Letter to the Editor

Folate Determination in Human Milk

Dear Editor:

With great interest, we read the article by Sakurai et al. (1) describing the longitudinal changes in nutrient contents of human milk. We, however, would like to point out that their method of measuring folate content may not be suitable. Sakurai et al. (1) used an HPLC method with fluorescent detection after alkaline extraction followed by permanganate oxidation. This method was originally developed by Allfrey et al. (2) in 1949 to measure pteroylglutamic acid (folate acid), a synthetic form of the vitamin not usually present in biological specimens. Although some forms of folate, including folic acid, are stable under alkaline pHs (3), alkaline extraction of folates in the absence of a reducing agent, such as ascorbate, is known to be unsuitable for biological samples that contain principally reduced forms of folate. When the method by Allfrey et al. (2) is used for biological samples, the values are generally lower than those obtained by other methods, such as microbiological assay (4). The use of a reducing agent during the extraction procedure to protect reduced folates is essential (5). Furthermore, C-9–N-10 cleavage by permanganate oxidation is suitable for detecting folic acid (and perhaps dihydrofolate and tetrahydrofolate); however, the cleavage is not complete when a sample contains other folates, such as 5-methyltetrahydrofolate and 10-formyltetrahydrofolate (6). These fully-reduced folates comprise the majority of folates found in biological samples.

Human milk folates consist of polyglutamates of reduced folate, particularly of 5-methyltetrahydrofolate (7–9). The microbiological assay using Lactobacillus rhamnosus preceded by trienzyme extraction (using α-amylase, protease and folate conjugase) is considered to be the most suitable means to determine human milk folate content (10). Trienzyme extraction is essential to release folate from carbohydrate or protein matrices before folate analysis, and this method is becoming widely used for folate assay not only for milk samples but also for foods (11).

Therefore, the method used by Sakurai et al. (1) is not appropriately applied to human milk for folate analysis. The method would be acceptable for measuring synthetic folic acid that is added to infant formulas, but would not allow for accurate detection of endogenous folates supplied by natural ingredients of the formulas. Because information on human milk nutrient contents is vital to our understanding of the nutritional needs of rapidly growing infants (12), it is essential that suitable methods for analysis be employed.

Sincerely yours,

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REFERENCES