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ORIGINAL ARTICLE

The negative effect of unloading exceeds the bone-sparing effect of alkaline supplementation: a bed rest study

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Abstract

Summary Potassium bicarbonate was administrated to an already alkaline diet in seven male subjects during a 21-day bed rest study and was able to decrease bed rest induced increased calcium excretion but failed to prevent bed rest-induced bone resorption.

Introduction Supplementation with alkali salts appears to positively influence calcium and bone metabolism and, thus, could be a countermeasure for population groups with an increased risk for bone loss. However, the extent to which alkalization counteracts acid-induced bone resorption or whether it merely has a calcium and bone maintenance effect is still not completely understood. In the present study, we hypothesized that additional alkalization to an already alkaline diet can further counteract bed rest-induced bone loss.

Methods Seven healthy male subjects completed two parts of a crossover designed 21-day bed rest study: bed rest only (control) and bed rest supplemented with 90 mmol potassium bicarbonate ($KHCO_3$) daily.

Results $KHCO_3$supplementation during bed rest resulted in a more alkaline status compared to the control intervention, demonstrated by the increase in pH and buffer capacity level (pH $p = 0.023$, HCO_3 $p = 0.02$, ABE $p = 0.03$). Urinary calcium excretion was decreased during $KHCO_3$ supplementation (control 6.05 ± 2.74 mmol/24 h; $KHCO_3$ 4.87 ± 2.21 mmol/24 h, $p = 0.03$); whereas, bone formation was not affected by additional alkalization (bAP $p = 0.58$; PINP $p = 0.60$). Bone resorption marker UCTX tended to be lower during alkaline supplementation (UCTX $p = 0.16$).

Conclusions The more alkaline acid-base status, achieved by $KHCO_3$ supplementation, reduced renal calcium excretion during bed rest, but was not able to prevent immobilization-induced bone resorption. However, advantages of alkaline salts on bone metabolism may occur under acidic metabolic conditions or with respect to the positive effect of reduced calcium excretion within a longer time frame.

Trial registration Trial number: NCT01509456

Keywords Bed rest · Bone metabolism · Calcium excretion · Potassium bicarbonate

Introduction

The role of acid-base balance in bone metabolism represents a scientific as well as popular public concern having implications for osteoporosis development. Acid-base balance may be the link between diet and bone metabolism, as this equilibrium is highly influenced by nutrient supply and interacts with many metabolic systems.

In vitro studies with isolated bone cells in acidotic milieu as well as the condition of a severe metabolic acidosis have shown damaging effects through activated bone resorption cells [1–5]. In addition, chronic low-grade metabolic acidosis or diet-induced acidosis appears to have

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similar albeit milder effects on calcium and bone metabolism which can lead to bone loss over extended periods of exposure [6–8]. The pH is kept constant by providing buffering substances deriving from bone [9]. Furthermore, there is growing evidence that a typical western-style diet with an acid load of 50–100 mEq/day presents an elevated risk factor for the development of osteoporosis due to excess acid intake as recently summarized in a review by Burckhardt [10, 11].

We have recently shown that a significant decrease in buffering capacity through decreased concentration of bicarbonate, induced by 14 days of high salt intake, increases calcium and bone resorption marker excretion [12, 13]. Consequently, increasing the buffer capacity, for example, with alkaline supplementation may protect the body from low-grade metabolic acidosis-induced bone loss. A recently published meta-analysis by Lambert et al. (2015) concluded that the alkaline salts potassium bicarbonate and citrate may be beneficial to bone health by lowering urinary calcium and bone resorption markers such as urinary collagen type 1 cross-linked N-telopeptide (NTX) [14]. In vitro, studies have already shown that alkalosis can directly decrease bone calcium efflux by suppressing osteoclasts and stimulating osteoblasts [15]. However, only few intervention studies in humans have demonstrated that bone resorption can be lowered with a more alkaline diet, via either supplements or high intake of alkalizing nutrients or an alkaline mineral water [16–22]. In the end, the question remains whether this effect is based on neutralization of an acidogenic diet or whether an alkaline status itself has a protective effect on bone metabolism. In either case, the consideration of dietary acid or alkaline load is of high importance for the prevention and therapy of bone disorders, especially in humans bearing an increased risk.

With respect to bone metabolism, astronauts represent a vulnerable group due to high bone loss in load-bearing bones during their stay in space. The effect is mainly induced by the physiological effects of unloading in microgravity but could be exaggerated by malnutrition. Consequently, optimizing nutrient intake during spaceflight to avoid further exacerbation of bone resorption could be complementary to the exercise countermeasures [23]. The mechanical unloading of bones during spaceflight can be simulated on Earth with head-down tilt (HDT) bed rest which has also been shown to rapidly induce bone loss [24] and therefore represents a useful model to study the various effects on inactivity on bone metabolism. Thus, the primary research question in the present study was to determine if additional alkalization to an already alkaline diet is effective in changing acid-base status and decreasing calcium excretion as well as bone resorption during 21 days of bed rest.

Materials and methods

Standardized study protocol

The study, named nutritional countermeasures study (NUC study), was conducted by the German Aerospace Center (DLR) and European Space Agency (ESA). The study protocol was approved by the local ethics commission (Aerztekammer Nordrhein, Duesseldorf, Germany) and conducted in accordance with the Declaration of Helsinki. The study was implemented and performed according to the standardized procedures and conditions of the "ESA (European Space Agency) Standardization Plan" issued by ESA for bed rest studies allowing the comparison and pooling of data collected from several ESA-funded studies (standardization of bed rest study conditions, Version 1.5, ESTEC contract no. 20187/06/NL/VJ). In addition, the study was registered at http://www.clinicaltrials.gov with the unique trial number: NCT01509456.

Subject recruitment

Healthy, male, non-smoking subjects with a height of 158–190 cm and a body weight of 65–85 kg, who were not participating in any kind of high-performance sports were recruited for the study. Subject recruitment was conducted in various stages including an information session as well as a medical and psychological screening. After an initial general assessment of suitability by a telephone interview, the interested subjects were invited to an information session, where the study and experiments were presented in detail together with the completion of the first part of the psychological screening. The successful participation warranted a medical screening, which included a complete blood count, fasting serum glucose, protein, albumin, kidney (creatinine, urea, uric acid) and liver (bilirubin, GOT, GPT, GGT, AP) parameters, thyroidal status (TSH, T3, T4), inflammatory parameters (CRP), serum lipids (cholesterol, HDL, LDL, TG), electrolytes, several vitamins and minerals, blood-gas analysis, urine analysis (pH, specific gravity, appearance, ketones, proteins, glucose, bilirubin, urobilinogen, nitrites, blood, and leukocytes), thrombosis sensibility screening (factor II and V, prothrombin mutants, lupus-PTT, activities of protein S and C), HIV and hepatitis screen (hepatitis B, C), stand test for orthostatic tolerance, 12-lead ECG, and a DXA-scan to assess for bone mineral density of the femur and the lumbar vertebra column. Subjects were excluded in case of discrepancies from general normal values. Suboptimal status of vitamins or minerals during the medical screening was adjusted with nutritional recommendations or supplementations. Subject exclusion criteria included drug and alcohol abuse, bone fractures within 1 year prior to the study, chronic back pain, migraines, history of mental illness and metallic, or osteosynthesis implants.

Subsequent to the medical screening, the psychological interview was scheduled to ensure the willingness and capability of completion of the whole study to minimize subject dropout in the crossover design experiment.

Test subjects

Eight healthy male subjects started and completed the first study campaign. Shortly, before the onset of the second campaign, one subject was excluded from the study due to medical reasons independent of the study experiments and measurements. The mean age (± SD) was 27.6 ± 3.3 years, a mean height of 180.7 ± 0.04 cm, and weight 78.6 ± 6.4 kg, $n = 7$. All volunteers gave written informed consent before starting the study protocol.

Study design and treatment

The nutritional countermeasure study (NUC study) was a single center, crossover design trial conducted in two campaigns at the Institute of Aerospace Medicine in Cologne, Germany. In the study, the 6° HDT bed rest model was applied, a well-established gold standard model to simulate the physiological changes that occur in weightlessness [25–27].

The order of subject assignment to the treatment group either first or the non-treatment group (control) in an alternating order. The study started February 22, 2010 and was finished October 15, 2010. The first campaign lasted from February to April 2010 and the second campaign from August to October 2010. Each campaign comprised a 7-day baseline data collection (BDC-7 to BDC-1) phase, a 21-day HDT bed rest phase (± administration of treatment) (HDT1 to HDT21), and a 6-day in-house recovery phase ($R + 0$ to $R + 5$) at the Metabolic Ward of the Institute of Aerospace Medicine. During the HDT phase, all physical activities of daily routine including eating, showering, using the lavatory, leisure activities like reading, watching TV, or studying were conducted in the 6° HDT lying position. Subjects were allowed to lie on their backs as well as on their sides; however, the legs had to be kept straight and the arms could not to be utilized as support for the head. Furthermore, all physical activities were reduced to a minimum level. Environmental conditions were kept constant across conditions.

Treatment ($KHCO_3$) and control (CON)

The treatment ($KHCO_3$) implemented during the HDT bed rest phase included the supplementation of 90 mmol potassium bicarbonate to the daily diet. Three times a day 30 mmol $KHCO_3$ each was administered as an effervescent tablet (Krueger GmbH & Co KG, Bergisch Gladbach, Germany), dissolved in 200 ml of tab water during the main meals. The control group subjects received 200 ml of tab water in addition to the daily diet during the HDT phase.

Nutrition

During the study, test subjects received a strongly controlled, standardized, and definite nutrient intake based on individually adapted limits for a range of specific nutrients. The implemented diet followed the ESA standardization plan specified regulations for the diet as well, namely the macronutrients and water, minerals, and vitamins, according to the present standards for dietary reference intakes.

An indirect calorimetry conducted on the first day of the study (Delta Trac™ II, Datex- Ohmeda, Datex, Engström GmbH, Germany) calculated the resting metabolic rate of each subject. Individual daily energy needs were calculated by adding the activity level (1.4 for BDC and $R +$ phase or 1.1 for HDT phase) with a 10% dietary-induced thermogenesis. These requirements were satisfied by proteins in an amount of 1.2 g/kg body weight/day, less than 30% fat, and 55–60% carbohydrates. Water was administered at 50 ml/kg body weight/day. Calcium intake was limited to 1000-1200 mg/day and vitamin D was supplemented once daily by 1000 I.U. (Vigantoletten 1000 I.E, Merck). The latter was started 1 month prior to study start and was continued during the time between both campaigns due to a general deficiency as well as a lack of sunlight exposure during the study.

The acid load of the diet, without considering the alkaline supplementation itself, was estimated by the potential renal acid load (PRAL) model [28] and was set to be alkaline and identical in both groups (CON − 52.82 ± 14.18 mEq/d, $KHCO_3$ − 52.20 ± 14.17 mEq/d, $p = 0.718$). The PRAL value of the supplementation added to the diet in the $KHCO_3$ group was 72.14 ± 12.5 mEq/d.

Thus, individual diets were compiled in accordance with these nutritional limits. Apetito AG (Rheine, Germany) supplied the main components of lunch, dinner, and snacks. Gravies together with all kinds of breads utilized for breakfast and dinner were homemade with standardized recipes in the metabolic kitchen of the institute. Considering natural deviations of ham and cheese, these products were analyzed at a local laboratory (Eurofins Analytik GmbH, Cologne, Germany). All other groceries were purchased at local shops. This approach minimized any major effect of nutrient intake on the main study objectives.

The nutrition software PRODI 5.6 (2009) (Nutri-Science, Germany) was applied for the composition and control of the menus. Each food item was weighed at an accuracy of 0.1 g and three main meals and two snacks (breakfast, lunch, snack, dinner, snack) as well as a late night drink of 300 ml tab water were served. To ensure the complete intake of all predefined nutrients, all meals had to be eaten completely which was supervised by trained staff.

Biological sample collection and processing

Blood

Blood samples were obtained from an antecubital vein through a short catheter in serum-monovettes (Sarstedt, Germany) at 7 a.m. after overnight fast. Samples were taken 6 and 2 days before bed rest (BDC-6 and BDC-2) in horizontal position as well as several times during 6° HDT bed rest on days 2, 6, 10, 14, and 21. Additional blood samples were taken after 2 ($R+2$) and 5 ($R+5$) full days of recovery following HDT bed rest. Whole blood was centrifuged (3000 rpm, 4 °C) after coagulation and serum was distributed in small aliquots and immediately frozen at − 20/− 80 °C until analysis.

Blood gases

Arterialized capillary blood for blood-gas analysis was collected in the fasting state immediately after blood sampling. The samples were collected from the pre-warmed fingertip in an anticoagulant tube by trained personnel and immediately analyzed.

Urine

Continuous urine was collected daily on a void-by-void basis from 7:00 (± 15 min) to 7:00 (± 15 min) on the following day. Single voids were stored under darkened and cooled conditions and subsequently pooled to 24-h volumes. Several aliquots of 24 h were stored in the freezer at − 20 °C for later analysis. For the analysis of calcium, specific samples were acidified before freezing (pH 3–4, pH electrode utilized, inoLab pH 720, WTM, Weilheim, Germany).

Biochemical analysis

Serum and urinary calcium were analyzed by flame photometry (EFOX 5053, Eppendorf, Germany). Serum concentrations of parathyroid hormone, bone formation marker bone-specific alkaline phosphatase (bAP) and N-terminal propeptide type I (PINP), and 24-h urinary bone resorption marker C- and N-terminal telopeptide (UCTX, UNTX) were determined with commercially available assays (PTH: Immunotech, Beckmann Coulter GmbH, Krefeld, Germany, bAP: Tandem R, Ostase, Hybritech, Liege, Belgium, PINP: RIA Orion Diagnostica, Finland; UCTX Urine crossLaps® EIA, Immunodiagnostic Systems, UK; UNTX: Osteomark®, Wampole Laboratories, USA) in the in-house laboratory of the Institute of Aerospace Medicine (Cologne, Germany). Intra- and inter-assay variation was as follows: intra PTH 6.4%; bAP 4.9%, PINP 1.9%, UCTX 2.0%, NTX 1.3%; inter bAP 6.0%, PINP 2.3%, UCTX 3.7%, UNTX 4.9%.

Blood gas samples were analyzed by an automated blood gas system (ABL 7, Radiometer, Germany) for pH, pCO_2, and pO_2. Bicarbonate concentration [HCO_3^-] and base excess (BE) was calculated according to the Henderson-Hasselbalch equation.

Statistical analysis

All statistical analyses were performed with the software package Statistica 10 (Statsoft, Tulsa, OK, USA). Anthropometric data are presented as mean ± SD and all results are expressed as means ± SEM. Comparability of baseline values was analyzed with a Students paired t test for dependent samples. If not otherwise stated, baseline data were identical so that effects of the intervention are considered to depend on the treatment. Differences in time and responses between the treatment vs. control groups were determined with repeated measures analysis of variance (ANOVA) and verified by the post hoc Tukey test. The minimum p value taken as significant was $p \leq 0.05$.

Results

Acid-base status

The dietary regime led to an alkaline state in all test subjects (Table 1). As hypothesized, dietary $KHCO_3$ supplementation resulted in a more alkaline status within the normal range reflected by an increased average capillary pH value inside regular pH values (CON 7.405 ± 0.006, $KHCO_3$ 7.410 ± 0.006, $p = 0.023$) and an increased blood buffering capacity (bicarbonate (HCO_3^-); CON 28.34 ± 0.33, $KHCO_3$ 28.79 ± 0.32, $p = 0.02$; actual base excess (ABE); CON 3.28 ± 0.26, $KHCO_3$ 3.74 ± 0.23, $p = 0.03$) in the intervention phase. Twenty-four-hour urinary pH was also more alkaline in the treatment group compared to the control group (CON 6.50 ± 0.10, $KHCO_3$ 7.15 ± 0.15, $p < 0.001$). There was no change in partial pressure of carbon dioxide and partial pressure of oxygen (pCO_2 $p = 0.64$, pO_2 0.89). Table 1 shows the values of all measuring time points.

Calcium excretion

Urinary calcium excretion was significantly decreased during HDT with supplemented $KHCO_3$ (control 6.05 ± 2.74 mmol/24 h; $KHCO_3$ 4.87 ± 2.21 mmol/24 h, $p = 0.03$). The usual increase in urinary calcium due to immobilization was only seen in the control group ($p < 0.001$). Figure 1 shows the course of the calcium excretion over time.

Table 1 Capillary pH, partial pressure of carbon dioxide (pCO_2), partial pressure of oxygen (pO_2), bicarbonate (HCO_3), actual base access (ABE) and urinary pH during head-down tilt bed rest (HDT), and HDT with potassium bicarbonate ($KHCO_3$) supplementation

		Pre-bed rest		HDT				After bed rest		*p*
		−6	−1	2	6	10	14	21	+2	+5	CON vs $KHCO_3$
pH	CON	7.407 ± 0.008	7.415 ± 0.006	7.404 ± 0.007	7.400 ± 0.007	7.402 ± 0.004	7.405 ± 0.005	7.413 ± 0.005	7.415 ± 0.004	7.427 ± 0.007	$p = 0.023$
	$KHCO_3$	7.406 ± 0.006	7.412 ± 0.005	7.408 ± 0.007	7.403 ± 0.004	7.405 ± 0.006	7.415 ± 0.004	7.4176 ± 0.008	7.419 ± 0.006	7.420 ± 0.005	
pCO_2 (mmol/L)	CON	45.17 ± 1.43	45.31 ± 1.04	45.51 ± 1.20	47.07 ± 1.13	46.17 ± 0.63	46.34 ± 0.61	45.7 ± 0.87	44.67 ± 0.52	44.58 ± 1.22	$p = 0.68$
	$KHCO_3$	44.66 ± 0.85	45.36 ± 0.93	45.99 ± 1.05	47.4 ± 0.69	46.29 ± 1.01	45.6 ± 1.00	46.66 ± 1.09	43.83 ± 0.98	45.34 ± 0.63	
pO_2 (mmol/L)	CON	69.22 ± 1.97	71.74 ± 2.40	74.87 ± 3.18	73.52 ± 1.74	71.19 ± 2.52	71.49 ± 2.17	72.37 ± 1.24	71.92 ± 1.54	71.64 ± 2.35	$p = 0.89$
	$KHCO_3$	72.98 ± 2.00	69.56 ± 2.88	73.7 ± 1.15	73.85 ± 1.89	71.04 ± 1.84	74.65 ± 1.27	71.6 ± 2.15	72.3 ± 2.28	70.32 ± 3.09	
HCO_3 (mmol/L)	CON	27.71 ± 0.55	28.5 ± 0.38	28.09 ± 0.45	28.43 ± 0.34	28.19 ± 0.28	28.34 ± 0.33	28.67 ± 0.26	28.12 ± 0.28	29.01 ± 0.59	$p = 0.02$
	$KHCO_3$	27.49 ± 0.40	28.4 ± 0.35	28.45 ± 0.28	28.98 ± 0.25	28.44 ± 0.27	28.66 ± 0.52	29.43 ± 0.28	27.75 ± 0.33	28.67 ± 0.26	
ABE (mmol/L)	CON	3.02 ± 0.42	3.63 ± 0.28	2.94 ± 0.33	3.30 ± 0.27	3.10 ± 0.23	3.40 ± 0.33	3.67 ± 0.14	3.41 ± 0.25	4.26 ± 0.47	$p = 0.03$
	$KHCO_3$	2.70 ± 0.35	3.48 ± 0.23	3.39 ± 0.19	3.72 ± 0.17	3.37 ± 0.13	3.75 ± 0.39	4.48 ± 0.25	3.24 ± 0.22	4.07 ± 0.23	
Urinary pH	CON	6.30 ± 0.10	6.80 ± 0.03	6.34 ± 0.08	6.50 ± 0.10	6.46 ± 0.12	6.50 ± 0.13	6.72 ± 0.09	6.41 ± 0.15	6.67 ± 0.09	$p < 0.001$
	$KHCO_3$	6.28 ± 0.15	6.76 ± 0.09	7.05 ± 0.14	7.25 ± 0.19	7.08 ± 0.15	7.13 ± 0.12	7.24 ± 0.16	6.42 ± 0.16	6.71 ± 0.10	

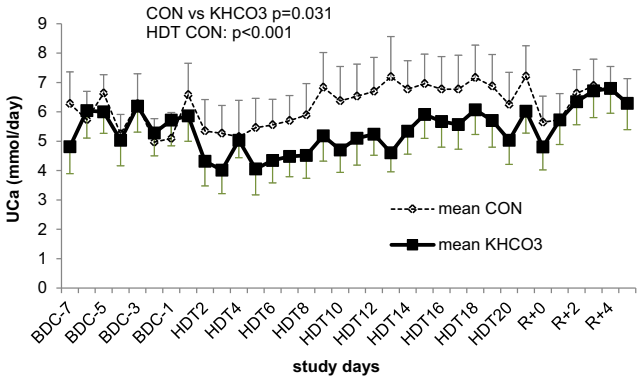

Fig. 1 Urinary calcium excretion (UCa) measured in 24-h urine pools during bed rest only (CON) and bed rest supplemented with potassium bicarbonate ($KHCO_3$). Shown are mean values ± SEM. vs, versus; BDC, baseline data collection; HDT, head-down tilt bed rest; R+, recovery phase

Bone marker

Immobilization during HDT bed rest reduced bone formation markers (bAP $p = 0.03$; PINP $p < 0.001$) and increased bone resorption marker UCTX and UNTX ($p < 0.001$) in both groups, as anticipated (Figs. 2 and 3).

Supplementing $KHCO_3$ had no additional effect on bone formation or bone resorption marker UNTX during HDT bed rest (bAP $p = 0.58$; PINP $p = 0.60$; UNTX $p = 0.91$). Bone resorption marker UCTX tended to be decreased during alkaline supplementation (UCTX $p = 0.16$) (Figs. 2 and 3).

Discussion

Dietary $KHCO_3$ supplementation to an already alkaline diet during 21-day HDT bed rest resulted in a more alkaline status which reduced renal calcium excretion during bed rest, but failed to counteract immobilization-induced bone resorption. Alkaline salts like potassium bicarbonate or potassium citrate are frequently used in studies aiming to reduce body acid levels and counteract the negative effects of dietary acid loading on calcium and bone metabolism. A recently published meta-analysis by Lambert et al. (2015) [14] concluded that potassium bicarbonate and potassium citrate have a calcium and bone-sparing property. Furthermore, a vegetarian diet with a low nutritional acid load has also been demonstrated to have a protective effect on bone in spite of usually low intakes of calcium. The positive effect on bone is attributed to the higher intake of potassium-rich and bicarbonate-rich food items of vegetarians [11, 29, 30].

In addition to alkali salt-induced reduced calcium excretion, several other studies with alkaline supplementation also observed a reduction of bone resorption marker

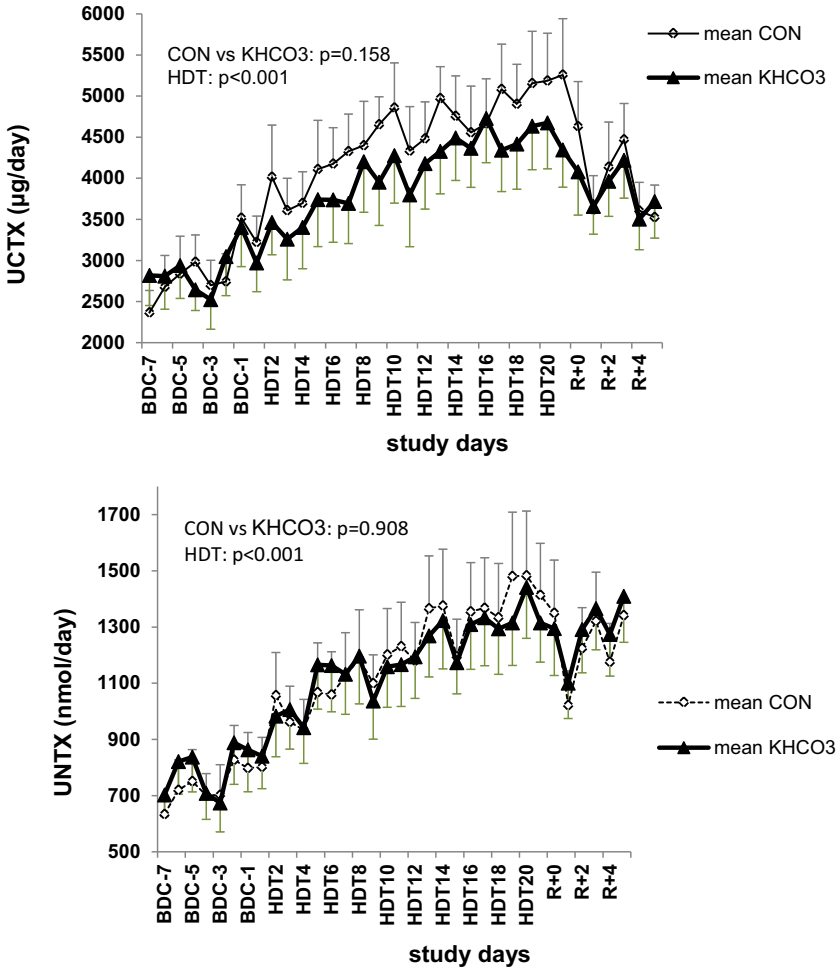

Fig. 2 Urinary bone resorption marker C-telopeptide (UCTX) and N-telopeptide (UNTX) excretion in 24-h urine pools during bed rest only (CON) and bed rest supplemented with potassium bicarbonate ($KHCO_3$). Shown are mean values ± SEM. vs, versus; BDC, baseline data collection; HDT, head-down tilt bed rest; R+, recovery phase

excretion NTX, not found in the present study. In a randomized controlled trial by Macdonald et al. [31], 2-year potassium citrate supplementation had no effect on bone turnover. As alkaline salts are supposed to neutralize dietary acids, most studies compare alkaline supplementation to acid-producing diets. However, a linear relationship between net acid excretion and changes in NTX and calcium excretion confirms that the reduction in acid load seems to be the active component of the alkaline supplementation [32].

In contrast to previous studies, we added alkaline salt to an already alkaline diet, reflected by a negative PRAL value, in order to investigate if alkaline supplementation provides an additional protective effect on bone. Due to bed rest-induced inactivity and resulting lower mechanical loading, osteoclasts are already activated and bone resorption markers are increased during bed rest. Thus, we aimed to examine whether alkalization by alkaline salt is effective in a bed rest study to reduce already activated bone resorption. The missing significant reductive effect in bone resorption by potassium bicarbonate in comparison to the control diet could be due to several factors.

First, due to the small sample size number decreased bone resorption marker, UCTX did not reach significance. Second, the additional supplementation to an already alkaline diet does not reduce immobilization-induced increases in bone resorption during HDT bed rest, indicating that the bone conserving effects of alkaline supplementation in other studies is based on the neutralization of dietary acid load. Third, the impact of exaggerated bone resorption due to mechanical unloading is higher than the effect of additional alkalization. Another important contributing factor could be the age of the test subjects. The EVAluation of Nutrients Intakes and Bone Ultra Sound Study on osteoporosis in Switzerland evaluated the influence of acid load on bone health in 401 ambulatory elderly women (> 75 years) with a validated FFQ and concluded that very elderly people with an already high fracture risk may be more sensitive to nutritional interventions. Bone metabolism of younger people, however, may be more influenced by other related factors such as muscle mass [33–35]. It is also known that the excretion of H^+ under acid load [36] is reduced with age, leading to a chronic status of low-grade metabolic acidosis, more often in elderly than in younger people. This could make them more sensitive to an alkaline supplementation.

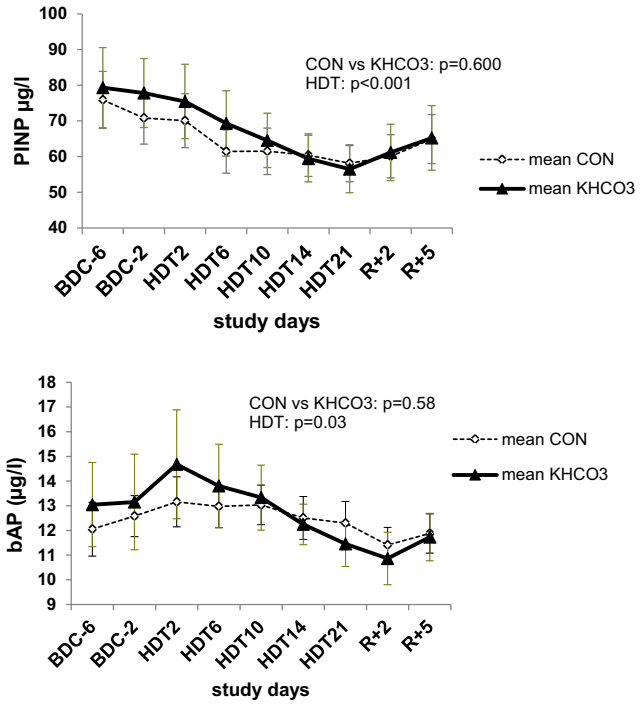

Fig. 3 Bone formation marker bone-specific alkaline phosphatase (bAP) and N-terminal propeptide type I (PINP) in fasting serum samples during bed rest only (CON) and bed rest supplemented with potassium bicarbonate ($KHCO_3$). Shown are mean values ± SEM. vs, versus;BDC, baseline data collection; HDT, head-down tilt bed rest; R+, recovery phase

Nevertheless, alkaline supplementation in the present study was able to reduce bed rest-induced calcium excretion and tended to decrease bone resorption marker UCTX, resulting in sustained improvements in calcium balance which may lead to bone conserving effects in the long run. However, only short-term studies with $KHCO_3$ administration are available confirming no change in intestinal calcium absorption [17, 37]. But no one has studied the effect of the long-term administration on intestinal calcium absorption. Only Ceglia et al. [38] assumed a reduction in calcium absorption after 41 days with potassium citrate supplementation.

Another important advantage of the alkali-induced urinary calcium reduction would be a reduced risk of renal stones which is of particular importance for the main target group of this study, the astronaut corps. It is well established apart from an increased bone loss, astronauts also suffer from an increased risk of renal stones [39–41]. The increase in urinary calcium and phosphate concentrations in spaceflight occur rapidly, sometimes accompanied by a reduced urinary volume, and may contribute to an increased saturation of calcium oxalate and brushite observed in astronauts during spaceflight [39, 40, 42]. Potassium citrate and potassium magnesium citrate supplementation has already been shown to decrease the relative saturation of calcium oxalate during bed rest and spaceflight [43, 44].

In addition to the reduction in urinary calcium, provision of alkali can increase urinary pH and therefore increase uric acid solubility. These findings could also have important therapeutic implications for kidney stones, which affect one in 11 people in the USA [45].

Furthermore, it is currently unknown where the additional calcium efflux out of bone goes during bed rest when it is not excreted through the renal system.

There were some limitations that should be considered when interpreting results from the study. First, the number of subjects in this study was operationally limited to eight test subjects with one dropout candidate, leading to only seven test subjects. Nevertheless, this was sufficient to show statistically significant difference in acid-base balance and calcium excretion, but not in bone resorption. This small sample size, however, may explain why the difference in bone resorption was not significant between the treatment group and the control group. Second, we studied only younger males, which are eventually less sensitive to acid-base imbalances compared to an elderly population or postmenopausal women.

Nevertheless, we found no effect of bicarbonate administration on bone turnover in young men during immobilization. It is out of the question that in general, an alkaline diet, as usually achieved by higher consumption of fruit and vegetables, is positively related to bone metabolism and that bone cells are very sensitive to changes in the acid-base balance.

As bone metabolism is highly influenced by several nutritional factors as well as physical and environmental factors, an interaction of all contributing factors on bone health should be considered.

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Compliance with ethical standards

Conflicts of interest The authors declare that they have no conflict of interest.

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