alone offer little benefit to middle-aged and older adults for building muscle mass and strength (4). In fact higher protein intakes in older adults could well be associated with adverse health effects, as indicated by a recent report identifying their association with higher serum concentrations of advanced glycation end-products in terms of carboxymethyl-lysine and its soluble receptor (5). Also higher protein intakes in elderly men, 1.71 compared with 0.75 g protein/kg body weight, are associated with a higher net protein loss in the fasted state, which might not be compensated for by the postprandial response to a high-protein meal (6) as would be predicted by the adaptive metabolic demand model for protein requirements (7). Although Szwiega et al. (1) are not arguing for excessive protein intakes in the elderly they do suggest that 1.0–1.2 g/kg/d of high-quality protein, or ingesting a leucine supplement with each protein-containing meal, particularly in those dependent on a plant-based diet, would be required to provide the 78.5 mg/kg/d leucine indicated as the estimated average requirement (EAR) for leucine in their study.

The authors argue that a limitation of the current study is that they did not include a young adult control group, but instead compared their data with the "comparable published data" in young adults, "which was done using carbon oxidation." In fact the study they quote (8) is in no way comparable to their study. That study was a conceptually very straightforward 24-h balance study in subjects who consumed 4 levels of leucine intake, albeit very laborious and requiring meticulous measurements. It involved a 24-h constant intravenous [1-^{13}C]leucine tracer-infusion protocol to determine leucine oxidation and daily leucine balance, as well as complete 24-h nitrogen balances, and identified 37 and 38 mg/kg/d leucine as the EAR from the leucine and nitrogen balance studies, respectively.

In contrast, the IAAO method deployed by Szwiega et al. (1), which involves a specific statistical analysis of an assumed biphasic response of an indicator amino acid oxidation to increasing test amino acid intake, identifies a breakpoint in the response curve. They argue that this identifies the maximum rate of protein synthesis as judged by no change in the flux of their indicator amino acid, [1-^{13}C]phenylalanine, which they administer by hourly oral doses with the small meals, and calculate the flux from urinary [1-^{13}C]phenylalanine enrichment. However their assessment of the [1-^{13}C]phenylalanine flux is so imprecise as a result of their "minimally invasive IAAO protocol," with CVs of individual mean values for a particular leucine intake appearing to be at least ±80% in some cases, that judging whether it remains constant with the varying leucine intakes becomes arguably meaningless. It is likely that the indicator oxidation breakpoint identifies how much leucine needs to be added to a leucine-free amino acid mixture, fed in hourly small meals, to maximize postprandial *net* protein deposition and minimize indicator oxidation. This would be the case whether or not the mechanism of the response to feeding involves a stimulation of protein synthesis or an inhibition of proteolysis, which is quantitatively the major response to feeding most often observed in the whole body (6, 9). How this breakpoint relates to the requirement for leucine over each 24-h period, as assessed by Kurpad et al. (8), is by no means transparent, but in any case the advocates of the IAAO method have only ever defined the amino acid requirement in operational terms by breakpoint analysis (10) and do not report any supporting information, such as the accompanying responses of plasma amino acid concentrations, which might help with the interpretation of their results. They state that "The IAAO method is widely accepted as a valid method for determining indispensable amino acid requirements" yet the method has not been adopted by other research groups. Furthermore in the current studies because the subjects were overweight and obese, the extent to which this influenced their results is an important issue, albeit difficult to judge given their likely increased splanchnic mass and high rate (possibly 50–60%), of splanchnic extraction of the meal amino acids (11). This means that with these subjects the IAAO method is to a large extent assessing postprandial splanchnic utilization of the meal amino acids.

What is clear, however, is that without measurements in both nonobese elderly and younger adults with an identical protocol, the authors are unable to claim that the leucine requirement of older adults is more than double the amount in current recommendations.

The author reports no conflicts of interest.

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Low vitamin B-12–high folate status in adolescents and pregnant women may have deleterious effects on health of the offspring

Dear Editor:

We read with interest the paper by Bailey et al. (1) and the associated editorial by Molloy (2). They report a significant interaction between low vitamin B-12 and high folate status in relation to lower cognitive functioning in elderly participants of the NHANES cohort. There is a suggestion that unmetabolized folic acid might be related to such

outcomes, raising a concern about dose of folic acid in fortified foods and supplements.

We wish to highlight the possible detrimental effects of maternal imbalance of these 2 vitamins (low vitamin B-12–high folate) on the health of the offspring, both in the short term and long term. Indians are predominantly vegetarian because of multigenerational cultural and socioeconomic influences. Low intake of animal-origin foods in a predominantly vegetarian population contributes to low vitamin B-12–high folate status. The national anemia-control program (now called Intensified-National Iron Plus Initiative) provides iron and folic acid but no vitamin B-12 to children, adolescents, and women of reproductive age. Obstetricians use large-dose folic acid supplements (5 mg) for the prevention of neural tube defects (NTDs) and other purported benefits (although the recommended dose for prevention of a first occurrence of NTDs is only 400 μg).

In the Pune Maternal Nutrition Study (PMNS), a preconceptional birth cohort established in 1993, two-thirds of pregnant mothers had vitamin B-12 deficiency (plasma vitamin B-12 <150 pmol/L) and 90% had elevated methylmalonic acid (>0.26 μM) (3). The mothers were folate replete (<1% had RBC folate <283 nmol/L) even before iron and folic acid were started. Higher frequencies of intake of green-leafy vegetables and fruits (both rich in folate) and maternal erythrocyte folate concentrations were associated with larger birth size of the offspring (4). However higher maternal folate concentrations in pregnancy were associated with higher adiposity in the offspring at 6 y of age (3). Offspring born to mothers with the lowest vitamin B-12 and highest folate concentrations had the highest insulin resistance at 6 y. In the Parthenon cohort from Mysore, maternal vitamin B-12 deficiency was associated with higher BMI, higher prevalence of gestational diabetes (GDM), and higher risk of permanent diabetes 5 y after delivery. Prevalence of GDM in vitamin B-12–deficient women progressively increased with higher folate concentrations (5). A hospital-based cohort study from Bangalore reported the highest risk of small-for-gestational-age infants in women who received high-dose folic acid supplements (>1000 μg/d) but were in the lowest tertile of vitamin B-12 to folate intake (6).

In the Pune studies we found a positive association between maternal vitamin B-12 status and neurocognitive performance of the child at 2 and 9 y of age (7). Controlled trials of vitamin B-12 supplementation (50 μg/d) during pregnancy from Bangalore demonstrated an improvement in neurocognitive development in the offspring at 9 and 30 mo of age (8). Preliminary findings from a preconceptional controlled trial of low-dose vitamin B-12 supplementation (2 μg/d) to adolescents showed an improvement in neurocognitive performance in the offspring at 2 y of age (9). A trial in children aged 6–30 mo from North India showed that children who received vitamin B-12 (1.8 μg/d) and folic acid (150 μg/d) for 6 mo showed better neurocognitive development than those who received only vitamin B-12 or only folic acid or placebo (10).

Bailey et al. and Molloy rightly comment that, although there is a biological plausibility, the current observational evidence does not support causality. Randomized controlled trials may provide an answer, but careful ethical considerations will be essential, and it will take considerable time and effort. Instead, it will be possible to use genetic markers for vitamin B-12 and folate status for a Mendelian randomization study. Genetic determinants for vitamin B-12 and folate are well known and fairly similar in different populations. This technique has been used to report a possible causal association of maternal vitamin B-12 dietary intakes with offspring intelligence at 8 y of age (11). We used Mendelian randomization analysis to support a causal association between maternal homocysteine concentrations and fetal growth restriction (12). We have also reported an association between maternal holo-transcobalamin concentrations (but not folate) and NTDs in Indians. Causality was supported by the association between maternal transcobalamin 2 genotype (but not methylenetetrahydrofolate reductase) with NTDs (13). Such an approach might help us overcome the current uncertainty, as highlighted by Molloy.

Thus, observations from India and other countries suggest that a low vitamin B-12–high folate pattern in early life may adversely impact neurodevelopmental and metabolic-endocrine processes. These observations expand the scope for improving the health of the population across the life course rather than only in the elderly. Improving vitamin B-12 status in deficient populations, and avoiding inadvertent vitamin B-12–folate imbalance, should be an important consideration for clinicians and public health specialists.

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