Homocysteine and B vitamins in dementia

Dear Sir:

Recently, Selhub et al (1) pointed out the importance of the B vitamins folic acid, vitamin B-6, and vitamin B-12 for the well-being and normal functioning of the brain. They also mentioned that the status of these vitamins is frequently inadequate in the elderly and that these inadequacies can result in hyperhomocysteinemia, a recently identified risk factor for atherosclerosis (2, 3) and Alzheimer disease (AD; 4). The authors further stated that these inadequacies can result in brain ischemia by way of occlusive vascular disease, stroke, or thrombosis.

Vascular disease risk factors such as hypertension are well recognized in AD (5). In our own series of 31 patients with cognitive decline (6), 7 of 19 AD patients had one or more vascular disease risk factors (hypertension, generalized atherosclerosis, atrial fibrillation, diabetes, and hypercholesterolemia). When we measured serum concentrations of vitamin B-12, folic acid, and homocysteine in these patients, both AD patients and those with vascular dementia had similarly elevated homocysteine compared with concentrations in healthy control subjects of similar age (**Table 1**), indicating that vascular disease risk factors may contribute to the pathophysiology of AD.

We found an inverse correlation between the degree of cognitive impairment as determined by the Mini-Mental State Examination (MMSE) and homocysteine ($r = -0.43$; Spearman rank correlation), an inverse correlation between folic acid and homocysteine ($r = -0.36$), a correlation between MMSE and folic acid ($r = 0.37$), and a correlation between folic acid and vitamin B-12 ($r = 0.60$) (all $P < 0.05$). These results agree well with recent data reported by Nourhashemi et al (7), who also found a correlation between cognitive skills and B vitamins including folic acid and homocysteine.

In our study, 9 of 31 patients with dementia and hyperhomocysteinemia were treated with 50 mg vitamin B-1, 50 mg vitamin B-6, 5 mg folic acid, and 0.05 mg vitamin B-12 (Beneuran compositum TM; Nycomed Austria GmBH, Linz, Austria). After 4 wk of treatment, serum homocysteine concentrations had returned to normal in all 9 patients, dropping from 17.3 ± 1.9 to 10.7 ± 3.5 μmol/L ($t = -7.72$, $P < 0.0001$; paired t test).

These data suggest that homocysteine, identified as an independent risk factor for vascular diseases (3) may also be of relevance in AD (4) and in vascular dementia, and that folic acid would be an additional therapeutic option in patients with dementia. We agree with the conclusion of Nourhashemi et al (7) that further studies must be performed to elucidate the association between vitamin status and homocysteine concentrations and the possible role of immune activation, free radicals, and oxidative stress in dementia (8).

TABLE 1
Serum concentrations of homocysteine in patients with Alzheimer disease (AD) or vascular disease (VD) and in age-matched healthy control subjects[1]

	Homocysteine
	μmol/L
AD patients ($n = 19$)	17.8 ± 6.6[2]
VD patients ($n = 12$)	18.5 ± 7.8[2]
Control subjects ($n = 19$)	13.8 ± 4.2

[1] $\bar{x}$ ± SD.
[2] Significantly different from control subjects, $P < 0.05$.

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Comment on the use of the term *intrinsic labeling*

Dear Sir:

Heaney et al (1) discussed problems encountered with isotopic labeling in their recent article, in which they compared the calcium bioavailability of fortified soy milk with that of cow milk. They added $^{45}CaCl_2$ to cow milk and soy milk and left it for 14 h to equilibrate with the native calcium. This technique is referred to as extrinsic labeling and was first applied to study iron absorption from different foods (2). It was shown previously that added isotope with native calcium in milk exchanges fully (3), but this is not true for all foods. When there is uncertainty about the validity of extrinsic labeling, the isotope must be incorporated into the food in the same form as the native calcium, otherwise referred to as intrinsic labeling.

The soy milk tested by Heaney et al was fortified with tricalcium phosphate (TCP), and this was labeled by the addition of $^{45}CaCl_2$ to a suspension of calcium hydroxide in water and precipitation of TCP with phosphoric acid. The [^{45}Ca]TCP was then used to replace unlabeled TCP in unfortified soy milk so that calcium absorption could be measured. Throughout the article the authors referred to the soy milk containing ^{45}Ca TCP as intrinsically labeled soy milk.

According to Weaver (4), "intrinsic mineral labeling of plants involves the biological incorporation of a form of the mineral of interest that can be distinguished analytically from the natural form of the element. The label may be substituted in tracer amounts or as a complete replacement of the naturally-occurring element . . . . The underlying assumption for use of intrinsic labeling techniques is that the label is deposited in the same manner and associated with the same constituents as occurs naturally."

It is generally accepted that the terms *intrinsic* and *biosynthetic* are interchangeable with respect to isotopic labeling of plant foods, including soy milk. The term *intrinsic* is associated with native calcium in the foodstuff, not added calcium salts. Thus, using the term *intrinsically labeled soy milk* to describe soy milk to which [^{45}Ca]TCP has been added is confusing or even misleading. The results section of the abstract refers to percentage calcium absorption from "intrinsically labeled soy milk," but the authors then conclude that "intrinsic labeling of the fortificant is required for such liquid suspensions." The lack of precision in terminology does not help crystallize the authors' arguments on method. We hope that this article will not set a precedent for misuse of the term *intrinsic* for describing isotopic labeling of foods.

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Reply to B Teucher and SJ Fairweather-Tait

Dear Sir:

We thank Teucher and Fairweather-Tait for the opportunity to clarify a terminologic issue and at the same time to make a point that we perhaps did not emphasize sufficiently in our article (1). Teucher and Fairweather-Tait are, of course, correct in stressing the importance of uniform distribution of tracer through all of the native ionic species. Otherwise, isotope movement could not be used validly as a quantitative marker, or tracer, for the native species, whether calcium, iron, or any other mineral or compound.

Moreover, we agree that the distinction between intrinsic and extrinsic labeling lies in whether the tracer is incorporated into the various native calcium species during their own synthesis, or whether it is added after the fact. We believe that, thus far, we are of one mind with Teucher and Fairweather-Tait. However, for use of the term *intrinsic*, they confine the notion of synthesis to biosynthesis, which, in the case of a calcium-enriched food, is not relevant. The article by Weaver (2) to which Teucher and Fairweather-Tait refer related not to engineered foods but to edible plants, in which synthesis and biosynthesis are one and the same.

Heaney et al (3) reported previously in the Journal on the bioavailability of soybean calcium, intrinsically labeled in exactly the sense preferred by Teucher and Fairweather-Tait. However, such intrinsic labeling of the plant calcium would not be useful in the case of fortified soy "milk," because virtually none of the native bean calcium ends up in the milk. Soy

milk, as it is commercially prepared, is a deficient food—that is why it is fortified. Consumers and the nutritional community need to know the bioavailability of the calcium in the end product, as consumed. The vast bulk of the calcium in fortified soy milk comes from the fortificant, and it is the fortificant that we have shown must be intrinsically labeled. The fact that the fortificant is extrinsic to the soy milk is beside the point. Moreover, the bioavailability of the fortificant must be tested, not in isolation but in the final food matrix. In our article, we used the terms *intrinsic* and *extrinsic* to refer to the labeling of the fortificant. We are sorry if we failed to make that point sufficiently clearly.

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Concentrations of sugars in high-carbohydrate diets

Dear Sir:

In Parks and Hellerstein's (1) extensive review article on carbohydrate-induced hypertriacylglycerolemia, there is ample discussion about future research, which probably will be influenced, at least in part, by their excellent article. In view of this probability, to avoid additional misleading studies that cannot be usefully compared (2, 3), it is important to integrate Parks and Hellerstein's data (1) with the recommendation that all future studies on the effects of high-carbohydrate diets should specify whether sugars are consumed in physiologic concentrations $<$ or $>$4.18 MJ/L. In fact, although sugars in concentrations $\leq$4.18 MJ/L leave the stomach progressively more slowly as concentrations increase, thereby allowing energy to be delivered to the intestine at physiologically constant rates per unit of time, sugars in concentrations $>$4.18 MJ/L leave the stomach more rapidly than do sugars in lower concentrations, thus causing the delivery of unphysiologically high amounts of energy to the intestine per unit of time (4). This recommendation applies especially to studies using liquid-formula diets (5).

Unfortunately, researchers have not yet realized that carbohydrate-induced hypertriacylglycerolemia may simply reflect the unnaturally high concentrations in which sugars are usually ingested and not the effects of a low-fat, high-carbohydrate diet per se (5). When starch accounts for most of the carbohydrates ingested, triacylglycerolemia can be drastically reduced, even in patients with familial endogenous hypertriacylglycerolemia (6).

Considering that the metabolic physiology of our ancestors, who practically lived on fruit for millions of years, was largely molded by the sugars present in fruit (4), which never exceed 4.18 MJ/L, it is hardly surprising that the consumption of sugars in concentrations above this natural limit can result in undesirable consequences (4, 5, 7).

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Reply to R Baschetti

Dear Sir:

We thank Baschetti for his positive comments. We strongly agree that many important questions remain about carbohydrate-induced hypertriacylglycerolemia and that feeding studies in humans are needed for them to be answered. Issues include the quantitative consequences of dietary fiber, the effects of carbohydrate composition, and the actual volume of food provided in experimental diets.

Baschetti raises the issue of the carbohydrate absorption rate and the role of ingested carbohydrate on the etiology of hypertriacylglycerolemia. Definitive data on these issues are lacking. Hudgins et al (1) reported 2 different patterns of de novo fatty acid synthesis in subjects fed low-fat, high-carbohydrate diets, depending on the ratio of complex to simple sugars provided. Different concentrations of ingested sugars and their relation to changes in serum triacylglycerol concentrations were not tested,

however. Although the simple sugar content of low-fat, high-carbohydrate diets appears to be an important factor in the risk of carbohydrate-induced hypertriacylglycerolemia, as we noted in our review (2), the mechanisms remain unclear. Baschetti's suggestion is interesting, but more research is needed before a causal role can be attributed to the glycemic index or carbohydrate absorption rate.

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Use of glycemic index in predicting risk of coronary heart disease

Dear Sir:

I am writing regarding the article by Liu et al (1) in the June issue of the Journal. In the results section, the authors made the statement that "Women with high dietary glycemic loads consumed more carbohydrates, dietary fiber, cereal fiber, vitamin E, and folate . . . ." This group apparently had lower intakes of fats and cholesterol and also smoked less than did women with low dietary glycemic loads. The authors did not appear to offer an explanation as to why these factors, which have been associated with a lower risk of coronary heart disease (CHD), occurred in the group that had the highest risk of CHD in their study. Are we to conclude that these other factors are unimportant relative to glycemic index in predicting the risk of CHD?

Liu et al used glycemic index to calculate what is referred to as glycemic load from food-frequency data. It is not clear what method was used to validate this approach, especially given that food-frequency data do not provide information on meal patterns, cooking methods, varieties of starchy foods, or other factors that may influence the actual glycemic index of carbohydrate-containing foods and meals, as consumed.

The authors observed that high glycemic load is most predictive of CHD risk in women with body mass indexes (in kg/m^2) >23. This observation suggests that metabolic factors associated with weight gain may be more important in explaining the role of carbohydrate intake and the risk of CHD than are the glycemic indexes of foods per se. Is glycemic index a tool for understanding of these metabolic factors, or is it an end in itself?

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Reply to BO Schneeman

Dear Sir:

We appreciate Schneeman's comments regarding our article. As noted, women with high dietary glycemic loads tended to be a health-conscious group (1). However, eating large amounts of low-quality carbohydrate, as reflected by a high dietary glycemic load, appeared to increase the risk of coronary heart disease (CHD) in these women, independent of healthy choices such as smoking less and consuming more dietary fiber and vitamins.

Any carbohydrate-containing food can induce plasma glucose responses, and dietary glycemic load (the amount of carbohydrate multiplied by its glycemic index) represents the quality and quantity of carbohydrate and the interaction between the 2. The interaction implies that carbohydrate quality, represented by glycemic index, should have a greater biological effect when the amount of carbohydrate consumed is large than when the amount is small. Also, because a common standard referent food—white bread—was used to standardize all carbohydrate-containing foods, we essentially compared the relative associations of different glycemic responses from these foods with CHD risk. Because a higher intake of dietary fiber, vitamin E, or folate was each independently associated with a lower risk of CHD (2), it is important to evaluate the association with glycemic load or glycemic index with adjustment for these other dietary factors. In relation to glycemic index, the Spearman correlation coefficients were –0.20 for dietary fiber, −0.23 for dietary folate, and –0.32 for vitamin E, indicating that the overall glycemic index was inversely associated with the intakes of these micronutrients that are thought to be protective against CHD. Thus, in a multivariate model that included dietary fiber, folate, and vitamin E, the independent association between glycemic load and CHD risk was even stronger (Table 2 of our article).

Cooking methods can have some influence on glycemic index; thus, we used average values for the ways that foods are

usually consumed (eg, potatoes are eaten cooked and apples are eaten raw). Meal patterns may affect the absolute glycemic response but do not affect the relative differences between foods (3–5). Metabolic studies using standardized methods indicated that the correlation between the glycemic index of mixed meals and the average glycemic indexes of individual component foods ranges from 0.84 to 0.99 (5–7). Even though the total quantity of the glycemic and insulinemic effects of foods may not be fully captured by dietary glycemic load, these measurement errors were likely to have been modest and unrelated to CHD because diets were assessed before disease occurred. Recently, in a random sample of 185 postmenopausal women in the Nurses' Health Study who provided fasting blood samples, we found a strong positive relation between dietary glycemic load assessed by a food-frequency questionnaire and fasting triacylglycerol concentrations (8), a well-established relation from metabolic studies (9).

Metabolic experiments suggested that the adverse metabolic responses to a high dietary glycemic load, including hyperinsulinemia, hypertriglyceridemia, and low HDL-cholesterol concentrations, are strongly related to an individual's underlying degree of insulin resistance (10). Thus, our observation of a stronger positive association between dietary glycemic load and CHD risk in overweight women highlights the importance of considering the physiologic effects of carbohydrate quality in the context of other metabolic variables. Judged by its abilities to predict physiologic responses as well as clinical endpoints, glycemic index appears to represent a more informative means than the conventional simple versus complex approach in classifying carbohydrates.

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Glycemic load and the risk of coronary heart disease

Dear Sir:

The results of the study by Liu et al (1) regarding glycemic load and risk of coronary heart disease (CHD) among participants of the Nurses' Health Study are both fascinating and timely. The debate over the role of carbohydrate in efforts to promote health and control weight has perhaps never been more intense. This study, suggesting that CHD risk is directly related to the glycemic load of the diet, could be used as evidence that recommendations for high intakes of complex carbohydrate are injudicious. However, the information provided is insufficient for reaching this conclusion.

First, although the study showed an association between glycemic load and CHD risk, no consideration is given to the potential bidirectionality of that association. Specifically, with prevailing recommendations to restrict fat intake in the advent of CHD or its risk factors, women who developed angina, anginal equivalents, or overt CHD risk factors might have subsequently reduced their fat intake and substituted carbohydrate. As a result, high glycemic load might appear to be linked to CHD risk, but the causality would actually be in the opposite direction. If the authors have pertinent information, this issue should be addressed.

Other limitations are worth noting. In citing references concerning the adverse effects of carbohydrate on the lipid profile (2–4), the authors were selective and potentially biased, omitting studies that showed beneficial effects of high carbohydrate intake when fiber content was high (5, 6). In Liu et al's study, fiber intake in proportion to total carbohydrate intake was lower in the higher quintiles of glycemic load, as shown in Table 1; this fact is inadequately discussed. Limited discussion is devoted to the neutral effects of dietary starch displayed in Table 3. Finally, the authors imply in their discussion that fruit and vegetables, because of their content of simple sugars, are sources of simple rather than complex carbohydrate. This questionable classification biases the study results against complex carbohydrate and in favor of the conclusions reached by the authors.

As is often true in nutritional epidemiology, this report raises more questions than it answers. That said, one might question whether it is prudent and justified to use the results of this observational study to challenge dietary guidelines that the bulk of evidence suggests would, if implemented, reduce the population burden of CHD.

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Reply to DL Katz

Dear Sir:

We appreciate Katz's interest in our article. He suggested that because women who had coronary heart disease (CHD), angina, or overt CHD risk factors may have changed their diet to a high-carbohydrate one, the positive association between dietary glycemic load and CHD risk observed in our study may be spurious because of confounding by these high-risk conditions. This concern, although theoretically possible, was not supported by findings in the Nurses' Health Study. First, higher intake of dietary carbohydrate, total starch, or total grain was not significantly associated with higher CHD risk (1, 2); neither was intake of total fat (3). However, higher intake of *trans* fat (3) as well as lower intakes of polyunsaturated and monounsaturated fats (3), whole grains (1), and fruit and vegetables (4) were significantly associated with higher CHD risk. Second, nurses who had CHD, angina, or diabetes at baseline were excluded from the main analyses and higher dietary glycemic load was not associated with a less favorable CHD risk profile (Table 1 in reference 2). Third, the positive association between dietary glycemic load and CHD risk became even stronger after adjustment for conventional risk factors, arguing against the possibility of residual confounding.

Our findings that the types of carbohydrate as measured by the glycemic indexes are important for predicting CHD risk are consistent with an inverse relation between dietary fiber intake and CHD risk that we (5) and others (6) reported previously. Incorporating the concept of glycemic index in our assessment of the physiologic effect of carbohydrates enabled us to measure both the total amount of carbohydrate and the quality of carbohydrate intake. As shown in our article, the positive association between dietary glycemic load and CHD risk was independent of dietary fiber intake and was particularly strong in overweight women. This finding is supported by metabolic data showing that the adverse metabolic effects of high carbohydrate intake on blood HDL-cholesterol and triacylglycerol concentrations depend directly on the degree of insulin resistance, which is largely determined by excess body fat (7).

Although a prevalent nutritional recommendation has been that a low-fat, high-carbohydrate diet can prevent heart disease, few empirical data support such a recommendation (6). Results from the Nurses' Health Study add to the growing body of evidence suggesting that neither total fat nor total carbohydrate in the range typically eaten is related to CHD risk and that a more complex picture exists relating to different types of fats and carbohydrates. As in any field of scientific pursuit, new data often generate new hypotheses to be tested. Future investigations are thus needed to confirm our findings in high-quality prospective studies of different populations in whom the range and nature of carbohydrate intake and the degree of insulin resistance may be different. We do not advocate that dietary recommendations be based on epidemiologic data alone but do note that our findings are consistent with results from metabolic studies, including those cited by Katz. Additional basic and experimental studies are also warranted to achieve an understanding of how different types of carbohydrates may affect CHD risk. For example, why does a high-carbohydrate diet typically produce high plasma triacylglycerol and low HDL-cholesterol concentrations characteristic of the insulin resistance syndrome? How do different types of carbohydrates affect insulin and other hormonal responses, particularly in individuals who are already prone to insulin resistance? What other hemodynamic or inflammatory markers may also be related to such a diet?

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Scientific thoroughness of human studies showing immune-stimulating properties of yogurt

Dear Sir:

We read with great interest the review article "Immunologic effects of yogurt" by Meydani and Ha (1), which was published recently in the Journal. Although these authors agree that there are strong indications that yogurt containing certain probiotics have immunostimulatory effects, they argue that most of the studies that substantiated this evidence lacked thoroughness and that the data from these studies were often misinterpreted. In particular, Meydani and Ha claim that most of these studies were poorly designed and lacked appropriate placebo groups and proper statistical analysis. We note that our human studies that showed several immunostimulating properties of *Lactobacillus johnsonii* strain La1 (reviewed in reference 2) are different. Even though our initial longitudinal studies that showed increased phagocytic activity and antigen-specific immunoglobulin A lacked placebo control groups, the data were analyzed by using repeated-measures analysis of variance, followed by least-significant-difference multicomparison procedures (3–5). More recent studies with La1 bacteria were carried out in a double-blind placebo-controlled fashion and differences within groups were tested by paired *t* tests (6–8).

A second concern of the authors is that many studies investigated immune stimulation by probiotics given to animals parenterally or in vitro in cell culture systems. It is true that immunization of animals with probiotics via a different route from that by which yogurt is usually consumed (ie, orally) is somewhat artificial. The results of these studies merely suggest that antigenic epitopes from probiotics have the potential to induce immune responses when put in direct contact with immunocompetent cells in vivo. This is why in all of our studies of both animals and humans (3–9), La1 bacteria were administered orally and, in most studies, in fermented milk. To ensure that La1 bacteria exert the above-mentioned functions in the final product (ie, yogurt), most observations made with the use of fermented milk were repeated in trials using a commercially available Lc1 yogurt that contained La1 bacteria and that included a placebo group that received an identical yogurt that did not contain La1 bacteria (6, 10). These studies, therefore, addressed effects that may have been due to bacteria used in the starter culture used for the fermentation process.

Last, Meydani and Ha criticize the short duration of most of the studies conducted with probiotics. Indeed, most studies were performed over relatively short periods and, in some studies, immune stimulation peaked after a few days and declined thereafter. According to Meydani and Ha, this result suggests that administration of probiotics has short-term adjuvant effects on the immune response. In our studies, La1 bacteria were usually administered over 3 wk, after which time all volunteers resumed a diet that did not contain probiotics or any other fermented product. In these studies, we showed that immune stimulation was maintained as long as the probiotic was administered, but declined 6 wk after administration ended (3, 5, 7). This finding suggests that, at least for La1 bacteria, immune stimulation is maintained as long as the bacteria is provided. Furthermore, we showed in a recent study of patients infected with *Helicobacter pylori* that the beneficial effects of La1 bacteria were measurable 6 wk after administration of the bacteria ended (8). Thus, even though the immune-stimulating properties of La1 bacteria may not be maintained in the absence of the bacteria, some beneficial effects are still observed long term. Whether these long-term effects are immune mediated remains to be shown. Nevertheless, the fact that innate immune responses are not only important for early containment of pathogens, but also crucial in shaping the subsequent acquired immune response that is responsible for long-term memory, makes this hypothesis conceivable. Because most studies of probiotics focused on innate immune markers, they may have overlooked long-term acquired immune functions because such functions are more difficult to address.

In summary, the review by Meydani and Ha provided excellent insight about all available tools and studies of immune modulation by probiotics and their components. These authors concluded that most of these studies conducted thus far were poorly designed and lacked scientific rigor. We hope that we have convinced Meydani and Ha that not all studies fall into this category.

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Erratum

Rush D. Nutrition and maternal mortality in the developing world. Am J Clin Nutr 2000;72(suppl):212S-40S. On page 240S, reference 218 is incomplete and should read as follows: Stoltzfus RJ, Chakraborty J, Rice A, de la Briere B, de Francisco A. Plausible evidence of effectiveness of an iron-supplementation programme for pregnant and post-partum women in rural Bangladesh. Food Nutr Bull 1998;19:197–204.