Effect of changes in fruit and vegetable intake on plasma antioxidant defenses in humans

Dear Sir:

In a recent issue of the Journal, Dragsted et al. (1) investigated whether fruit and vegetable intake affects biomarkers of oxidative stress or antioxidant defenses. They conducted a well-designed, 25-d, randomized, partly blinded intervention trial. Some of their conclusions related to an apparent lack of effect on markers of total antioxidant capacity (TAC; namely, the ferric-reducing ability of plasma (FRAP) and Trolox-equivalent antioxidant capacity (TEAC)), most of the enzymatic antioxidant defenses (superoxide dismutase, catalase, glutathione reductase, and glutathione S-transferase), and lipid oxidation (isoprostanes and malondialdehyde) in the fruit and vegetable (fruveg) group compared with the placebo group.

TAC measurement, representing the cumulative action of all electron-donating antioxidants present in body fluids, is increasingly being used to monitor redox status in vivo in intervention, bioavailability, and epidemiologic studies (2, 3). However, different studies have indicated that there may be a physiologic modulation of the redox status of body fluids (4, 5), and results from the S. V. M. A. X. intervention trial indicate the importance of baseline plasma concentrations on the effectiveness of antioxidant supplementation (6). Therefore, dietary effects on the redox status of healthy subjects may be small and difficult to discern, especially if nonoptimized assay conditions are used. We suggest that the lack of significant variation in plasma antioxidant defenses observed by Dragsted et al. may be a consequence of these factors. First, the dietary change failed to modify the redox status of the healthy subjects during the experimental period (see Table 6 in reference 1) and, second, the plasma TAC data could have been adversely affected by suboptimal measurement conditions.

The data of Dragsted et al. clearly show that none of the measured redox markers were affected by the withdrawal of fruit and vegetables from the control diet. A decrease in plasma antioxidant concentrations was observed only with vitamin C and carotenoids, which in humans are modest contributors to plasma TAC (7, 8). We speculate that this indicates that 25 d was not an adequate time period to impair plasma TAC in healthy subjects. Because of the ability to cope with light dietary stress, plasma antioxidant defenses may need >25 d or specific and stronger dietary stresses, such as a high-fat diet, to be challenged significantly. We believe that the lack of change in plasma TAC concentrations in the placebo and fruveg groups could have been due to a physiologic regulatory mechanism that in the short term buffers against significant variation in plasma TAC in healthy young subjects (26 ± 6 y for the fruveg group and 29 ± 8 y for the placebo group).

The lack of observed changes in plasma FRAP and TEAC could also be the result of a decrease in the sensitivity of the TAC measurements as the result of nonoptimized assay techniques. The wavelength used by Dragsted et al. to measure both FRAP and TEAC was 620 nm. The correct reference wavelengths are 595 nm for the FRAP assay and 734 nm for the TEAC assay (9, 10). Experiments conducted in our laboratories indicate that measurement at 620 nm results in a decrease in sensitivity of ≈40% and 66% for TEAC and FRAP, respectively. This is borne out by the uncharacteristically high CVs (16.6% and 8.8%, respectively, for TEAC and FRAP) obtained by Dragsted et al. compared with reference studies (9, 10). The difference in vitamin C concentration between the fruveg and the placebo group at the end of the supplementation period was ≈60 μmol/L (Figure 2 in reference 1). The expected relative difference in TAC, based on the stoichiometry of ascorbic acid, should have been ≈10% for FRAP (10). This small, but generally discernable, effect on TAC, may have been masked by the reduced sensitivity of the TAC protocols applied in this study.

In conclusion, this interesting and valuable study by Dragsted et al. (1) highlights both a requirement for optimized assay conditions and the need to consider the possibility of dynamic mechanisms of control of the body’s redox defenses when designing human intervention studies with dietary antioxidants. Measurements of TAC (the sum of the parts) and of single antioxidants (parts of the sum) are useful biomonitoring tools in supplementation and health-related studies of redox balance. However, an understanding of the physiologic mechanisms of control of the body’s redox defenses is an important issue that must be addressed to clarify the role of dietary antioxidants in disease prevention.

None of the authors had any conflict of interest.

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Dear Sir:

We appreciate the comments on our paper (1) made by Serafini et al, who highlight some important problems in the interpretation and power of biomarker-based human intervention studies. Serafini et al’s letter contains 2 major points of criticism. The first concerns the possibility that our intervention period of 25 d was insufficient to observe a change in fasting measures of antioxidant capacity without an added dietary oxidative stress, such as increased fat. Relatively few human intervention studies have actually been able to show differences in antioxidant capacity, and as far as we are aware, all of these found only postprandial effects. This is the case for studies of tea and chocolate, which have been shown to result in short-term increases in markers of antioxidant capacity equivalent to the increased plasma concentration of catechins (2–6). The tomato study mentioned by Serafini et al contains 2 major points of criticism. The first concerns the possibility that our intervention period of 25 d was insufficient to observe a change in fasting measures of antioxidant capacity without an added dietary oxidative stress, such as increased fat. Relatively few human intervention studies have actually been able to show differences in antioxidant capacity, and as far as we are aware, all of these found only postprandial effects. This is the case for studies of tea and chocolate, which have been shown to result in short-term increases in markers of antioxidant capacity equivalent to the increased plasma concentration of catechins (2–6). The tomato study mentioned by Serafini et al also came to this conclusion (7). In another study, the intervention of 20–25% changes in fat or total energy intake for 12 wk was insufficient to elicit observable changes in plasma antioxidant capacity (8).

Thus, we can speculate that prolonged dietary changes are necessary to affect antioxidant capacity. For example, the lifestyle factors leading to type 2 diabetes also result in chronic decreases in plasma antioxidant capacity, apparently as the result of changes in uric acid metabolism (9, 10). Whether fruit and vegetables would counteract this effect in the long run remains to be investigated. Therefore, our conclusion that a large intake of fruit and vegetables does not affect fasting plasma measures of antioxidant capacity seems valid and in accordance with the literature.

The second criticism concerns our method for measuring plasma antioxidant capacity. According to Serafini et al, an increase in the measurement error may have resulted in our failure to detect minor changes, such as the 10% change calculated from the drop in ascorbate concentrations. Our automated assay of the ferric-reducing ability of plasma (FRAP) and Trolox-equivalent antioxidant capacity (TEAC) was optimized within the boundaries of our equipment, eg, with the absorbance filters available. This decreased sensitivity offset the absolute values of TEAC and increased intra-assay variability compared with the same assays on other equipment. We agree that the intra-day CV of our standard plasma sample was high and understand the concerns of Serafini et al. We have reinvestigated the cause of this and found that other samples and our calibrators had much lower variability, indicating some unidentified problem with our standard plasma. In these other samples, our intra-assay variation was still higher (6.7% for TEAC and 3.9% for FRAP) than the reference values cited in the literature (11, 12). However, this is unlikely to have caused a type I error in our study because the interindividual variability in FRAP and TEAC was still much higher than the assay variability. The measurement error therefore has relatively little influence on the actual power to detect differences. We observed an overall interindividual CV at baseline of 11.2% for TEAC (\( \bar{x} \pm SD: 885 \pm 99 \mu M/L \)) and 22.5% for FRAP (\( \bar{x} \pm SD: 693 \pm 156 \mu M/L \)) in the fasting samples (\( n = 43 \)). In the postprandial samples, the variation was 17.0% and 26.7% (\( n = 28 \)), respectively. In papers by others, including those cited by Serafini et al, the interindividual CVs for plasma antioxidant activities are variable but similar to ours, eg, 20.6% for FRAP (\( n = 141 (11) \)), 21.7% for total radical-trapping antioxidant potential (\( n = 11 (7) \)), 9.6% for TEAC (\( n = 312 (12) \)), and 18.3% for oxygen radical absorbance capacity (\( n = 60 (13) \)).

In our study (1), we tried to increase power by looking at the time course during the intervention with a repeated-samples analysis of covariance (ANCOVA) that used each volunteer’s value at baseline as a covariate. In this analysis, the analytic error becomes more important for the power because the interindividual differences are balanced out. However, it still depends on the intra-individual (inter-day) variation, which in our study was 9.3% for FRAP and 11% for TEAC. This leads to a power of 70% to detect a significant 10% change in TEAC or FRAP [determined by G-power (14) as a post hoc analysis]. In addition to the values at baseline and at the end of intervention (25 d), we measured plasma antioxidant capacity 3, 9, and 16 d after the start of the intervention and 8 and 29 d after the volunteers had resumed their habitual diet. As seen in Figure 1, there is no indication of deviations from the initial or post-intervention values, as we also confirmed by repeated-samples ANCOVA. In the case of FRAP, the known difference of 25% between men and women (11) was readily observable at all time points, which indicates to us that we would have seen some indication of a 10% change in fasting blood samples. Moreover, the groups with higher initial values were stable throughout.

In conclusion to this point, we agree that our assay sensitivity was probably not optimal and that our absolute values for TEAC may have been offset by the shortcomings of our automated equipment. However, we disagree that this seriously affected our power to detect a real change in measures of antioxidant capacity. The major source of noise in the measurement of plasma antioxidant capacity is the interindividual variation, which was similar in our study to that of noise in the measurement of plasma antioxidant capacity is the interindividual variation, which was similar in our study to that.
observed by others, including the cited reference studies. Consequently, we still conclude that there was no significant effect of fruit and vegetables on fasting plasma antioxidant capacity within the 25-d study period.

None of the authors had any conflict of interest related to the results and discussion published in this letter.

FIGURE 1. Mean (±SE) ferric-reducing ability of plasma (FRAP) and fasting plasma Trolox-equivalent antioxidant capacity (TEAC) determined according to reference 1 in samples collected before (day 0), during (days 3–25), and after (days 33 and 54) intervention with 600 g fruits and vegetables (●); a corresponding supplement containing nutrients, vitamins, and minerals (▲); or a placebo pill plus an energy-balancing drink (■). The start and end of the intervention are marked with vertical arrows. None of the groups differed significantly at any time point by repeated-samples analysis of covariance.

None of the authors had any conflict of interest related to the results and discussion published in this letter.

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Body mass index and survival in incident dialysis patients: the answer depends on the question

Dear Sir:

In a recent issue of the journal, Johansen et al (1) examined an important question—What is the association of body size with survival adjusted for muscle mass in incident dialysis patients? However, there are really 3 questions: 1) What is the independent association between muscle mass and mortality, 2) What is the independent association between BMI and mortality, and 3) How does mortality vary across different levels of BMI and muscle mass combined. Based on the answer to the question posed by Johansen et al., inferences on whether body composition influences the survival of incident dialysis patients with a high BMI could not be drawn.

We reexamined the data from our earlier study (2), which the authors graciously discussed. Details on study population, inclusion criteria, data collection, and statistical methods were described earlier (2). In 70 028 incident hemodialysis patients in the United States, from 1 January 1995 to 31 December 1999, the associations of BMI categories described by Johansen et al with survival were examined in a multivariable parameteric proportional hazards survival model adjusted for urinary creatinine, serum albumin, and functional status. The results (Figure 1) are similar to those reported by Johansen et al.

To further examine the influence of body composition on survival in high-BMI patients, each of the BMI groups was divided into groups on the basis of muscle mass: low (urinary creatinine /L50140, normal, or high (urinary creatinine /L50872 g/d) subgroups. The hazard ratios from the multivariable parametric proportional hazards survival model, adjusted for all of the above factors except urinary creatinine, are presented in Figure 2.

At first glance, Figures 1 and 2 appear contradictory, but, in reality, they are not. Adjustment for urinary creatinine in the multivariable model (Figure 1) does not mean that the hazard of death is constant across the spectrum of urinary creatinine values in any
given BMI group (Figure 2). Whether the association of BMI with survival is confounded by muscle mass is examined in Figure 1. Whether those with a large body size but low muscle mass have a survival advantage over “healthy” patients with a normal BMI and a normal or high muscle mass is examined in Figure 2.

In our study we summarized the findings in Figure 2 as “the survival advantage conferred by high BMI in dialysis patients is limited to patients with normal or high muscle mass.” We understand the concerns of Johansen et al that this could be construed as independence. We rephrase our conclusions as follows. Patients with a
Dear Sir:

We appreciate the comments of Beddhu et al regarding our recent publication that examined the relation between body size and outcomes among incident hemodialysis patients (1). In particular, we agree with the idea that body composition, and perhaps muscle mass in particular, is important to consider for patients receiving dialysis. However, it is important that, in our discussion of the “best” way to adjust for muscle mass in these patients, we not lose sight of the larger issues at hand. First, although analyses using large data sets are subject to confounding, the results used to determine which components are most important to patient survival.

In conclusion, the questions addressed in the 2 studies were related but had different emphases. We absolutely agree with Johansen et al that body size is an important determinant of survival in incident dialysis patients. However, we stand by our earlier conclusion that, in incident dialysis patients, body size and body composition influence survival. In incident dialysis patients, adiposity confers a survival advantage over undernutrition, but higher muscle mass is better than higher body fat. We agree that, given the current data, incident dialysis patients should not be encouraged to lose weight but should be encouraged to increase muscle mass rather than fat mass.

None of the authors had a conflict of interest.

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Reply to S Beddu et al

Dear Sir:

We would like to point out some problems with the interesting article by Rastogi et al (1) that was recently published in the Journal. In this study, only 12% of the subjects were women; the remaining 88% were men. Generally speaking, in India, men are not involved in cooking. Hence, the men in this study may not have been able to correctly specify the amount of cooking oil that would be used.

In Table 4, there are some factors that were not significant in the univariate analysis but that were significant in the multivariate analysis because of an interaction among the variables. A better way of presenting these data would have been to present data for only those variables that were significant in the univariate analysis and then subjected to multivariate analysis to determine the variation in relative risk.

None of the authors had a conflict of interest.

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Diet and risk of ischemic heart disease in India

Dear Sir:

We would like to point out some problems with the interesting article by Rastogi et al (1) that was recently published in the Journal. In this study, only 12% of the subjects were women; the remaining 88% were men. Generally speaking, in India, men are not involved in cooking. Hence, the men in this study may not have been able to correctly specify the amount of cooking oil that would be used.

In Table 4, there are some factors that were not significant in the univariate analysis but that were significant in the multivariate analysis because of an interaction among the variables. A better way of presenting these data would have been to present data for only those variables that were significant in the univariate analysis and then subjected to multivariate analysis to determine the variation in relative risk.

None of the authors had a conflict of interest.
Letters to the Editor

Reply to TV Chacko et al

Dear Sir:

We appreciate the comments provided by Chacko et al regarding our recent article on diet and risk of ischemic heart disease in India (1). Most of our study participants were indeed men, despite the fact that we recruited only incident myocardial infarction cases from 8 different hospitals in New Delhi and Bangalore. Although it is true that women in India are primarily involved in cooking foods, we qualitatively observed that men are often involved in the shopping for food items, particularly those items purchased in bulk, such as flour, rice, and cooking oils. Thus, men are knowledgeable about the oils used in food preparation. Moreover, our analysis did not consider the amount of oil used in cooking but simply the type of oil used.

Concerning the comment about Table 4, it is important to consider the possibility that some variables may not have been significant in the univariate analyses because of confounding. Ignoring variables that are not significant in univariate analyses from further consideration could, thus, lead to biased results. Because this was one of the first epidemiologic studies of diet and heart disease in India, we believed it prudent to present the findings for all food groups because these preliminary findings could stimulate further research.

Stress is an important factor in coronary heart disease risk. Unfortunately, we did not have the opportunity to examine the association between stress and risk of coronary heart disease in our study. However, we believe it unlikely that stress would have confounded our results because the diets of individuals in our study population were determined, to a large extent, by the overall dietary pattern of their families.

None of the authors had a conflict of interest.

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Serum cholesterol and visuomotor speed: inverse or direct association?

Dear Sir:

The article by Zhang et al (1) contains an apparent contradiction in its findings. In the abstract, the authors first state that “...we found inverse linear associations of serum total cholesterol and non-HDL cholesterol with visuomotor speed...”. However, the conclusion of the abstract then states, “Low serum total cholesterol and non-HDL cholesterol are associated with slow visuomotor speed...” which implies a direct, not an inverse, association. Similarly, a portion of the title of the article, “Serum cholesterol concentrations are associated with visuomotor speed,” and the statement in the discussion that “...we documented that low serum TC [total cholesterol] and low serum NHDLC [non-HDL cholesterol] concentrations are significantly associated with slow visuomotor speed...” imply a direct association.

The source of the confusion is the incorrect way in which Zhang et al define “visuomotor speed.” They state that “Visuomotor speed was measured by the SRT [simple reaction time] test,” and that “The measured response was the latency (in ms)...”. Consistent with this definition, their Table 2 reports latency values in ms under the heading “Visuomotor speed.” However, the term “speed” refers to rate of change, conventionally defined as distance traveled divided by time (2); thus, visuomotor speed would be the reciprocal of latency (ms⁻¹), not latency itself. In the first statement quoted above in which Zhang et al declare their finding an inverse relation, they use “visuomotor speed” as they have incorrectly defined it, namely as latency. However, in the remaining quoted statements, they seem to be using it in its conventional and correct sense, as the reciprocal of latency. Hence, their statements appear contradictory.

The solution is to consistently use the term “visuomotor speed” to refer to the reciprocal of latency. Then their finding, when clearly stated, is that the higher the cholesterol concentration, the higher the visuomotor speed—a direct association. This is an interesting and provocative finding. It would be regrettable if their inconsistent use of an incorrect definition prevented this important finding from being readily appreciated.

The author had no conflict of interest to declare.

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LETTERS TO THE EDITOR 537
Reply to SL Black

Dear Sir:

We appreciate Black’s comments and thank him for providing a detailed explanation. It is true that many neuropsychological tests involve timed responses, such that longer response latencies correspond to poorer performance (1). In describing test results, however, there is a tendency to refer to the “speed” of the subject’s response, which is essentially the inverse of response latency. Black’s suggestion that data be scored and reported as the inverse of response latency, corresponding more directly to the concept of speed, is quite appropriate and would have enabled us to avoid confusion among readers (2).

None of the authors had a conflict of interest to declare.

Matthew F Muldoon

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Erratum


In Table 4 of this article, the Month 0 LDL-cholesterol concentrations are incorrect. The correct concentrations (all in mmol/L) are as follows: Placebo group, 3.7 ± 1.15; CLA-FFA group, 3.3 ± 0.80; and CLA-triacylglycerol group, 3.6 ± 0.97.

Erratum


The first author’s name is incorrectly printed in the journal. It should appear as SoJung Lee.