tion systems, such as electrochemical or mass spectrometry, have been developed (6).

There is also the possibility that the bacteria used in the MA gave unequal responses to the different vitamers or can be affected by some nonfolate compounds in the samples. However, the latter was evaluated on the basis of the combined LC-MA results. The data clearly show the absence of any nonfolate peaks with organism activity.

We conclude that the MA is preferred for the determination of the total folate content of foods, especially if data on individual folates is not considered necessary. Furthermore, we stress the need for careful validation of HPLC methods. Given the considerable, yet unidentified, difference between the microbiological and HPLC data, we advise caution when estimating dietary folate intakes at the population level.

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Reply to Kariluoto et al

Dear Sir:

We appreciate the reaction from Kariluoto et al regarding our paper (1), particularly on the population intakes, which are based on newly established food folate data. We completely agree that this is an important issue. Kariluoto et al referred to an estimated intake of 182 ± 119 μg/d for a population aged 1–92 y and compared these data with data for adults. Our estimated intakes were 173 and 215 μg/d for women and men, respectively.

Kariluoto et al reported that as a part of the European Union’s fifth framework project, baseline folate intakes for populations across 7 European countries were studied by using data based on each country’s food-composition data. An important problem with estimating folate intakes is the lack and the unreliability of the data on folate content in food-composition tables (2). Additionally, in 1996, a European working group, “COST 99,” compared folate data in food-composition tables for some vegetables, milk, bread, and cereals from 7 European countries. Total folate was measured by microbiological assay (MA). These food folate data showed a 2–3-fold variation (PM Finglas, unpublished observations, 1996), which indicates that folate intakes cannot be compared between countries.

In an intercomparison study that analyzed 4 certified reference materials (CRMs), estimated folic acid and 5-methyltetrahydrofolate contents were comparable in 4–5 laboratories, but no agreement in the other vitamers was found. Other than our previously published results (3), no other participating laboratory used affinity chromatography as a cleanup step before the final determination of all vitamers with HPLC. Selhub (4) and Pfeiffer et al (5) used this technique successfully earlier. It must be stressed that only this cleanup procedure is suitable for determining the folate content in food-sample extracts when HPLC is used. Anion-exchange purification alone or solid-phase extraction leaves many interfering compounds in the chromatogram, which hampers interpretation and accurate quantitation (3, 6).

It is not correctly stated by Kariluoto et al that 5-formyltetrahydrofolate, 10-formyltetrahydrofolate, or both convert to 5,10-methyltetrahydrofolate under acidic conditions. In particular, 10-formyltetrahydrofolate could be converted to 5,10-methylenetetrahydrofolate rapidly (i.e., on column conversion) because of the low pH of the mobile phase (5).

5-Formyltetrahydrofolate also undergoes formation of 5,10-methylenetetrahydrofolate in acidic media, although at a much slower rate (5). Thus, the acidic mobile phase used is fully suitable for separation and quantification of 5-formyltetrahy-
drofolate, as shown previously by Gregory et al (7). The presence of 5,10-methylenetetrahydrofolate in our HPLC procedure was not determined by fluorescence detection as suggested by Kariluoto et al but was quantified by diode-array detection when present (3). 10-Formyltetrahydrofolate is completely oxidized to 10-formylldihydrofolate or 10-formylfolic acid during food preparation (8). The purpose of our study was to analyze food products in the form they are consumed. Both vitamers could be quantified with the system we used (1, 3).

It is not clear from Kariluoto et al’s letter why the absence of bacterial growth by nonfolate compounds was based only on the results of 2 CRMs instead of the 4 CRMs used in the intercomparison study. This does not necessarily mean that the response of bacteria to nonfolate compounds is absent in all food matrices. According to Kariluoto et al, liquid chromatography–microbiological assay (LC-MA) showed the absence of any nonfolate peaks with organism activity, and all other peaks with microbiological activity add up to the same amount as found by HPLC in other analyses (Figure 1 of Kariluoto et al’s letter). This does not explain the discrepancy between the results by HPLC and MA. One would expect equal amounts between results of LC-MA and MA if MA is preferred as the standard method. According to these results, HPLC is preferred as the standard method.

The determination of folates by MA is grounded on biological activity, whereas the determination by HPLC is grounded on the actual detection of separate folate vitamers. Problems with the response of *Lactobacillus casei* to different folate vitamers, as reported by Phillips and Wright (9), and the influence of nonfolate compounds on the bacterial growth response are still not refuted and might explain the difference between the HPLC and MA results.

New, accurate food-composition data for folates are needed. Our study (1) was the first comprehensive assessment of a large range of folate-containing foods. Furthermore, this study showed the effect of new analytic data for folates, including identification and quantification. In light of current knowledge, we determined all relevant folates in foods with reliable and reproducible results (3).

With regard to the relative importance of accurate food folate data, because they are frequently used in epidemiologic analyses in which intakes are related to disease endpoints, we recommend the standardization of methods for the determination of folate in foods to ensure accurate quantification of total folate contents. HPLC should be the method of choice.

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Lessons learned in iron intervention trials

Dear Sir:

The December 2001 issue of the Journal featured the coincident publication of 3 articles related to anemia and its partition between iron deficiency and non-iron deficiency origins among African preschoolers living in areas of endemic malaria (1–3). Our attention was drawn to the article concerning an iron-supplementation intervention trial (3), which reported difficulties and caveats reminiscent of an experience of ours in Guatemala (4).

In the planning of their study, Zlotkin et al (3) approached their power and sample-size assumptions in the following manner: “On the assumption of 90% cure rates in the drops group and 80% in the sprinkles group and with a type I error set at 0.05 and a 0.9 probability of detecting a true difference, the final sample size estimate was 286 subjects per group.” They expected up to 90% of anemic Ghanaian infants and toddlers to have iron deficiency and iron-responsive anemias. This expectation raises 2 points in our minds. The first of these points is that such an expectation conflicts with the sense of the introductory section of their paper, where they state, “…we tested the hypothesis that the response to treatment of anemia would be better with 2 mo of treatment with microencapsulated ferrous fumarate sachets daily than with ferrous sulfate drops…” (3). Both of these statements use wording that seems to refer to a one-tailed test, but with the latter statement inclining toward superiority for the sprinkles and the former statement inclining toward a 10% better efficacy for the drops. Thus, it is important that the authors clarify whether the study was powered for a one-tailed or two-tailed test of the hypothesis of dif-