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## Ad Libitum Choline Intake in Healthy Individuals Meets or Exceeds the Proposed Adequate Intake Level,<sup>1,2</sup>

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### Abstract

Choline is an essential nutrient for humans that is used to synthesize membrane phospholipids and the neurotransmitter acetylcholine. Betaine, a metabolite of choline, functions as a methylgroup donor in the conversion of homocysteine to methionine, and is important for renal function. Accurate analysis of choline intake was previously not possible because the choline content of most foods was not known. Using new and recently published data on the concentrations of choline in common foods, we measured the choline content of diets consumed ad libitum by healthy adult volunteers housed in a clinical research center and compared these with estimates of choline intake derived from 3-d food records kept by subjects immediately before study enrollment. Mean choline intake in this subject population met or slightly exceeded the current Adequate Intake (AI) of 7 mg/(kg · d) set by the Institute of Medicine. Men and women consumed similar amounts of choline per day (8.4 and 6.7 mg/kg, respectively;  $P = 0.11$ ). Choline intakes estimated from the 3-d food records were significantly lower than this (when expressed as mg/kg, or as total mg, but not when normalized to energy intake), suggesting underreporting of food intake. Intake of betaine, which may spare choline utilization as a methylgroup donor, was 5.3 mg/(kg · d) in men and 4.7 mg/(kg · d) in women. Intake of folate, vitamin B-12, and methionine + cysteine, were similar and sufficient in all subjects. The current recommended AI for choline seems to be a good approximation of the actual intake of this nutrient.

### Keywords

choline; lecithin; betaine; folate; humans

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Choline, an essential nutrient, plays a number of vital roles in the body. It is used to synthesize phosphatidylcholine, a molecule needed for the structural integrity and signaling functions of cell membranes, as well as for lipid transport and metabolism (1). Choline directly affects cholinergic neurotransmission via synthesis of the neurotransmitter, acetylcholine (1). Finally, choline functions as a major source of methylgroups in the diet (1). Betaine, used by the kidney as an osmolyte (2), is a metabolite of choline and participates in the methylation of homocysteine to form methionine (3). Methyltetrahydrofolate is the alternative dietary methylgroup donor and is metabolically interrelated with betaine. Both regulate the formation of *S*-adenosylmethionine, and thereby influence methylation reactions. Diminished folate

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availability increases the demand for choline as a methylgroup donor and decreases choline availability for other functions in the body (4,5).

Many foods eaten by humans such as eggs, meats, cruciferous vegetables, and legumes contain substantial amounts of choline and choline esters (6). This ubiquitous distribution of choline in foods likely prevents the majority of healthy individuals from becoming choline deficient. The Institute of Medicine (IOM)<sup>4</sup> of the National Academy of Sciences set an Adequate Intake (AI) level for choline at 550 mg/d for adult men and 425 mg/d for adult women [both equivalent to 7 mg/(kg body weight · d)] (7), based on experimental data in humans showing that this amount prevents liver damage and fatty liver associated with choline-deficient diets (8). Although the exact Dietary Reference Intake (DRI) for choline remains to be established experimentally, setting such a requirement will be informed by data on the range of usual dietary intake of choline in humans. Because the choline content of foods was not included in major nutrient databases until quite recently, such calculations have never been made. In this study, using new and recently published data on food choline values (6,9) [(also available from the USDA Nutrient Data Laboratory (10)], we describe self-reported and measured ad libitum intake of choline, betaine, folate, vitamin B-12, and methionine in healthy adult men and women.

## SUBJECTS AND METHODS

### Human subjects

Subjects described in this paper were healthy male and female volunteers, recruited by advertisement, and housed in the University of North Carolina General Clinical Research Center (GCRC). Before acceptance into this study, the volunteer's health was verified by medical history and physical examination by a licensed medical doctor. The subjects provided written, informed consent, and the study was conducted in accordance with the guidelines of the Institutional Review Board of the School of Medicine at the University of North Carolina (UNC) at Chapel Hill.

The study included 16 adult men, and 16 adult women, ages 18–67 (demographic data are presented in Table 1). Some of these subjects were participants in a larger clinical study in which they also ingested experimental diets differing in choline content during periods other than the one in which they consumed ad libitum the diet described here.

### Diet analysis

All subjects were asked to keep a complete 3-d food record, reflective of their usual intake, immediately before enrolling in the study. Subsequently in the GCRC, all subjects consumed ad libitum a freely chosen diet of normal foods for at least 3 d. Specifically, on each day, subjects were allowed to choose from 53 prepared and single food items, 18 beverage items, and 14 condiment items offered by the GCRC metabolic kitchen. Moreover, subjects were permitted to request prepared food items that were not specifically offered, if so desired. Subjects were free to choose any of these food items in any quantity desired, approximating food choices that they would make at home (while off-study). All meals were prepared in the GCRC metabolic kitchen, and weighed before serving. All returned, uneaten foods and beverages were similarly weighed and deducted from the amount served. The net weight of foods and beverages, reflecting the exact amount consumed, was used for the dietary analyses. Subjects were constantly monitored and had no access to food other than that provided by the GCRC metabolic kitchen.

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<sup>4</sup>Abbreviations used: AI, adequate intake; DRI, Dietary Reference Intake; EAR, Estimated Average Requirement; GCRC, General Clinical Research Center; IOM, Institute of Medicine.

Daily food intake records were analyzed using the Food Processor SQL program (Version 9.2, 2003, ESHA Research). This software contains nutrient information based on the USDA National Nutrient Database for Standard Reference, Release 16, including food values for folate, vitamin B-12, methionine, and cysteine, but not including values for choline and betaine. Hence, all known food choline and betaine values (6,9) were manually entered into the database before conducting the analyses, and referenced according to the 5-digit Nutrient Databank Number (NDB\_No). The total choline content of a food was calculated as the sum of amounts of choline, phosphocholine, glycerophosphocholine, lysophosphatidylcholine, phosphatidylcholine, and sphingomyelin in the food. Betaine, a metabolite of choline, was calculated independently. Data on the total choline or betaine content of several food items, reported primarily in the 3-d food records, were unavailable. Whenever possible, a nutritionally equivalent food was substituted in the analysis. For example, white bread was substituted for a hamburger bun. (See Supplemental Table 1 for a list of all substitutions made in the diet analyses.) Very few of the substituted foods were high in choline and these substitutions were not expected to significantly perturb the results. For some food items, such as mango and okra, no obvious food substitution existed. For these foods, the choline, choline esters, and betaine contents were measured by our laboratory using a LC-MS-based method as previously described (6,11). All food items were purchased locally, prepared in the manner in which they are most commonly consumed, and homogenized using a food processor or blender. Dry foods were frozen and then ground with a mortar and pestle. (The results of these analyses are presented in Supplemental Table 2.) The total choline and betaine values for these food items were also added to the Food Processor SQL database before conducting the dietary analyses. Each day of the 3-d food record or measured 3-d food intake was analyzed individually, and then averaged for each subject.

### Statistics

Men and women were compared using *t* tests. Food records were compared with measured means using paired *t* tests (SAS/STAT®, Version 8; SAS Institute). All statistical tests were conducted using a two-sided significance level of 0.05. Values in the text are means  $\pm$  SD.

## RESULTS

Measured total choline intake relative to body weight by men was  $8.4 \pm 2.1$  mg/(kg  $\cdot$  d), 120% of the current AI (7 mg/kg body weight), whereas women consumed  $6.7 \pm 1.3$  mg choline/(kg  $\cdot$  d), 96% of the AI (not different from men,  $P = 0.11$ ) (Table 2). Twelve of 16 men, and 6 of 16 women met or exceeded their estimated choline requirement with their measured dietary intake (data not shown).

Measured choline intake, expressed as total mg choline/d was greater in men than in women ( $P = 0.02$ ) (Table 2). Measured choline intake normalized to energy intake did not differ between men and women ( $P = 0.52$ ).

Daily choline intakes estimated from the 3-d food records were lower than the measured intakes when expressed as mg/kg body weight [ $P = 0.0001$  (men) or 0.003 (women)] or total mg consumed/d ( $P = 0.0001$  (men) or 0.004 (women); Table 2). However, choline intake relative to energy ingested did not differ in the food records compared with the measured intakes consumed ad libitum [ $P = 0.08$  (men) or 0.25 (women)].

Measured total betaine intake relative to body weight was less than choline intake and did not differ between men and women ( $P = 0.55$ ; Table 2). As with choline, daily betaine intakes estimated from the 3-d food records were lower than the measured intakes in men ( $P = 0.02$ ), and tended to be lower in women ( $P = 0.05$ ). There is currently no dietary requirement established for betaine.

Daily intake of total folate equivalents and vitamin B-12 did not differ between the sexes [folate ( $P = 0.17$ ); B12 ( $P = 0.06$ )], and exceeded the current DRI for both nutrients. For methionine, the Recommended Dietary Allowance is set for the combination of methionine + cysteine in the diet; however, only methionine in the diet can spare choline requirements. Measured methionine + cysteine intake did not differ between men and women ( $P = 0.13$ ). Intakes by both men and women greatly exceeded the Estimated Average Requirement (EAR) of 13 mg/kg. As with estimates of choline intake, measured folate, vitamin B-12 (men only), and methionine + cysteine intakes were significantly higher than estimates calculated from reported food intakes.

## DISCUSSION

Previously, it was not possible to calculate dietary choline intake in humans, and there are currently no nationally representative estimates of the intake of choline from food or food supplements (7). This is because the choline content of foods had not been included in major nutrient databases; moreover, until quite recently, extensive food choline data were either lacking or unreliable due to older, imprecise assay procedures.

Using new and recently published data on choline levels in a large number of common foods, we report here that healthy men and women consumed amounts of choline that were at or slightly higher than the current AI level, but some individual subjects, especially women, consumed slightly less. These data are of use to nutrition scientists considering the likely validity of the current dietary recommendation. If we had found that healthy individuals consumed significantly less than the recommendations, it would have led to the conclusion that the recommended amounts were likely too high. Our data suggest that the recommended intake is very close to the actual intake of this nutrient, although only 6/16 women met or exceeded this recommended intake of choline.

Betaine intake was substantial in all subjects and may have spared conversion of dietary choline to betaine for methylgroup donation. Vitamin B-12, and methionine + cysteine intakes were well above the current established requirements, and folate intake was adequate in men and women.

We obtained a measurement of dietary intake of choline using in-patient subjects whose total food intake was observed directly for at least 3 d, and we compared these measurements with estimates derived from self-reported intakes during another period. In an earlier study, choline levels calculated from the analysis of individual components of the diet were compared with values obtained from analyses of all foods combined into a single sample (12). The laboratory analyses of choline and betaine in the whole diet aliquots matched the estimated amounts in the diets that were calculated from the analyses of individual foods. We are thus confident that our calculated estimates of choline intake based on the observed and measured diet phase with ad libitum consumption accurately represent actual choline intakes.

Much debate surrounds the accuracy of current methods to assess dietary intake; 7-d weighed food records were historically considered to be the best for estimating dietary exposure; however, 3- or 4-d records are commonly used in research studies (13). Validation studies of various dietary assessment instruments, including food records, against doubly labeled water, the most widely accepted biomarker of energy intake, revealed that self-report intake instruments consistently underestimate energy intake (14). Here we found that self-report in 3-d food records significantly underestimated daily choline intake (as well as betaine, folate, vitamin B12, and methionine + cysteine intakes). There are many possible explanations to account for this discrepancy. Because the amount of choline ingested relative to energy status was similar in the measured and estimated analyses, subjects likely underreported total food

intake or serving sizes. Subjects may also have selected a different variety of foods in the hospital-like situation than they would have at home. Although this may be the case, it is difficult to believe that differences of the magnitude that we observed can be explained solely by this factor. Finally, due to the wide variety of foods reported and the limited nature of the database, a number of substitutions had to be made in the analyses. We do not think it is likely that these substitutions contributed to the observed discrepancy because they occurred in both the reported and measured intake analyses. Our data suggest that self-report using 3-d food records should be used with caution when assessing choline or other nutrient intakes.

In summary, we report for the first time the range of ad libitum dietary consumption of choline and betaine in healthy humans. The observed values are similar to the recommended AI for choline.

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## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

**TABLE 1**Demographic characteristics of study subjects<sup>1</sup>

	Men	Women
<i>n</i>	16	16
Age, <i>y</i> ( <i>range</i> )	19–65	18–67
Body weight, <i>kg</i>	76.0 ± 11.9	64.7 ± 10
Height, <i>cm</i>	174.4 ± 10.1	162.9 ± 4.7
Ethnicity/Race, %		
White	81.25	50
Black	18.75	25
Asian	0	18.75
Other	0	6.25

<sup>1</sup> Values are means ± SD unless noted otherwise.

**TABLE 2**

Measured and estimated dietary intakes of choline, betaine, folate, vitamin B-12, and methionine + cysteine in humans consuming diets ad libitum<sup>1</sup>

	Total measured in diet		Total reported in food record	
	Men	Women	Men	Women
Choline, <i>mg/(kg · d)</i>	8.4 ± 2.1	6.7 ± 1.3	5.3 ± 2.1 <sup>*</sup>	3.9 ± 1.4 <sup>*</sup>
% AI	120	96		
Choline, <i>mg/d</i>	631 ± 157	443 ± 88 <sup>#</sup>	398 ± 152 <sup>*</sup>	258 ± 91 <sup>*</sup>
Choline, <sup>2</sup> <i>mg/kcal</i>	0.22 ± 0.05	0.2 ± 0.06	0.18 ± 0.06	0.17 ± 0.06
Betaine <i>mg/(kg · d)</i>	5.3 ± 3.2	4.7 ± 2.5	3.2 ± 1.6 <sup>*</sup>	2.8 ± 1.8
Folate, <i>μg/d</i>	568 ± 124	493 ± 97	403 ± 141 <sup>*</sup>	340 ± 146 <sup>*</sup>
% DRI	142	123		
B-12, <i>μg/d</i>	6.5 ± 2.5	4.7 ± 1.9	4.3 ± 2.0 <sup>*</sup>	3.8 ± 2.3
% DRI	269	195		
Methionine + Cysteine, <i>mg/(kg · d)</i>	61.5 ± 14.8	49.9 ± 11.2	48.2 ± 17.8 <sup>*</sup>	34.9 ± 14.1 <sup>*</sup>
% EAR	473	384		

<sup>1</sup> Each value represents the mean of 3 d of dietary intake ± SD, *n* = 16.

\* Different from measured mean, *P* < 0.05.

<sup>#</sup> Different from men, *P* < 0.05.

<sup>2</sup> 1 kcal = 4.184 kJ.