Relations Between Dietary Choline or Lecithin Intake, Serum Choline Levels, and Various Metabolic Indices*

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Choline administration increases blood choline, brain choline, and brain acetylcholine levels in rats. It also increases blood choline levels in humans and appears to be a useful treatment for some patients with tardive dyskinesia, a brain disease probably associated with deficient cholinergic tone. In order to characterize other possible metabolic and hormonal effects of choline-containing compounds, we measured changes in serum choline, glucose, insulin, cortisol, prolactin, cholesterol, and triglyceride levels resulting from ingestion of low- or high-choline meals in 16 normal human subjects. After the consumption of a single meal containing 3 g choline chloride, serum choline rose by 86% ($p < 0.01$), attaining peak values after 30 min. When the same subjects ate a meal containing an equivalent amount of choline in the form of lecithin, serum choline levels rose by 33% after 30 min, and continued to rise for at least 12 hr, to 265% over control values ($p < 0.001$). Serum choline concentrations were related to the amount of choline in the diet: they did not vary significantly during 24-hr periods when the subjects consumed a low-choline diet for two consecutive days, but rose substantially ($p < 0.01$) after each high-choline meal. Serum glucose, insulin, cortisol, and prolactin levels were not significantly modified by choline or lecithin ingestion. Lecithin consumption increased serum triglyceride levels and lowered serum cholesterol concentration.

ADMINISTRATION OF CHOLINE to rats by injection,[1,2] stomach tube,[3] or dietary supplementation[4] produces sequential elevations in serum choline, brain choline, and brain acetylcholine (ACh) levels. Choline apparently accelerates the rate at which neurons synthesize ACh; since the effects of giving choline and physostigmine are additive,[4] release of the neurotransmitter is probably enhanced as well.[5-8] These observations prompted speculation that choline ingestion might benefit patients with brain diseases, e.g., Huntington's disease and tardive dyskinesia, that may be associated with deficient central cholinergic tone.[9,10] In humans, choline ingestion was found to cause dose-dependent elevations in plasma choline levels[11,12] and to increase choline concentrations in the cerebrospinal fluid.[11] In a double-blind, cross-over study, we found that choline, taken orally for 14 days, suppressed choreiform movements in 9 of 20 patients with tardive dyskinesia.[13] Choline also suppressed choreic movements in some patients with Huntington's disease,[14] but not in others.[15,16]

Therapeutic trials of choline, and such choline-containing compounds as lecithin, will probably continue as physicians seek new ways to treat diseases that may be associated with deficient cholinergic tone. While consumption of

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choline or lecithin has been shown to be a reliable means of elevating serum choline levels in human subjects,[17] other possible metabolic and hormonal effects these compounds might produce remain to be characterized. In the present study, we measured changes in serum choline, glucose, insulin, cortisol, prolactin, cholesterol, and triglyceride levels resulting from various amounts of free and lecithin-bound choline in the diets of normal human subjects.

MATERIALS AND METHODS

Subjects

Sixteen healthy, paid volunteer subjects, ages 18–30, participated in the studies. Each received a physical examination and gave informed consent according to a protocol on choline and lecithin ingestion approved by the Massachusetts Institute of Technology Committee on the Use of Humans as Experimental Subjects.

Protocol

Single meal study. After a 12-hr fast, 10 subjects consumed a single low-choline meal supplemented with 3 g choline chloride (ChCl; Aldrich Chemical Co., Metuchen, N.J.) and, then, fasted again for the following 12 hr. Blood samples were drawn before the meal (8:00 a.m.—fasting) and 30 min, 1, 4, 8, and 12 hr after the meal; sera were separated and assayed for choline, glucose, insulin, cortisol, and prolactin. One week later, the protocol was repeated with the same 10 subjects who, in the meantime, had been released and allowed to resume their normal dietary habits) consuming a single low-choline meal supplemented with 100g lecithin granules (Sigma Chemical Co., St. Louis, Mo.); the granules contain 10%–20% lecithin and 80%–90% mixed neutral lipids, and the supplier reports that the only phospholipid in this preparation is lecithin. Blood samples were collected and assayed as before. The following week, the protocol was repeated with four subjects eating a control meal: the same low-choline meal, but unsupplemented with ChCl or lecithin. (Prolactin assays were not performed on controls.) The unsupplemented meal contained a base level of less than 15 mg choline; the two supplemented meals each supplied a base level of about 2.3g choline (3g choline chloride salt; lecithin granules contained 2%–3% choline, as measured using the assay described below).

Two-day study. In another study, 6 additional subjects, who had fasted overnight, consumed 3 high-choline meals (at 8:30 a.m., 12:00 p.m., and 5 p.m.) on each of 2 consecutive days (5g choline/day). Beginning at 8:00 a.m. on the second day, 7 blood samples were drawn at 4-hr intervals; sera were separated and assayed for choline. Some samples were assayed for cholesterol and triglycerides. One week later, the protocol was repeated with the same subjects consuming the low-choline diet; sera were assayed for choline, but not for cholesterol or triglycerides.

Diets

Meals were prepared and served in the Massachusetts Institute of Technology Clinical Research Center. The low-choline meal (a cereal-based drink—"MIT meal"—Junket Danish Dessert, applesauce, and cornstarch cookies) contained 782 kcal/meal, distributed as 0% protein, 35% fat, and 65% carbohydrate. (The MIT meal is a homogenized liquid oatmeal containing the following ingredients: oatmeal, corn oil, dextrimaltose, pectin, methylcellulose, vanilla, lemon juice, sodium chloride, calcium and potassium salts, and water; it contains 0.3% protein/100-g serving. Junket is a tapioca-based dessert consisting largely of corn oil, cornstarch, and flavorings. The cookies contain cornstarch, dextrimaltose, sucrose, Crisco, sodium chloride, and flavorings.) The total choline content of the diet was less than 50 mg/day (15 mg/meal, 3 meals/day, based on assays of total choline in food samples).

The high-choline meal (5 egg yolks, 12g lecithin granules, MIT Meal, Junket Dessert, applesauce, and cookies contained 830 kcal/meal, distributed as 8% protein, 52% fat, and 40% carbohydrate, and supplied 5.0 g choline/day (4 g from egg yolk and 1 g from lecithin granules). All subjects in both studies consumed the test meals completely.

Analytical Methods

Blood samples were obtained by venipuncture or through an indwelling venous catheter; sera were separated and frozen within 30 min of collection. Serum free choline levels were determined by a radioenzymatic method.[18] To determine the choline content of the lecithin granules, samples of lecithin granules (10 mg) were incubated with 10 ml of 1*N* potassium hydroxide for 16 hr at 37°C to liberate the choline moiety from the lecithin molecule.[19,20] This incubation procedure would be expected to liberate choline from glycerophosphorylcholine, or from any related molecule—e.g., lecithin—containing a phosphorylcholine moiety. Samples of a 98% pure synthetic dipalmitoyl lecithin (2 or 5 mg; mol wt 734.1) were treated similarly and served as external standards for the completeness of the alkaline hydrolysis and the survival of the choline. Aliquots of the hydrolysate were assayed for free choline using the radioenzymatic method.[18] The recovery of the choline from the synthetic dipalmitoyl lecithin was 90%; this figure was used to correct for the amounts of choline in the lecithin granules. Glucose levels were assayed using the O-toluidine procedure.[21,22] Radioimmunoassay techniques were used to measure blood cortisol (New England Nuclear Kit NEA-066, Boston, Mass.), insulin[23] and prolactin (NIAMDD Kit, Bethesda, Md.) levels. Enzymatic methods were used to determine serum levels of triglyceride (Worthington Biochemicals Kit, Freehold, N.J.) and cholesterol (Boehringer Mannheim Corp., Danbury, Conn.).

Data were analyzed with the paired t test, 2-way and 3-way analysis of variance; each parameter was compared by subject, time of day, and type of diet.

RESULTS

Single Meal Study

The mean fasting (8:00 a.m.) serum choline level was 11.7 ± 0.6 nmoles/ml; 30 min after ChCl ingestion, it had risen by 82% to 21.3 ± 2.3 ($p < 0.01$; Table 1). Serum choline levels remained elevated 1 hr after the meal (19.0 ± 1.0 nmoles/ml; $p < 0.001$) and approached control levels by 4 hr after the meal. When the same subjects consumed a meal containing an equivalent amount of choline in the form of lecithin, serum choline rose by 33% after 30 min ($p < 0.05$) but continued to rise for at least 12 hr (265% over control values; $p < 0.001$). Consumption of a single choline-supplemented meal failed to affect serum glucose, insulin, or cortisol concentrations or to modify serum prolactin levels (Table 2).

Table 1. Effects of Choline Chloride and Lecithin Ingestion on Serum Free Choline Levels in Normal Subjects

Hours After Meal	Serum Free Choline (nmoles/ml)	
	Choline Chloride	Lecithin
0	11.7 ± 0.6	8.2 ± 0.6
½	21.3 ± 2.3*	10.9 ± 1.2‡
1	19.0 ± 1.0†	17.4 ± 2.5*
4	16.7 ± 2.6	26.4 ± 3.6†
8	12.9 ± 1.4	29.4 ± 2.5†
12	10.6 ± 0.9	30.0 ± 2.5†

Nine subjects consumed the single low-choline meal supplemented with either 3 g choline chloride or 100 g lecithin granules, both equivalent to 2.3 g choline base. Values represent means ± SEM. Data were analyzed using the paired t test.

*$p < 0.01$.

†$p < 0.001$.

‡$p < 0.05$.

Table 2. Effects of Choline Chloride and Lecithin Ingestion on Serum Glucose, Insulin, Cortisol, and Prolactin Levels in Normal Subjects

	Hours After Meal				
	0	$\frac{1}{2}$	1	4	8
Glucose (mg/100 ml)					
Control	87 ± 10	84 ± 25	96 ± 17	97 ± 09	89 ± 18
ChCl	70 ± 21	115 ± 45	102 ± 38	85 ± 11	90 ± 14
Lecithin	83 ± 18	110 ± 27	92 ± 34	91 ± 19	88 ± 17
Insulin (μU/ml)					
Control	18 ± 5	49 ± 17	34 ± 17	18 ± 5	17 ± 4
ChCl	10 ± 1	54 ± 29	45 ± 16	15 ± 6	13 ± 4
Cortisol (μg/100 ml					
Control	15 ± 4	12 ± 3	11 ± 3	10 ± 5	11 ± 7
ChCl	14 ± 3	15 ± 4	12 ± 5	8 ± 6	6 ± 2
Lecithin	16 ± 4	16 ± 5	11 ± 4	9 ± 5	7 ± 1
Prolactin (ng/ml)					
Control			(not measured)		
Choline	10 ±2	8 ± 2	9 ± 2	8 ± 1	8 ± 2
Lecithin	9 ± 2	8 ± 2	8 ± 2	8 ± 1	8 ± 2

Subjects consumed the single low-choline meal supplemented with 3 g ChCl or 100 g lecithin granules, both equivalent to 2.3 g choline base; control subjects consumed the unsupplemented low-choline meal (<15 mg choline base). Values represent the means ± SEM. Data were analyzed using 3-way ANOVA; there were no significant variations between data from subjects consuming the control diet and those from subjects consuming ChCl or lecithin.

Two-Day Study

The mean fasting (8:00 a.m.) serum choline level was 9.8 ± 1.8 nmoles/ml. Four hours after the fourth high-choline meal (breakfast on day 2) the mean serum choline level had risen to 36.6 ± 3.9 nmoles/ml. Choline levels remained significantly elevated through the remaining two meals ($p < 0.01$) and declined slowly with fasting overnight (Fig. 1A). By 8:00 a.m. the following day, serum choline levels were not significantly different from control (8:00 a.m. on day 1) values. When, 1 wk later, the same subjects consumed the low-choline diet for 2 days, serum choline levels during day 2 did not vary significantly from control (8:00 a.m. on day 1) values, ranging from 5.5 ± 0.9 to 9.3 ± 1.8 nmoles/ml (Fig. 1 B). Thus, when only low levels of dietary choline were consumed, blood choline content did not fluctuate diurnally or in response to eating. Serum cholesterol and triglyceride levels were measured at 8:00 a.m. on day 1 (fasting) and twice on day 2, i.e., 4 hr after the first meal (12:00 p.m.) and 3 hr after the last meal (8:00 p.m.) (Table 3). Consumption of the high choline diet significantly elevated serum triglyceride levels at both times ($p < 0.01$) and depressed serum cholesterol at 8:00 p.m. ($p < 0.01$).

DISCUSSION

These studies show that the consumption by humans of lecithin (the usual source of choline in the diet) elevates serum free choline levels more effectively than an equivalent amount of choline salts. Serum choline levels appear to be related to nutritional state: consumption of a diet rich in choline causes prolonged elevations in serum choline concentrations, whereas consumption of

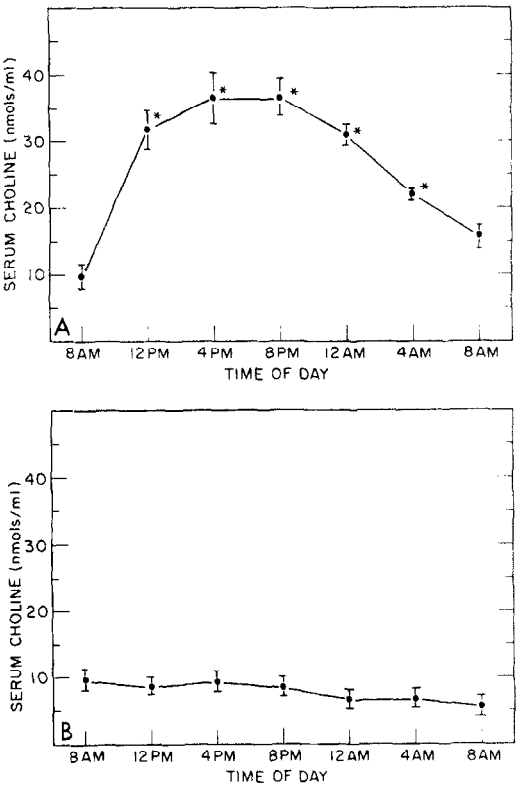

Fig. 1. Serum choline levels during consumption of a high-choline (A) and a low-choline (B) diet by 6 normal human subjects for 2 days. Each point represents the mean ± SEM. The high-choline diet contained 50 mg/day, or about 0.67 mg/kg. Meals were eaten starting at 8:30 a.m., 12:00 p.m., and 5:00 p.m. Blood samples were obtained at 8:00 a.m. on day 2 and every 4 hr thereafter to 8:00 a.m. the following day. Data were analyzed by 2-way ANOVA and paired t test. *$p < 0.01$.

a diet lacking choline is associated with relatively constant low levels of blood choline. Serum glucose, insulin, cortisol, and prolactin levels apparently are not affected by single meals containing choline or lecithin.

Choline is the physiologic precursor of acetylcholine (ACh); since the brain probably cannot synthesize choline de novo, all of the choline used for ACh biosynthesis must come from the systemic circulation, entering the brain via a low-affinity blood–brain barrier uptake system that is normally unsaturated.[24] A portion of the choline in blood is synthesized by the liver;[25] the rest is supplied through the diet (eggs, meat, vegetables). Less than 1% of the choline in the diet is present as the free base; most is ingested as phosphatidylcholine

Table 3. Serum Cholesterol and Triglyceride Levels Following Consumption of a High-Choline Diet for 2 Days

Sample	Cholesterol (mg/100 ml)	Triglyceride (mg/100 ml)
Fasting	179 ± 14	73 ± 14
12:00 p.m.	178 ± 15	139 ± 20
8:00 p.m.	154 ± 09	158 ± 35

Fasting samples were obtained from six subjects before they ate the high-choline diet (8:00 a.m. on day 1). Experimental samples were obtained on day 2 of the high-choline diet, 4 hr (12:00 p.m.) and 3 hr (8:00 p.m.) after meals. Data are expressed as means ± SEM. Analysis by 2-way ANOVA indicated an elevation in serum triglyceride ($p < 0.01$) and a reduction in serum cholesterol levels ($p < 0.01$).

(lecithin)—a choline molecule covalently bound to a glycerol chain containing two fatty acids. Presumably, most of the free choline in blood is derived from the absorption and subsequent hydrolysis of the lecithin molecule.

Our observation that lecithin more effectively elevates serum free choline levels than an equimolar amount of choline salts (Table 1) is consistent with observations that oral choline is rapidly metabolized to di- and trimethylamines by intestinal bacteria[26] and that urinary trimethylamine levels are much lower in people consuming lecithin than in those consuming choline salts.[27] Since both choline salts and lecithin affect brain ACh by elevating blood choline levels, our data suggest that lecithin may be more effective than choline chloride as a therapeutic agent.

The estimated average daily intake of choline by adults ranges from 0.5 to 0.9 g;[28] however, if a person were to consume, for example, a pound of meat, several egg yolks, and a cup of beans during one particular day, his choline intake would exceed 5 g. Thus, day-to-day variations in food choice can normally produce 10-fold or greater variations in daily choline consumption, and ingestion of dietary lecithin in the varying amounts that Americans might normally consume probably causes shifts in serum choline levels. The amount of choline supplied by our single test meals supplemented with lecithin or choline chloride, 2.3 g, was well within this normal range, as was the 5 g choline/day in the 2-day study.

Virtually no information has been available regarding the metabolic and hormonal consequences of choline consumption by humans. Our data indicate that choline salts or lecithin doses that elevate serum choline levels do not modify serum glucose or insulin levels among subjects consuming our high-carbohydrate test meal (Table 2). Because of the relatively high fat and cholesterol contents of our high-choline meal (15 egg yolks/day for 2 days), we measured serum levels of triglycerides and cholesterol. Although triglycerides increased and cholesterol decreased significantly (Table 3), neither change was of sufficient magnitude to fall beyond normal postprandial ranges. (A decline in serum cholesterol levels after consumption of lecithin has been noted previously.[29]) The consumption of pure lecithin might be expected to have less effect on serum triglyceride levels than the 20% lecithin granules used in our study.

The participation of central cholinergic neurons in the neuroendocrine control of cortisol[30,31] and prolactin[32–34] has been suggested by observations made using a number of animal models; hence, we measured serum cortisol and prolactin levels in subjects eating the single choline-supplemented meal. The fact that we detected no significant increases in either hormone suggests that this mode of choline administration does not constitute a significant stress to normal subjects.

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