Serum Unmetabolized Folic Acid: The Straw That Broke Dihydrofolate Reductase’s Back?1,2

Rima Obeid*

Aarhus Institute of Advanced Studies, University of Aarhus, Aarhus, Denmark

We have grown accustomed to the idea that a tiny amount of unmetabolized folic acid (UMFA)3 in serum is an unwanted effect of consuming folic acid–containing supplements or fortified foods, even though there is no evidence so far for any health implications for UMFA. Folic acid is reduced via dihydrofolate reductase (DHFR) before entering the folate cycle and being converted later into 5-methyltetrahydrofolate (5-methylTHF). The liver (1, 2) and the intestine (3) appear to participate in folic acid reduction after oral intake, but there is also a highly variable displacement of endogenous folate into different tissues after acute administration of the vitamin (4).

The elimination of folate from the blood occurs via different ways (5, 6). Alcohol increases urinary elimination of folic acid (6) or inhibits apical renal reabsorption of folate (7). Plasma 5-methylTHF reached a peak ~2.5–3.0 h after oral administration of a single dose of folic acid (200 μg to 20 mg) in people who were not regularly exposed to folic acid (8), but this apparent bioavailability has also been related to the immobilization of tissue folates. Fasting concentrations of serum folates represent the product of a balance between all of the processes mentioned above. An article in this issue of The Journal of Nutrition by Pfeiffer et al. (9) reports data on UMFA in >2700 serum samples from the NHANES 2007–2008, almost 1 decade after mandatory fortification began in the United States in 1998 (9). They showed that almost everyone who chronically consumes folic acid–fortified foods or additional supplements will have a small fraction of total serum folate as UMFA, which is the non-coenzyme form (9). The strongest and most expected predictors of the presence of UMFA in serum were taking supplements containing folic acid in addition to consumption of folic acid–fortified foods and having fasted for <3 h at the time of blood collection. Several important issues can be learned from this study.

Figure 1 shows a theoretical model based on data presented by Pfeiffer et al. [Table 1 in (9)] concerning the effects of vitamin use and fasting conditions on folate forms. In non–vitamin users, the difference in mean serum 5-methylTHF between the non-fasting (< 3 h, 32.5 nmol/L) and the fasting (≥8 h, 29.7 nmol/L) conditions was 2.80 nmol/L (within ±5 h). In vitamin users, the mean values for serum 5-methylTHF were 48.8 nmol/L after <3 h and 48.0 nmol/L after ≥8 h of fasting (Δ5-methylTHF = 0.8 nmol/L). The corresponding differences in serum UMFA according to fasting status were 0.269 nmol/L (0.936 – 0.667 nmol/L) in non–vitamin users and 1.174 nmol/L (2.140 – 0.966 nmol/L) in vitamin users. Therefore, it can be assumed that, in vitamin users, 1.174 nmol/L of UMFA was metabolized or eliminated from the circulation within ≥5 h, despite the high concentration of serum 5-methylTHF (mean: 48.0 nmol/L). In contrast, in non–vitamin users, less UMFA (i.e., 0.269 nmol/L) appeared to be reduced or eliminated within ≥5 h when mean serum 5-methylTHF was 29.7 nmol/L. Pfeiffer et al. reported a higher overall total folic acid intake in vitamin users than in nonusers (median: 508 vs. 145 μg/d). Therefore, the average intake of folic acid from supplements was ~363 μg/d (508 – 145 μg/d). From this, it follows that at least 5 h (the difference between 3 and 8 h) may be needed to eliminate or immobilize an average of 363 μg/d from an additional source of folic acid on top of folic acid from fortified foods (143 μg/d). Despite the theoretical nature of this model, it shows that after ingesting supplements containing folic acid, a higher amount of UMFA disappears from the circulation when people have a high baseline of serum 5-methylTHF (~48.0 nmol/L), when compared with the eliminated amount of UMFA at a lower concentration of serum folate (~29.7 nmol/L).

In a similar way, the effect of vitamin usage on UMFA is modeled in Figure 1. According to Pfeiffer et al. (9), approximately half of the population consumed ≥363 μg folic acid/d. In the nonfasting state, 5-methylTHF and UMFA differed between supplement users and non–supplement users by 16.3 and 1.204 nmol/L, respectively. In fasting individuals, 5-methylTHF and UMFA differed according to supplement usage by 18.3 and 0.299 nmol/L, respectively. Therefore, a larger difference in serum 5-methylTHF between supplement users and nonusers (48.0 vs. 29.7 nmol/L) was translated into less proportional difference in UMFA (0.966 vs. 0.667 nmol/L; ΔUMFA = 0.299) when people were fasting for ≥8 h. The NHANES 2007–2008 also clearly showed that non–supplement users who had a rather low mean serum 5-methylTHF of 18.1 nmol/L (first quartile, ≤25 nmol/L) had a UMFA fraction of 3.6% of serum folate [Supplemental Table 5 in (9)]. In contrast, vitamin users who had a mean serum 5-methylTHF of 72.2 nmol/L (fourth quartile, ≥52 nmol/L) had a similar UMFA fraction of total serum folate (3.3%). The proportion of UMFA to the sum of serum folates was remarkably consistent in supplement users and nonusers across all concentrations of serum folate [Supplemental Table 5 in (9)]. Therefore, the UMFA fraction (% of total) is probably less related to folate input or RBC or serum folate.

In Figure 2, data from the NHANES III (1988–1994; prefortification) (10) were proposed as a baseline for the NHANES 2007–2008 (9). Figure 2 illustrates folate markers prefortification (no additional folic acid), postfortification (+ 145 μg folic acid/d), and postfortification plus 363 μg folic acid/d from multivitamins (+508 μg/d total folate acid intake). It was proposed that UMFA was zero, because it had not been reported in the prefortification
period. This assumption might slightly underestimate the baseline UMFA, which is related to supplement usage in the prefortification period. However, mean total serum folate (12.1 nmol/L) and RBC folate (391 nmol/L) in the NHANES III (1988–1994) (10) are similar to those in European populations in whom there is no fortification (11, 12). Figure 2 shows that

FIGURE 1 A theoretical model showing changes in concentrations of folic acid and 5-methyltetrahydrofolate in serum in response to the fasting state and folic acid supplementation. Results are based on data from the NHANES 2007–2008 [Table 1, original publication (9)]. The model proposes that the average folic acid intake from supplements was 363 μg/d (derived from 508 – 145 μg/d) (9). The numbers in the black boxes refer to serum 5-methyltetrahydrofolate, and those in the white boxes refer to unmetabolized folic acid. The model depends on data from independent groups, and thus it does not represent intraindividual changes after fasting or supplementation. The model proposes that dihydrofolate reductase and other elimination methods operate to remove folic acid from the circulation. DHFR, dihydrofolate reductase; UMFA, unmetabolized folic acid; vit, vitamin; 5-methylTHF, 5-methyltetrahydrofolate.

FIGURE 2 Modeling changes in RBC folate and serum 5-methyltetrahydrofolate and unmetabolized folic acid starting from prefortification (NHANES III, 1988–1994) (10) to postfortification (NHANES 2007–2008) [based on Table 1 in (9)] without additional supplement and postfortification (NHANES 2007–2008), with an estimated additional folic acid intake of 363 μg/d. 5-Methyltetrahydrofolate in the 1988–1994 survey was not available. For modeling purposes, 5-methyltetrahydrofolate was estimated to be 11.5 nmol/L, because total folate was 12.1 nmol/L (10). Gray areas represent the temporary increase in serum 5-methyltetrahydrofolate or unmetabolized folic acid within <3 h after the meal. The figure is only for simplification; thus, the y axis (concentrations of folates, nmol/L) is not exactly proportional to the changes or concentrations that are plotted in the individual lines. UMFA, unmetabolized folic acid; 5-methylTHF, 5-methyltetrahydrofolate.
serum 5-methylTHF changed from 11.5 nmol/L (estimated value from a total folate concentration of 12.1 nmol/L in NHANES 1988–1994) to 29.7 nmol/L (NHANES 2007–2008) (i.e., by 158% or 18.2 nmol/L). UMFA showed less difference between the 1988–1994 and 2007–2008 surveys (by 67% or 0.667 nmol/L).

In fasting participants from the NHANES 2007–2008, serum 5-methylTHF was 62% higher in supplement users when compared with nonusers (mean: 48.0 vs. 29.7 nmol/L; Δ5-methylTHF = 18.3 nmol/L), whereas UMFA showed less proportional increase between the same groups.

The recent data from the NHANES 2007–2008 provide an important insight into the issue of UMFA. With chronic high total folate intake (diet plus supplement and/or fortification, 200–1000 mg/d), the stores of folate are already high, but the proportional increase in UMFA fraction is less than the increase in serum folate.

Figure 3A shows that in non–supplement users, 145 mg folic acid/d may be metabolized or eliminated through different mechanisms, leading to 2.20% of total serum folates as UMFA. In contrast, at an intake of 508 mg folic acid/d, 1.97% of total serum folates were measured as UMFA (Figure 3B). Therefore, immobilization of folic acid from or into the liver and elimination or metabolism into 5-methylTHF might be enhanced at high concentrations of serum folate. The results also strongly suggest that even when serum 5-methylTHF was >52.0 nmol/L, folic acid (i.e., the substrate UMFA = 3.3% of 5-methylTHF) disappeared from the circulation at least as effectively as when serum 5-methylTHF was, on average, 75% lower (<25.0 nmol/L). Potential mechanisms that need further investigation include the following: displacement of folate between tissues, deposition of folic acid in tissues that metabolize it, sequestration of folic acid by tissue DHFR (i.e., lower dissociation at higher folic acid concentrations), induction of DHFR by its substrates, and an enhanced elimination of folic acid via the kidney or bile upon supplementation.

Acknowledgments
The sole author had responsibility for all parts of the manuscript.

References

