Dear Sir:

We read with interest the article by Detopoulou et al (1) that reported dietary choline and betaine intakes and associations with inflammatory markers in healthy free-eating adults enrolled in the ATTICA Study. In that article, subjects with higher dietary choline and betaine intakes had significantly lower markers of inflammation in the blood. The report and the accompanying editorial by Zeisel (2) discussed possible mechanisms by which betaine and choline may be involved in reducing inflammation, including their important role as a source of one-carbon units for the metabolism of homocysteine.

We would like to add 2 points to the discussion of this article and editorial. First, we stress the importance of betaine itself to human well-being; second, we express caution about combining betaine intake data obtained in different parts of the world.

We suggest the possibility of a direct impact of dietary betaine on health because of its role as an osmolyte. Aside from being an important source of methyl groups for one-carbon metabolism, betaine functions as a near-ubiquitous intracellular osmolyte to regulate cell volume by counteracting changes in extracellular tonicity and to stabilize macromolecules against a variety of physiologic perturbations (3, 4). It is actively accumulated in most tissues in response to osmotic stress and is centrally important in cell volume regulation, and, as such, betaine is a bodily requirement. Tissue betaine concentrations are higher than circulating concentrations, by more than an order of magnitude in some tissues. Dietary betaine reduces the amount of dietary choline required because less choline oxidation is needed to maintain betaine tissue concentrations.

We have shown that plasma betaine concentration and urinary excretion are tightly regulated and are controlled around individual set points (5). An increase in betaine intake, both acutely and persistently, significantly increases plasma betaine concentrations but does not result in increased betaine excretion (6, 7), nor are there large changes in plasma homocysteine or dimethylglycine concentrations relative to the dose of betaine supplied. This implies that a substantial amount of the ingested betaine is accumulated by the tissues rather than being metabolized; thus, dietary betaine is important for replenishing tissue betaine concentrations and is likely to have an important role in maintaining osmotic control. Inadequate osmolyte accumulation during times of osmotic stress causes a loss in cell volume control, which induces apoptosis and, thus, possibly increases tissue damage. Dietary betaine may thus have a more direct influence on health, and it is important to consider this when discussing the effects and potential mechanisms that dietary betaine and choline intakes may have on inflammation.

In both the article and editorial, it was also noted that dietary betaine intake in populations consuming a Mediterranean diet (those in the ATTICA study) was significantly higher than in populations that consumed an American diet. This was largely attributed to the higher consumption of fruit, vegetables (particularly spinach), legumes, and red meat in the Greek population. We previously measured the betaine content of New Zealand foods (8, 9) and estimated the intake in the New Zealand diet based on >4000 respondents to the New Zealand National Nutritional Survey (9).

We found that the mean betaine intake was 298 mg/d (median: 227 mg/d), and it is closer to that in populations that consume a Mediterranean diet. However, unlike the Greek population, >65% of the New Zealand dietary betaine intake is attributed to grain-based products (mostly bread), with only 4% contributed by vegetables. Similarly, whereas the betaine intake in the Greek population increases with age, betaine intake in the New Zealand population decreases with age, a reflection of the lower consumption of bread by older New Zealanders.

We would also like to make the point that the level of betaine in the diet is affected by the source of the food. Betaine is also a plant osmoprotectant and cryoprotectant, and the level of betaine in plants may be significantly different depending on the conditions in which they are grown. For example, Australian wheat (which is widely consumed in New Zealand), because it is grown in comparatively arid conditions, has a higher betaine content than wheat grown elsewhere. Similarly, because betaine is a small highly water-soluble molecule, the way in which food is processed or cooked can have a large impact on betaine content. Although the US Department of Agriculture food content database is an invaluable resource for estimating the betaine intakes of various populations, its use may lead to an under- or overestimation of betaine intake in populations outside the United States.

No conflicts of interest were reported.

Sandy Slow
Jane Elmslie
Michael Lever

Clinical Biochemistry Unit
Canterbury Health Laboratories
PO Box 151
Christchurch
New Zealand
E-mail: sandy.slow@cdhb.govt.nz

REFERENCES

Reply to S Slow et al

Dear Sir:

The comments presented by Slow et al highlight some mechanisms linking the dietary intake of betaine to concentrations of inflammatory markers. In our article (1), we referred in some detail to the role of betaine as an osmolyte. Betaine’s fundamental role in human health is well recognized (2), and its action as an osmolyte may provide an additional antiinflammatory mechanism. Indeed, betaine can have immunomodulating effects on osmotically stressed cells (such as suppression of cyclooxygenase 2), can protect cells against premature apoptosis, and can regulate cell volume (3). This may also explain the extra benefit of diets high in both choline and betaine in comparison with diets that are only choline rich, as we found in our study (1).

Regarding the measurement of betaine and choline intakes, no national values are available, as in the case of New Zealand (4, 5); therefore, we used international food-composition tables (6). In this case, the generalization of reference values for these markers to the total population is difficult; however, the strength of the effect sizes of the investigated associations is, mainly, unbiased. Nevertheless, this does not mask the emerging need for national values of various health-related markers. Moreover, it can be hypothesized that the relations of betaine intake with other lifestyle and anthropometric characteristics may be influenced by the specific particularities of the studied population. Thus, additional multiethnic studies are needed to shed light on the relatively new field of physiologic ranges of dietary betaine intake and health-related variables.

No conflicts of interest were reported.

Paraskevi Detopoulou
Demosthenes Panagiotakos
Smaragdi Antonopoulou
Department of Dietetics and Nutrition Science
Harokopio University
46 Paleon Polemiston St
Attica 166 74
Greece
E-mail: d.b.panagiotakos@usa.net

Christos Pitsavos
Christodoulos Stefanadis
First Cardiology Clinic
School of Medicine
University of Athens
Athens
Greece

REFERENCES

Triglyceride concentrations and endotoxemia

Dear Sir:

In a recent article in the Journal, Erridge et al (1) provided data to suggest that circulating endotoxin concentrations increase after a high-fat meal. One other report in the literature described elevated endotoxin concentrations after 4 wk of a high-fat diet (2).

Given the scientific interest in gut permeability to lipopolysaccharide (LPS; endotoxin) and to inflammatory disease processes and metabolism (3, 4), we pursued the measurement of LPS by using methods similar to those described by Erridge et al. Using the chromogenic limulus amebocyte lysate (LAL) QCL-1000 assay (Cambrex Bioscience, Walkersville, MD), we analyzed 40 clinical serum samples. The assay is reported as a quantitative test for gram-negative endotoxin (ie, LPS). Samples are measured spectrophotometrically at 405–410 nm after the addition of LAL. The absorbance of the sample is in direct proportion to the amount of endotoxin present in the sample, and it can be calculated by using a standard curve. We intentionally selected samples with a range of triglyceride values (mean: 287 mg/dL; range: 22–1067 mg/dL). Triglycerides and endotoxin values were highly correlated ($r^2 = 0.73$). Endotoxin concentrations ranged from 0.198 to 1.22 endotoxin units/mL. This association could be explained by the affinity of endotoxin for the VLDL particle as a carrier, or it could represent cross-reactivity of the assay for another lipid, such as triglyceride or phospholipid.

To test the hypothesis that triglycerides may bias the endotoxin assay, we performed the assay with reaction buffer and Intralipid (Fresenius-Kabi, Mississauga, Canada), with or without plasma. Intralipid is a sterile, nonpyrogenic fat emulsion containing phospholipids and triglycerides, and it is approved for clinical use via intravenous administration. Briefly, we performed the assay with both sterile pyrogen-free water and serum samples spiked with Intralipid; dilutions of Intralipid varied from 1:30 to 1:10 000. The manufacturer recommends heat inactivation of the samples before assay; we varied the duration at 37 °C from 5 to 60 min. In all cases,