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Choline status is not a reliable indicator of moderate changes in dietary choline consumption in premenopausal women

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Abstract

For the prevention of liver dysfunction in women, a choline adequate intake of 425 mg/day was established. To date, the relationship between dietary choline intake and plasma concentrations of choline moieties remains relatively unexplored. As an extension of our previous work, this 14-week controlled feeding study investigated the relationship between moderate changes in dietary choline intake and blood indicators of status. The influences of folate intake and the methylenetetrahydrofolate reductase (MTHFR) C677T genotype were also considered. Healthy premenopausal women (n=45, 18–46 years) with the MTHFR 677CC (n=28) or TT (n=17) genotype consumed a folate-restricted diet for 2 weeks followed by randomization to one of four dietary treatments (n=6–9/group) differing in total choline (344–486 mg/day), betaine (122–349 mg/day) and/or folate (400–800 μg dietary folate equivalents/day) content for 12 weeks. Responses to treatment were assessed as changes in the plasma levels of choline moieties (i.e., betaine, choline, phosphatidylcholine and sphingomyelin) and/or leukocyte global DNA methylation between pretreatment (Week 2) and posttreatment (Week 14) values. No significant changes were detected in the measured variables in response to dietary increases in choline (i.e., 41% increase) or betaine (i.e., 286% increase) intake. However, the MTHFR C677T genotype, alone or together with a diet, influenced betaine (P=.03) and phosphatidylcholine (P=.03). These data suggest that choline status is not a reliable indicator of moderate changes in dietary choline intake possibly due to the engagement of compensatory mechanisms. In addition, the MTHFR C677T genotype appears to influence the direction and use of choline moieties in this group of women.

Keywords: Choline; Phosphatidylcholine; MTHFR; Betaine; Women; Folate

1. Introduction

While the nutritional importance of choline was described over 70 years ago [1], the dietary essentiality of this nutrient was only recently recognized with an establishment of an adequate intake (AI) level of 425 and 550 mg of choline/day for women and men, respectively [2]. Phosphatidylcholine is the primary form of choline in food and in the body. In mammalian cells, phosphatidylcholine may be synthesized from choline via the cytidine diphosphate (CDP) choline pathway in which cytidine triphosphate (CTP): phosphocholine cytidylyltransferase (CT) serves as the rate-limiting and regulating enzyme (reviewed in Ref. [3]). In addition, the liver and a few other tissues make phosphatidylcholine via the methylation of phosphatidylethanolamine, a reaction catalyzed by phosphatidylethanolamine N-methyltransferase (PEMT) and involving the sequential transfer of three methyl groups from *S*-adenosylmethionine [4].

Phosphatidylcholine is the primary phospholipid of all classes of lipoproteins in mammals [5]. The active synthesis of phosphatidylcholine appears to be required for the secretion of very low-density lipoprotein and high-density lipoprotein [6,7], although gender-specific differences have been described [7–11]. In addition to its important role as a phospholipid precursor, choline can be acetylated to the

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Table 1
Study design

Description	Dietary treatment group			
	I	II	III	IV
MTHFR C677T genotype	7 CC	6 CC	7 CC, 8 TT	8 CC, 9 TT
Dietary choline (mg/day)[a]	169	169	237	311
Supplemental choline (mg/day)[b]	175	175	175	175
Total choline (mg/day)	344	344	412	486
Betaine (mg/day)	122	122	267	349
Folate intake (μg DFE/day)	400	800	400	800

[a] Includes dietary phosphatidylcholine, glycerophosphocholine, choline and lysophosphatidylcholine.
[b] Choline (350 mg/day) (from choline bitartrate; TwinLab, Twin Laboratories) was consumed every other day, yielding an average supplemental choline intake of 175 mg/day.

neurotransmitter acetylcholine or oxidized to the methyl donor betaine [12].

Folate and choline are interrelated as either metabolite may serve as a methyl donor for the conversion of homocysteine to methionine. In a depletion–repletion study, folate restriction was associated with significant declines in plasma concentrations of phosphatidylcholine, whereas folate treatment with 800 μg/day as dietary folate equivalents (DFE) was linked to significant increases [10]. Although the roles of folate in disease and developmental conditions have been extensively investigated (reviewed in Ref. [13]), fewer studies have assessed the role of choline in these disease processes. Nonetheless, choline and/or betaine insufficiency is associated with the accumulation of lipid in liver and liver dysfunction [8], neural tube defect risk [14], hyperhomocysteinemia [15], DNA damage [16], muscle dysfunction [17] and altered DNA methylation patterns [18,19].

Given the establishment of a choline AI and a growing body of literature suggesting that suboptimal choline intake/status may increase the risk of certain diseases, it is essential to delineate the relationship between the dietary intake of choline moieties and blood status indicators. Thus, as an extension of our previous work [20,21], this 14-week controlled feeding study conducted in healthy premenopausal women sought to investigate the relationship between moderate changes in dietary choline/betaine intake and blood status indicators. In doing so, we considered the influences of folate intake and methylenetetrahydrofolate reductase (MTHFR) C677T genotype as these factors may influence choline status [10].

2. Methods

2.1. Subjects

Healthy premenopausal female subjects aged 18 to 46 years, preselected for the MTHFR 677CC or TT genotype, were recruited between January 2002 and April 2003 from the staff and student population at Cal Poly Pomona, Pomona, CA, as well as the surrounding community as previously detailed [20,21]. The study was approved by the Cal Poly Pomona's institutional review board for human subjects, and informed consent was obtained from each participant.

2.2. Study design

This was a 14-week controlled feeding study. During the first 2 weeks of the study, participants consumed a folate-restricted diet that, together with supplements, provided 133 μg/day of DFE, 344 mg/day of choline (169 and 175 mg/day from the diet and supplement, respectively), 122 mg/day of betaine and all other nutrients in recommended amounts. For the remaining 12 weeks of the study (i.e., Week 3 through 14), subjects with the MTHFR 677CC genotype were randomized to one of four treatment groups: Group I and II consumed 344 mg of total choline, 122 mg of betaine and 400 or 800 μg of DFE/day respectively; Group III consumed 412 mg of total choline, 267 mg of betaine and 400 μg of DFE/day; and Group IV consumed 486 mg of total choline, 349 mg of betaine and 800 μg of DFE/day (Table 1). In addition, subjects with the MTHFR 677TT genotype were randomized to groups III or IV (Table 1). For every treatment group, supplemental choline provided 350 mg every other day or an average of 175 mg of choline/day. The 400 and 800 μg of DFE/day were derived from varying amounts of supplemental folic acid (prepared in-house) and/or naturally occurring food folate as previously described [20,21].

2.3. Diets and supplements

The 5-day diet described in detail previously [20,21] provided 169±11 mg/day of total choline, 122±9 mg/day of betaine and 133±8 μg/day of naturally occurring food folate

Table 2
Choline, glycerophosphocholine, phosphocholine, phosphatidylcholine, lysophosphatidylcholine sphingomyelin, betaine and folate content of the menus[a,b]

Metabolite	Menus		
	A	B	C
Choline (mg/day)	25.6±1.5	50.9±9.1	105.0±11.7
Glycerophosphocholine (mg/day)	18.2±1.7	29.5±5.1	42.0±5.5
Phosphocholine (mg/day)	5.6±0.9	7.6±1.7	7.2±0.5
PtdCho (mg/day)	63±7	79±6	99±5
LysoPtdCho (mg/day)	47±5	61±13	51±7
Sphingomyelin (mg/day)	8.2±1.2	8.5±1.7	7.3±1.2
Total choline (mg/day)	169±11	237±22	311±18
Betaine (mg/day)	122±9	267±42	349±57
Folate (μg DFE/day)	133±8	426±44	835±33

PtdCho, phosphatidylcholine; LysoPtdCho, lysophosphatidylcholine.
[a] Values are means±S.E.M., n=5 days.
[b] Menu A was consumed by all subjects during the first 2-week baseline phase of the study. During the 12-week treatment phase, Menu A was consumed by Groups I and II, Menu B by Group III and Menu C by Group IV.