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Letters to the Editor

Microalbuminuria, glomerular filtration rate, and dietary fat and protein intakes in type 1 diabetes

Dear Sir:

We read with interest the cross-sectional study by Riley and Dwyer (1), who reported a positive association between microalbuminuria and dietary saturated fat intake and a negative association between microalbuminuria and dietary protein intake in people with type 1 diabetes. They also reminded us that protein intake might be involved in renal hyperfiltration preceding the loss of nephron units in type 1 diabetes (2). Glomerular hyperfiltration may be another early marker of diabetic nephropathy (3, 4). We previously studied the relation between dietary macronutrient intake and glomerular hyperfiltration in 110 patients with type 1 diabetes (5). The patients' mean (±SD) age was 34.6 ± 11.2 y, their mean duration of diabetes was 13.9 ± 9.0 y, and their mean body mass index (in kg/m^2) was 22.9 ± 2.9. None had hypertension. Mean glycated hemoglobin was 8.3 ± 1.4%, or 0.083 ± 0.014 (normal: ≤5.6%, or ≤0.056). The glomerular filtration rate (GFR) was assessed by the plasma disappearance of 3.7×10^4 Bq [^{51}Cr]EDTA and corrected by 1.73 m^2 body surface area. Hyperfiltration was defined as a GFR > 137 $mL \cdot min^{-1} \cdot 1.73\ m^{-2}$ (mean value + 2 SDs of age-matched healthy subjects) (6). Food intake was recorded with a computer-assisted program (5).

The distribution of dietary intake was similar to that observed by Riley and Dwyer (1): the percentage of energy from fat, protein, and carbohydrate, respectively, was 40.2 ± 7.2%, 18.2 ± 3.2%, and 41.6 ± 6.9%. Our cross-sectional study outlined a positive relation of both protein and fat intakes with GFR, indicating that subjects with type 1 diabetes with glomerular hyperfiltration ($n = 15$) ingested higher amounts of protein (1.60 ± 37 compared with 1.38 ± 0.34 $g \cdot kg$ body $wt^{-1} \cdot d^{-1}$; $P = 0.032$) and fat (1.70 ± 0.54 compared with 1.39 ± 0.44 $g \cdot kg$ body $wt^{-1} \cdot d^{-1}$; $P = 0.0022$) than did patients with normofiltration, although total energy intake was not significantly different between the 2 groups. Saturated fat intake was significantly higher in the group with hyperfiltration ($P = 0.05$), whereas no difference was found for polyunsaturated fat intake. Univariate regression analysis showed that GFR was positively related to both protein intake ($r = 0.28$, $P = 0.003$) and fat intake ($r = 0.25$, $P = 0.007$). In a stepwise multivariate regression analysis, fat intake was an independent determinant of GFR ($F = 13.15$, $P \leq 0.002$), whereas protein intake no longer appeared to be so ($F = 1.36$, $P \leq 0.08$). No relation was found between dietary intakes and serum total cholesterol, HDL cholesterol, apolipoprotein A-I, apolipoprotein B, or serum triacylglycerol. Thus, we found an independent, positive relation between hyperfiltration and fat intake, suggesting that fat intake may play a role in the development of renal disease.

Fifteen of the 110 patients had microalbuminuria (defined as a urinary albumin excretion rate of 30–300 mg/24 h in ≥2 of 3 timed, overnight urine collections) (7). Protein (1.50 ± 0.45 compared with 1.40 ± 0.33 $g \cdot kg$ body $wt^{-1} \cdot d^{-1}$; $P = 0.30$) and fat (1.25 ± 0.41 compared with 1.46 ± 0.47 $g \cdot kg$ body $wt^{-1} \cdot d^{-1}$; $P = 0.10$) intakes were not significantly different between patients with microalbuminuria and patients with normoalbuminuria. However, the study was not designed as a case-control study with microalbuminuria as a selection criterion. The results from Riley and Dwyer (1) and our own study support the hypothesis that dietary fat intake may play a role in the development of nephropathy in patients with type 1 diabetes. Prospective follow-up studies are required to strengthen this hypothesis further.

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Whole-body bone mineral content in premature infants measured at full-term age

Dear Sir:

We read with great interest the paper published recently by Wauben et al (1). They determined the whole-body bone mineral content (BMC) of full-term premature infants by dual-energy X-ray absorptiometry (DXA) and also studied the influence of diet on the bone mineralization in these infants. As mentioned by these authors, whole-body BMC has been measured for many years with several other methods, such as single-photon absorptiometry; however, they did not refer to our study of the use of DXA published 4 y ago, which was the first study of its kind (2). We showed that preterm infants had a lower whole-body BMC (43.3 ± 30.8 g) at full-term age than did infants born at full term (62.4 ± 18.3 g). No significant influence of diet was shown; however, a lower mean whole-body BMC (35.8 ± 33.8 g) was observed in the group fed the preterm formula than in the group fed their own mother's milk that had been enriched with a fortifier (54.6 ± 22.9 g). We were pleased that Wauben et al confirmed some of our results, especially that premature infants had a lower whole-body BMC at full-term age than did full-term infants at birth. We fully agree with the suggestion by Wauben et al that these findings appear to represent a characteristic of preterm infants rather than a lack of influence of nutritional intervention.

However, we think it most important to interpret DXA-measured whole-body BMC according to weight and not according to length or lean mass as the authors did (*see* their Figure 1). We showed, in several studies of different groups of infants [eg, appropriate-for-gestational-age infants (3), small-for-gestational-age infants (3), and infants of diabetic mothers (4)], that whole-body BMC correlates well with gestational age and body length, but even more closely with weight. We concluded from these studies that the interpretation of body-composition data of infants should therefore take into account birth weight rather than gestational age or length. Especially in premature infants, variations in whole-body BMC observed at full term could be related to variations in the individual weights of the infants. We effectively showed that whole-body BMC in premature infants at full-term age is more closely correlated with weight than with length. Therefore, this correlation could explain, in part, the lower BMCs observed in the group fed preterm formula (mean weight: 3179 g) than in the group fed enriched mother's milk (mean weight: 3580 g). The data obtained by Wauben et al seem to confirm this hypothesis. In their study, the group with the lowest weight at full term (ie, the group that was fed mother's milk fortified with calcium glycerophosphate) had the lowest whole-body BMC and 2 of these infants (15%) had a BMC below the normal range, whereas the group with the highest weight at full term (ie, the group fed a preterm formula) also had the highest whole-body BMC.

The body-composition data reported by Wauben et al showed that fat mass in premature infants at full-term age was higher than that in full-term infants at birth (16%), regardless of diet, reaching a mean value of 20–21% of body weight. They postulated that postnatal, age-matched infants may be more appropriate reference models of body composition for preterm infants than are gestational age–matched infants. On the other hand, we showed previously that the body composition of preterm infants at full-term age is close to the reference body composition of full-term infants at birth (5). The differences between the 2 studies could be explained by differences in either the nutritional management of these infants or in the software used (6, 7).

We conclude that interpretation of data obtained by DXA in premature infants remains difficult and it is not clear whether these data should be compared with those of age-matched or weight-matched infants. Nevertheless, we postulate that whole-body BMC in healthy, premature infants is closely related with weight gain during the first months of life and that osteopenia observed at the time of hospital discharge or at full-term age could disappear rapidly during the first 6 mo of life independent of diet (2).

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Reply to A Lapillonne and BL Salle

Dear Sir:

Interpretation of measurements of bone mineral content (BMC) in prematurely born infants is difficult; confounding variables such as gestational age, body size, body composition, and dietary intake must all be considered. It is gratifying that Lapillonne and Salle share our concern about this difficulty. The issue of size-related artifacts in the analysis of bone mineral measures was addressed previously by Prentice et al (1) in adults and by Molgaard et al (2) in children. These authors share our view that measures of body size, other than total

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TABLE 1
Whole-body bone mineral content (BMC) expressed as a function of age, body weight (BW), length, or lean mass in preterm infants at 6 mo corrected age fed breast milk or standard formula (5) and at 3 mo corrected age recovering from bronchopulmonary dysplasia and fed standard or nutrient-enriched high-energy formulas (6)[1]

Study	BMC	BMC by BW	BMC by length	BMC by lean mass
	g	*g/kg*	*g/cm*	*g/kg*
Wauben et al (5)				
Breast-fed (n = 6)	132 ± 10[2]	19 ± 0.7[2]	2.1 ± 0.7[2]	29 ± 6
Formula-fed (n = 6)	159 ± 14	22 ± 1	2.4 ± 0.2	31 ± 3
Brunton et al (6)				
Standard-formula–fed (n = 18)	85 ± 20[2]	19 ± 2[2]	1.6 ± 0.3[2]	26 ± 5
Nutrient-enriched-formula–fed (n = 24)	104 ± 30	20 ± 2	1.8 ± 0.4	26 ± 4

[1] $\bar{x}$ ± SD.
[2] Significant difference between diet groups within each study, $P < 0.05$.

body mass [eg, lean mass or height-(length)-for-age], are important variables to consider when expressing the whole-body BMC of subjects for research purposes.

Lapillonne and Salle give the example that, in their study, preterm infants fed a preterm formula or enriched mother's milk had a mean difference in BMC of 18.6 g that was in part explained by a mean difference in body weight of 401 g (3). Our data (4), however, do not confirm the hypothesis of these authors that body weight is the best predictor of BMC in infants. For example, in our study (4), the preterm infants with the lowest body weight at full-term age (3.164 kg), who were fed mother's milk fortified with calcium glycerophosphate, had a BMC only 4.6 g less than that of the preterm infants with the highest body weight (3.462 kg) at full-term age, who were fed preterm formula. Thus, despite a mean difference in body weight of 398 g (similar to the difference in body weight between the diet groups in Lapillonne et al's study), whole-body BMC was not significantly different between the 2 groups. In addition, whereas BMC was lower in preterm infants than in infants born full term, albeit by only 13.9 g, the mean body weight of the preterm infants was actually greater (3.642 kg compared with 3.405 kg). In this example, differences in length and in the proportions of lean body mass and fat mass distinguished the preterm from the full-term infants. Thus, we predict that in such instances BMC is best expressed as a function of lean mass or body length.

The results of 2 recent studies that showed that lean mass may be the preferred basis on which to express BMC in preterm infants when body composition is variable between dietary treatment groups are summarized in **Table 1**. Differences in BMC of sometimes >20 g between diet groups (breast-fed compared with formula-fed infants or standard formula compared with nutrient-enriched formula), when expressed as a function of corrected age, cannot be explained by differences in body weight. In both of the studies noted in Table 1, the percentage fat mass of the infants was significantly different between the diet groups at the ages noted: breast-fed infants had a higher fat mass than formula-fed infants (5) and infants fed the standard energy-enriched formula had a greater percentage body fat mass than infants fed the enriched formula (6). In these studies, when BMC was expressed as a function of the lean compartment rather than of whole body weight, the significant difference in BMC between the diet groups disappeared.

Although body weight appears to "correct" for differences in BMC if infant groups differ in body weight, we believe that it is more accurate to express BMC as a function of lean mass because body weight does not necessarily reflect body composition, as supported by the above-mentioned examples. If a difference in body composition is overlooked, diet may be seen as a major factor contributing to a lower BMC (eg, breast-feeding compared with formula-feeding); however, differences in body composition also contribute significantly to the differences in whole-body BMC.

Lapillonne et al's (3) observation that BMC in preterm infants at full-term age is lower than that of infants born at full term was not referenced in our paper (4) because it did not address our initial research question. Lapillonne et al's data (3) were difficult to compare with our data because of differences in the study population (their full-term infants had a BMC of only 62.4 g, which was similar to the BMC of our preterm infants at full-term age), in the study design (lack of randomization), and in dietary management. Both Lapillonne et al's study (3) and our recent study (5) showed that the infant's diet after discharge from the hospital appears to have more influence on BMC during the first year of life than does the infant's diet while in the hospital.

We agree that interpretation of BMC in preterm infants remains a complicated task. However, because it is possible to measure BMC, fat mass, and lean mass with DXA, it is our view that DXA-derived data should be compared on the basis of body composition rather than on the basis of weight alone because body-composition data provide a more realistic comparison between preterm infants receiving different dietary treatments.

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Evaluation of vitamin E potency

Dear Sir:

Vitamin E (α-tocopherol) is available as both *RRR*-α-tocopheryl acetate (*RRR*-α-TA) and *all-rac*-α-tocopheryl acetate (*all-rac*-α-TA). Differences in biological activity of these preparations are expressed by an officially accepted potency factor, according to which *RRR*-α-TA is 1.36 times as potent as *all-rac*-α-TA (1). Burton et al (2) suggested deriving the potency factor from the relative availability of *RRR*- and *all-rac*-α-tocopherol and concluded that the ratio should be closer to 2:1. In most of their studies, an equimolar mixture of d_3-*RRR*-α-TA and d_6-*all-rac*-α-TA was ingested before biokinetics in human plasma and tissue concentrations in patients were monitored. Single and multiple dosing studies with comparatively high loads of α-TA were carried out. Relative availability was expressed as the ratio of d_3-α-tocopherol to d_6-α-tocopherol. It seems premature to conclude that the ratio is 2:1, however, because of the following 2 unresolved issues.

1) Is relative bioavailability an indicator of biopotency? The weight potency factor of vitamin E is a relative measure of the functional activity of α-tocopherol and its homologues and stereoisomers. Potency reflects the functional response of a compound and usually is expressed in terms of the EC_{50} (concentration or dose of an effector that produces 50% of the maximal possible effect). The weight potency factor for *RRR*-α-TA is the ratio of the EC_{50} of *RRR*-α-TA to the EC_{50} of *all-rac*-α-TA and was assessed by the resorption-gestation assay in rats. Assessment of the weight-potency ratio included administering and testing either *RRR*-α-TA or *all-rac*-α-TA, a design denoted as the 2-independent-experiments approach. Additional assays based on the prevention or cure of muscular dystrophy in rats, chicks, and rabbits and on the prevention of encephalomalacia in chicks provided almost identical values for the factor. I appreciate the difficulties of conducting similar studies in humans.

Availability of the active compound at the target site is a prerequisite for its efficacy, and therefore potency is dependent on availability. Concentrations in tissues and plasma may be easily related to biopotency, provided the relation between concentration and effect can be described linearly. However, a sigmoidal concentration-effect response curve restricts proportionality to a range of intermediate concentrations only. At high doses, absorption or transport usually becomes a limiting factor and as a result availability does not parallel potency. Thus, changes in bioavailability can result in varying efficacy. Such data are not meaningful for the assessment of potency, which is defined as a property of the compound. Moreover, the functional dose response of the individual α-tocopherol stereoisomers was found to be synergistic and not additive (3). These synergistic effects are probably difficult to relate to availability. Obviously, several factors may complicate the evaluation of biopotency from data on relative bioavailability and appropriate validation of such an approach is mandatory.

2) Is the method valid for assessing relative availability? Burton et al (2) concluded that bioavailability of *all-rac*-α-tocopherol is roughly half that of *RRR*-α-tocopherol, at least in the long term. However, the new method provided a range of values for relative availability because the d_3-d_6 ratio changed with time from ≈1.5 to 2.0.

The classic approach to assessing relative availability is based on 2 separate applications: 1 for the test and 1 for the reference compound (4). Therefore, classic relative availability has to coincide with availability as determined by the competitive dosing approach, thereby demonstrating the validity of the new approach. For *RRR*-α-TA and *all-rac*-α-TA, both approaches would be expected to provide identical relative availabilities under conditions of first-order (or dose linear) kinetics.

There are, however, several indications in the paper that the tocopherol plasma kinetics were not linear (eg, lack of dose proportionality in the multiple dosing experiments, washout kinetics of unlabeled tocopherol after administration of high doses of labeled tocopherol, and variation of the d_3-d_6 ratio with time after multiple dosing). These findings are not surprising because at high doses nonlinear kinetics would be expected for a system exerting biodiscrimination. Stereoselectivity is usually mediated by proteins displaying binding saturation. For the discrimination of the 8 stereoisomers of α-tocopherol, the hepatic tocopherol transfer protein (TTP) is assumed to play a major role in adjusting plasma and tissue tocopherol concentrations (5). I agree with Burton et al (2) that the configuration at the C-2 position of the molecule is the major determinant of biological differences between the α-tocopherol stereoisomers. Discrimination between 2*S* and 2*R* forms of α-tocopherol is assumed to take place because of lower binding affinity of the 2*S* forms to TTP (6). Thus, presentation of both *RRR*- and *SRR*-α-tocopherol to TTP will result in preferential binding of *RRR*-α-tocopherol. Increasing the relative abundance of *RRR*-α-tocopherol will lower the probability of *SRR*-α-tocopherol binding to TTP, thereby enhancing the probability for *SRR*-α-tocopherol discrimination.

The dose administered in the studies by Burton et al (2) would contain 50% d_3-2*R*-α-TA, 25% d_6-2*R*-α-TA, and 25% d_6-2*S*-α-tocopherol, so that 75% of the 2*R* forms would compete with 25% of the 2*S* forms (ratio of 3:1) for TTP binding. By contrast, if the 2 compounds were given separately, administration of *all-rac*-α-TA would result in 50% of the 2*R* forms competing with 50% of the 2*S* forms (ratio of 1:1). Thus, because of the relatively high abundance of the 2*R* forms when the 2 forms are given simultaneously, the 2*R* forms would compete more effectively and bioavailability of the 2*S* forms would be underestimated compared with that in experiments in which the compounds are given independently.

Because binding to TTP is saturable and stereoselective, the competitive dosing approach is not applicable unless tracer doses are used. At high doses the competitive dosing approach will tend to overestimate relative availability. I agree, however, with the authors that new methods have to be developed to reassess the potency factor in humans, perhaps based on an enhanced understanding of vitamin E function. Nevertheless, revision of

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the currently accepted potency factor has to rely on an appropriate and validated method.

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Reply to W Cohn

Dear Sir:

In reference to our recent publication (1), Cohn questions the validity of our method of measuring relative bioavailability of natural (*RRR*) and synthetic (*all-rac*) α-tocopherols in human tissues and whether our results are predictive of relative biopotency. In particular, Cohn states that giving both forms of vitamin E together in equal amounts, providing 75% 2*R* and 25% 2*S* forms, could overestimate the relative bioavailability of the 2*R* forms.

Cohn suggests that interactions between *all-rac*-α-tocopherol stereoisomers (eg, synergism) could invalidate the competitive method. However, it must be recognized that for many people supplementation with *all-rac*-α-tocopheryl acetate will itself be an uncontrolled, competitive exercise, carried out under conditions that are quite different from those used to determine animal biopotency. That is, the *all-rac*-α-tocopherol stereoisomers must compete with an unknown and variable amount of circulating, endogenous *RRR*-α-tocopherol, making it difficult to see how there could be a useful bioavailability ratio if the interactions are significant. We agree that validation of our method is important and welcome this opportunity to provide additional supporting evidence.

The official biopotency ratio for the acetate esters of natural and synthetic α-tocopherol (*RRR*-α-tocopheryl acetate:*all-rac*-α-tocopheryl acetate = 1.36) is based on a small number of biological responses (primarily fetal gestation-resorption, as well as muscular dystrophy and encephalomalacia) measured in assays in a few species of vitamin E–deficient animals (rats, chicks, and rabbits). The general similarity between the different assay results, coupled with support from some high-dose, short-term bioavailability studies in humans, has been taken as evidence that a single number can adequately represent the relative biopotency of natural and synthetic vitamin E for most, if not all, animal species, including humans. Our recent study (1) provides evidence that the currently accepted biopotency value may significantly underestimate tissue bioavailability in humans, at least over the long term (ie, 1–2 y and beyond).

We applied the competitive dosing technique in a companion study of relative bioavailability in male rats (N Hidiroglou, DO Foster, GW Burton, KU Ingold, unpublished observations, 1989). Concentrations of *RRR*-α-tocopherol and *all-rac*-α-tocopherol were measured at various times in plasma and tissues of rats fed exclusively a 1:1 mixture of the acetates (at a relatively high dose of 36 mg/kg diet) over a 5-mo period. The ratio of *RRR*-α-tocopherol to *all-rac*-α-tocopherol (*RRR:rac*; averaged over 10 animals) varied little between tissues (except liver and plasma) and over time: 1.40 ± 0.06 in brain, 1.46 ± 0.06 in heart, 1.36 ± 0.07 in kidney, 1.47 ± 0.06 in lung, 1.46 ± 0.07 in muscle, 1.41 ± 0.07 in testis, 1.16 ± 0.06 in liver, and 1.62 ± 0.08 in plasma.

Recently, the Hoffmann–La Roche group published the results of their application of chiral chromatographic methods to determine the relative concentrations of all 8 stereoisomers of *all-rac*-α-tocopherol in plasma and several tissues of vitamin E–deficient female rats dosed only with *all-rac*-α-tocopheryl acetate daily for ≤3 mo (2). Averaging their calculated *RRR:rac* over time gives 1.38 for brain, 1.41 for adipose tissue, 1.18 for liver, and 1.61 for plasma. Clearly, there is good agreement between the 2 methods when comparisons are possible, despite the fact that, as Cohn remarked (1), the 2*R*-2*S* dose ratio is 3:1 in our competitive method and 1:1 in the traditional method. Thus, the suggestion that the competitive method overestimates the relative *RRR:rac* bioavailability is not supported by these experimental findings.

Furthermore, it is apparent that the relative bioavailability values in nonhepatic rat tissues are all close to rat biopotency values [eg, 1.26–1.41 for fetal resorption and 1.43–1.52 for myopathy (3)]. The closeness of these tissue bioavailability ratios to the biopotency ratios is consistent with bioavailability being the dominant cause of the difference in biopotency in rats. This strengthens the observation of Weiser et al (2), with respect to their own findings, that "the results are in agreement with the hypothesis that for α-tocopherol stereoisomers, biopotency differences are related to corresponding differences of α-tocopherol concentrations."

In rats the maximum *RRR:rac* is observed in plasma (1.5–1.7), in both continuous feeding and single-dose, competitive studies, and tissue values do not exceed ≈1.5 over a period of ≥5 mo of continuous dosing. In humans, the ratio in plasma starts at ≈1.5 during dosing and rises to a limiting value of ≈2 several days after dosing, regardless of the total dose (30, 150, or 300 mg/d) and dosing period, and is maintained over a wide range of plasma concentrations (up to the analytic sensitivity of the method, ≈3 mo after 8 continuous days of dosing at 300 mg/d) (1). Nonhepatic tissue *RRR:rac* values (≈1.4–1.6) after short-term dosing (<5 wk) generally reflect the lower plasma values observed during dosing and the proportion of labeled α-tocopherol greatly lags behind the corresponding proportion in plasma. In contrast, the results obtained from 2 terminally ill patients indicate, especially for the patient receiving 300 mg/d for almost 2 y, a long-term trend toward equilibration of the labeled α-tocopherol between plasma and tissues, with regard both to the *RRR:rac*, which tends to a maximum value of ≈2, and to the fraction of labeled α-tocopherol. Also noteworthy is

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an even higher ratio (3.4) observed in fetal cord blood obtained from women fed a 1:1 mixture of *RRR*- and *all-rac*-α-tocopheryl acetate just before giving birth (4).

The higher *RRR:rac* observed in human plasma, and eventually in tissues, implies that a greater degree of discrimination occurs in humans than in rats. The rapid disappearance of *SRR*-α-tocopherol from human plasma after a single 1:1 dose of *RRR*- and *SRR*-α-tocopheryl acetate (ie, ambo-α-tocopherol) (5, 6) suggests that most, if not all, of the 2*S* forms are eventually eliminated because the behavior of *SRR*-α-tocopherol more or less approximates that of the four 2*S* stereoisomers (2). If the four 2*R* forms behave like *RRR*-α-tocopherol (2), a limiting *RRR:rac* value of ≈2 will result.

The main source of discrimination is likely the liver tocopherol transfer protein (TTP), which is of major importance for vitamin E homeostasis in humans (7). Indeed, rat TTP shows an ≈2-fold greater affinity for *RRR*- than for *all-rac*-α-tocopherol (8). Determination of the extent to which TTP controls recycling and redistribution, as well as uptake, of vitamin E would reveal its importance in controlling tissue bioavailability. We suggest that reassessment of relative bioavailability for human nutritional and health needs is appropriate, bearing in mind that most benefits of the vitamin are of a long-term nature.

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Definition of *sensitivity* and *specificity*

Dear Sir:

I noticed a technically incorrect definition in an otherwise interesting and potentially important article published recently in the Journal (1). The authors state in the introduction that "Sensitivity (proportion of subjects classified as 'positive' by both the screening and reference tests) and specificity (proportion of subjects classified as 'negative' by both tests) are calculated…" The wording "proportion of subjects" implies that the total number of subjects was the denominator.

Reference to any standard epidemiologic text will reveal that *sensitivity* is correctly and unambiguously defined as the proportion of reference test positive (diseased) subjects who test positive with the screening test. Note that the denominator is the number of reference test positive subjects, not the total number of subjects. Similarly, *specificity* is defined as the proportion of reference test negative (healthy) subjects who test negative with the screening test. Judging from the results, it seems likely that the authors used the correct denominator, but readers should be aware that the text is not technically correct.

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Reply to RM Lazarus

Dear Sir:

Lazarus pointed out that the wording we used to define sensitivity and specificity in a recent article could be misinterpreted (1). He is of

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course correct that the sensitivity of a screening test refers to the number of subjects who are classified as positive by the screening test divided by the number of subjects who test positive by the reference test. The sentence preceding that quoted by Lazarus noted, "ROC [receiver operating characteristic] curves determine the efficacy of a screening measure to correctly identify subjects on the basis of their classification by a reference or gold standard test." The reader could infer from this sentence that the correct denominator was indeed used. We acknowledge, however, that the wording in our introduction may not have been entirely clear to readers. We assure readers of the Journal that we applied the correct denominator throughout our paper and we thank Lazarus for bringing this matter to our attention.

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Seasonal vitamin D in African American and white infants

Dear Sir:

Thank you for publishing the article on seasonal vitamin D in 51 black and 39 white individuals by Harris and Dawson-Hughes (1) and the accompanying editorial by Norman (2). In a 1986 study in 198 subjects <18 mo of age, we found that differences in vitamin D status by race were significant in winter but not in summer (3). What was also surprising was that African American infants had significantly higher 1,25-dihydroxyvitamin D concentrations than white infants, both in winter and in summer. The study was uniformly blocked by sex, race, age, season, and diet (formula-fed compared with breast-fed infants). We advanced the thesis (4) that the renal 1-hydroxylase enzyme is particularly active in infancy as an adaptive mechanism in conditions in which serum 25-hydroxyvitamin D concentrations are low, such as during the winter. High 1,25-dihydroxyvitamin D concentrations coincide with periods when serum phosphate concentrations are significantly lower [in winter and in African American infants (3)], which could serve as the stimulus for 1,25-dihydroxyvitamin D production during these periods. What may be little known is that in human milk-fed infants who do not receive vitamin D supplementation (5), there can be a marked 2- to 3-fold seasonal alteration in serum 25-hydroxyvitamin D concentrations.

Thus, in infancy there are *1*) marked seasonal variations in vitamin D status, *2*) marked differences in vitamin D status by race (African Americans compared with whites), and *3*) higher serum 1,25-dihydroxyvitamin D concentrations in the winter than in other seasons and in African Americans than in whites. These findings provide an additional perspective on the issue of seasonal variations in vitamin D status.

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