High sodium chloride intake exacerbates immobilization-induced bone resorption and protein losses

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According to the World Health Organization osteoporosis is regarded as a global healthcare burden and currently affects some 200 million people (14). The number of sufferers is expected to increase steadily with the advancing longevity in the world’s population. Besides postmenopausal women who have developed osteoporosis as a result of estrogen deficiency, immobilized people such as space travelers and bedridden patients are associated with alterations in protein turnover (1, 2, 11, 15, 41, 44, 66). Specific disuse-induced changes include a negative nitrogen balance, a decrease in whole body protein synthesis, and an increase in lower-limb proteolysis leading to decreased amounts of contractile protein in skeletal muscle (11, 20, 44, 57, 67).

Lowered mechanical load is the dominant stimulus leading to bone and muscle loss in immobilization, and the results of several clinical trials have shown the significance of weight-bearing activities in the maintenance of bone mass, bone strength, and muscle mass (13, 40, 56, 59, 63, 68). However, high dietary NaCl intake is also considered as a risk factor for osteoporosis (34) and as such might exacerbate immobilization-induced bone loss. Although there is no doubt that increasing NaCl intake causes high calcium excretion (5, 34, 50), the mechanism that leads to calcium mobilization out of bone remains unknown. In a metabolic balance study, we have recently shown that a very high NaCl intake leads to increased bone resorption in ambulatory test subjects, most likely by inducing a low-grade metabolic acidosis (25). Besides this effect on bone, metabolic acidosis has also been demonstrated to contribute to increases in whole body protein turnover and concomitant decreases in whole body protein content (9, 10, 54). In certain groups such as patients with renal failure and obese subjects who were on a weight-loss diet, metabolic acidosis has also been linked to protein wasting (26, 32); in acidotic rats, impaired growth, abnormal nitrogen utilization, and increased muscle protein degradation were found (48, 71). Several investigations have shown that the correction of acidosis compensates for those losses (27–29, 45, 53).

The sodium intake of astronauts as well as of the general population is much higher than the recommended intake of <85 mmol Na+ per day (nearly 5 g NaCl/day) (17, 22). Nutritional analysis of astronaut diets on various space missions has shown that the intake of astronauts is between 200 and 400 mmol Na+ per day (35). The average industrialized Western population consumes nearly 200 mmol, which is twice as much as recommended (22).

In the study we present here, we hypothesized that immobilization, such as 6° head-down-tilt bed rest (HDBR), a model used to simulate microgravity-induced bone and muscle loss, high NaCl intake with concomitant low-grade metabolic acidosis leads to exacerbated bone resorption and protein wasting. We compared the effects of high and low NaCl intake on calcium and bone metabolism, nitrogen balance, and acid-base status during 14 days of HDBR.
MATERIALS AND METHODS

Subjects. Eight healthy male volunteers (mean age 26.3 ± 3.5 yr, body weight 78.0 ± 4.3 kg) participated in the study called “Salty Life 7.” The “Salty Life” studies are a series of several metabolic ward studies at the German Aerospace Center (DLR) dealing with the impact of NaCl on several physiological systems. Approval for the study was obtained from the Ethical Committee of the “Aerztakammer Nordrhein,” Duesseldorf, Germany, and was conducted in accordance with the Declaration of Helsinki. The study is registered on http://www.clinicaltrials.gov with the unique trial number: NCT01183299 and registration date 08/13/2010.

The subjects were confined for the entire study period to our metabolic ward at DLR, Institute of Aerospace Medicine, Cologne, Germany. Volunteers gave their written informed consent after receiving detailed information about the study protocol and the resulting risks. Inclusion criteria for subjects were passed medical and psychological tests. Exclusion criteria were any history of hypertension, diabetes, obesity, rheumatism, hyperlipidemia, hepatic disease, bone disease, physical exercise more than four times per week, smoking, consumption of drugs, or alcohol excess. Moreover, subjects needed to have negative results of a thrombophilia screening panel (AT III, S-Akt, Lupus-PTT, ferritin, Factor V Leiden, Factor IV, and Factor II) since they would be exposed to immobilization during the study.

Design. The Salty Life 7 study consisted of two study parts, each part divided into a 4-day adaptation phase (days 1–4, ambulatory), a 14-day intervention phase (days 5–18, immobilized in bed rest), and a 3-day recovery phase (days 19–21, ambulatory). During the bed rest periods subjects received either a high- [7.7 meq Na+/kg body wt (BW) per day] or a low- (0.7 meq Na+/kg BW per day) NaCl diet in a crossover design. The two parts of the study were performed separately with a 6-mo washout period between them. Immobilization was accomplished with 6° HDBR, a valid ground-based model to simulate microgravity-induced bone loss and cardiovascular changes (6, 37, 42; 61). Subjects were kept in bed for 24 h and were not allowed to elevate their head >30° from horizontal. The lying position was confirmed by constant video monitoring. All daily activities (such as showering, weighing, reading, watching television) and all lavatory activities were carried out in this position. During the non-bed-rest phases the subjects were allowed to walk around in the ward without doing any physical exercise. For the whole duration of the study a study nurse made sure they adhered to the study rules.

Temperature and humidity were kept constant and controlled during the entire experiment (mean temperature 22.8 ± 0.82°C, mean relative humidity 57.7 ± 4.8%).

Diet. During the whole study the subjects received an individually tailored, standardized, and strictly controlled nutrient intake. The energy intake (total energy expenditure, TEE) was calculated by multiplying basal metabolic rate, measured by indirect calorimetry with the Delta Trac device (DeltaTrac II MBH 200 metabolic monitor, Datex-Ohmeda), by a physical activity level of 1.4 [for light physical activities, according to the German reference intake values (16)] during the ambulatory phase and by 1.1 during immobilization, and adding 10% of TEE for energy expenditure associated with thermogenesis. The daily diet was also constant for fat (<30% of TEE), carbohydrates (50–55% of TEE), protein (1.3 g/kg BW), calcium (1,000–1,150 mg), potassium (3.0–4.5 g), and water (40 ml/kg BW) intake.

The sodium intake varied according to the study part and was mainly ingested as NaCl, which represents the typical intake form of sodium. Because of the large difference in NaCl intake it was impossible to supply the same food composition in both study parts. For this reason the retrospectively calculated potential renal acid load (PRAL) according to Remer et al. (55) was higher during low NaCl intake than during high NaCl intake (mean PRAL during high NaCl intake: −41.39 meq/day; during low NaCl intake: 17.89 meq/day).

Standardization and control of the diet, especially the sodium intake, was carried out as described in detail in Frings-Meuthen et al. (25).

Blood and urine sampling. Fasting blood samples were taken in the supine position under standardized conditions at 7:00 AM before breakfast in each study phase: on each of the last 2 days of the adaptation phase (study days 3 and 4), 6 times during the intervention phase (study days 6, 9, 11, 14, 16, and 18), and on the first 2 days of the recovery phase (study days 19 and 20). Whole blood was immediately centrifuged and than aliquoted.

Urine was collected as 24-h urine pools on all study days. The first morning sample was scheduled each day at 7:00 AM. Each void was kept dark and cold until final pooling to the 24-h urine pool. Aliquots of blood and urine were stored at −20 or −80°C respectively, according to the requirements for later analysis.

Analysis. Serum and urinary sodium and calcium concentrations were analyzed in duplicate by flame photometry (EFOX 5053, Eppendorf, Germany). Serum chloride concentration was analyzed by an automated analyzer (COBAS Integra 400, Roche Diagnostics, Mannheim, Germany).

As markers for bone resorption we used 24-h urinary NH₄⁺- and CL⁻-terminal telopeptide (NTX and CTX). For the assessment of bone formation we used the markers bone-specific alkaline phosphatase (bAP) and NH₄⁻-terminal propeptide type I (PINP). All biomarkers of bone metabolism were analyzed by commercially available assays (bAP: Tandem R, Ostase, Hybritech, Liege, Belgium; PINP: Orion Diagnostica, Finland; NTX: Osteomark, Wampole Laboratories, Princeton, NJ; CTX: Crosslapps, Osteometer BioTech, Herlev, Denmark). Parathyroid hormone (PTH) was determined in fasting morning blood samples using kits from Diagnostic System Laboratories (DSL) (Sinsheim, Germany). Interassay and intra-assay variation were as follows. Interassay were bAP, 7.6%; PINP, 1.65%; NTX, 14%; CTX, 8.0%; PTH, 6.0%; intra-assay were bAP, 4.5%; PINP, 1.34%; NTX, 12%; CTX, 8.3%; PTH, 2.1%.

Total urinary nitrogen was determined by highly sensitive chemiluminescence (30, 70) with an ANTEC automated analyzer (ANTEC 7000v, CTC Analytics AG, Zwingen, Switzerland). Coefficient of variation of this method was 1.6% within a series of analyses performed on one day using freshly prepared calibrators. Nitrogen balance was estimated as nitrogen intake (protein/6.25) minus urine nitrogen excretion. Because nitrogen losses through skin and feces are very low and regarded as constant (33), these were not taken into account when calculating nitrogen balance.

Metabolic parameters of acid-base status (pH, bicarbonate, and base excess concentrations) were determined in capillary blood with a clinical blood gas analyzer (Radiometer ABL5, Willich, Germany). Blood gas analysis was scheduled after venous blood sampling, done twice in the adaptation phase, 6 times in the intervention phase, and twice in the recovery phase. Capillary blood was arterialized with a hot cherry-pit pillow. The bicarbonate and base excess concentrations were calculated according to the Henderson-Hasselbalch equation (31) and the actual hemoglobin concentration, which was measured on the same morning with a hemogem (Coulter ACT 5diff CP, BeckmanCoulter, Germany). All samples were collected by trained medical-technical assistants in anticoagulant tubes without air bubbles and immediately analyzed. In addition, acid-base status was determined in 24-h urine samples on the last 2 days of the adaption and intervention phases. Titratable acid (TA), ammonium (NH₄⁺), and bicarbonate (HCO₃⁻) were measured according to the method of Lithy et al. (46). Net acid excretion (NAE) was calculated as the sum of the TA and NH₄⁺ minus HCO₃⁻.

Statistics. Data on subjects’ age and weight are presented as means ± SD, all other values as means ± SE. The mean values of each test subject for each study phase were statistically compared by a mixed linear regression model, which takes into account the requirements of the crossover design (SAS, PROC MIXED). Comparison of NAE values was done with Student’s t-test for dependent variables. The t-test also showed that baseline levels at the end of the adaptation...
phase were identical for the high- and low-NaCl-intake phases. An effect of NaCl consumption was considered significant when the difference between the two treatments, low and high NaCl intake, was significant. Mean values of calcium excretion and bone resorption markers in the intervention periods were also compared with the mean baseline levels (second-to-last days of the preambulatory phase) with Student’s t-test for dependent variables. Seasonal and overhang effects were tested and could be excluded. The minimum P value taken as significant was P < 0.05.

RESULTS

Serum sodium and calcium concentrations did not differ between low and high salt intake (Na⁺: P = 0.63; Ca²⁺: P = 0.06), whereas serum chloride concentration was significantly higher during high than during low salt intake (high, 103.88 ± 0.91 mmol/l; low, 99.95 ± 1.00 mmol/l; P < 0.001). Results are presented in Table 1.

As expected, 24-h urinary calcium excretion increased significantly (P < 0.001) with high NaCl intake, and during immobilization it was 74% higher with high NaCl than with low NaCl intake (high NaCl: 8.26 ± 0.79 mmol/day; low NaCl: 4.75 ± 0.43 mmol/day, P < 0.001) (Fig. 1). Compared with the preambulatory (4.00 ± 0.3 mmol/day) phase, in which the common NaCl intake was 2.8 meq Na/kg BW per day, low NaCl and HDBR increased calcium excretion by 19% (P < 0.001), high NaCl and HDBR by 107% (P < 0.001).

Both of the urinary bone resorption markers NTX and CTX (Fig. 1) were significantly different between high and low NaCl intake during immobilization (both P < 0.001); high NaCl intake caused exacerbated excretion of these markers. The average excretion during high salt intake was 991 ± 63 nmol/day for NTX and 5,273 ± 628 μg/day for CTX; during low NaCl intake only 657 ± 55 nmol/day for NTX and 3,706 ± 424 μg/day of CTX were excreted. On average, urinary NTX was 50% higher and CTX was 43% higher with high NaCl intake and HDBR than on low NaCl and HDBR. Compared with the preambulatory phase (NTX: 610 ± 83 nmol/day; CTX 2,994 ± 167 μg/day), immobilization with low NaCl intake led to an increase of only 8% in NTX (P = 0.03) and 24% in CTX (P < 0.001), whereas high NaCl intake caused an increase of 62% in NTX (P < 0.001) and 76% in CTX (P < 0.001) (Fig. 1). Concentrations of serum calcium, bone formation markers (Table 1), and fasting PTH were identical in both study parts (bAP: P = 0.74, PINP: P = 0.50) (Table 1).

Increased calcium, NTX, and CTX excretion rates were accompanied by reduced capillary blood bicarbonate (P = 0.018) and base excess (P = 0.009) levels (Table 1), as already shown in ambulatory test subjects (25). During HDBR, bicarbonate concentration with high NaCl intake (25.21 ± 0.33 mmol/l) was lower than during low NaCl intake (26.40 ± 0.47 mmol/l); the same was observed for base excess levels (high NaCl: 0.83 ± 0.31 mmol/l; low NaCl: 2.0 ± 0.43 mmol/l). Capillary blood pH (Table 1) did not show any significant difference between high and low NaCl intake (P = 0.26 for HDBR). PO₂ and PCO₂ were also not different between low and high salt intake during bed rest (PO₂: P = 0.57, PCO₂: P = 0.07). Net acid excretion during HDBR (Fig. 2) was significantly lower with high NaCl intake than with low NaCl intake (high: 39.53 ± 3.74 meq/day, low: 69.26 ± 4.72 meq/day, P < 0.001). This difference was attributable mainly to a reduction in titratable acid (high NaCl intake: 10.93 ± 1.39 mmol/day, low: 27.03 ± 2.26 mmol/day, P < 0.001) and an increase in bicarbonate excretion (high: 22.41 ± 1.65 mmol/day, low: 10.43 ± 1.02 mmol/day, P < 0.001). Ammonium excretion was not affected by the difference in NaCl regimens (P = 0.54).

Table 1. Electrolytes, PTH, bone formation markers, and blood gases during low and high salt intake

<table>
<thead>
<tr>
<th>Electrolytes</th>
<th>Low NaCl</th>
<th>High NaCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium, mmol/l</td>
<td>140.32 ± 1.17</td>
<td>140.10 ± 0.80</td>
</tr>
<tr>
<td>Calcium, mmol/l</td>
<td>2.39 ± 0.02</td>
<td>2.34 ± 0.02</td>
</tr>
<tr>
<td>Chloride, mmol/l</td>
<td>99.95 ± 1.00</td>
<td>103.38 ± 0.91</td>
</tr>
<tr>
<td>Plasma PTH, μg/ml</td>
<td>43.57 ± 4.01</td>
<td>41.22 ± 4.27</td>
</tr>
<tr>
<td>bAP, μg/l</td>
<td>11.25 ± 1.25</td>
<td>10.87 ± 0.89</td>
</tr>
<tr>
<td>PINP, μg/l</td>
<td>46.09 ± 4.76</td>
<td>49.80 ± 3.71</td>
</tr>
<tr>
<td>Capillary pH</td>
<td>7.426 ± 0.007</td>
<td>7.420 ± 0.006</td>
</tr>
<tr>
<td>Capillary bicarbonate, mmol/l</td>
<td>26.40 ± 0.47</td>
<td>25.21 ± 0.33</td>
</tr>
<tr>
<td>Capillary base excess, mmol/l</td>
<td>2.00 ± 0.43</td>
<td>0.83 ± 0.31</td>
</tr>
<tr>
<td>Capillary PO₂, mmHg</td>
<td>69.79 ± 2.46</td>
<td>70.71 ± 2.27</td>
</tr>
<tr>
<td>Capillary PCO₂, mmHg</td>
<td>40.23 ± 0.80</td>
<td>39.73 ± 0.76</td>
</tr>
</tbody>
</table>

Values are means ± SE, n = 8 for low and high NaCl intake in crossover design. PTH, parathyroid hormone; bAP, bone-specific alkaline phosphatase; PINP, NH₂-terminal propeptide of type I procollagen. †Different from low NaCl (P < 0.05). ‡Different from low NaCl (P < 0.001).
DISCUSSION

Increasing salt intake during 14 days of bed rest led to exacerbated bone resorption and nitrogen losses. As in ambulatory test subjects (25), increased bone resorption was accompanied by changes in acid-base status characterized as a so-called low-grade metabolic acidosis. According to Vormann and Daniel (69), low-grade metabolic acidosis is characterized by a slight shift of blood pH within the normal range accompanied by a reduction of total buffer capacity. Clinical symptoms are not detectable at this stage, but other metabolic systems such as muscle and bone play an essential role in correction of the imbalance.

As expected, high salt intake for 14 days led to increased excretion of calcium and bone resorption markers, with no effect on bone formation. This is the first study combining high salt intake and mechanical unloading induced by 14 days of bed rest. Both are well-known risk factors for osteoporosis. To determine the additional effect of a high salt intake, low salt intake and immobilization was chosen as the control. Bone resorption was increased shortly after bed rest started, as expected and as shown in previous studies in our laboratory (6). The increase was more pronounced with the high-salt diet than the low-salt diet. In immobilization the increase in CTX was 43% percent higher with the high-salt diet than the low-salt diet. Calcium excretion was 74% higher. Disregarding a potential additional positive effect of the low-salt diet on bone, immobilization itself led to an increase in bone resorption of 24% (preambulatory phase to intervention with low NaCl) during low salt intake and HDBR, as stated by other authors previously (4, 21, 37, 42, 60). Most notably, the effect of the high salt intake in HDBR—an increase in one bone resorption marker (CTX) of 76% (preambulatory to intervention with high NaCl)—seems to have been much more pronounced than the effect of immobilization itself and highlights the importance of diet for immobilized persons, space travelers, and people with a sedentary lifestyle.

Moreover, our data provide the first evidence that the application of a short-term very high NaCl intake can cause additional protein losses in HDBR. Consistent with the results of others (20, 57), we showed that during the first days of HDBR, nitrogen balance trends toward being negative, irrespective of the level of NaCl intake. However, in the low-NaCl intake phase, nitrogen balance recovered toward the end of the HDBR period, suggesting that in 2 wk of HDBR the overall protein loss caused by immobility is rather low. When subjects ingested a high-NaCl diet, nitrogen balance became more negative, indicating a whole body nitrogen loss. These data suggest that during low NaCl intake in short-term bed rest the reduced mechanical loading causes only initial protein loss. However, when decreased mechanical loading is combined with high NaCl intake, protein wasting is exacerbated. As each gram of nitrogen represents about 6.25 g of protein (65), the cumulative protein losses with high NaCl intake after 14 days of HDBR total 110 g protein, whereas with a low NaCl intake only 39 g protein was lost.

Regarding reduced capillary bicarbonate and base excess concentrations during high salt intake, the results of these studies support the previously stated hypothesis that a low-grade metabolic acidosis may cause NaCl exaggerated bone resorption. Evidence exists that the additional protein losses we found with high NaCl intake can also be attributed to these NaCl-induced changes in the acid-base system. In rats, ammonium chloride-induced metabolic acidosis has been shown to 1) stimulate the ubiquitin-proteasome system for muscle proteolysis (8, 49), and 2) activate branched-chain ketocacid dehydrogenase, leading to excessive oxidation of branched-chain amino acids in skeletal muscle (7, 47). In contrast, our test subjects suffered from mild disturbances of acid-base balance within the normal range (69). However, our results suggest that even a low-grade metabolic acidosis may induce a catabolic effect on protein metabolism. Additionally the aforementioned observations indicate that the protein losses we found were mainly derived from skeletal muscle and therefore exaggerated muscle weakening during immobilization. When protein losses are expressed in terms of equivalent muscle mass (with a muscle protein content of 25%) and summed over the duration of the HDBR period of 2 wk, the exacerbation of disease-induced muscle losses would account for muscle loss of about...
70 g with high NaCl and only 25 g with low NaCl. However, the SE shows that not all test subjects reacted to the same extent, which needs to be further investigated with methods of protein turnover analysis.

Still, only presumptions exist about the mechanisms behind the effect of NaCl on acid-base balance. One possible mediator is the chloride ion: Frassetto et al. (24) have reported an inverse correlation of plasma HCO₃⁻ and dietary chloride intake in the form of NaCl. They found increasing degrees of hyperchloremic acidosis with increasing NaCl intake and evidence that the chloride intake independently predicts the plasma HCO₃⁻ concentration. Frassetto et al. (24) explained these findings in terms of the Stewart approach (64) as a “strong ion acidosis.” Thus a high chloride load necessarily leads to a compensatory release of HCO₃⁻ to maintain electroneutrality in plasma. Our results support the findings of Frassetto et al.: the high NaCl intake during HDDR led to a low-grade metabolic acidosis, which was at least partially caused by ion movement of chloride and HCO₃⁻. But still other possible mechanisms may be involved, as previously mentioned (25). Enforced activity of cellular Na⁺/H⁺ exchangers (18, 36) as well as Na⁺/H⁺ ion exchangers on the extracellular glycosaminoglycans (19) and/or a Na⁺-induced extracellular electrolyte, resulting in a decrease in HCO₃⁻ reabsorption (3, 38, 52, 58), can contribute to mediation of the effect of NaCl on acid-base balance. Summarizing, with high NaCl intake, renal loss of bicarbonate but also excess acid production could be reasonably expected to affect acid-base status. The lowering of net acid excretion with high NaCl intake seems to support the hypothesis of renal loss of bicarbonate. However, with respect to the lower potential renal acid load in the high-NaCl diet, these results could be confounded by differing acid loading of the diet.

In summary our data suggest that high NaCl intake during immobility, to an even greater extent than in ambulatory test subjects, enforces disuse-induced bone and protein losses. In a space-related context, but also in day-to-day life, it therefore seems very advisable to improve nutrient supply by regimenting NaCl intake, to protect not just bone but the whole musculoskeletal system. These results are also very important for more and more people living a sedentary lifestyle, which is by itself suggested as a significant factor contributing to the increased prevalence of osteoporosis. Frequent high salt intake in these people will potentiate their risk of developing musculoskeletal problems. With regard to the underlying mechanism, further studies should focus on the question of whether acid-base balance and/or electrolyte imbalances are the mediator of NaCl-induced losses of bone and protein.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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