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

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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 (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 (145 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 folic acid intake). It was proposed that UMFA was zero, because it had not been reported in the prefortification comments.

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3 Abbreviations used: DHF, dihydrofolate; DHFR, dihydrofolate reductase; THF, tetrahydrofolate; UMFA, unmetabolized folic acid; 5-methylTHF, 5-methyltetrahydrofolate.
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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

In non-vit-users: 0.269 nmol/L UMFA appear to be reduced or eliminated within ≥ 5 h, (i.e., \( \Delta = 22.54 \) pmol/(L·h) when fasting for 8-12 h); though 5-methylTHF is lower than in vit-users

In vit-users: 1.174 nmol/L UMFA were metabolized or eliminated within ≥ 5 h, (i.e., \( \Delta = 98.235 \) pmol/(L·h) when fasted for 8-12 h); though serum 5-methylTHF is already very high
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 3** Translation of the NHANES 2007–2008 results into a whole-system model for unmetabolized folic acid and folate metabolism and elimination from the circulation in fasting individuals who were consuming only 145 μg folic acid/d from fortified foods (A) and who consumed 508 μg folate acid/d from fortified foods plus folic acid-containing supplements (B) (data based on Table 1 in [9]). The model suggests that dihydrofolate reductase activity alone cannot explain variations in serum unmetabolized folate acid. At higher folic acid intakes (B), the elimination from the circulation may be enhanced via different ways. DHF, dihydrofolate; DHFR, dihydrofolate reductase; RBC, RBC folate; THF, tetrahydrofolate; UMFA, unmetabolized folate acid; 5-methylTHF, 5-methyltetrahydrofolate.

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**References**


