The second point raised by Remer is a critical one, given the current controversy concerning the relation between dietary protein and bone (3, 4). We have examined our extensive data set to determine the percentage of women with an intake of protein below the reference nutrient intake (RNI), ie, <45 g/d (5), and we examined their respective bone mineral density (BMD) values. Only 24 women (23%) were below the RNI for protein and had a mean estimate for NEAP of 3.94 ± 0.95 mEq·d⁻¹·MJ⁻¹ (range 2.04–5.50 mEq·d⁻¹·MJ⁻¹) and a mean estimate of potential renal acid load (PRAL) of −7.90 ± 9.44 mEq/d (range −33.6 to 4.58 mEq/d). Of these subjects, 50% had a lumbar spine BMD below the median value for the population. Figures for the other BMD sites were as follows: 67% (n = 16) for femoral neck BMD; 50% (n = 12) for femoral trochanter BMD; and 71% (n = 17) for femoral Ward’s BMD. The numbers of subjects with low protein intake are too small to allow us to comment further on the growing body of evidence that long-term low dietary protein consumption may be harmful to skeletal integrity. It is important also to note that subjects with a low dietary protein intake are likely to be deficient in other nutrients that may be of benefit to bone.

In response to the third point, we estimated the PRAL by using Remer’s calculation model (6), and these estimates are shown in Table 1. Furthermore, we investigated the association between estimates of PRAL and measurements of bone metabolism and BMD. The correlation between NEAP and PRAL was 0.93 (P < 0.001). Lower estimates of PRAL were associated with a higher peripheral total bone mass and pyridinoline excretion (P < 0.048), with similar nonsignificant trends for peripheral total bone mass and pyridinoline excretion (P < 0.07 and P < 0.1, respectively). However, correlations were weaker than those we reported for NEAP and bone. There was a nonsignificant trend for the lumbar spine and hip BMD to decrease across increasing tertiles of PRAL (P < 0.1), with similar findings for forearm bone mass.

We thank Dr Remer for providing us with this opportunity for extensive discussion of estimates of renal net acid excretion and its subsequent effects on bone health, and we encourage other groups to reanalyze existing dietary intake and bone health data sets to enable further exploration of the effect of dietary acidity and alkalinity on skeletal integrity.

Table 2. Net endogenous (noncarbonic) acid production (NEAP) estimates for quartile classification

<table>
<thead>
<tr>
<th>Quartile 1</th>
<th>Quartile 2</th>
<th>Quartile 3</th>
<th>Quartile 4</th>
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</table>

1 Estimate of NEAP obtained by using Frassetto et al (2) algorithm in mEq·d⁻¹·MJ⁻¹.

### Iron and zinc interactions

**Dear Sir:**

In a letter to the Editor in the December issue of the Journal, Sreedhar (1) raises the question whether iron supplementation has a negative effect on zinc concentrations, because the data from various studies seem to be conflicting. Although Lind et al (2) already pointed out several reasons that could explain the differences between the studies, we think that there is a more important reason for such differences.

Note that the results of the study by Lind et al (3) and of our study (4) were similar. Both studies reported a significant effect of interaction between iron and zinc supplementation on hemoglobin concentrations, because the data from various studies seem to be conflicting. Although Lind et al (2) already pointed out several reasons that could explain the differences between the studies, we think that there is a more important reason for such differences.

Note that the main difference between the studies is that iron supplementation had a modest negative effect on the prevalence of low zinc concentrations in the study by Lind et al, whereas iron supplementation appeared to have a slight positive effect in our study.

Whereas Lind et al attribute this difference to differences in initial zinc status, we believe that the most important reason for this difference is that we controlled for the effect of the acute phase response by excluding infants with a C-reactive protein concentration > 10

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**References**


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**LETTERS TO THE EDITOR 787**

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mg/L in the analysis of the prevalence of deficiency and by controlling for the acute phase response in the analysis of covariance. The acute phase response strongly influences the concentrations of many indicators of micronutrient status, including ferritin and zinc concentrations (5). In the study by Lind et al, it is conceivable that the morbidity in the iron group was higher than in the placebo group, which led to lower zinc concentrations in the iron group. Iron supplementation is known to affect morbidity (6, 7).

Rather than the results, it is the interpretation of the outcomes that differs between the reports because of the importance given to outcomes by the 2 sets of authors. For example, Lind et al report that the prevalence of iron deficiency anemia was reduced to 2% and 3% after supplementation with iron and iron combined with zinc, respectively; these prevalence values are similar to those reported by us (3% and 8%, respectively). Our conclusion is that supplementation with iron and zinc combined is as effective in reducing iron deficiency anemia as is supplementation with iron alone. Thus, combined supplementation should be recommended in populations with a high risk of both iron and zinc deficiency. However, Lind et al concluded that combined iron and zinc supplementation is not optimal, because the increase in hemoglobin concentrations in the combined supplementation group was not significant. From a physiologic point of view, this is completely correct. However, note that in the study by Lind et al, the prevalence of low serum zinc after supplementation was 9% higher in the iron-only group and 24% lower in the iron plus zinc group than in the placebo group. To us, this provides clear evidence of the benefit of combined supplementation with iron and zinc.

The discussion between Sreedhar and Lind et al again shows the need for defining what is meant by interactions, because several different definitions are currently used. One definition is based on statistical arguments, with an interaction being signficant when the combined effect of 2 interventions on an outcome variable does not equal the additive effect of the 2 interventions alone. To take a hypothetical example, iron and zinc interact according to this definition when combined supplementation reduces the prevalence of anemia by 30%, while the single supplements reduce anemia prevalence by 40% and 15%, respectively. If the reduction of anemia prevalence had been 55% after combined supplementation, there is no interaction in the statistical sense. However, interactions can also be physiologically defined. This means that 2 nutrients are surmised to play a role in the same metabolic pathway. Using the same example, a 55% reduction in anemia prevalence by the combined supplement would signify an interaction in the physiologic sense, because both nutrients contribute to the effect.

However, with Bob Dylan in mind, it is clear that “all definitions can’t be right all of the time.” On the basis of the first definition, how should the occurrence of an effect of interaction between iron and zinc on ferritin concentrations in the absence of an effect on hemoglobin concentrations be interpreted? It would be very unsatisfactory if only the outcome measured would determine whether an interaction between 2 nutrients exists. In contrast, in the example of iron and zinc, a physiologic interaction is surmised, but we are not sure. Iron and zinc may contribute to higher hemoglobin concentrations via 2 completely separate pathways.

It is not surprising that this situation leads to confusion in discussions and interpretation of results. The term interaction needs to be clearly defined, not only whether it is used in the statistical or physiologic sense but also whether the interaction is antagonistic, synergistic, or additive in nature.

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**Reply to FT Wieringa et al**

Dear Sir:

I appreciate the comments of Wieringa et al in response to my letter to the Editor (1). In their letter, Wieringa et al search for explanations of the conflicting evidence of iron and zinc interactions (2, 3).

In my letter, I agreed with the convincing evidence that exclusion of acute phase reactants can correct for underestimation or overestimation of serum ferritin, retinol, and zinc, which are the most commonly used indicators of iron status, vitamin A status, and zinc status, respectively. Wieringa et al have rightly pointed out the caveats of using serum ferritin, retinol, and zinc as indicators of their respective micronutrient status during inflammation. However, it should be emphasized that other factors like age, sex, and a variety of other host and environmental factors, such as pregnancy, genetic condition, overall nutrition, and force of infection, may influence the inflammatory process and hence micronutrient status. Plasma concentrations of these micronutrients may bear little relation to tissue status or the storage pool during inflammation, and data on nutritional status should be carefully examined and interpreted. In this respect, it is essential to understand the dynamics of both inflammation and micronutrient indexes.

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