Accelerated uptake of an intravenously administered dose of choline chloride in choline-deficient humans

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The role of choline in the human diet continues to be debated, in part due to the lack of an appropriate assessment technique. Information regarding the turnover of this nutrient in various body pools in humans is lacking. An intravenous infusion of ($d_9$methyl)-choline chloride was administered over 1 hour to human subjects fed either a choline-containing (5 mmoles/day choline chloride) or a choline-deficient diet for 3 weeks. Blood samples were collected during the infusion and for 1 hour postinfusion. Plasma levels of choline, ($d_9$methyl)choline, and phosphatidylcholine were measured. The uptake of ($d_9$methyl)-choline from plasma was calculated by nonlinear regression analysis. In control subjects (n = 4), the half-life of ($d_9$methyl)-choline in plasma was 7.0 ± 0.85 minutes, while in deficient subjects (n = 6) it was 3.5 ± 0.42 minutes (P < 0.004). Extracellular choline pools were also decreased in deficient subjects (mean ± SEM; control: 2.6 ± 0.2 mmoles; deficient: 2.0 ± 0.2 mmoles; P < 0.05). The rate of appearance of unlabeled choline into the plasma was unaffected by the level of dietary choline. We conclude that intravenously administered choline chloride is cleared more rapidly in humans fed a choline-deficient diet than in control subjects, and that choline deficiency decreases choline pools in the body. Our results also indicate that an intravenous load test, similar to the one used in these studies, may be useful as a method of measuring choline nutriture. (J. Nutr. Biochem. 5:303–307, 1994.)

Keywords: choline deficiency; intravenous supplementation; stable isotopes

Introduction

Choline is a water-soluble compound that is necessary for the synthesis of acetylcholine and the phospholipids phosphatidylcholine and sphingomyelin. It also contributes methyl groups to the labile methyl pool.[1] Although the role of choline in the diet of humans continues to be debated, recent studies suggest that under several clinical conditions[2–7] significant decreases in plasma choline concentration, a pool which mirrors tissue choline levels,[8] occur. Healthy male subjects who consumed a choline-deficient diet for 3 weeks exhibited a decline in plasma choline concentration associated with liver dysfunction,[9] further supporting the hypothesis that choline may be an essential nutrient for humans. Recently, Buchman et al.[10] demonstrated that an exogenous source of choline increased plasma levels and decreased liver steatosis in patients receiving long-term total parenteral nutrition. Because a significant proportion of patients who receive total parenteral nutrition have compromised gastrointestinal tracts, the best way to administer choline to this population is the intravenous route. Although the administration of large doses of choline orally has been shown to have minimal side effects,[11] intravenous choline can be toxic. The LD_{50} for choline chloride in rabbits is 0.01 mmol/kg and in cats it is 0.25 mmol/kg.[11] Studies in mice suggest that the LD_{50} for intravenous choline chloride is 0.5 mmol/kg.[12]

In the present study, we measured body pools of choline as well as the appearance of unlabeled choline and the uptake of labelled choline from the plasma of healthy subjects who had been fed a synthetic diet with or without choline for 3 weeks. Our hypothesis was that body choline pools would be diminished and that choline would be taken up more

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rapidly in deficient subjects. Our findings also provide information regarding the turnover of the plasma choline pool in healthy, human subjects maintained on a constant diet for several weeks and suggest that an intravenous load test may be useful as a tool to measure changes in choline nutriture.

Methods and materials

Subjects

As previously described,[9] 15 healthy male volunteers were recruited for a study investigating the effects of a choline-deficient diet. All subjects gave written consent to participate in accordance with the guidelines of the Institutional Review Board-Human Studies Committee of Boston University. Intravenous administration of the stable isotope, ($d_9$methyl)-choline chloride was approved by the Office of Biologics Research and Review, Center for Drugs and Biologics of the Food and Drug Administration. Subjects were randomly assigned to the control group (n = 7) or the choline deficient group (n = 8). The mean age of the control group was 26.8 years (±1.5, SEM); mean age of the deficient group was 29.1 years (±1.8). The mean weight for the control group was 74.4 kg (±4.9), while the choline-deficient group had a mean weight of 72.4 kg (±4.5).

Study design

Subjects were admitted to the Boston University Clinical Research Center (CRC) where they lived under constant observation during the 5 weeks of the study. Two control and one deficient subject were permitted to attend class or to work at the medical center during the daytime. However, they consumed all meals at the CRC and slept there. Adherence to the diet was monitored by weekly blood choline levels.

All subjects received the same liquid formula, which delivered the daily protein requirement (0.95 g/kg/d) along with a portion of the daily energy requirement. Subjects ingested the formula in three isocaloric meals each day. Choline-free, between-meal snacks of protein-free cookies and/or ginger ale were provided to meet the remaining energy needs. Calorie and protein requirements were estimated by a registered dietitian and were calculated to provide adequate energy to support weight maintenance. Subjects were required to consume all of the protein-containing formula provided to them. Weight was monitored and caloric intake adjusted such that all subjects maintained their body weight throughout the 5-week study.

Subjects consumed approximately 40 kcal/kg/day (9.5% protein, 35% fat, 55.5% carbohydrate). The diet provided the recommended daily allowance for all amino acids, vitamins, and minerals.[9] The total choline content of the diet, as analyzed in our laboratory, was 124 μmoles. During weeks 1 and 5, all subjects received 5 mmoles of choline chloride per day (or 5 mmoles of free choline) in capsule form. This was provided in a single dose and is approximately one-half of the usual daily intake of humans.[1] During the 3-week experimental period (weeks 2 through 5), deficient subjects received a placebo containing cellulose.

Infusion studies

Infusion studies were performed at the end of the experimental period. A sterile saline solution (0.9% sodium chloride) containing ($d_9$methyl)-choline chloride was infused into the antecubital vein of one arm at a calculated rate of 12.5 to 15.0 μmoles choline chloride/kg for 1 hour. Blood was collected by venipuncture from the antecubital vein of the other arm immediately prior to the beginning of the infusion; every 10 minutes during the infusion; and at 5, 10, 20, 30, 40, 50, and 60 minutes following the cessation of the infusion. Heparinized blood samples were immediately placed on ice, then centrifuged, and the plasma frozen at −80° C until biochemical analysis could be performed. Electrocardiograms and heart rate were monitored continuously throughout the 2-hour study.

This amount of stable isotope was administered based on calculations of steady state, assuming a total body pool of free choline of 5.4 mmoles.[13] This estimated body pool was calculated from available data regarding organ size of the reference male (70 kg) and tissue choline concentrations in guinea pigs, rats, and humans. Adjustments due to differences in body composition were not made because no data exists regarding its effect on total body choline concentrations. Average body weights were not different between groups, thus any error in our original estimate would be the same between groups.

We estimated a half-life of approximately 10 minutes and arrived at an estimate of approximately 15 μmoles/kg of choline chloride for 1 hour as the dose that would permit six half-lives. This calculation was based on studies in the guinea pig, which demonstrated that the half-life of choline in the liver and kidney was 10 minutes.[14] Thus, a 1-hour infusion would approximate six half lives in the two major organs involved in choline metabolism.

Biochemical analysis

The level of both ($d_9$methyl)-choline and unlabeled choline were measured using gas chromatography and a mass selective detector.[15] Plasma phosphatidylcholine was measured in a subset of samples collected at the end of the hour-long infusion and 60 minutes later. Following extraction of serum by the method of Bligh-Dyer,[16] phosphatidylcholine was isolated by thin-layer chromatography and then cleaved by acid hydrolysis to form free choline, which was measured as described above.

Data analysis

We determined that isotopic enrichment had achieved steady state by nonlinear regression analysis (Systat, Evanston, IL USA). Data points from the hour-long infusion period were fit to the equation: $\text{Enrichment}_t = (F/(V*C*k))\ (1 - e^{-kt})$, where F is the infusion rate (μmol/min), V is the volume of distribution (L), C is the concentration of choline in the pool (μmol/L), and k is the rate constant of elimination. For these studies, we assumed that the volume of distribution was the extracellular pool.

The rate of appearance of unlabeled choline was estimated using the equation: R_a (μmol/min) = $(F/\text{Enrichment}_{ss}) - F$, where F is the infusion rate (μmol/min) and enrichment_{ss} is isotopic enrichment at steady state (($d_9$methyl)-choline (μmole)/total choline (μmole); calculated as described above).

We assumed that following a 1-hour constant infusion of ($d_9$methyl)-choline the appropriate volume of distribution was the extracellular fluid. Therefore, pool size was calculated using the equation: Body Pool = Total amount of isotope infused (μmol/hr)/Enrichment at steady state. We made the arbitrary assumption that the volume of distribution was the extracellular fluid. We realize that this is an underestimation, but the same assumptions were made for both groups. It seems reasonable to assume that choline first distributes in the plasma volume, then the extracellular volume, and then in some volume less than total body water. Given the brief period of our study, we chose to use extracellular volume.

The disappearance rate of ($d_9$methyl)-choline from plasma was analyzed using nonlinear regression analysis (Systat). Data were fit to the function: $\text{nmoles} = B + A(e^{-k(\text{Time})})$. The half-life of ($d_9$methyl)-choline in plasma was estimated using the equation $t_{1/2} = \ln 2/k$. This model is based on the assumptions that: (1) labeled choline is adequately mixed with the unlabeled choline

pool; and (2) it is transported at the same rate as the unlabeled molecule.

Results are expressed as the mean ± SEM. An independent *t* test was performed to compare group means. Statistical significance was defined as $P < 0.05$.

Results

Plasma choline and phosphatidylcholine concentrations declined 30% in deficient subjects over the course of the 3-week experimental period when compared with controls ($P < 0.01$ and $P < 0.05$, respectively).[9] Signs of choline deficiency included increased serum alanine aminotransferase levels.[9]

Intravenous infusion of ($d_9$methyl)-choline was carried out on the last 11 subjects (n = 4 control; n = 7 deficient) who were enrolled in a controlled study of the effects of choline deficiency in humans. One deficient subject was omitted from statistical analysis because blood samples from two postinfusion timepoints were unusable. In a second deficient subject, isotopic enrichment did not reach steady state. Therefore, estimates of pool size and rate of appearance of unlabeled choline could not be accurately calculated for this subject.

The average dose of choline that was infused did not differ between groups (control = 17.3 ± 1.17; deficient = 13.8 ± 1.48 μmoles/minute). Isotopic enrichment (($d_9$methyl)-choline/total plasma choline) was not different between groups at the end of the infusion period (control = 0.39 ± 0.01; deficient = 0.40 ± 0.05) and reached a steady state in nine of 10 subjects after 20 minutes of infusion.

No adverse physical symptoms were observed during or following the infusion studies. Both heart rate and electrocardiographs remained unchanged from baseline.

Table 1 Plasma half-life of intravenously administered choline, estimate of extracellular choline pool size, and plasma choline and phosphatidylcholine concentrations in human subjects following ingestion of a choline-containing or choline-deficient diet*

Subject	Half-life (minutes)	Pool size (mmoles)	Choline (μM)	PtdylChol (mM)
Control				
1	9.0	2.5	7.5	0.93
2	6.1	2.4	11.8	1.60
3	5.2	3.2	10.0	1.66
4	7.7	2.3	8.6	1.09
Mean ± SEM	7.0 ± 0.9	2.6 ± 0.2		
Deficient				
1	2.3	1.6	6.6	0.87
2	3.7	2.0	6.4	0.98
3	2.4	1.9	7.5	1.23
4	4.7	nd†	7.9	0.94
5	3.5	2.1	8.7	0.94
6	4.6	2.4	8.3	0.95
Mean ± SEM	3.5 ± 0.4[a]	2.0 ± 0.2[b]		

*Half-life (minutes) and pool size were calculated as described in Methods and materials. Subjects had been on their respective diets for 3 weeks.

[a]$P < 0.004$; [b]$P < 0.05$.

†Unable to calculate pool size because steady state was not achieved after 1-hour infusion.

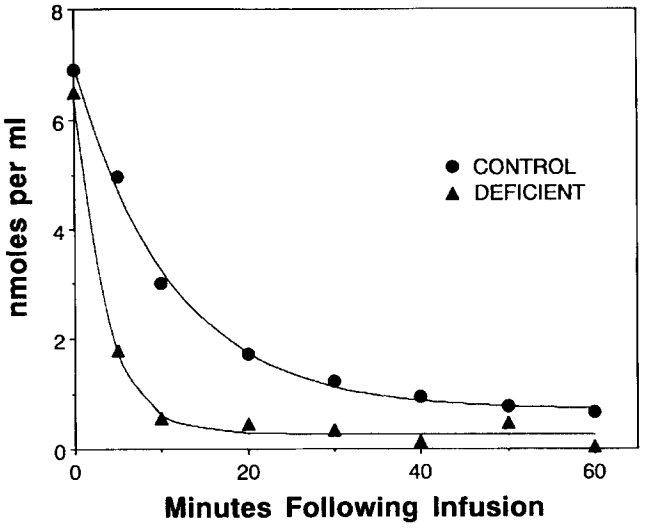

Figure 1 The disappearance of choline from plasma following the intravenous administration of ($d_9$methyl)-choline chloride. Subjects ingested either a choline-containing or choline-deficient diet for 3 weeks. At the end of this period, an intravenous dose of ($d_9$methyl)-choline chloride was administered over 1 hour and blood samples collected at 0, 5, 10, 20, 30, 40, 50, and 60 minutes postinfusion. Isotopic enrichment was determined and disappearance from the plasma was calculated using nonlinear regression. Shown are data collected from control subject #4 and deficient subject #3.

The rate of appearance of unlabeled choline was not different between groups (control = 25.6 ± 2.8; deficient (n = 5) = 19.9 ± 3.5 μmoles/minute). The half-life of choline in plasma as measured by the disappearance of ($d_9$methyl)-choline chloride was 7.0 ± 0.85 minutes in controls and 3.53 ± 0.42 minutes in deficient subjects (n = 6) ($P < 0.004$; *Table 1; Figure 1*). The 95% confidence limits were: control = 5.33 to 8.67 minutes versus deficient = 2.68 to 4.32 minutes.

Estimates of the extracellular choline pool indicated that deficient subjects (n = 5) had decreased levels in this compartment compared with controls (*Table 1*; $P < 0.05$).

No ($d_9$methyl)-choline was found in the plasma phosphatidylcholine pool at the end of the infusion period nor 1 hour postinfusion (data from 10 infusion studies).

Discussion

The plasma half-life of an intravenously administered dose of choline was decreased by 50% in deficient subjects, supporting our hypothesis that choline uptake increases during choline deficiency. During infusion of choline at the rates administered in this study, the rate of appearance of unlabeled choline from tissues was not significantly altered due to a choline-deficient diet despite a 30% decline in plasma choline concentrations during the 3-week experimental period. Thus, although the rate of appearance and the infusion rate were not different between groups, the disappearance rate of choline was two times greater in deficient subjects. This may be explained by the drop in the extracellular pool size we observed. Our data also suggest that an intravenous load test similar to the one used in these studies could be

useful in assessing choline status in humans, although a larger sample size would be necessary to validate such a technique.

Our findings also provide new information regarding the turnover of the plasma choline pool in healthy subjects consuming a constant choline intake for 4 weeks. The finding of a half-life of 7 minutes in human subjects is in agreement with previous studies in the rabbit,[17] guinea pig,[14] cat,[18] and dog,[19] which indicated that the plasma half-life in these species is also short (approximately 1 to 2 minutes).

With the exception of plasma phosphatidylcholine level, we did not collect data regarding the metabolic fate of intravenously administered choline. In the guinea pig,[14] the major choline metabolite found in urine and plasma 1 hour after choline infusion was betaine. In tissues, major metabolites included betaine, phosphorylcholine, and phosphatidylcholine. Our data do not permit us to state which tissues were responsible for the increased uptake of choline in deficient humans. We did not measure urinary excretion of choline during the infusion period and cannot rule out the possibility that the rapid clearance from the plasma pool was due to increased renal uptake and excretion, resulting in limited retention of choline in deficient subjects. Data from both animal and human studies suggest that choline is indeed rapidly taken up by the kidney, where it is converted to betaine and excreted.[20] Thus, it seems reasonable to hypothesize that a pattern of metabolism and distribution similar to that observed in animals is true for humans. However, additional studies will be necessary to clarify this point.

The data presented have relevance to the clinical management of patients receiving total parenteral nutrition. Depressed plasma choline levels have been reported in this population in several studies, suggesting inadequate choline status.[2,3,5,7] Buchman et al.[10] have also demonstrated that the oral administration of phosphatidylcholine to patients receiving long-term total parenteral nutrition increases plasma choline concentration and decreases fatty infiltration of the liver. However, large doses of choline (as phosphatidylcholine) were required to achieve these results due to the decreased absorptive capacity of their subjects. The use of a parenteral choline supplement would provide more accurate delivery of choline and might also be better tolerated by such patients. Preliminary data were recently presented suggesting that intravenous choline may be of use in these patients.[21]

Choline can be supplemented in a variety of forms: the two most common are choline salts and phosphatidylcholine. Intravenous lipid emulsions contain small amounts of free choline and greater quantities of phosphatidylcholine.[2] A positive correlation between the amount of lipid administered and the plasma choline level has been observed.[2] However, this association could also be due, in part, to improved nutritional status and increased de novo choline synthesis and not solely from exogenous phosphatidylcholine. Indeed, Buchman et al.[7] have recently reported that phosphatidylcholine obtained from lipid emulsions was not sufficient to raise the free plasma choline level, indicating that phosphatidylcholine administered parenterally is metabolized differently from that supplied orally. These findings suggest that choline salts may be the most effective means of increasing plasma choline concentration when supplementation occurs intravenously.

Our findings indicate that the intravenous administration of a single dose of choline at a rate of 1 mmole per hour was not associated with toxicity. This dose, which represents approximately 10 to 15% of the usual daily choline intake of healthy humans,[22] was not sufficient to significantly raise the plasma choline level in either deficient or control subjects.

In summary, body choline pools were decreased in healthy, human subjects following 3 weeks of a choline-deficient diet. The half-life of a single dose of choline administered over an hour was also decreased by 50% in these subjects. The fate (i.e., metabolism, excretion) of intravenously administered choline was not addressed by these studies. However, the administered choline was not converted to phosphatidylcholine in plasma during the first 60 minutes following the infusion. In the present study, the rate of appearance of unlabeled choline was not altered by the ingestion of a choline-deficient diet for 3 weeks.

These results are important for several reasons. First, these data support the hypothesis that choline uptake is accelerated and that body pools are diminished following 3 weeks of a choline-deficient diet. Second, they provide information regarding the rapid turnover of plasma choline in healthy human subjects. Third, they suggest that an intravenous load test may be useful in assessing choline stores. Fourth, they indicate that choline can be administered safely in humans via the parenteral route, although more data are necessary to determine both the amount and the rate of administration of choline that will safely increase body pools of this nutrient.

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References

1. Zeisel, S.H. (1980). Choline. In *Modern Nutrition in Health and Disease*, (M. Shils and V.R. Young, eds.), p. 440–452, Lea & Febiger, Philadelphia, PA USA
2. Sheard, N.F., Tayek, J.A., Bistrian, B.R., Zeisel, S.H., and Blackburn, G.L. (1986). Plasma choline concentration in humans fed parenterally. *Am. J. Clin. Nutr.* **43,** 219–224
3. Burt, M.E., Hanin, I., and Brennan, M.D. (1980). Choline deficiency associated with total parenteral nutrition. *Lancet* **2,** 638–639
4. Shapira, G., Chawla, R.K., Berry, C.J., William, P.J., Roy, R.G.B., and Rudman, D. (1986). Cysteine, tyrosine, choline, carnitine supplementation of patients on total parenteral nutrition. *Nutr. Int.* **2,** 334–339
5. Chawla, R.K., Berry, C.J., Kutner, M.H., and Rudman, D. (1985). Plasma concentrations of transsulfuration pathway products during nasoenteral and intravenous hyperalimenation of malnourished humans. *Am. J. Clin. Nutr.* **42,** 577–584
6. Chawla, R.K., Wolf, D.C., Kutner, M.H., and Bonkovsky, H.L. (1989). Choline may be an essential nutrient in malnourished patients with cirrhosis. *Gastroenterology* **97,** 1514–1520
7. Buchman, A.L., Moukarzel, A., Jenden, D.J., Roch, M., Rice, K., and Ament, M.E. (1993). Low plasma free choline is prevalent in patients receiving long term parenteral nutrition and is associated with hepatic aminotransferase abnormalities. *Clin. Nutr.* **12,** 33–37
8. Haubrich, D.R., Wang, P.F.L., Chippendale, T., and Proctor, E. (1976). Choline and acetylcholine in rats: effect of dietary choline. *J. Neurochem.* **27,** 1305–1313
9. Zeisel, S.H., DaCosta, K.A., Franklin, P.D., Alexander, E.A., LaMont, T., Sheard, N.F., and Beiser, A. (1991). Choline, an essential nutrient for humans. *FASEB J.* **5,** 2093–2098
10. Buchman, A.L., Dubin, M., Jenden, D., Moukarzel, A., Roch, M.H., Rice, K., Gornbein, J., Ament, M.E., and Eckhert, C.D. (1992).

Lecithin increases plasma free choline and decreases hepatic steatosis in long-term total parenteral nutrition patients. *Gastroenterology* **102,** 1363–1370

11. FASEB Life Sciences Research Office (1975). Evaluation of the health aspects of choline chloride and choline bitartrate aspects as food ingredients. Report #PB-223 845/9. Bureau of Foods, Food and Drug Administration, Department of Health, Education, and Welfare, Washington, DC USA
12. Agut, J., Font, E., Sacristan, A., and Ortiz, J.A. (1983). Dissimilar effects in acute toxicity studies of CDP-choline and choline. *Arzneim.-Forsch./Drug Res.* **33,** 1016–1018
13. Zeisel, S.H. (1987). Phosphatidylcholine: Endogenous precursor of choline. In *Lecithin,* (I. Hanin and G.B. Ansell, eds.), p. 107–120, Plenum Press, New York, NY USA
14. Haubrich, D.R., Wand, P.F., and Wedeking, P.W. (1975). Distribution and metabolism of intravenously administered choline(methyl-^{3}H) and synthesis in vivo of acetylcholine in various tissues of guinea pigs. *J. Pharm. Exp. Ther.* **193,** 246–255
15. Pomfret, E.A., DaCosta, K.A., Schurman, L.L., and Zeisel, S.H. (1989). Measurement of choline and choline metabolite concentrations using high-pressure liquid chromatography and gas chromatography-mass spectrometry. *Anal. Biochem.* **180,** 85–90
16. Bligh, E.G. and Dyer, W.J. (1959). A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* **37,** 911–917
17. Gardiner, J.E. and Gwee, M.C.E. (1974). The distribution in the rabbit of choline administered by injection or infusion. *J. Physiol.* **239,** 459–476
18. Gardiner, J.E. and Paton, W.D.M. (1972). The control of the plasma choline concentration in the cat. *J. Physiol.* **227,** 71–86
19. Bligh, J. (1953). The role of the liver and the kidneys in the maintenance of the level of free choline in plasma. *J. Physiol.* **120,** 53–62
20. Rennick, B., Acara, M., and Glor, M. (1977). Relations of renal transport rate, transport maximum, and competitor potency for tetraethylammonium and choline. *Am. J. Physiol.* **232,** F443–447
21. Buchman, A.L., Dubin, M. Moukarzel, A.A., Jenden, D., Roch, M., Rice, K., Gornbein, J., and Ament, M.E. (1993). *Gastroenterology* **104,** A881
22. Zeisel, S.H., Growdon, J.H., Wurtman, R.J., Magil, S.G., and Logue, M. (1980). Normal plasma choline responses to ingested lecithin. *Neurology* **30,** 1226–1229