to be adequate to optimize the counteractive probability of artificial gravity. Using 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, i.e. 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.

## 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 the rest 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 wk) bed rest when muscle mass is known to decrease (Coburn et al. 1995). Based on data from 4-6 month space flights, there is no change in red

blood cell transaminase activation (Smith *et al.* 2005); however, plasma PLP has not been determined after long-duration space flight.

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

Because both oxidative stress and decreased muscle mass are observed during spaceflight and during simulated weightlessness (head-down-tilt bed rest) (Ferrando *et al.* 2006;LeBlanc *et al.* 2000;Zwart & 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.

## *3. Minerals and artificial gravity*

- Calcium & Vitamin D

Calcium and vitamin D play are essential to built up bone mass. During most of the missions calcium intake, as well as vitamin D supply was below the recommended intake for space (Bourland et al. 2000). Although calcium intake has been improved during the ISS missions and was during ISS missions one to eight at a level of about 1000 mg/d (Smith *et al.* 2005). However, calcium intake was very low in previous missions and is still of concern for long-duration spaceflight (Bourland *et al.* 2000;Heer *et al.* 1999;Smith & 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 & Woo 1998;Cumming & Nevitt 1997;Ilich & 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. (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. High serum calcium concentration and low 25-hydroxyvitamin D levels are found in astronauts (Smith *et al.* 2001) as well as 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 P. *et al.* 1999) so that increased calcium intake above the recommended level will not be absorbed. In short-term 6- and 14-day head-down bed rest studies it was shown that bone turnover was unchanged by increasing calcium intake from 1000 mg/d to 2000 mg/d (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 because of exercise. Now, artificial gravity is a form of isometric exercise and might activate bone forming cells during the respective hypergravity periods. 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 artificial gravity it therefore remains the question if calcium intake above the recommended level is necessary to keep up bone mass and strength under these study conditions.

In addition to the effect of vitamin D on calcium homeostasis it 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 M et al. (Snijder *et al.* 2006) showed that low physical performance is associated with low serum 25-hydroxyvitamin D levels. Thinking about artificial gravity the supply of vitamin D in a sufficient amount might be preventive to achieve muscle strength as well.

- 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 space flight. Urinary magnesium and phosphorus were about 45% less after landing than before launch in 11 ISS crew members (Smith *et al.* 2005). Results of previous space flight studies are consistent with a significant decrease in urinary magnesium (Leach & 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 space flight 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.

- **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 hypergravity by centrifugation as described above may act as a resistive exercise and if so might lead to anaerobic processes and consequently reduce pH by increased 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 protocol 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 BMD (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. (Arnaud *et al.* 2000) in a seven 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 increased (Frings *et al.* 2005). This supports the notion of Arnett 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 (Arnett 2003) and may exacerbate bone loss in bedrest. 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.

- 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 & 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/day (Perez & Delargy 1988). Moderate depletion of potassium in humans is associated with clinically significant cardiovascular risks (Srivastava & Young 1995). During long-duration space flight, serum potassium is decreased and potassium balance is negative, suggesting potassium loss from the body (Johnston R.S. & Dietlein L.F. 1975;Johnston R.S. & Dietlein L.F. 1977;Leach-Huntoon & 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 arm 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.

- 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 space flight, with concerns over a "space flight 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 d of launch. At this level – 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 & 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 concentration 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.

### *4. Impact of artificial gravity on GI-tract (absorption, transit time, constipation)*

Gastrointestinal 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 & 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 – 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 application, 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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