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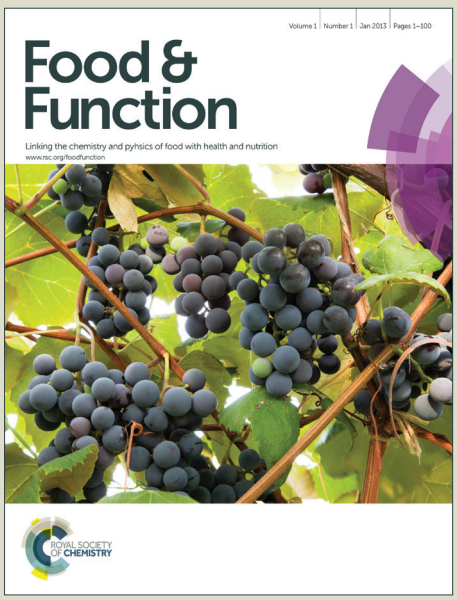

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ARTICLE

Cheese 'refinement' with whey B-vitamin removal during precipitation potentially induces acute 'functional' dietary shortage: homocysteine as a biomarker

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Cheese 'refinement' with massive B-vitamin losses (≈70-84%) through whey removal during precipitation may potentially induce an acute imbalance between protein/methionine load and temporal inadequacy/shortage of nutrients critical for their metabolism, i.e. B6 and B12. Acute effect of cheese consumption was evaluated by increased plasma homocysteine as a B-vitamin shortage marker. In a double-blind study, healthy, normal-weight (BMI=22-27), premenopausal women aged 25-45y were first given a methionine load (100mg/kg, n=15); then cottage cheese alone (500g, ≈50g protein, ≈1200mg methionine, n=49) at breakfast, then with added B6 (2mg, n=8), and/or B6+folate (1mg+200mcg, n=7). Plasma homocysteine was measured preprandially (t_0), then postprandially 5h (t_5) and ≥6-8h. Cheese-induced homocysteine increased 28.7% ($p \leq 0.001$), ≈60% the free methionine response, remaining higher through ≥6-8h. Co-supplementation with B6 lessened the Hcy increase by 45.0% (to 14.9%, p=0.025), and B6+folate 72.3% (to 7.5%, p=0.556, NS). Homocysteine increased more in participants with lower baselines (<5μM vs. ≥5μM, $p \leq 0.001$), following cheese, ≈3-fold (54.8% vs. 18.5%) or methionine, 47.3% (266.7% vs. 181.1%). Cheese B-vitamin depletion – i.e. to B6≈2.0-4.0μ/g protein, far below women's metabolic requirement (15-20μ/g) – here appeared to induce acute relative shortage vs. methionine/protein loads, exemplified by greater homocysteine increases than with other animal proteins (previous data), more so with lower baseline homocysteine. Smaller increases following re-supplementation demonstrated potential for 'functional fortification'/co-supplementation. Unnoted cheese 'refinement', like white bread, potentially induces episodic vitamin shortage effects, warranting consideration for acute/cumulative implications, compensatory technologies, and food combinations, especially for at-risk populations (i.e. with genetic, hormonal/gender, or aging-related predispositions), and for cardiovascular, bone, and brain health.

Keywords: cheese, dairy products, whole food, functional food, B-vitamins, homocysteine, women's nutrition

Introduction

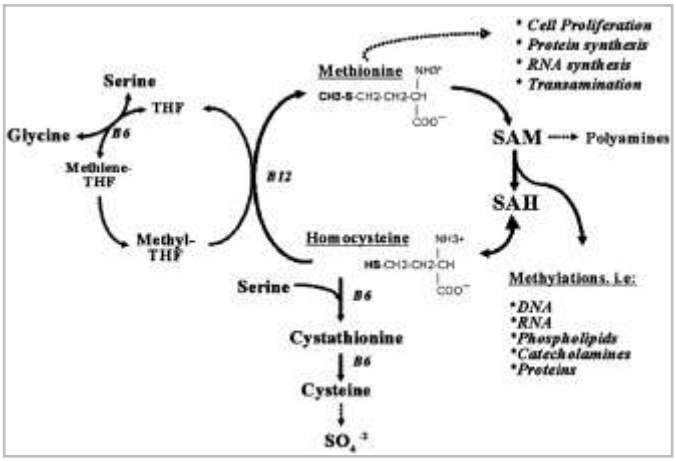

Figure 1. Pathways of homocysteine and methionine metabolism

Promotion of the consumption of whole foods is based on epidemiological studies showing the cumulative advantage, i.e. of whole grain products – including inverse association with risk of type 2 diabetes mellitus and cardiovascular diseases (CVD) [1-5], and clinical studies showing positive intervention outcomes [6, 7]. Also the post-prandial state, an additional important contributing factor to the development of atherosclerosis and metabolic impairments [8], has demonstrated an acute benefit of whole grain products/breads on blood glucose, insulin, and incretins [9], potentially lasting up to 16 hours following consumption of indigestible carbohydrates from whole barley [10]. Further benefits of increased plasma B-vitamins such as folic acid from whole grain consumption was manifested by inversely decreased plasma homocysteine (Hcy) in a Norwegian population [11].

Cheese potentially becoming a refined food following precipitation and removal of whey, with massive losses of innate whey B-vitamins [12] relative to retained protein/methionine (MET) content (United States Department of Agriculture [USDA] Food Composition Database), may also induce metabolic/health disadvantages, similarly to those associated with refined grains. Cheese losses ≈70% of B1, B2, and B3; ≈76% of B12 – essential for protein metabolism and re-methylation of Hcy back to MET, for brain function, and DNA and hemoglobin production; and up to 84% loss of B6 – required in proportional amounts to protein for its metabolism [13] and further for cardiovascular [14], bone [15-17] and brain health [18].

While vitamin B6, being a key cofactor in Hcy metabolism (Fig. 1) [19], is especially critical under high-protein conditions [20, 21], cottage cheese – having a high protein/MET content – may yield a very low B6 level of ≈1.86μg/g protein (≈6.2-fold lower than in milk, ≥11.52μg/g) (USDA data). This level is far below the minimum B6:protein ratio considered sufficient for women (15-20μg/g) [13], and lower than the threshold (<0.5 μg/g) used for experimental induction of a vitamin B6-depleting diet [13, 22]. Thus, cottage cheese can correspondingly be perceived as a B6-depleting food, which may induce an acute/temporal dietary flow shortage, potentially reflected by increased Hcy.

Interestingly, though animal protein foods such as meat are high in all essential amino acids – including high MET content, the only dietary source of Hcy – increasing animal protein intakes did not consistently raise plasma Hcy [23, 24], and in certain cases even decreased it [25]. This is because most animal proteins are also rich in B-vitamins, i.e. B6 and B12, which can lower plasma Hcy concentrations [26] (see Fig. 1). Here cheese is a notable exception, as it may potentially induce increased Hcy, due to its high ratio of protein/MET to B-vitamins, i.e. B6 and B12. As episodic micronutrient shortage was recently suggested to cause cumulative degenerative effects that may expressed later in life [27], cheese potentially inducing temporal B-vitamin shortage may warrant increased awareness. This is especially relevant facing the long-existing evidence showing that high Hcy levels have been associated with risk of CVD [21, 28-30], cerebrovascular damage [31], cognitive dysfunction [32, 33], metabolic syndrome [34], hepatic steatosis [35], impaired pregnancy outcomes [36, 37], osteoporosis, and bone fractures [38-41].

Cheese, potentially having differential nutritional effects compared to other dairy products – being significant dietary sources of high-quality protein, minerals, and B-vitamins, and highly recommended (3-4 servings daily) by nutrition authorities [42-44] – emphasizes the importance of increasing attention to the specific effects of cheese processing associated with B-vitamin removal, especially for sensitive populations. Those, include elderly individuals, who are at high risk for B-vitamin deficiencies, especially vulnerable to B6 and B12 insufficiencies [45], and highly predisposed to increased Hcy-related risks, that potentially associated with vascular dementia [46] and psychogeriatric disorders [47, 48]. Women may also be particularly vulnerable to acute B-vitamin deficiency, due to hormone-related tendency toward reduced plasma B6 [49, 50], and their tendency to consume more cheese and other dairy products than men [51].

The present study was undertaken to evaluate cheese-induced acute B-vitamin shortage as manifested by increased plasma Hcy, and potential of re-supplementation in healthy, well-nourished, premenopausal women.

Methods

Study population

Participants were healthy female hospital employees (n=49) of normal body mass index (BMI = 22-27), young (aged 25-40 years), recruited from two locations (Rambam Health Care Campus, Haifa; and Chaim Sheba Medical Center, Tel HaShomer, Israel).

Study protocol

The study protocol was approved by the Helsinki Committee of Rambam Medical Center and the Israel Ministry of Health, and informed consent was obtained from and corresponding forms signed by all participants.

In a preliminary phase, one week before the active study, a subgroup of participants (n=7) received 500g of 5% fat cottage cheese (Tnuva Ltd., Israel), providing ≈50g protein and ≈1200mg MET (B-vitamin contents are shown in Table 1, based on USDA

Food Composition Database data) with a standard hospital staff breakfast (two slices of whole meal bread, tomato, cucumber, and coffee or tea), and were followed for up to 24 hours to assess optimal time for Hcy response. Blood samples (10ml) were collected preprandially at baseline (t_0) and postprandially at 4, 6, 8, and 24 hours. A second subgroup of participants (n=15) was tested for Hcy response to free MET load (0.1g/kg body weight, average ≈5670mg MET) at the same time intervals. Peak postprandial Hcy levels were observed between 4 and 6 hours, and thus a timepoint of 5 hours (t_5) was selected for testing in the remainder of the study.

In the active study phase, the entire group of participants (n=49) received the standard breakfast with 500g of cottage cheese, and underwent preprandial (t_0) and postprandial (t_5) blood testing.

An additional group (n=8) received the same cottage cheese breakfast, together with tablets of 2mg vitamin B6 (Solgar®; Netanya, Israel); another group (n=7) received the same cottage cheese breakfast and a powdered mixture containing 1mg vitamin B6 and 200µg folic acid. All participants were asked to avoid protein intake between baseline (t_0) and the following blood sampling at t5.

Study analysis

Sample size calculation

Power analysis for paired t-test of the difference in plasma Hcy preprandially (t_0) vs. postprandially 5h (t_5) following cottage cheese and MET loads indicated a sample size of a minimum of 10 subjects with 2 measurements each for a probability of at least 80% that the study will detect a significant difference ($p<0.05$, two-tailed) of approximately 1.5 µmol/L, with assumed standard deviation of 1.5 µmol/L [24].

Endpoint calculation

Blood samples were drawn into vacutainers containing EDTA, and immediately refrigerated at 4°C. Within two hours of collection, the whole blood was spun in a refrigerated centrifuge, and the separated EDTA plasma was aliquoted and kept at -70°C until analyzed. Total plasma Hcy was determined by HPLC with fluorescence detection, using a modification of the method originally described by Araki and Sako [52].

Inter- and intra-assay coefficients of variation for this assay were both <5%. Statistical significance of differences in the Hcy responses among the treatments was assessed by Wilcoxon non-parametric test.

Results

The time course of plasma Hcy levels following the ingestion of cottage cheese (Fig. 2), similar to that of the MET load, shows that the largest increases in Hcy occurred after approximately 5 hours (t_5), after which they gradually declined, though some were still higher than baseline after 24 hours.

Compared to a mean t_5 Hcy increase of 227.0% (n=15) following 0.1g/kg free MET loads with average intake ≈5670mg, equivalent to an increase of ≈0.04%/mg MET ($p\leq0.001$, Fig. 3a), the Hcy increase of 28.7% (n=49) following consumption of cottage cheese-bound MET was equivalent to 0.024%/mg, ≈60% the free MET response.

Participants with lower vs. higher baseline plasma Hcy exhibited differential Hcy responses, shown following a free MET load, by a 47.3% higher mean increase of 266.7% vs. 181.1% in participants with lower baseline Hcy of <4.0 µM/L vs. ≥4.0 µM/L

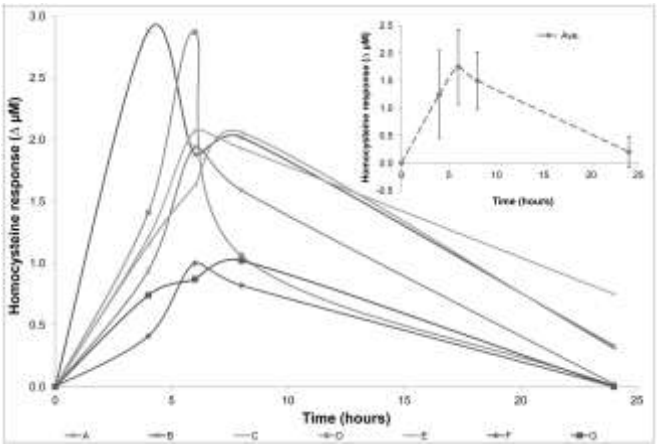

Figure 2. Postprandial plasma homocysteine curve following cottage cheese meal 500g (n=7)

($p\leq0.001$, Fig. 3c); and following the cheese load, those with lower baseline Hcy <5.0 µM/L had significantly higher t5 Hcy, up 54.8% vs. 18.5% in those with higher ≥5.0 µM/L ($p\leq0.001$, Fig. 3d).

Co-supplementation of cottage cheese with vitamin B6 (2mg in tablet form) reduced the Hcy response by 45.0%, from 27.1% to 14.9% (p=0.025) (Fig. 4a), and by 83.4% to 7.5% above baseline

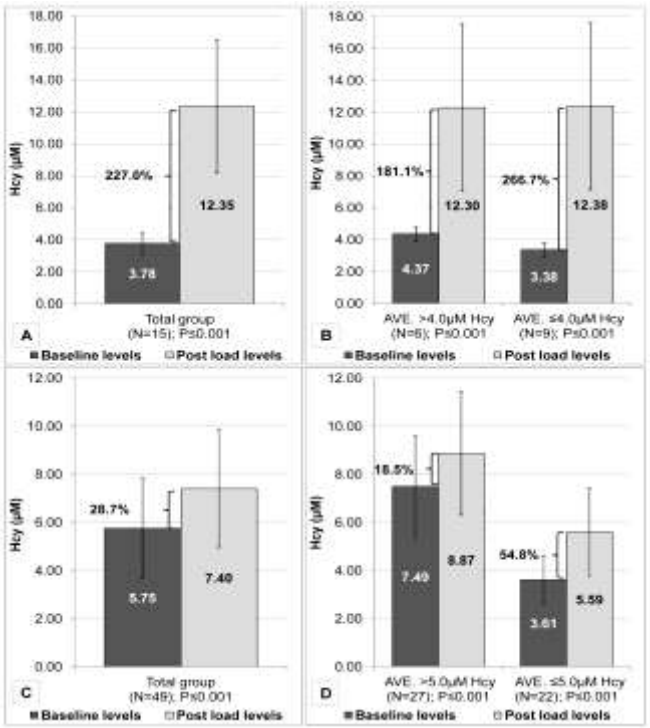

Figure 3. Plasma homocysteine response to free methionine load in the total group (**A**) and subgroups according to baseline levels of <4.0 and ≥4.0 µM homocysteine (**B**), and to cottage

cheese load (500g) (**C**) and subgroups according to baseline homocysteine of <5.0 and ≥5.0 µM Hcy (**D**)

(p=0.556, non-significant [NS] vs. baseline) following a powdered mixture of B6 (1mg) + folic acid (200µg) mixed with the cottage cheese (Fig. 4b). Wilcoxon test on differential responses in the three treatments confirmed significance of the increases in Hcy following both cottage cheese alone and after cottage cheese with B6 tablet, but a non-significant increase following cottage cheese with B6 + folic acid powdered mixture.

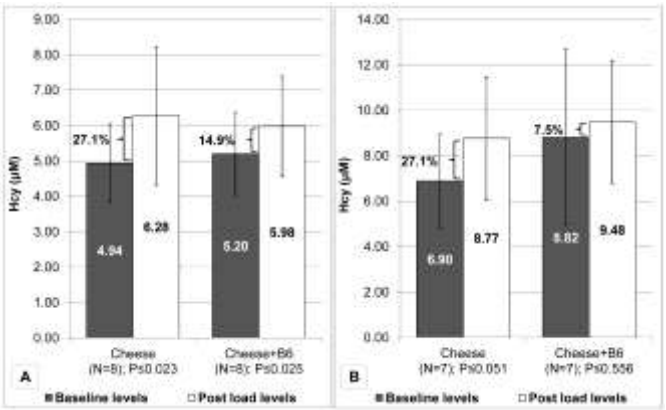

Figure 4. Plasma homocysteine response to cottage cheese, alone and with co-supplementation of vitamin B6 (**A**) or vitamin B6+folic acid (**B**)

The effect of cheese B-vitamin depletion, resulting from massive losses during precipitation [53], with potential retention of extremely low levels (Table 1), is demonstrated in cottage cheese 2% milk fat, which retains only ≈16% of the original milk B6 amount (relative to protein content) and ≈24% of the vitamin B12 (Table 1, Fig. 5).

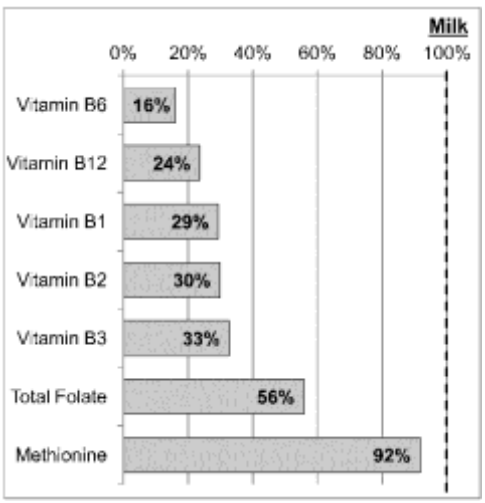

Figure 5. Methionine and B-vitamin % retained, cottage cheese (USDA ID: 01015) vs. milk

Table 1. Protein, methionine, and B vitamins in milk, yogurt, and various cheeses, showing the percent (% vitamins/g protein) retained following whey removal in cheese precipitation as compared with other dairy products.

Item		Protein (g)	MET (mg)	B1 (µg)	B2 (µg)	B3 (µg)	B6 (µg)	B9* (µg)	B12 (µg)
Milk (1.98% fat)	Per 100g	3.3	87	39	185	92	38	5	0.53
	Per 1 g protein	—	26.36	11.82	56.06	27.88	11.52	1.52	0.16
Whey (0.36% fat)	Per 100g	0.85	16	36	158	74	31	1	0.28
	Per 1 g protein	—	18.82	42.35	185.88	87.06	36.47	1.18	0.33
	% retained	—	71.40	358.37	331.57	312.28	316.72	77.65	205.11
Yogurt (1.55% fat)	Per 100g	5.25	155	44	214	114	49	11	0.56
	Per 1 g protein	—	29.52	8.38	40.76	21.71	9.33	2.10	0.11
	% retained	—	111.99	70.92	72.71	77.89	81.05	138.29	66.42
Cottage Cheese (2% fat)	Per 100g	11.83	286	41	198	108	22	10	0.45
	Per 1 g protein	—	24.18	3.47	16.74	9.13	1.86	0.85	0.04
	% retained	—	91.70	29.33	29.86	32.75	16.15	55.79	23.68
Cottage Cheese (4.3% fat)	Per 100g	11.12	269	27	163	99	46	12	0.43
	Per 1 g protein	—	24.19	2.43	14.66	8.90	4.14	1.08	0.04
	% retained	—	91.76	20.55	26.15	31.93	35.92	71.22	24.08
Ricotta Cheese (12.98% fat)	Per 100g	11.26	281	13	195	104	43	12	0.34
	Per 1 g protein	—	24.96	1.15	17.32	9.24	3.82	1.07	0.03
	% retained	—	94.66	9.77	30.89	33.13	33.16	70.34	18.80
Gouda Cheese (27.44% fat)	Per 100g	24.94	719	30	334	63	80	21	1.54
	Per 1 g protein	—	28.83	1.20	13.39	2.53	3.21	0.84	0.06
	% retained	—	109.35	10.18	23.89	9.06	27.86	55.57	38.45

*Total folate

Discussion

The present study supports the expected acute effect of cottage cheese-induced episodic B-vitamin supply shortage, manifested by significant Hcy increases, and further confirmed by representative groups showing smaller responses following co-supplementation with B6±folic acid. The potential of cheese to induce an episodic

B6 shortage is related to its very low B6:protein ratio post whey removal to well below the depleting threshold [13], with resultant induced 'B6-debt' relative to protein ingested. Similarly with B12 – which also plays a key role in the Hcy metabolic reactions, and is required in amounts proportional to protein consumption. Thus, in contrast to the expectation of dairy products (as animal protein) to be sources of B12, certain cheeses may induce an episodic dietary B12 shortage, potentially conferring metabolic risks, though with delayed health manifestations [27]. The 28.7% increase in Hcy found here (Fig. 3a) was much higher than shown in previous studies with other comparable animal proteins, i.e. meat [54], and some studies showing no increase. This is because most animal proteins such as meat, poultry, and fish, retain their innate B-vitamin content, resulting in cottage cheese having much higher protein:B-vitamin ratios, i.e. a ≈4-fold higher MET:B6 ratio and ≈2-fold higher MET:B12 ratio than beef (USDA Food Composition Database).

The long hours of sustained increased Hcy levels (Fig. 2) corroborate previous findings showing moderately increased postprandial plasma Hcy throughout the day following a high dose of protein-bound MET [24, 55]. Extension of elevated Hcy to the next morning after the cheese load (24 hours) may further suggest that high, repeated, and habitual consumption of certain types of cheese may increase the cumulative effects of exposure to B-vitamin shortages, when high protein/MET intake is unopposed by B-vitamin co-supplementation/compensation.

The meaning of these findings is emphasized by recent studies suggesting that low micronutrient intake is associated with accelerated degenerative diseases of aging, assumed to reflect a preference of short-term survival at the expense of taking long-term health risks [27]. Similarly here, the episodic Hcy increase (for >5 hours), may not be an acutely clinically meaningful risk, but could potentially impose cumulative risk that will be expressed later in life, as shown by later DNA damage associated with earlier states of various micronutrient deficiencies [27].

The potential of B6±folic acid co-/administration to reduce the plasma Hcy response indicated here supports consideration of functional cheese fortification and/or recommendation for combinations with complementary foods high in B-vitamins, i.e. high-folate green leafy vegetables and/or oranges, to be consumed with cheese meals. The greater effectiveness of B6+folate powder may be related to their combined/additive biochemical effects and/or higher bioavailability in powder form as compared to tablet form of B6 given separately. This is despite a 284.55% ($p \leq 0.01$) increase in plasma pyridoxal phosphate from baseline 5 hours after B6 tablet supplementation, compared to a 5.71% (NS) increase after cheese alone (unpublished data).

The selection of female participants for the present study is justified by their expected high responsiveness to cheese-induced acute/temporal B6 shortage, due to their predisposition to hormone-induced lowered plasma B6 levels, as seen in epidemiological studies [56], with oral contraceptive use [49, 50], hormone replacement therapy (≈ 50%) [57], and with other hormone combinations (≈25-40%) [19]; another reason is that women also tend to consume higher amounts of cheese than men. Additional at-risk groups are lactovegetarians, subjects adhering to the DASH diet against hypertension, and those genetically predisposed to high Hcy, i.e. with a MTHFR mutation, who may be more predisposed to risk of cheese-induced B6 and B12 deficiencies.

This could result in later manifestation of typical corresponding B-vitamin deficiency symptoms, especially B6-related, as previously shown for CVD [58], oxidative stress [59], inflammation [60], diabetes sequelae [61, 62], age-related neural decline [63], bone risk [64, 65], and impaired pregnancy outcomes [66, 67].

The significantly higher Hcy response in participants with lower baseline Hcy levels following both free MET and cheese protein-bound MET loads may reflect habitually low animal protein intake, whereby lower Hcy could result from low protein/MET consumption, and the greater increase in Hcy response could have resulted from low B6 and B12 intake, both of which may be associated with a low animal protein diet [68]. This pattern is highly reflective of the Israeli diet – low in red meat, B12, and folic acid [69].

The main limitations of the present study were the small size of groups exposed to the cheese co-supplementation interventions and a shortage of information regarding plasma vitamins. The results of supplementation can therefore be perceived as a preliminary indication rather than conclusive evidence, warranting research of cheese effects and potential implications of co-supplementation and/or fortification.

Conclusions

A major outcome of this study is increasing the awareness of cheese being a refined food with massive B-vitamin losses during production to levels close to and even below a depletion diet, potentially inducing an episodic shortage effect, despite having high animal protein nutritional value. The higher Hcy response to cheese compared to other animal proteins (previous data) indicate the importance of protein/MET to B6/B12 ratios, and the relative micronutrients' insufficiencies of cheese compared to other whole milk products, due to whey vitamin depletion. The Hcy response was shown to be a relevant biomarker for indicating increased protein/MET:B6 ratio, the extent of 'B6-debt,' and the potential for an acute vitamin shortage effect. Increased Hcy levels for an extended period of time may incur a cumulative effect with corresponding risk, especially in people with a genetic, gender/endocrinological, and/or aging-related predisposition. The smaller Hcy response with B6±folate co-administration suggests potential for functional fortification, co-supplementation, and/or compensatory meal/menu/diet planning.

Conflicts of Interest

Niva Shapira is the sole author of this paper. There were two United States patents associated with this research, which have expired: United States Patent Office no. WO1997034497A1 and no. WO1999013737A1. There were no conflicts of interest.

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Notes and References

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