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Dietary choline supplementation in rats increases carnitine concentration in liver, but decreases plasma and kidney carnitine concentrations

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Choline and choline-containing compounds are used in the treatment of certain neurological disorders. Limited information is available regarding the metabolic consequences of choline supplementation. The effect of both acute and chronic dietary choline supplementation on carnitine concentration in plasma, urine and tissues was studied in adult, male Sprague-Dawley rats. Eight groups of rats (n = 6/group) were assigned to four timepoints and two diets. Carnitine concentrations in choline supplemented (CS) (10 g choline chloride/kg diet) rats were measured after 1, 3, 21, and 42 days and results compared to control (C) animals (1 g choline chloride/kg). The carnitine concentration of plasma, urine, liver, heart, kidney, and gastrocnemius muscle was determined by radioenzymatic assay. Urinary choline and betaine levels were analyzed by single proton NMR spectroscopy. Both choline and betaine excretion were higher in CS animals throughout the study period. A single choline supplemented feeding (one 3 h meal) produced no significant changes in carnitine concentrations 3 h post prandial. Plasma and kidney carnitine concentrations increased over time in both dietary groups (P = 0.0001, P = 0.0004, respectively) and choline supplementation depressed concentrations in both of these pools when compared to controls (P = 0.0001, P = 0.006, respectively). No effect on urinary carnitine excretion was observed. Heart carnitine concentration increased over the course of the study in both dietary groups (P = 0.006). Carnitine concentration in liver was increased due to supplementation (P = 0.016). We conclude that the administration of a single, large dose of choline in rats does not alter carnitine concentrations during the first 3 h post prandial. Chronic choline administration decreased plasma and kidney carnitine concentrations and increased liver concentration. Choline supplementation influences the distribution of carnitine in specific body compartments, perhaps, by influencing the the transport of carnitine into or out of the liver. (J. Nutr. Biochem. 8:68–73, 1997.) © *Elsevier Science Inc. 1997*

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Introduction

Choline and the phospholipid phosphatidylcholine are used in the treatment of diseases of the nervous or cardiovascular system.[1] These compounds are also a popular nutritional supplement among members of the lay public. Ingestion of these compounds results in increased blood and brain choline levels and augmented synthesis of acetylcholine by neurons.[2] Choline supplementation, however, is beneficial only in certain ranges, and megadoses of oral choline are not benign.[3] Established side effects include gastrointestinal intolerance and cholinergic symptoms.[1]

Recently, several investigators have reported that long-term choline supplementation results in decreased urinary carnitine excretion in healthy adults, although plasma carnitine concentration was not altered in these subjects.[4,32] In guinea pigs,[4] choline supplementation also decreased urinary carnitine excretion, but increased plasma carnitine. In

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choline deficiency, the concentration of carnitine in the liver, heart, and muscle are decreased, whereas plasma and urine carnitine concentrations are elevated.[5,6] These data support the hypothesis that dietary choline alters carnitine metabolism and/or tissue distribution.

The objective of the present study was to investigate the effect of both short- and long-term oral choline supplementation on the concentration of carnitine in urine, plasma, and tissues of the adult rat. Animals were fed either a control diet (C) or a choline supplemented (CS) diet (10 times the choline concentration of control diet) for varying lengths of time (1, 3, 21, or 42 d), in 3-hr mealtime settings. Urine, plasma, liver, heart, kidney, and gastrocnemius muscle were collected and the carnitine concentration determined. Changes in urinary choline and betaine excretion were also measured.

Methods and materials

Animals and diets

Forty-eight, male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA, USA), 40 days of age were placed in individual wire-bottom cages in a 23°C room. To permit a more convenient feeding pattern, the 12-hr light cycle was reversed (21:00 hr - 09:00 hr light). All animals were fed the control diet (*Table 1;* ICN Biochemicals, Cleveland, OH, USA) for 2 weeks after their arrival. Meals were time restricted. Water was provided 24 hr per day throughout the study. During the initial 2-week period, animals adapted to the synthetic diet and were trained to reduce their feeding time from 24 hr to 3 hr (between 09:00 hr and 12:00 hr). On day 14, the animals were divided into four groups (days 1, 3, 21, and 42). Each group was subdivided into either a control (C; 1 g choline chloride/kg diet; 7.16 mmol free choline/kg diet) or a CS group (10 g choline chloride/kg diet; 71.6 mmol free choline/kg diet), with six animals per subgroup. This protocol was approved by the Institutional Animal Care and Use Committee at the University of Massachusetts.

Animals from each dietary treatment group ($n = 6$) were killed after 1, 3, 21, or 42 days. Except for rats in the acute study (day 1), each rat was placed in a metabolic cage for 24 hr before killing; urine was collected and food intake was recorded. Day 1 rats were placed in metabolic cages at the beginning of the 3-hr meal, and for 3 hr after the meal. Three hr after the 3-hr feeding ended, animals were anesthetized by injection with sodium pentobarbital (13 mg/100 g body wt (BW); Anthony Products Co., Arcadia, CA, USA). Animals were exsanguinated by cardiac puncture. Heparinized blood and urine were kept on ice until centrifugation (1400 × *g* for 13 min at 10°C). Liver, heart, kidney, and gastrocnemius muscle were removed, rinsed in ice cold saline (9 g/L sodium chloride), trimmed of fat, weighed, and immediately frozen in liquid nitrogen. Urine and plasma were stored at −20°C and tissues were kept at −80°C until analysis.

Table 1 Diet composition

Ingredient	g/kg
Vitamin-free casein	100
Sucrose	600
Lard	200
Alphacel	50
Mineral mix[1]	40
Vitamin mix[2]	10

[1]Mineral mix supplied the following (g/kg mineral mixture): Calcium carbonate, 2.10, copper sulfate, 0.0039; ferric phosphate, 0.0147; manganous sulfate, 0.0035; potassium aluminum sulfate, 0.0009; potassium chloride, 1.20; potassium phosphate monobasic, 3.10; potassium iodide, 0.0005; sodium chloride, 1.05; sodium flouride, 0.0057; tricalcium phosphate, 1.49; zinc chloride, 0.0002; chromium potassium sulfate, 0.0006; sodium selenite, 0.00001.
[2]American Institute of Nutrition[31]; Control diet contained 1 g choline chloride/kg and an additional 9 g sucrose/kg of diet; choline supplemented diet contained 10 g choline chloride/kg of diet.

Carnitine analysis

Tissue samples were cut into small pieces on a watch glass and gently ground. Eighty mg of broken tissue were weighed into a plastic scintillation vial and 1.5 mL of formic acid/ acetone solution (0.4 mol/L) were added to extract free and esterified carnitine. This mixture was homogenized, using a Virtis homogenizer (Virtis Company, Gardiner, NY, USA). The homogenate was incubated for 12 to 24 hr at −20°C. After centrifugation, duplicate samples were drawn from each vial and dried under vacuum (Savant Vacuum Drier, Savant Instruments Inc., Framingdale, NY, USA). Samples were resuspended in potassium phosphate buffer. Carnitine esters were hydrolyzed using potassium hydroxide and an aliquot was analyzed, along with carnitine standards. Plasma and 24-hr urine samples were centrifuged (1400 × *g* for 13 min at 10°C), carnitine esters were hydrolyzed, and total carnitine was assayed using the radioenzymatic method described by Borum et al.[7]

Other biochemical analysis

Liver lipid concentration was measured gravimetrically using the method of Folch et al.[8] Liver and muscle protein were determined spectrophotometrically by the method of Bradford.[9] Urinary creatinine was quantitated by a colorimetric assay that utilized the Jaffé reaction (Catalog no. 555, Sigma, St. Louis, MO, USA).

Choline and betaine determination

Urinary choline and betaine were quantitated using ^{1}H-NMR spectroscopy as described by Iles et al.[10] and Davies et al.[11] Urine samples were thawed and centrifuged (6500 × *g* for 10 min). Five hundred μL of supernatant were mixed with 50 μL of 99.8% D_2O (New England Nuclear, Boston, MA, USA) to lock the magnetic field, along with 20 μL of 500 mmol/L 3-trimethylsilyl-2,2,3,3-tetradeuteropropionate sodium salt ($TMSPd_4$) (New England Nuclear), which acted both as a chemical shift reference and as an internal standard for integration. The mixture was transferred to a 5-mm NMR tube (Kontes, Vineland, NJ, USA) for injection into the spectrometer. External standards of creatinine, choline, and betaine were prepared in water; the pH was adjusted with potassium hydroxide to agree with the pH of the urine samples. Samples were run at room temperature on a Bruker AC200 spectrometer (Bruker, Karlsruhe, Germany) operating at a frequency of 200.133 MHz. A single 15-us pulse was used with a pulse angle of 90° and an acquisition time of 2.67 sec. The water signal was suppressed by presaturation during the relaxation delay (2 sec).

Statistical evaluation

Data are expressed as mean ± SEM of six observations, unless otherwise specified. The data from day 1 were compared using the Student's *t*-test, whereas all other timepoints were analyzed by two-way analysis of variance (ANOVA) (main effects of diet and time) using SAS general linear model program (SAS Institute Inc.,

Cary, NC, USA). A value of $P \leq 0.05$ was used to determine statistical significance.

Results

Animal growth and choline intake

Although the rate of growth slowed during the initial period of adjustment to meal feeding, average growth rates during the experimental period were 5 to 6 g/day regardless of dietary regimen. Average daily food intake in the present study (range 9 to 18 g/day over the course of the experimental period) was not different from our previous studies in which animals ingested a synthetic diet containing 10% protein. Final mean BW were not different between C and CS subgroups (*Table 2;* day 1: C = 220 ± 4.6, CS = 216 ± 3.7 g), nor did average daily food intake differ between C and CS animals (data not shown). Choline intake for the CS groups ranged between 3.93 ± 0.21 and 4.54 ± 0.31 mmol/kg BW, and was approximately 10 times the intake of C groups (range: 0.38 ± 0.01 to 0.58 ± 0.09 mmol/kg BW). On day 1, kidney wet weight (g/100 g BW) was greater in CS animals (C = 0.73 ± 0.02, CS = 0.81 ± 0.02; *t*-test, $P = 0.013$), although dry weight was not different between dietary groups. Similarly, liver wet weight was also increased in CS animals on day 1 (C = 3.78 ± 0.10; CS = 4.07 ± 0.08; *T*-test, $P = 0.048$). Mean organ weights from day 3, day 21, and day 42 are shown in *Table 2*. BW increased with time ($P = 0.0001$), whereas liver and kidney weights (expressed as g wet weight/100 g BW) decreased over time in both groups ($P = 0.0002$ and $P = 0.0001$, respectively). Heart weight also decreased over time, although this effect did not reach statistical significance ($P = 0.07$). CS rats demonstrated a trend toward higher kidney ($P = 0.07$) and heart ($P = 0.053$) weights (g/100 g BW).

Fat concentration of liver and protein concentration of liver and muscle

Liver fat decreased with the age of the animals (2-way ANOVA; main effect of time; $P < 0.001$), but no effect of diet was observed ($P = 0.153$). Values in younger animals (day 1:198; day 3:197 mg/g dry weight; $n = 12$ per group) were higher than those in older age groups (day 21:136, day 42:153 mg/g dry weight; $n = 12$ per group). Liver and muscle protein concentrations were not different between C and CS subgroups at any timepoint (data not shown).

Urinary creatinine, choline, and betaine

Creatinine excretion (mg 100 g $BW^{-1}24\ hr^{-1}$) did not differ between C and CS subgroups (*Table 3*). Chemical shifts obtained by our choline and betaine standards were consistent with the results of Davies et al.[11] Choline supplementation increased choline excretion at every timepoint (*Table 3*). Betaine excretion (μmol/mg creatinine) in choline supplemented rats was 10-fold greater than in control rats on day 1 and day 42, and 20-fold on day 3 and day 21 (*Table 3*).

Carnitine concentrations in urine, plasma and tissues

Carnitine concentrations in urine, plasma and tissues were not altered by a single feeding of a CS diet. In chronically fed rats, urinary carnitine excretion was not affected by either time or dietary treatment (*Table 4*). Plasma carnitine concentration (μmol/L) increased with time ($P = 0.0001$) and was lower in CS animals ($P = 0.0001$; *Table 4*).

Choline supplementation increased liver carnitine concentration (nmol/g wet wt; $P = 0.016$) (*Table 5*). A trend toward increasing carnitine concentration with time was also observed in this organ ($P = 0.059$). Carnitine concentration increased over time in kidney ($P = 0.0004$) and was lower in CS animals ($P = 0.006$; *Table 5*). Heart carnitine concentration increased with time ($P = 0.006$; *Table 5*). Neither time nor dietary treatment altered muscle carnitine concentration.

Discussion

In the present investigation, we supplemented a synthetic diet with choline chloride and examined the effects on car-

Table 2 Body and organ weights of rats fed a control (C) or choline supplemented (CS) diet for 3, 21, or 42 days

Group	Body Weight g	Liver	Kidneys g/100 g BW	Heart
Day 3				
C	209	3.86	0.70	0.32
CS	211	3.81	0.77	0.32
Day 21				
C	242	3.60	0.66	0.30
CS	241	3.53	0.68	0.32
Day 42				
C	278	3.24	0.64	0.28
CS	267	3.24	0.64	0.31
Pooled SEM	6.5	0.12	0.018	0.012
ANOVA (2-way)	Time	Time	Time	
P	0.0001	0.0002	0.0001	

Values are means ± SEM of six animals.
BW, body weight.

Table 3 Concentration of creatinine, choline, and betaine in urine

Group	Creatinine mg/100g wt/24 hr	Choline μmol/mg creatinine	Betaine μmol/mg creatinine
Day 1			
C	3.25 ± 0.55	0.35 ± 0.08 ($n = 4$)	0.45 ± 0.08 ($n = 4$)
CS	2.79 ± 0.30	6.71 ± 4.25 ($n = 5$)	4.23 ± 1.75 ($n = 5$)
Day 3			
C	2.65 ± 0.23	0.17 ± 0.02 ($n = 3$)	0.40 ± 0.20 ($n = 4$)
CS	2.62 ± 0.26	0.56 ± 0.09 ($n = 5$)	9.87 ± 3.21 ($n = 6$)
Day 21			
C	3.22 ± 0.22	0.10 ± 0.02 ($n = 3$)	0.18 ± 0.07($n = 3$)
CS	2.90 ± 0.13	1.38 ± 0.77 ($n = 4$)	5.28 ± 1.66 ($n = 6$)
Day 42			
C	3.13 ± 0.13	0.09 ± 0.03 ($n = 3$)	0.16 ± 0.01 ($n = 3$)
CS	3.11 ± 0.29	1.12 ± 0.77 ($n = 5$)	2.29 ± 0.93 ($n = 6$)

Values are means ± SEM for six rats, unless otherwise noted.

Table 4 Urine and plasma carnitine concentration in rats fed a control or choline-supplemented diet for 3, 21, or 42 days

Group	Urine nmol/mg creatinine	Plasma μmol/L
Day 3		
C	38.2	58.3
CS	27.7	39.6
Day 21		
C	34.9	52.3
CS	31.6	46.8
Day 42		
C	42.6	77.7
CS	40.7	60.7
Pooled SEM	6.69	3.73
ANOVA (2-way)		Diet, time
P		0.0001, 0.0001

Values are means ± SEM of six rats.

nitine concentrations in urine, plasma, and various tissues. We also examined the effect of duration of choline supplementation on carnitine concentration in tissues. Four different timepoints were chosen in an attempt to characterize acute and chronic effects of choline supplementation.

In this study, we used mature rats (age 54 days at the beginning of experimental period) that were still rapidly growing. We chose this age group because we wished to study the effects of choline supplementation on carnitine concentrations in a variety of tissues, including muscle. Use of older, more slowly growing animals might have limited our ability to observe an effect of this dietary treatment on tissues, such as muscle, which contain large pools of carnitine. Thus, our results may not be generalizable to all adult rats.

Rats were trained to eat the experimental diets over a 3-hr period. We chose to use meal feeding so that we would be able to examine whether the effect of an acute/single dose (day 1) differed from the effects of chronic (days 3, 21, and 42) supplementation. In addition, meal feeding the CS diet is more similar to human supplementation studies in which choline is provided in a single, large dose.

The level of dietary choline supplementation used in our study was within the range of previous oral supplementation studies in rats, but greater than others.[4,12,13] In the present study, the average daily intake of choline was 7 to 10 times greater in supplemented animals compared to controls (4.0–4.5 versus 0.4–0.6 mmol/kg body wt). Choline supplementation at this level had no adverse effect on the animals as measured by their appearance, food and water intake, or BW. This finding is consistent with a previous study[14] in which rats received 10 mmol/kg BW and did not exhibit any toxic side effects of choline supplementation. Cohen and Wurtman,[2] however, observed decreased food intake in rats consuming larger doses of choline (14 mmol/kg BW). Large doses of orally administered choline can saturate intestinal transport systems, leading to decreased absorption and increased degradation by colonic microorganisms, resulting in less choline available for tissues.[15] However, orally administered choline at doses less than 7 mmol/kg BW increase trimethylamine and trimethylamine oxide excretion in the urine only modestly.[13] Our largest intake averaged 4.5 mmol/kg BW, thus making it unlikely that much of the dose administered was wasted by gut microflora.

Approximately 65% of a single dose of choline chloride (1.5 or 15 mmol/kg BW), administered orally, is absorbed from the intestinal lumen after 3 hr.[13] Animals in our study were killed 3 hr after the CS meal ended. Thus, we examined the metabolic response to choline supplementation after 2/3 of the choline dose had been absorbed and a marked elevation (50 to 100%) in plasma choline concentration had been achieved.[2,16]

The urinary excretion of both choline and its metabolite betaine was greater in CS animals than in controls. Although the kidney rapidly clears choline from the blood, choline is not commonly found in the urine.[17,18] Most choline transported into the kidney is metabolized to and excreted as betaine.[19] Only when choline oxidase is saturated are large amounts of choline found in the urine.[20] Our results indicate that on day 1 kidney choline oxidase was saturated in supplemented animals as significant amounts of free choline were excreted in the urine. By day 3, however, more betaine than choline was excreted in the urine of supplemented rats, suggesting that either choline oxidase activity in the kidney had increased or, alternatively, that plasma betaine concentration was increased, most likely from choline oxidation in the liver. Such a hypothesis is consistent with previous investigations that demonstrated that betaine production varies with the level of dietary choline.[21,22]

In the present study, some osmotically active compound (choline, betaine, methylamines) may have accumulated in the kidney of CS animals on day 1, resulting in increased water retention in this organ.[23] This hypothesis would explain the greater kidney wet weight that we observed in the supplemented group on this day. We did not observe this finding at later timepoints.

Plasma carnitine concentrations increased with age in both dietary groups. This finding is consistent with the results of Borum et al.,[24] who described a steady increase in plasma carnitine concentration between 50 and 90 days of age in male rats maintained on a carnitine-free diet. Animals in the present study were 54 to 96 days of age at the time our

Table 5 Liver, kidney, heart, and muscle carnitine concentrations in rats fed a control or choline-supplemented diet for 3, 21, or 42 days

Group	Liver	Kidney	Heart nmol/g wet weight	Muscle
Day 3				
C	272	612	741	778
CS	270	362	856	769
Day 21				
C	300	401	749	762
CS	367	413	806	739
Day 42				
C	257	626	900	761
CS	348	560	1063	825
Pooled SEM	25.1	41.3	68.0	34.9
ANOVA (2-way)	Diet	Diet, Time	Time	
P	0.016	0.006, 0.0004	0.006	

Values are means ± SEM of six rats.

measurements were made. Carnitine concentration in plasma was higher in controls than supplemented rats, although plasma values in supplemented animals were still within the normal range. This finding is opposite of the effect of choline deficiency on carnitine concentration in plasma, ie., increased plasma carnitine concentration in deficient rats.[5,6]

Changes in renal carnitine concentration paralleled those observed in plasma, ie., kidney carnitine concentration increased with time (age) and decreased with choline supplementation. Renal carnitine response is closely related to changes in plasma carnitine concentration.[25] Thus, the decrease in plasma carnitine concentration that we observed is the likely cause of depressed kidney carnitine concentration in supplemented rats.

We observed no effect of either diet or time on urinary carnitine excretion. This finding is somewhat paradoxical in light of the significant effects of choline supplementation on plasma and kidney carnitine concentrations. However, it might be explained by the ability of the kidney to conserve carnitine when dietary intake is low.[25] In the present study, both dietary treatments were devoid of carnitine and only small amounts of carnitine were excreted in both C and CS animals. Therefore, it seems reasonable to suggest that in both groups, the kidney was actively conserving carnitine, thereby minimizing any potential effect of choline supplementation on urinary carnitine excretion. The finding of similar carnitine excretion rates indicates that plasma carnitine concentrations in CS rats were not lowered due to increased renal loss of carnitine.

Our findings of decreased plasma carnitine concentration and no change in urinary carnitine excretion are not in agreement with the data of Daily et al.[4] These investigators observed a decrease in urinary carnitine excretion after 20 days of choline supplementation and no effect on plasma carnitine concentration. The lack of agreement between the two studies can be explained, in part, by the differences in experimental design. In the present study, animals received a synthetic diet devoid of carnitine and the level of choline supplementation was approximately 4 times that of Daily et al.[4] Indeed, when we fed rats a diet that contained a lower amount of choline (0.45 g/kg), we also found no effect on plasma or urinary carnitine concentrations.[26] In addition, the current study involved meal feeding and was of longer duration (6 weeks versus 3 weeks).

Heart carnitine concentration increased with time. Within 6 weeks, heart carnitine nearly doubled, a finding that agrees with the data of Borum.[24] Choline deficiency depresses heart carnitine concentrations[5,6] and choline supplementation in deficient animals normalizes it.[5,30] No effect of choline supplementation on carnitine concentration in muscle or heart was observed in the present study.

Dietary choline that is absorbed enters the circulation through the portal vein.[3] Liver has a very active transport system for choline that is not saturated at normal plasma choline concentrations.[27,28] Therefore, the effects of oral choline supplementation might be expected to occur first in this organ. We found no effect of a single, high-choline meal on liver carnitine concentration; however, an overall increase in liver carnitine due to choline supplementation was observed. Previous reports have shown that choline deficiency decreases carnitine concentration in liver.[5,6] Thus, our data suggests that, in the liver, dietary choline supplementation may have an effect opposite to that observed during choline deficiency.

In the rat, carnitine is synthesized predominantly by the liver, and to a limited extent in the testes, or can be acquired from the diet.[29,33] In the present study, there was no dietary intake of carnitine and therefore, liver was the primary supplier of carnitine for plasma and tissues. The increase in liver carnitine concentration that we observed could result from an increase in the de novo synthesis of carnitine by this organ, or from a change in the transport into or out of this tissue. The precursor for carnitine is protein-bound trimethyllysine, which is the product of protein turnover in tissues (predominantly muscle). Thus, for an increase in the biosynthesis of carnitine to occur, an increase in the rate of protein breakdown would be required. Alternatively, an increase in the concentration of protein-bound trimethyllysine could result in an increase in the amount of precursor for carnitine synthesis. However, we are aware of no data that suggest or support either of these hypotheses. In addition, the findings of decreased plasma carnitine concentration and no change in heart and muscle carnitine content indicate that carnitine synthesis was not increased due to choline supplementation. Rather, the increase in liver and concomitant decrease in plasma carnitine concentration suggest that carnitine transport into or out of the liver was altered by choline supplementation. Such an effect of choline on carnitine transport is supported by findings in choline-deficient animals in which a single dose of choline increased the carnitine concentration in liver and decreased the concentration in plasma within 1.5 hr.[5] However, in the present study, we did not observe an acute effect of choline supplementation on liver carnitine levels as these authors did. It may be that their findings are specific to choline deficient animals. We propose that the increase in liver carnitine concentration observed in this study during chronic choline supplementation is the result of a change in membrane structure (via phosphatidylcholine) that altered carnitine transport, or, alternatively, that a metabolite of choline, such as betaine, mediated the effect that we observed.

In conclusion, we found that oral supplementation with choline chloride did not influence dietary intake, growth, or liver fat in adult rats. Urinary choline and betaine excretion were greater in supplemented animals. A single, high-choline feeding produced no changes in carnitine concentrations in liver, plasma, urine, kidney, heart, or gastrocnemius muscle. Carnitine concentrations in plasma, kidney, and heart increased over time in both C and CS animals, and a similar trend was noted in liver. Choline supplementation lowered carnitine concentrations in plasma and kidney, and increased levels in liver. No effect on urinary carnitine excretion or muscle or heart carnitine concentration was observed. Although the finding of increased carnitine concentration in liver is consistent with the hypothesis that carnitine synthesis is increased due to chronic choline supplementation, depressed plasma and kidney and normal heart and muscle carnitine concentrations are not consistent with such a theory. It is more likely that chronic choline supplementation alters carnitine transport into or out of

liver. Further studies are necessary to determine the mechanism of choline's effect on carnitine distribution in rats.

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