Diet-Dependent Net Endogenous Acid Load of Vegan Diets in Relation to Food Groups and Bone Health-Related Nutrients: Results from the German Vegan Study

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Key Words
Acid load • Bone health • Estimated diet-dependent net acid load • Renal acid load • Vegan diets

Abstract
Background/Aims: Dietary composition has been shown to affect acid-base homeostasis and bone health in humans. We investigated the potential renal acid load (PRAL) and the estimated diet-dependent net acid load (net endogenous acid production, NEAP) in adult vegans and evaluated the relationships between NEAP, food groups and intake of bone health-related nutrients.

Methods: The German Vegan Study (GVS) is a cross-sectional study. Data from healthy men (n = 67) and women (n = 87), aged 21–75 years, who fulfilled the study criteria (vegan diet for ≥1 year prior to study start; age ≥18 years, and no pregnancy/childbirth during the last 12 months) were included in the analysis. NEAP values were calculated from diet composition using two models: one based on the protein/potassium quotient and another taking into account an anthropometry-based loss of urinary organic anions. Results: Mean daily intakes of phosphorus, potassium, sodium, magnesium and vitamin C were above, and vitamin D and calcium below Dietary Reference Intake (DRI). Regardless of the model used, the diet in the GVS was characterized by a nearly neutral NEAP. A strong correlation was observed between the NEAP values of the two models (r s = 0.873, p < 0.001). Only the consumption of fruits decreased constantly across the increasing quartiles of NEAP.

Conclusions: It can be hypothesized that vegan diets do not affect acid-base homeostasis. With respect to bone health, the significance of this finding needs further investigation.

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Introduction

Since the beginning of the 20th century, when the physiologists Sherman and Gettler [1] as well as Blatherwick [2] published their experimental data, it is well known that dietary composition affects urine pH and acid-base homeostasis in man [3–5]. In the meantime, evidence indicates that even small diet-induced disturbances in acid-base homeostasis have pathogenic effects [6]. In particular, there is growing epidemiological evidence showing negative associations between the diet-dependent net acid load (net endogenous acid production, NEAP) and bone health [7–10]. Furthermore, in vitro studies have shown that even a small decrease in pH resulted in a remarkable increase in bone resorption as a result of osteoclast stimulation [11–
Additionally, net acid-producing diets increase urinary calcium excretion in a dose-dependent manner [14]. Accordingly, it has been hypothesized that net acid-producing diets will induce osteoporosis in the long run [15]. Consequently, alkaline supplements decrease bone absorption in humans [16–18].

In this context, benefits of plant-based diets on bone health have received particular attention since fruits, vegetables, tubers and roots are net base-producing foods [4]. Indeed, diets rich in fruits and vegetables have a positive effect on the calcium economy and markers of bone metabolism in both males and females [15]. Because vegetarians and especially vegans tend to consume more minimally processed plant foods like fresh fruits and vegetables [19, 20], it seems plausible that such diets are characterized by a net base load and hence are of particular benefit to the bones. However, the interpretation of the effect of vegan diets on acid-base homeostasis and bone metabolism merits further consideration in light of the fact that vegans also generally consume high amounts of cereal grains [20, 21], which are net acid producers [4]. Until now, the question of whether a vegetarian or a vegan diet has any positive or negative effect on bone health is a contentious issue [22, 23].

Although a large number of studies on vegetarian [24–25] and vegan nutrition [20, 21, 36–41] have been published during the last years, only one small study has examined the effect of vegan diets on acid-base homeostasis [42]. Whereas the effect of diets on human acid-base status has significant implications on skeletal health [7–10, 43, 44], the primary aim of this cross-sectional study was to investigate how vegan diets affect acid-base balance. Furthermore, we evaluated the associations between NEAP, food groups and the intake of bone health-related nutrients in 154 apparently healthy adult vegans.

Methods

Ethical Considerations

The German Vegan Study (GVS) was conducted in accordance with the Helsinki Declaration of 1964 as amended in 1996. Since there was no intervention, the Ethics Commission of the State of Lower Saxony decided that an ethical approval was not required. All subjects participated voluntarily, gave written consent prior to participation and were free to withdraw from the study at any time.

Recruitment and Screening of Subjects

Participants were recruited by advertisements in eight German magazines. Of initially 868 potential participants, a total of 154 persons took part in all study segments [pre-/main questionnaire and food frequency questionnaire (FFQ)] and fulfilled the following study criteria: vegan diet for ≥1 year prior to the start of the study; age ≥18 years; resident in Germany, and no pregnancy or childbirth during the last 12 months. Exclusion criteria were: severe illness during the last 12 months (such as malignant or cardiovascular diseases, renal failure, or severe diseases of the gastrointestinal tract), diagnosis of a blood coagulation disorder, treatment with inhibitors of blood coagulation, alcohol and/or drug abuse, participation in another study during the last month and stating the ingestion of fish or meat in any of the study questionnaires.

Life course factors (except alcohol consumption; FFQ) were evaluated by means of the main questionnaire of the study, in which frequency, duration and kind of physical activity as well as amount of smoking (number of cigarettes per day) were assessed. During the (physical) examinations, the participants were screened for health status by a general practitioner [standardized questionnaire with questions regarding (severe) diseases and minor complications] in order to identify any of the exclusion criteria. Body height and weight were determined by trained personnel. Body weight was measured without shoes and with light clothes on using a calibrated SECA scale and recorded to the nearest 0.1 kg, and body height was determined without shoes using a tape measure and recorded to the nearest centimeter. Body mass index (BMI) was calculated from body weight (in kg) divided by height squared (in m²).

Definition of ‘Vegan’

As previously described [20], persons were included in the study when they were adhering to either a strict vegan diet (SV, n = 98) or a near-vegan diet [moderate vegan diet (MV, n = 56)]. In MV diets, a maximum of 5% of the ingested food energy came from eggs, milk and/or dairy products. Regarding ‘moderate vegans’, it was discussed that on the one hand, the consumed amounts of eggs (0.88 ± 3.31 g/day), milk and dairy products (7.95 ± 10.1 g/day) in the MV subgroup were so low that it seemed not appropriate to call them restricted lacto-ovo-vegetarians. On the other hand, the consumption of eggs, milk and dairy products makes it difficult – if not impossible – to call those persons (strict) vegans. Therefore, we decided to call them moderate vegans rather than restricted lacto-ovo-vegetarians or strict vegans.

Collection of Dietary Data

A slight modification of the validated FFQ used in the Giessen Raw-Food Study [45] was used in the GVS for data collection. The original FFQ was complemented for vegan foods; foods of animal origin – except eggs, butter, milk and dairy products – were excluded. The GVS-FFQ included 199 vegan foods and 7 non-vegan foods such as dairy products and eggs. In the GVS-FFQ, common household measures and their equivalents in grams or milliliters were given for each food item. In some cases, portion sizes were made clear with photos or specific comments (i.e. 9 strawberries of a size of walnuts weigh 150 g; an orange of the size of a tennis ball equals 250 g). In addition, participants were asked for copies of recipes of homemade vegan dishes.

In order to minimize seasonal differences, the FFQ was administered twice: once in autumn and once in spring. In each of the two FFQ, food consumption was documented over a period of 9 consecutive days. However, the first 2 days of each 9-day FFQ were regarded as a training phase and excluded from the analyses; only the following 7 days were evaluated. On the basis of the German nutrient data base (BLS II.2), we developed our own software (Par-
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Computation of the Estimated NEAP and the Potential Renal Acid Load

Since direct measurement of acid-base balance via 24-hour urine collection is not practical in larger epidemiological studies, several algorithms for estimating the daily NEAP from the food composition have been developed [46]. Frassetto et al. [3], for example, estimated NEAP from the protein/potassium ratio of the diet. In contrast, Remer and Manz [4] as well as Remer et al. [47] calculated NEAP from average intestinal absorption rates of ingested proteins and minerals as well as an anthropometry-based estimate for organic acid excretion. Based on the fact that each of the algorithms has its merits and drawbacks [46], we applied both models and examined how they alter NEAP (in mEq/day) in vegan diets.

The algorithm formulas for the calculations of the two models are given in the following:

\[
\text{NEAP}_{\text{Frassetto}} = 54.5 \times \frac{\text{protein}}{\text{potassium}} - 10.2
\]

This algorithm considers the diet contents of protein (an acid precursor; in g/day) and potassium (an index of base precursors; in mEq/day) from organic anions [3].

\[
\text{NEAP}_{\text{Remer-Manz}} = \text{PRAL} + \text{OA}_{\text{est}}
\]

where PRAL (in mEq/day) denotes the dietary potential renal acid load and OA_{est} (in mEq/day) represents anthropometry-based estimated urinary organic anions.

PRAL was calculated as follows [4, 47]:

\[
\text{PRAL} = 0.49 \times \frac{\text{protein}}{\text{phosphorus}} + 0.037 \times \frac{\text{phosphorus}}{\text{magnesium}} - 0.021 \times \frac{\text{magnesium}}{\text{potassium}} - 0.026
\]

with protein given in g/day and phosphorus, potassium and magnesium in mg/day. Divergent from the original model of Remer and Manz [4], but in accordance with Alexy et al. [48], calcium was not included in the PRAL algorithm because its absorption is highly variable.

The computation of OA_{est} (in mEq/day) was carried out using the following formula [49]:

\[
\text{OA}_{\text{est}} = 0.007184 \times \text{height}^{0.725} \times \text{weight}^{0.425} \times 41 / 1.73
\]

with height given in cm and weight in kg.

Classification of Food Groups

Referring to Alexy et al. [48] and Gannon et al. [50], several food groups were formed and classified:

- fruits: fresh fruits and fruit products (frozen, dried and canned fruits);
- vegetables: fresh, frozen and canned vegetables;
- cereals and staple foods: rice, wheat, rye, millet, oat and oatmeal, pasta, breakfast cereals and cereal bars;
- bread and pastries: white and wholemeal bread, soft grain, rolls and other bread products;
- potatoes: boiled, mashed, fried/roasted potatoes, potato salads and dishes;
- legumes: beans, lentils and soy products;
- nuts and seeds: hazelnuts, walnuts, cashew nuts, almonds, and sunflower and pumpkin seeds;
- milk and dairy products: whole, semi-skimmed and skimmed milk; yoghurts, and other fermented milk products;
- cheese: curd, fresh and cottage cheese, and hard and soft cheese;
- eggs: eggs and egg dishes;
- coffee: instant, coffee bean and decaffeinated preparations,
- tea and herbal tea: infusions, instant and decaffeinated preparations.

Statistical Methods

Statistical analysis was performed using SPSS 17.0 (SPSS, Chicago, Ill., USA). Results are shown as means ± SD or as medians with 25th and 75th percentiles (interquartile range). The following two-tailed tests at the 5% level of significance were employed to evaluate the data: to analyze differences in skewed data between two subgroups (SV and MV; sex), the Mann-Whitney U test was used. For normal distributions, the independent sample t test was chosen. Normal data distribution was checked visually and using the Kolmogorov-Smirnov test. Dealing with nominal data, the \( \chi^2 \) test was employed to evaluate significant differences. Differences in nutrient intakes as well as intake of different food groups between persons within the lowest and the highest NEAP per MJ quartiles were tested with the Mann-Whitney U test.

Associations between NEAP_{Frassetto} and NEAP_{Remer-Manz}, NEAP values and energy-adjusted nutrients were examined using the Spearman rank correlation.

Results

Subject Characteristics

Of the subjects recruited, 56.5% were females (SV: 51.0% and MV: 66.1%; \( p = 0.07 \)). The two subgroups (SV and MV) did not differ in mean age (SV: 43.4 ± 15.4 years and MV: 45.7 ± 14.2 years; \( p = 0.358 \)) and their mean BMI (SV: 21.3 ± 2.73 and MV: 21.3 ± 2.20; for anthropometric details of the whole study population see Waldmann et al. [20]).

Food and Nutrient Intakes and Bone-Related Nutrient Interrelationships

Due to subgroup definition, strict and moderate vegans differed significantly in their egg, milk and dairy product consumption (\( p < 0.001 \)). Intake of legumes, especially soy and soy products, was higher in strict vegans (SV: 102 ± 99.1 g/day and MV: 56.8 ± 85.4 g/day; \( p < 0.001 \)). The daily intakes of total energy, macronutrients and bone-related micronutrients of the two vegan subgroups are shown in table 1. Mean daily intakes of vitamin C, phosphorus, magnesium, sodium and potassium were above Dietary Reference Intake (DRI) recommen-
dations, while intakes of vitamin D and calcium were below DRI [51]. There were significant differences regarding intakes of protein, phosphorus, magnesium, and sodium between SV and MV groups, with lower consumption in the MV group. Data on nutrient density for micronutrient intake are also shown in table 1. Table 2 lists high and significantly positive correlations ($r_s \geq 0.6$) between magnesium and calcium, magnesium and phosphorus, vitamin C and potassium, protein and phosphorus as well as protein and sodium.

**Estimates of the Diet-Dependent Net Endogenous Acid Load**

Besides anthropometric data and PRAL, table 3 shows the findings of the computed NEAP in the GVS with respect to the two different algorithms established by Remer and Manz [4] as well as Frassetto et al. [3]. Regardless of the model used, both the entire vegan cohort and the two subgroups (SV and MV) were characterized by nearly neutral NEAP values. However, NEAP differed significantly between the dietary subgroups. In the total cohort, women had lower NEAP values (median: 15.1 mEq/day) than men (median: 16.7 mEq/day), but the difference was only significant for NEAPFrassetto ($p = 0.008$).

**Comparison of the Two NEAP Algorithms**

As shown in figure 1, the NEAP values calculated with the different methods were significantly correlated ($r = 0.873, p < 0.001$). However, the modified formula of Remer and Manz [4] revealed a greater range and lower NEAP values (median: 7.26, range: −113 to 48.3) than the formula of Frassetto et al. [3] (median: 15.6, range: −0.08 to 33.8).

**Association between Specific Food Groups and NEAP**

Table 4 displays a comparison of the median values for specific food groups categorized according to NEAP.
quartiles. Across the quartile of NEAPRemer-Manz only the median intake of fruits decreased significantly and above all constantly. Opposed to this, there was no clear and consistent association between the intake of cereals and staple foods, potatoes, nuts, seeds and legumes across the increasing NEAP quartiles.

**Association and Correlation of Macro- and Micronutrient Intake and Dietary Acidity**

The median values of macro- and bone-related micronutrients across the quartiles of NEAP are given in table 5. Protein, phosphorus and sodium intake increased significantly, while intake of vitamin C and potassium decreased significantly across the increasing quartiles of NEAP.

Table 6 shows Spearman rank correlation coefficients between energy-adjusted nutrient intakes (nutrient intake per MJ) and NEAP according to Remer and Manz in the total GVS cohort. NEAP was positively associated with phosphorus, sodium and protein intake, but negatively associated with calcium, potassium and vitamin C intake.
The role of nutrition in human acid-base homeostasis has been a subject of controversy for a long time. Actually, because of the high capacity of the kidney to excrete acid prevents any serious diet-induced alterations in blood pH, the pathophysiological relevance of the so-called low-grade metabolic acidosis in healthy adults has long been ignored [52]. However, currently, there is an increasing body of evidence from observational [7–10], clinical [16–18] and mechanistic studies [11, 12] suggesting that even small diet-induced acid-base disturbances result in negative effects on bone health. Based on this background, the primary aim of the present analysis was to answer the following questions: Which NEAP is characteristic for vegan diets? Are the NEAP values obtained using the two NEAP algorithms from Remer and Manz

**Discussion**

The role of nutrition in human acid-base homeostasis has been a subject of controversy for a long time. Actually, because of the high capacity of the kidney to excrete acid prevents any serious diet-induced alterations in blood pH, the pathophysiological relevance of the so-called low-grade metabolic acidosis in healthy adults has long been ignored [52]. However, currently, there is an increasing body of evidence from observational [7–10], clinical [16–18] and mechanistic studies [11, 12] suggesting that even small diet-induced acid-base disturbances result in negative effects on bone health. Based on this background, the primary aim of the present analysis was to answer the following questions: Which NEAP is characteristic for vegan diets? Are the NEAP values obtained using the two NEAP algorithms from Remer and Manz.
and Frassetto et al. [3] related? Do bone health-related nutrients and specific food groups affect NEAP and how? Since an interpretation of bone-related effects on NEAP should be made in the context of the total diet [43], our secondary aims were to analyze bone health-associated nutrient intake and interrelationships among nutrients. In the following, those topics are discussed.

**NEAP of Vegan Diets**

In the present paper, NEAP of a larger vegan cohort was calculated for the first time. A comparison of the NEAP values computed with the two different methods showed that they were highly correlated (r_s = 0.873). This result is in accordance with findings from earlier studies. For example, Prynn et al. [43] and Gannon et al. [50] calculated for both methods a correlation coefficient of r = 0.76 and r = 0.72, respectively. Given the fact that the formula of Remer and Manz [4] seems to have a higher capacity to predict renal acid excretion [47], the results generated with the NEAP Remer-Manz algorithm should reflect a more realistic situation than the results obtained with the NEAP Frassetto method. Therefore, it seems plausible that vegans consume a more alkaline diet than calculated with the NEAP Frassetto method. However, from a physiological point of view, the differences in the NEAP values calculated with the two methods are apparently not relevant. Actually, regardless of the model used, vegan diets are characterized by virtually neutral NEAP values. This observation is in accordance with the findings of a small cross-sectional study (n = 10 vegans) indicating

### Table 5. Daily intake of macro- and bone-related micronutrients across the quartiles of dietary-dependent net endogenous load (NEAP) in the total study group

<table>
<thead>
<tr>
<th></th>
<th>Q1 (n = 37)</th>
<th>Q2 (n = 41)</th>
<th>Q3 (n = 38)</th>
<th>Q4 (n = 38)</th>
<th>P (Q1 vs. Q4)^b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>median</td>
<td>percentiles</td>
<td>median</td>
<td>percentiles</td>
<td>median</td>
</tr>
<tr>
<td>Energy, MJ/day</td>
<td>8.8</td>
<td>6.5 - 10.1</td>
<td>8.7</td>
<td>6.9 - 10.9</td>
<td>7.9</td>
</tr>
<tr>
<td>Protein, En%</td>
<td>9</td>
<td>8 - 10</td>
<td>12</td>
<td>11 - 13</td>
<td>12</td>
</tr>
<tr>
<td>Fat, En%</td>
<td>29</td>
<td>24 - 37</td>
<td>31</td>
<td>25 - 35</td>
<td>29</td>
</tr>
<tr>
<td>Carbohydrates, En%</td>
<td>59</td>
<td>51 - 64</td>
<td>55</td>
<td>51 - 60</td>
<td>58</td>
</tr>
<tr>
<td>Vitamin C, mg/day</td>
<td>487</td>
<td>391 - 669</td>
<td>367</td>
<td>293 - 422</td>
<td>235</td>
</tr>
<tr>
<td>Vitamin C, mg/MJ</td>
<td>57.2</td>
<td>50.1 - 74.9</td>
<td>41.1</td>
<td>32.3 - 55.6</td>
<td>26.7</td>
</tr>
<tr>
<td>Phosphorus, g/day</td>
<td>1.1</td>
<td>9.3 - 1.4</td>
<td>1.5</td>
<td>1.1 - 1.8</td>
<td>1.5</td>
</tr>
<tr>
<td>Phosphorus, mg/MJ</td>
<td>136.5</td>
<td>110.9 - 145.5</td>
<td>169.7</td>
<td>149.0 - 183.3</td>
<td>176.4</td>
</tr>
<tr>
<td>Magnesium, mg/day</td>
<td>571</td>
<td>491 - 721</td>
<td>683</td>
<td>521 - 808</td>
<td>632</td>
</tr>
<tr>
<td>Magnesium, mg/MJ</td>
<td>69.7</td>
<td>64.9 - 78.3</td>
<td>75.2</td>
<td>66.5 - 89.0</td>
<td>79.3</td>
</tr>
<tr>
<td>Calcium, mg/day</td>
<td>854</td>
<td>761 - 1036</td>
<td>872</td>
<td>703 - 1092</td>
<td>803</td>
</tr>
<tr>
<td>Calcium, mg/MJ</td>
<td>104.7</td>
<td>93.5 - 127.0</td>
<td>98.3</td>
<td>86.2 - 119.2</td>
<td>94.3</td>
</tr>
<tr>
<td>Potassium, g/day</td>
<td>6.4</td>
<td>4.8 - 7.3</td>
<td>5.5</td>
<td>4.2 - 6.1</td>
<td>4.4</td>
</tr>
<tr>
<td>Potassium, g/MJ</td>
<td>0.74</td>
<td>0.65 - 0.82</td>
<td>0.61</td>
<td>0.55 - 0.66</td>
<td>0.55</td>
</tr>
<tr>
<td>Sodium, g/day</td>
<td>1.3</td>
<td>0.6 - 1.8</td>
<td>2.2</td>
<td>1.4 - 3.1</td>
<td>2.3</td>
</tr>
<tr>
<td>Sodium, g/MJ</td>
<td>0.14</td>
<td>0.07 - 0.22</td>
<td>0.26</td>
<td>0.17 - 0.31</td>
<td>0.29</td>
</tr>
</tbody>
</table>

En% = % of energy.  
^a Calculated by the modified formula of Remer and Manz [4] (see also Alexy et al. [48]). NEAP values were calculated as mEq/MJ.  
^b Mann-Whitney U test was used to test for differences in nutrient intakes between the groups with the lowest and the highest NEAP quartile.

### Table 6. Spearman’s rank correlation between energy-adjusted bone-related nutrient intakes and NEAP values

<table>
<thead>
<tr>
<th></th>
<th>Calcium</th>
<th>Phosphorus</th>
<th>Potassium</th>
<th>Sodium</th>
<th>Magnesium</th>
<th>Vitamin C</th>
<th>Vitamin D</th>
<th>Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>NEAPRemer-Manz</td>
<td>-0.182</td>
<td>0.568</td>
<td>-0.792</td>
<td>-0.506</td>
<td>0.070</td>
<td>-0.087</td>
<td>0.011</td>
<td>0.563</td>
</tr>
<tr>
<td>r</td>
<td>0.024</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.387</td>
<td>&lt;0.001</td>
<td>0.890</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

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a NEAP of 17.3 ± 14.5 mEq/day for a vegan diet [42]. In contrast, most modern, omnivore, Western diets show net acid-producing effects in the range of >50 mEq/day [43, 50, 53, 54]. With respect to preventive medicine, the near neutral characteristic of the vegan diet is of interest, since it is known that neutral or net base-producing diets have shown multiple health benefits, e.g. decreased urinary calcium excretion and bone resorption [14, 16–18]. Because vegans tend to consume lower amounts of calcium, lower intake of that bone-related nutrient may be compensated by the ‘calcium-saving effects’ of the neutral character of vegan diets. This phenomenon would at least partly explain why (Western) vegetarians and vegans differ from omnivores clinically [22, 55]. In spite of this, the effect of a vegan diet on bone health needs further examination [23].

The associations between bone health-associated nutrients and NEAP found in this study parallel those reported in other studies. Prynne et al. [43], for example, showed that NEAP<sub>Remer-Manz</sub> was positively associated with intake of calcium, phosphorus, sodium and protein and negatively to intake of potassium and vitamin C. These associations were also found in our study in that protein and sodium intake increased, whereas potassium and vitamin C intake declined with increasing quartiles of NEAP. Again, our results corroborate reports of other researchers. For instance, Gannon et al. [50] observed that protein and phosphorus intakes were significantly associated with increasing quartiles of NEAP, however, there was a marked reduction in vitamin C intake with increasing quartiles of NEAP.

Since nutrient intake reflects the ingestion of specific foods, we also analyzed associations between food groups and NEAP, and between increasing NEAP quartiles and intake of bread and pastries – a finding consistent with other results [50]. This reflects the sum of sulfuric and organic acid production rates exceeding the bicarbonate production rates of the metabolism of those foods [4]. Furthermore and in accordance with other studies [43, 50], we found a clear and consistent association between decreasing fruit intake with increasing NEAP quartiles. Since fruits and vegetables are foods rich in potassium, which possesses alkaline effects [15], our finding is not surprising.

**NEAP of Vegan Diets in the Context of Bone Health-Associated Nutrients**

As mentioned above, an interpretation of bone-related effects on NEAP should be carried out in the context of the total diet [43]. As our analysis showed, the nutrient intakes of bone health nutrients in vegans met the current DRI recommendations, except for calcium and vitamin D. Especially the vitamin D supply in vegans seems to be a serious problem since foods providing the highest amounts of vitamin D naturally are, except for mushrooms and avocados, all from animal sources. Indeed, there are reports identifying vitamin D deficiency as a problem for vegans [35]. In Finnish vegans, Outila et al. [56] showed that dietary intake of vitamin D was insufficient to maintain serum concentrations of 25-hydroxycholecalciferol (25-OHD) within normal ranges during winter. However, since vitamin D from the diet has only a marginal effect on serum 25-OHD in humans, oral intake of 1 µg vitamin D per day increases the 25-OHD level by only 0.66 [57] and 1.96 nmol/l [58], the vegetarian status is not a significant risk factor for vitamin D deficiency per se [59, 60]. In vegans, other factors, such as vitamin D supplementation, degree of skin pigmentation, and amount and intensity of sun exposure, have a greater impact on 25-OHD than the diet [56]. Especially solar UVB irradiation is a major determinant for endogenous biosynthesis of vitamin D and, therefore, the main indicator of 25-OHD serum levels [60]. Accordingly, the marginal vitamin D content of vegan diets is only relevant if sun exposure is low. In this case, vegans should take vitamin D supplements [35, 61]. With respect to multiple health outcomes, including bone health, a serum 25-OHD level of about 75–110 nmol/l (corresponding to oral doses in the range of 45–100 µg vitamin D/day) seems to be optimal [62].

The fact that mean calcium intake in vegans was about 16% below the DRI recommendations, which is in accordance with other studies [61, 63], seems to be problematic with respect to bone health. However, based on the findings of several meta-analyses of observational as well as interventional studies [64, 65], the optimal calcium intake for bone health in adults is not well known. Given the fact that the low acidity effect of vegan diets lowers urinary calcium loss, vegans may require less calcium than recommended for omnivore adults. In addition, because higher phosphate intake was associated with decreased urinary calcium and increased calcium retention [66], the phosphate content of vegan diets could result in a lower net need of calcium in vegans.

**Limitations of the Study and Conclusion**

As we have stated elsewhere [21], the most important limitation that should be mentioned when examining the results of this study is the use of an FFQ. Actually, the
overall accuracy of the evaluation of food intake via FFQs has substantial limitations [67] and has therefore been questioned [68]. However, up to now, the FFQ is the standard tool for dietary assessment in all large epidemiological studies, and the results of those studies are still relevant [69, 70]. Similarly to other studies [43, 44, 51], we have not assessed bone mineral density or markers of bone turnover. Therefore, it is unknown whether vegans consuming diets with higher acidity compared to those eating more alkaline diets differ with respect to their bone health. A further limitation of our analysis was the fact that we did not measure acid-base status via 24-hour urine collection. Therefore, our estimates of NEAP do not allow us to draw strong conclusions on how vegan diets affect acid-base homeostasis effectively.

In summary, based on our data, it can be hypothesized that vegan diets do not affect acid-base homeostasis. With respect to bone health, further investigation including all dietary components is needed.

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Disclosure Statement

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