Can we consider plasma alkyresorcinols as a potential biomarker of whole-grain food?

Dear Sir:

We read with great interest the article by Ross et al (1). In their article, one of the main conclusions was that plasma alkyresorcinol concentrations were correlated with whole-grain (WG) intake and could be used to distinguish between low- and high-WG consumers. On the basis of the above findings, Ross et al proposed plasma alkyresorcinol concentrations as a potential biomarker for WG intake. However, there are several issues in their study that reduce the efficacy of the final results. We think their idea could be very useful in discussing the functions of alkyresorcinols if the following issues are addressed.

First, 316 overweight and obese participants were recruited at the beginning of the trial, but only 266 subjects completed the intervention trial. For a randomized, parallel dietary intervention study, the dropout rate (15.82%) was high, which may have caused disruption of random grouping (2), because dropout rates that differ between groups will cause a selection bias to any type of long-term follow-up (3). Therefore, the balance between the 2 groups could not be ensured. Furthermore, the research conclusions would not be precise. For example, if all of the subjects who dropped out of the intervention group had high alkyresorcinol concentrations, the results would be underestimated.

Second, the authors tried to obtain a balance between different groups by using quartiles methods. However, this statistical method is unreasonable because random grouping was disrupted. Therefore, we suggest that propensity score matching (4, 5) should be used to achieve balance, which has the advantage of dealing with nonrandom or observational data (6). As we know, obtaining a good balance or random condition can make the study results more valuable.

Third, an ideal biomarker must be specialized, stable, and early-predictive. The results of Ross et al (1) showed that the correlations with plasma alkyresorcinols for WG, WG wheat, and alkyresorcinol intakes were 0.32, 0.39, and 0.38 respectively (Table 8 in their article). From a statistical perspective, these are weak correlations. Other researchers (7, 8) have reported that plasma alkyresorcinol concentration is not the only factor that correlates with WG intake. Another article published by Aubertin-Leheudre et al (7) indicated that 3,5-dihydroxybenzoic acid also correlates with WG intake ($r = 0.37$). This is to say that 3,5-dihydroxybenzoic acid may become another potential biomarker, although it has some limitations. Therefore, alkyresorcinol concentration is not an ideal biomarker. Similarly, the reproducibility of plasma alkyresorcinol concentrations was not high enough. The results of Landberg et al (9) and Andersson et al (10) indicated a moderate reproducibility for plasma alkyresorcinol concentrations, which suggests that we cannot achieve a stable value at 2 time points.

In summary, the data from this article were not sufficient to support the authors’ conclusion that plasma alkyresorcinol concentration is a good biomarker for WG intake. Further studies need to be conducted to improve the efficacy of the study.

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Reply to L Zheng et al

Dear Sir:

We thank Zheng et al for their interest in our article published in the Journal earlier this year on the relation between plasma alkylresorcinols and whole-grain intake (1). They query a number of aspects of the study and whether alkylresorcinols can really be considered to be biomarkers of whole-grain intake. They raise a number of points related to the random nature of the population of the WHOLEheart study and the correlations between reported whole-grain intake and plasma alkylresorcinols.

The relatively high dropout rate from the WHOLEheart study has been previously addressed (2). Approximately two-thirds of those who dropped out did so after random allocation through minimization (3) but before they had completed their first study visits (ie, the point at which they would receive instructions on what was required for their arm of the study), and one-third dropped out between randomization and the start of the study. Notwithstanding this early elective withdrawal, the dropout rate was lower than that factored into the power calculation (based on at least 250 to complete, 266 actually completed), which was based on a reduction in LDL cholesterol. In terms of age, sex, and BMI, there was no skewing of the population due to dropouts. Although a propensity score matching analysis might be suitable in a nonrandom sample, as described above, we still consider that the population taking part in the study was suitably random on the basis of the minimization criteria balanced on age, sex, and BMI.

Zheng et al state that a biomarker must, among other things, be “early-prediction.” Although this may be desired for a biomarker of disease, it is not the case for a biomarker of food intake. A biomarker of food intake should reflect recent food intake. Whereas correlations of 0.32–0.39 may appear to be low, these are relatively high for biomarkers of intake because they are highly affected by the type of dietary recall instrument used. Although food-frequency questionnaires lead to lower correlations (0.2–0.4), more accurate measures such as weighed food records result in correlations between alkylresorcinol or whole-grain intake and plasma alkylresorcinols of 0.55–0.6 (4–6). Thus, recall bias and nonspecificity of dietary recall instruments are a major factor in determining the results, along with factors determining plasma alkylresorcinol concentration (eg, alkylresorcinol intake, food matrix effects, absorption efficiency, sex). One of the proposed uses of biomarkers of food intake is not to replace other measures of food intake but to complement subjective methods to improve estimation, as the associated errors are different (7).

A recent review article addressed the issue of intact alkylresorcinols compared with alkylresorcinol metabolites (4). Correlations with measures of alkylresorcinol intake are similar, but there were too few studies on the metabolites, especially at low intakes of alkylresorcinols to clearly establish whether there is an advantage of one over the other. Zheng et al also query whether alkylresorcinols are sufficiently reproducible to act as biomarkers. This appears to depend greatly on the control of food intake: under controlled conditions, alkylresorcinol measurements are highly reproducible [intraclass correlation of 0.88–0.9 (8)], whereas under free-living conditions the intraclass correlation is moderate (9). This would suggest that part of the problem lies in the assumption that people maintain a constant cereal intake, rather than that alkylresorcinols themselves have an abnormally erratic response in plasma. We need to adapt our expectations of biomarkers around people, rather than the other way around.

Zheng et al suggest that the data from our work were not sufficient to support the conclusion that plasma alkylresorcinols are a good biomarker of whole-grain intake and that greater “efficacy” is required in this study. The suggestion that plasma alkylresorcinols and their metabolites might be biomarkers of whole-grain intake is not new: to date there are 19 published studies in which plasma alkylresorcinols have been measured, with all finding that an increase in alkylresorcinol intake leads to an increase in plasma alkylresorcinols. An earlier editorial in the Journal outlined some of the opportunities and issues for the use of alkylresorcinols as biomarkers of whole-grain intake (10). Biomarkers are not established in single studies and take many years and many different study designs and approaches before they can be accepted. Our work is only one part of this effort that helps us understand how alkylresorcinols respond as biomarkers under different situations. Knowing the limitations helps us to best apply alkylresorcinols and dietary intake data in epidemiologic and intervention studies for ultimately getting the most accurate possible picture of whole-grain intake.

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Comparison of vitamin D2 and vitamin D3 supplementation in increasing serum 25-hydroxyvitamin D status: a systematic review and meta-analysis

Dear Sir:

We thank Tripkovic et al (1) for their recent comprehensive review, which will be of particular interest to those involved in recommending vitamin D supplementation. The authors reviewed and updated the evidence with regard to the effectiveness of vitamin D2 (ergocalciferol) and vitamin D3 (cholecalciferol) in increasing serum 25-hydroxyvitamin D [25(OH)D]. However, it appears that the conclusion paragraph in the Abstract is based on bolus administration only as there was no difference between vitamin D2 and vitamin D3 at increasing serum 25(OH)D concentration from daily supplementation. Whereas the conclusion is valid when considering treatment options where a loading dose may be required before prescribing a maintenance dose in the longer term, it may not be applicable where recommendations are based on daily intakes. We believe that in the interest of public health, the message should be that there is an option for those who for religious reasons and strong vegan principles may prefer to take vitamin D2. Also, although unlikely to be a major problem, at least in the short term when considering vitamin D sources, there could be issues around sustainability in the future. Ultraviolet irradiation of plant-based foods to produce ergocalciferol would be an alternative option that might need to be considered when deciding on large-scale fortification (2).

The issue of the different metabolic fates of vitamin D2 and vitamin D3 is an interesting one. Vitamin D2 appears to be metabolized more rapidly (3), which may underlie the rationale that vitamin D3 is less effective in increasing serum 25(OH)D concentration. It has been inferred that vitamin D2 may be less toxic than vitamin D3 when given in large amounts (3).

Our data from the VICtORy (Vitamin D and Cardiovascular Risk) study (4) during which participants [healthy postmenopausal women; mean (±SD) age: 64 ± 2 y] were supplemented for 1 y with vitamin D3 (400 or 1000 IU) or placebo daily in a parallel-group, double-blind randomized controlled trial of cardiovascular disease risk outcomes shows that increases in mean serum 25(OH)D3 concentration resulted in concomitant decreases in mean serum 25(OH)D2 concentration (Figure 1). An important feature of


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this study is that all participants were randomized simultaneously between January to March in the same year. These data suggest there may be interaction between the 2 forms of vitamin D, with a dose-dependent response. This is not caused by assay interference because the addition of increasing concentrations of 25(OH)D3 into samples containing varying concentrations of 25(OH)D2 does not result in a decrease in the measured 25(OH)D2 concentration. These effects, although small, may have implications in the assessment of the benefit-risk ratio of different vitamin D treatment regimens and in furthering our understanding of vitamin D metabolism.

We agree with the authors that further research is required to examine other metabolites of vitamin D. Developments in tandem mass spectrometry may allow for their more accurate and precise measurement, which could be particularly important after administration of high doses of vitamin D either orally or intramuscularly.

The authors declared no relationships with industry or financial associations that might pose a conflict of interest.

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Reply to HM Macdonald et al

Dear Sir:

We appreciate the comments made by Macdonald et al regarding our article, which is the first-ever systematic review and meta-analysis of the current evidence available comparing the relative efficacy of vitamin D2 and vitamin D3 in increasing serum 25-hydroxyvitamin D [25(OH)D] concentrations.

Because this particular area of the vitamin D field is controversial due to the conflicting opinions on the likelihood of equipotency between vitamins D2 and D3, the purpose of this meta-analysis was to highlight that, at the moment, the research is not consistent in either study design or outcome and is also underpowered. Therefore, the article was written to describe and explain what data are currently available, stimulate discussion within the field, and show the urgent need for a cohesive approach to investigating the comparative effects of vitamins D2 and D3. It was initially hoped that the meta-analysis would unequivocally determine whether vitamin D2 or vitamin D3 was more efficacious; however, it rapidly became apparent that the field is far too underdeveloped at the moment to be able to achieve that aim.

Overall, when all available studies were included and a random-effects model was used to account for heterogeneity, it was found that vitamin D3 appeared to be more effective at increasing serum 25(OH)D concentrations than vitamin D2. The conclusion that vitamin D3 appears to be more efficacious than vitamin D2 was clearly drawn from this main analysis of the data, which included all studies that were available at the time that directly compared vitamins D2 and D3.

The data within the meta-analysis to which Macdonald et al are referring is the latter subanalysis, which was executed to explore the data available and to see whether further insight was possible when the frequency of dosage administration was considered. It is at this point that there appears to be some discrepancy between the properties of vitamins D2 and D3—there was no significant difference between the 2 vitamins when they were taken in a daily dosage. However, there still appeared to be a nonsignificant trend (P = 0.10) toward vitamin D3 retaining preference in increasing 25(OH)D concentrations (1). Vitamin D1 was significantly more efficacious than vitamin D2 (P = 0.0002) when given as a bolus dose (1).

This result was intriguing, yet there is still little evidence available within the included studies or beyond to explain the mechanism behind how and why the body appears to differentiate between vitamins D2 and D3. This brings us back again to the call for further studies that are specifically designed to determine the mechanism between vitamin D2 and D3 metabolism and that are highly powered to help prevent uncertainty in the outcome.

Macdonald et al make a valid point that vitamin D2 is a valuable source to those individuals unable to consume vitamin D3 sourced from animals, because of various cultural, religious, and ethical reasons. This is not disputed in our article; vitamin D2 is clearly effective in increasing serum 25(OH)D concentrations, as shown by the data collated in the meta-analysis. Indeed, we state that it should always remain an option when seeking to improve vitamin D intake from dietary sources for those who require it. However, it is also important to note that there are now nonanimal vitamin D3 supplement sources available on the market (Vitashine; ESB Developments Ltd).

The data presented by Macdonald et al from the VICtORy study provide interesting, yet when compared with other studies, generally contradictory data in terms of the metabolic fates of vitamins D2 and D3 and how they affect 25(OH)D metabolites. From the data, it appears that the vitamin D3 supplementation initiated an increase in 25(OH)D3, yet a decrease in 25(OH)D2; however, contradictions to this result are present within the literature. The study by Glendenning et al (2) was the only study involved in the meta-analysis that published complete 25(OH)D metabolite data. It was found that after vitamin D3 supplementation of 1000 IU/d for 3 mo,
there was an increase in both 25(OH)D$_2$ and 25(OH)D$_3$. However, the vitamin D$_2$ group experienced a different effect, showing an increase in 25(OH)D$_2$ but a decrease in 25(OH)D$_3$ (2). In a recent study conducted by Stephensen et al (3), UV-treated white button mushrooms (containing ergocalciferol) were given to 2 intervention groups who received an average of either 8.8 or 17 µg/d; the supplement group received 28.2 µg ergocalciferol/d. After the intervention, all 3 groups receiving ergocalciferol experienced the expected increases in 25(OH)D$_2$; however, 25(OH)D$_3$ concentrations significantly decreased compared with baseline concentrations. This meant that with any gains from improving ergocalciferol intake, increases in 25(OH)D$_2$ were cancelled out by the detrimental effects on 25(OH)D$_3$ concentrations; thus, overall 25(OH)D status was not affected (3).

The data presented by all parties show that interindividual discrepancies in vitamin D metabolism could be present within the population. Whether these differences are falsely highlighted because of variation in study design (ie, dosage, whether participants are supplemented directly or given fortified food products) in addition to technical aspects such as timing of blood samples and analysis techniques is yet to be determined. There is also the possibility that studies are simply underpowered, with any interindividual differences causing undue influence without correct participant numbers. We are currently seeking to close this gap by completing the first large-scale ($n \approx 400$) double-blind, placebo-controlled trial comparing different food products fortified with vitamin D$_2$ with those fortified with vitamin D$_3$ (4). We encourage the vitamin D research community to pursue participant numbers in human studies that are truly meaningful and which will produce outcomes that are much more reflective of the wider population.

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