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Choline supplementation increases tissue concentrations of carnitine and lowers body fat in guinea pigs

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It has been documented that choline supplementation results in urinary conservation of carnitine in both humans and guinea pigs. This conservation in guinea pigs is associated with increased concentrations of carnitine in skeletal muscle for which no functional consequences have been reported. The objective of this study was to evaluate changes in fat metabolism and body composition as a consequence of the increased tissue carnitine in choline-supplemented guinea pigs. Guinea pigs were given free access to commercial diet without or with 3 g choline/kg diet. Using indirect calorimetry, the respiratory exchange ratios (RER) of the animals were determined under normal, exercise, and unfed conditions. There were no differences in RER between supplemented and nonsupplemented groups under any of the conditions. The RER data lead to the conclusion that choline–carnitine did not promote oxidation of fat over carbohydrates for energy. However, proximate analysis of carcass revealed significantly lower total body fat and higher body proteins in the choline-supplemented animals compared with the nonsupplemented animals. These apparently contradictory results are explained by the hypothesis that the acetates generated by the β-oxidation of fatty acids are transferred to carnitine and not oxidized to carbon dioxide, resulting in little or no shift in RER. (J. Nutr. Biochem. 9:464–470, 1998) © *Elsevier Science Inc. 1998*

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Introduction

Relationships between choline and carnitine have been studied for many years, but the focus has been primarily on the impairment of carnitine status in choline deficiency.[1–4] Choline deficiency decreases fatty acid oxidation and carnitine concentrations in liver, heart, and skeletal muscle.[1–3] The plasma and urinary carnitine concentrations are elevated at the expense of tissue carnitine in choline deficiency.[2,4] On the contrary, our studies have shown that in choline sufficient humans and animals, choline supplementation [20 mg/kg body weight (BW)] results in a significant decrease (50% of controls) in urinary excretion of carnitine.[5,6] Plasma carnitine concentrations are variably affected in humans,[5] but are increased in guinea pigs.[6] The carnitine concentrations are increased significantly in the skeletal muscle of guinea pigs.[6] The functional consequences of these changes have yet to be determined.

Various dosages and duration of carnitine supplementation have been used in attempts to improve athletic performance in several studies; however, the results are conflicting with regard to the effects on energy substrate utilization in normal individuals.[7–13] Vukovich et al.[7] reported that carnitine supplementation (6 g/d) for 2 weeks had no effect on either muscle carnitine concentrations or respiratory exchange ratio (RER) during 1 hour of submaximal exercise at 70% maximal oxygen consumption (VO_2max). However, Arenas et al.[8] demonstrated effects of carnitine supplementation (2 g/d) in a 120 day placebo-controlled experiment. In that study, long-distance runners had significantly decreased muscle carnitine concentration, but subjects given carnitine (2 g/d) had a significant increase in muscle carnitine after 6 months of training. A similar effect was seen in sprinters. The different effects on muscle carnitine

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between the two studies may be an indication that supplementation with smaller doses (2 g/day) over a long period of time (120 days) is more effective than higher doses (6 g/day) for a short time (14 days). Unfortunately, Arenas et al.[8] did not determine any functional effects such as RER, endurance, or other indicators of oxidative capacity. Other investigators have reported an effect of carnitine supplementation on exercise capacity or RER, but did not determine effects on muscle carnitine concentrations.[10–13]

The results of a recent study showed no effect of "moderate" carnitine depletion on exercise capacity in young rats.[13] In this study, the control group was fed a commercial pellet diet, and the carnitine-devoid group was given a carnitine free isocaloric liquid diet. The muscle carnitine concentration in the carnitine-devoid group was 48% of that in the control animals. However, the lower muscle carnitine did not result in a reduced swimming exhaustion time or in a reduced oxidation of ^{14}C-palmitate after an 8 hour fast. The authors concluded that moderate carnitine depletion does not affect exercise capacity or fatty acid oxidation. However, in this study only free and short-chain acylcarnitines were measured, and long-chain acylcarnitines were not determined. If there were a higher percentage of long-chain acylcarnitines in the carnitine-devoid group, then the differences in muscle carnitine content would be correspondingly smaller than reported values. In addition, if the body fat were higher in the carnitine-devoid group, it would have made them more buoyant, hence requiring less work to swim and no difference in endurance. The body composition data may help address this issue.

The objective of our study was to determine if choline-induced carnitine accretion in guinea pig muscle affects body composition and energy substrate utilization under normal, starvation, and exercise conditions as determined by indirect calorimetry and biochemical indices.

Methods and materials

The study protocol was approved by the Animals Care and Use Committee of the University of Tennessee. Twenty male Hartley guinea pigs (SASCO, Omaha, NE USA) weighing 514 ± 14 g BW were divided into two groups, nonsupplemented (NS) and choline supplemented (ChS) groups. All animals were given, free of choice, water and ground commercial diet (Purina Guinea Pig Chow no. 5025, Purina Mills, Richmond, IN USA) with its endogenous choline content (1.85 g/kg diet); the ChS group was given the same diet supplemented with choline (3 g/kg diet) as choline chloride. The same batch of commercial diet was used throughout the study to avoid differences in nutrients from one batch of diet to another. Thus, the choline content of the ChS diet was 4.85 g choline/kg diet. Animals were ordered in pairs and carried through the study as pairs, so that there would be minimal differences in age (9.7 ± 1.2 weeks). The animals that began as pairs for the starvation study were then used in the exercise study as a pair.

Experimental design: Starvation study

This study monitored gas exchange in all animals over an 8 hour period prior to and following the experimental diets under fed and unfed conditions. Animals had free access to food at all times except when being monitored for gas exchange during the unfed state. At 09:00 hours gas measurements were begun and continued for the next 8 hours. Each pair of guinea pigs was treated according to the following schedule:

Week 1: Animals were monitored for gas exchange twice for 8 hours each under fed conditions. Urine was collected for 24 hours following the gas measurements.
Week 2: Animals were monitored for gas exchange once for each 8 hour period under unfed conditions. Urine was collected for 24 hours following the gas measurements.
Weeks 3–4: Animals were fed the respective experimental diets.
Week 5: Animals were monitored for gas exchange twice for each 8 hours under fed conditions.
Week 6: Animals were monitored for gas exchange once for each 8 hours under unfed conditions.

Animals were placed in the sealed metabolic chamber for 20 minutes and expired gases were collected after 10, 15, and 20 minutes. The O_2 and carbon dioxide (CO_2) content of each gas collection was determined using an Ametek S-3A/II oxygen analyzer and Ametek CD-3A carbon dioxide analyzer (Ametek, Inc., Paoli, PA USA). Values for the three collections were averaged and used as the value for that 1 hour period. After each 8 hour gas exchange measurement the guinea pigs were placed in metabolic cages for 24 hour urine collections. Urine was collected and frozen at −20°C for analysis of carnitine and urea nitrogen.

Experimental design: Treadmill study

The treadmill study was designed to evaluate choline-induced changes in RER, VO_2, and endurance during submaximal exercise. The average age of the pairs of guinea pigs was 8.1 ± 1.3 months, and the average time on experimental diets was 5.5 ± 0.9 months. All animals were trained to run on the treadmill prior to starting the treadmill study. Each pair was trained on the treadmill for 10 minutes and at the same intensity to insure that no differences in training altered the endurance parameters between the groups for reasons other than treatment.

The treadmill (Columbus Instrument International, Inc., Columbus, OH USA) was set at a 5% incline with a speed of 15 m/min. This incline and speed was determined during trial runs to be equivalent to a "fast walk," which was an exercise work rate that could be tolerated for extended periods. Animals were placed in the still treadmill for 15 minutes prior to the running at the air-flow rate of 2.1 L/min through the unit. After 15 minutes equilibration time, the first gas sample was collected (time 0) and analyzed. Immediately following the first gas collection, the air flow through the chamber was increased to 3.8 L/min, and gas samples were collected and analyzed at 5 minute intervals throughout the entire running period (2 hours or until animal refused to run any longer). All animals were given a minimum of 3 days of recovery time between the exercise periods. The expired gases were analyzed as described earlier, and assumption and conversion factors were as suggested by Bursztein et al.[14]

After completion of the treadmill studies the animals remained on their respective diets for a minimum of 2 weeks before being sacrificed. The animals were anesthetized with methoxyflurane, blood was taken by cardiac puncture, and the animals were sacrificed by decapitation. Heart, liver, brain, kidney, and muscle were collected for carnitine assay and frozen at −80°C. Liver samples were processed for the carnitine palmitoyltransferase-I (CPT-I) activity.

Carnitine, urea nitrogen, and CPT-I assays

Samples were fractionated for free and acylcarnitines, and carnitine was determined by the radio-enzymatic method described previously.[15,16] Urea nitrogen was determined spectrophotometri-

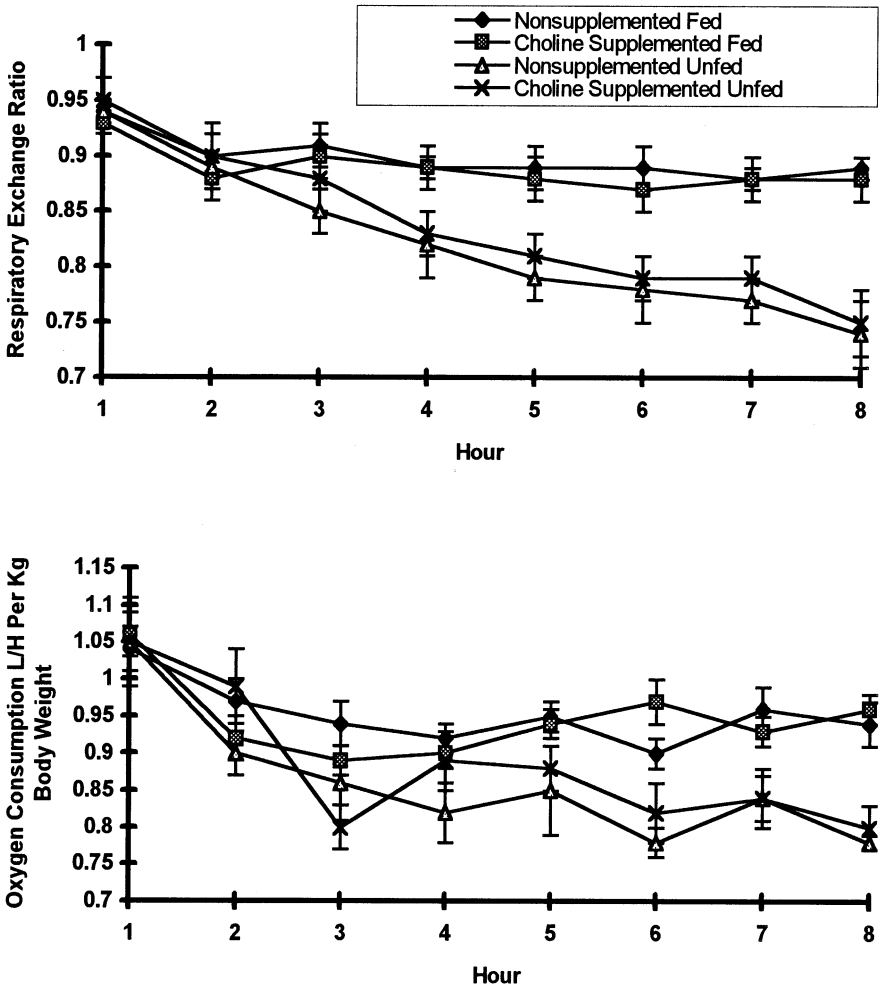

Figure 1 Postexperimental diet respiratory exchange ratios (RER; top) and oxygen consumption (bottom). Animals were either choline supplemented (ChS) or nonsupplemented (NS). Values are means for the group ± SEM. There were no differences between NS and ChS groups but RER and oxygen consumption were significantly lower after 4 hours and 6 hours, respectively, when food was withheld compared with fed states, as determined by analysis of variance and Student-Newman-Keuls test ($P \leq 0.05$, $N = 10$ when food withheld, $N = 20$ when fed).

cally (Sigma kit 66-20, Sigma, St. Louis, MO USA). The activity of CPT-I was determined in intact mitochondria from liver processed immediately following its removal from the animals. Mitochondria were prepared according the method of Johnson and Lardy,[17] protein was determined by Biuret assay, and the suspension was adjusted to 10 mg protein/mL. CPT-I activity was determined according to the method of Bremer.[18]

Proximate analysis of guinea pig carcasses

Guinea pig carcasses were thawed and shaved to remove hair. Large intestines, stomach, and caecum were removed. The carcasses were homogenized in a blender and the homogenate lyophilized for moisture determination. Aliquots of the dried homogenate were assayed for protein by the Kjeldahl method.[19] Percent fat was determined by Soxhlet extraction for 12 hours using hexane as the solvent. The percent fat in the sample was determined both by the amount of the fat recovered in the flask and by the loss of weight from the sample after extraction. The values obtained from the two methods were averaged for the reported fat percent. Mineral percent was determined by ashing an aliquot of the lyophilized sample in a muffle furnace at 650°C.

Statistics

The urinary excretion of carnitine in fed and unfed states was compared using an unpaired Student's *t*-test. Tissue carnitine concentrations, urinary excretion and plasma concentrations of carnitine, and CPT activity were compared using analysis of variance (ANOVA) and Student-Newman-Keuls test when the F-test indicated differences among groups. Running time between groups was compared using Student's *t*-test.

Results

There was no significant effect of choline supplementation on feed intake or BW during the study periods. The differences in the RER and oxygen consumption of the NS and ChS groups were not significant (*Figure 1*). However, the RER of both groups decreased from 0.95 to approximately 0.79 over the 8 hour starvation period.

The NS and ChS guinea pigs were run on a treadmill for either 2 hours or to exhaustion to evaluate if the added stress of exercise would reveal differences in energy substrate

Table 1 Urinary excretion of carnitine in nonsupplemented (NS) and choline-supplemented (ChS) guinea pigs at various states[1]

Treatment group	Carnitine (μmol/day)			
	NEC	ASAC	AIAC	Total[2]
Prior to starting on experimental diets				
NS-Fed (*N* = 8)	4.6 ± 0.7	2.8 ± 0.8	0.5 ± 0.1	7.8 ± 1.2
NS-Unfed (*N* = 8)	1.5 ± 0.3	19.4 ± 2.5	1.0 ± 0.2	22.0 ± 2.6
While on experimental diets				
NS-Fed (*N* = 8)	4.8 ± 0.7	2.5 ± 0.5	0.4 ± 0.4	7.6 ± 1.1
ChS-Fed (*N* = 8)	2.6 ± 0.7*	1.5 ± 0.4	0.4 ± 0.1	4.5 ± 1.0*
NS-Unfed (*N* = 8)	1.6 ± 0.2	21.7 ± 1.6	1.5 ± 0.5	24.7 ± 1.8
ChS-Unfed (*N* = 8)	2.2 ± 0.3	19.5 ± 1.8	1.3 ± 0.4	23.0 ± 1.7
2 Weeks following exercise				
NS-Fed (*N* = 8)	4.8 ± 1.0	2.1 ± 0.8	0.4 ± 0.1	7.2 ± 1.4
ChS-Fed (*N* = 4)	2.7 ± 1.1*	1.5 ± 0.4	0.2 ± 0.1	4.4 ± 1.3
US-Unfed (*N* = 8)	3.4 ± 0.6	13.8 ± 2.6	0.6 ± 0.1	17.9 ± 3.3
ChS-Unfed (*N* = 8)	4.3 ± 0.9	13.3 ± 2.9	0.7 ± 0.1	17.7 ± 2.6

[1]Nonsupplemented animals were fed ground commercial guinea pig diet; choline-supplemented animals were fed the same diet with 3 g/kg added choline from choline chloride. Food was withheld for 8 hours (for gas measurements) prior to placing the animals in the metabolic cages when unfed for 24 hour urine collections. Values are expressed as means ± SEM for the group of specified animals. Change in numbers per group occurred due to the loss of samples.
[2]NEC—nonesterified carnitine. ASAC—acid-soluble acylcarnitine. AIAC—acid-insoluble acylcarnitine. Total—total carnitine.
*Significant differences by Student's *t*-test.

utilization or endurance between the two groups. The RER was not different between the NS and ChS; however, their average running times were 55.7 ± 7.5 minutes and 72.4 ± 8.4 minutes, respectively (statistically not significant).

The urinary excretion of carnitine fractions under different conditions is shown in *Table 1*. Withholding of all diets (chow, NS, ChS) for 8 hours regardless of exercise resulted in higher urinary excretion of short-chain, or acid soluble, acylcarnitine (ASAC); long-chain, or acid insoluble, acylcarnitine (AIAC); and total carnitine (TC). The urinary excretion of nonesterified carnitine (NEC) and TC in the fed state was lower in the ChS group than NS group, which is consistent with our earlier findings. However, this effect of choline was masked when food was withheld for 8 hours. There were no significant differences in 24 hour nitrogen excretion of the NS and ChS groups (524 ± 53 and 471 ± 20 mg nitrogen/d/kg BW, respectively) during the starvation state.

Plasma carnitines were not significantly different between the NS and ChS groups under fed or unfed conditions; however, starvation resulted in lower NEC and higher ASAC with no change in TC (*Table 2*). ASAC in brain and TC in muscle were higher; however, the ASAC and TC were lower in the kidneys of guinea pigs in the ChS group compared with those in the NS group (*Table 3*).

The mitochondrial CPT activity in the NS unfed animals was higher than in the fed animals; however, this difference was not significant in the ChS animals (*Figure 2*). As an indication of how much of the activity was due to CPT-I as opposed to CPT-II, the malonylCoA inhibition was determined in all samples. The average maximum inhibition (Imax) for malonylCoA in the liver samples was 83%, indicating that most of the activity was due to CPT-I. The body composition data shows a significantly lower carcass fat but higher protein and ash content in the ChS than NS animals (*Table 4*).

Discussion

The primary objective of this study was to determine functional consequence of choline-mediated carnitine accretion in guinea pigs, an animal model of choice for studying the novel interactions of choline and carnitine. Guinea pig was preferred over rat because it responded akin to humans at equivalent doses of choline with regard to carnitine conservation,[6] perhaps because of lower choline oxidase activity in guinea pig than other species.[20] Choline supplementation resulted in a significant decrease in body fat (21.5%) and a significant increase in body protein (16%) and ash (19%) without significantly altering the live BW (*Table 4*). This is an important and unique consequence of choline–carnitine interactions because there was selective accretion of nitrogen and depletion of fat. These results

Table 2 Plasma carnitine in nonsupplemented (NS) or choline-supplemented (ChS) guinea pigs under fed or unfed states 2 weeks following exercise and 24 hours before sacrifice[1]

	Carnitine (μmoles/L)			
	NEC[2]	ASAC	AIAC	Total
NS-Fed	30.4 ± 3.5	9.0 ± 2.2	2.2 ± 0.3	41.6 ± 3.9
ChS-Fed	31.3 ± 4.0	8.0 ± 2.1	2.0 ± 0.3	41.3 ± 5.1
NS-Unfed	20.5 ± 1.3	17.2 ± 1.6	2.0 ± 0.2	39.7 ± 1.0
ChS-Unfed	20.7 ± 2.4	16.7 ± 2.5	1.8 ± 0.2	39.2 ± 4.7

[1]All animals were fed commercial guinea pig diet with or without 3 g/kg added choline from choline chloride. Food was withheld from half of the animals (Unfed) in each dietary treatment 24 hours prior to sacrificing them. The remainder were given free access to food. (Fed). All animals had completed the exercise study at least 2 weeks before being sacrificed. Values are expressed as means ± SEM for the group (*N* = 5) for all groups except for the fed ChS group (*N* = 4). The differences were not significant ($P < 0.05$), by Student's *t*-test.
[2]NEC—nonesterified carnitine. ASAC—acid-soluble acylcarnitine. AIAC—acid-insoluble acylcarnitine. Total—total carnitine.

Table 3 Tissue concentrations of carnitine in nonsupplemented (NS) and choline-supplemented (ChS) guinea pigs[1]

Tissue	Carnitine (nmol/g wet tissue)			
	NEC[2]	ASAC	AIAC	Total
Brain				
NS	85.2 ± 15.3	9.6 ± 3.5	20.0 ± 1.6	114.3 ± 20.9
ChS	100.3 ± 22.4	30.8 ± 6.7*	17.5 ± 3.1	148.6 ± 27.9
Liver				
NS	262.6 ± 50.5	5.8 ± 2.6	18.4 ± 4.8	286.8 ± 49.8
ChS	231.6 ± 32.9	8.4 ± 4.2	28.8 ± 2.2	268.8 ± 31.7
Heart				
NS	667.0 ± 84.6	719.9 ± 75.5	147.4 ± 27.4	1534.3 ± 74.8
ChS	660.9 ± 74.8	664.9 ± 111.1	163.6 ± 52.5	1489.2 ± 131.1
Kidney				
NS	444.8 ± 31.4	65.7 ± 14.3	22.7 ± 4.4	533.2 ± 24.4
ChS	393.4 ± 50.0	19.5 ± 9.3*	17.0 ± 4.2	430.7 ± 53.4*
Skeletal muscle				
NS	415.9 ± 44.6	215.7 ± 42.7	91.0 ± 16.2	722.6 ± 54.9
ChS	497.5 ± 80.5	299.1 ± 54.1	97.5 ± 21.4	894.1 ± 90.7*

[1]All animals were fed ground commercial guinea pig diet with or without 3 g/kg added choline from choline chloride. All animals had completed the exercise study at least 2 weeks prior to being sacrificed. Values are expressed as means ± SEM for the group ($N = 10$ for the NS group and $N = 9$ for the CHS group).
[2]NEC—nonesterifeid carnitine. ASAC—acid-soluble acylcarnitine. AIAC—acid-insoluble acylcarnitine. Total—total carnitine.
*Significant differences between the groups by Student's *t*-test ($P < 0.05$).

suggest that nutritional intervention may be better evaluated by a combination of BW and body composition than either parameter alone.

The decrease in body fat would have been expected to be a result of preferential utilization of fat for energy. However, the RER was not significantly altered by choline supplementation. The apparent contradiction may be due to lack of sensitivity of indirect calorimetry under the ChS conditions. It is quite possible that while β-oxidation of fatty acids was indeed promoted by carnitine-modulated entry of long-chain fatty acids into muscle mitochondria, the resulting acetyl moiety of the acetylCoA did not enter the TCA cycle, because it was scavenged by carnitine in the ChS group. Thus, the acetylcarnitine formed exited mitochondria, and as a consequence there was minimal oxidation of fatty acid carbons to CO_2 and no change in RER in the ChS group. This is possible because the fed ChS animals, unlike the unfed animals, did not develop critical deficiency of energy necessitating complete oxidation of fatty acids beyond acetate. This hypothesis is supported by a 39% increase in the concentration of ASAC in the skeletal muscle of the ChS animals (*Table 3*). Both skeletal muscle and brain of the ChS animals accumulated approximately 24 to 30% more total carnitine compared with the NS animals (*Table 3*). However, there were no corresponding changes in the plasma ASAC of the ChS animals (*Table 2*). It is important to note that tissue changes in carnitine fractions are not reflected in the plasma carnitine (*Table 2*), which is

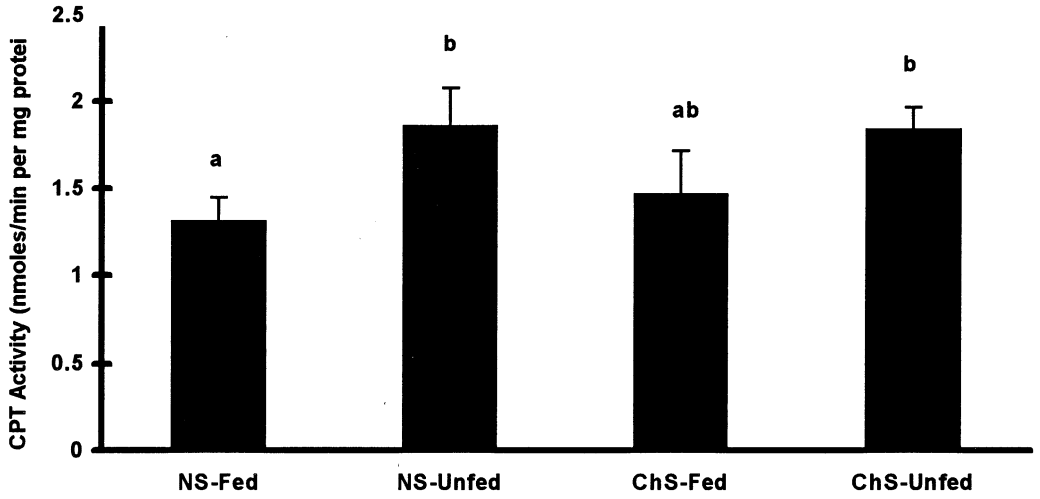

Figure 2 Activity of carnitine palmitoyltransferase-I (CPT-I) in liver of nonsupplemented (NS) or choline-supplemented (ChS) guinea pigs fed or unfed for 24 hours. Values are means for the groups ± SEM. Differences were evaluated by analysis of variance and Student-Newman-Keuls test ($P \leq 0.05$, $N = 5$). Different letters above bars indicate significant difference.

Table 4 Body composition of nonsupplemented (NS) and choline-supplemented (ChS) guinea pigs[1]

% Body mass	NS	ChS	*P*-value
Moisture	54.48 ± 1.01	55.13 ± 2.13	ns
Fat	26.34 ± 1.47	20.66 ± 1.57	0.006
Protein	16.17 ± 0.68	18.76 ± 1.19	0.03
Ash	3.86 ± 0.08	4.62 ± 0.22	0.002
Total	100.85 ± 0.75	99.17 ± 1.79	ns

[1]Values are the mean for the group ± SEM, $N = 10$ for the NS group and 9 for the ChS group. Statistical difference between groups was determined by Student's *t*-test.
ns—not significant.

often used for diagnostic purposes. On the other hand, the tissue carnitine accretions are reflected in a greater than 40% decrease in urinary carnitine of the fed ChS animals (*Table 1*).

When animals were unfed, energy derived from fat oxidation increased in magnitude. As a result urinary and plasma ASAC increased (*Table 1* and *Table 2*) and RER decreased during 8 hour starvation of both NS and ChS groups. Thus, the impact of supplementation, if any, is minor compared with the stress of starvation with regard to the magnitude of fat utilization.

The lack of effect of choline-mediated carnitine accretion on RER is similar to that in some of the carnitine supplementation studies reported by other investigators.[7–9,13] It may be a simplistic rationalization that a decrease in body fat is the result of increased fatty acid oxidation in mitochondria for which carnitine is essential. Carnitine has been shown to have additional functions including translocation of short- and medium-chain acyl groups from one body pool to another.[21,22] The intracellular carnitine pool helps maintain an optimal free/acylCoA ratio, which is important in the regulation of several important enzymes involved in intermediary metabolism, including pyruvate dehydrogenase.[21] Choline-induced increase in carnitine concentrations would affect the free/acylcarnitine ratio, which may affect both fat and carbohydrate oxidation,[21,23] resulting in little or no change in the RER.

Whether the guinea pig is a good animal model for the study of fatty acid oxidation is debatable. How efficiently guinea pigs utilize fat as an energy substrate is not well documented. The hepatic carnitine palmitoyltransferase-I (CPT-I) activity reported here for guinea pigs (*Figure 2*) is only approximately 25 to 30% of that reported for rat liver using the same assay.[24] This is not surprising, considering that the reported Km for carnitine in guinea pig liver CPT-I is 311 μmol/L, as compared with 37 μmol/L in rat and 39 μmol/L in human fetal liver.[25] Guinea pig liver CPT-I is also more sensitive to malonyl-CoA inhibition than is the CPT-I of rat liver (I_{50} = 1.38 vs. 2.7 μmol/L). The higher Km for carnitine and the greater sensitivity to malonylCoA suggest that guinea pigs may not be able to utilize fat for energy as efficiently as rats and humans. However, rats did not respond to choline supplementation in the same way as humans and guinea pigs.[6,26] This is most likely because choline oxidase activity in rat liver is 18-fold higher than guinea pig liver and 60-fold higher than human liver.[20]

It would be of great practical significance if the 30% increase in the running time of the ChS (72.4 ± 7.4 min) compared with the NS (55.7 ± 8.4 min) animals was significant because similar results have been reported for human athletes given carnitine. Veechiet et al.[9] found that carnitine supplementation increased work capacity without increasing RER in humans, which suggests that carnitine may be affecting performance in ways other than energy production from fatty acid oxidation. Carnitine in skeletal muscle may play an important role in the synthesis of acetylcholine through an interaction with choline. Acetylcholine is an important neurotransmitter both in nervous tissue and at the neuromuscular junction. It has been reported that carnitine and acetylcarnitine facilitate the production of acetylcholine in the brain and that carnitine acetyltransferase activity is reduced in patients with Alzheimer's disease.[27] In cultured neuroblastoma cells, the combination of choline and carnitine has been demonstrated to synergistically increase the production of acetylcholine.[28] Whether the combination of choline and carnitine in muscle cells could synergistically increase acetylcholine signaling at the neuromuscular junction remains to be investigated.

In summary, supplementary choline (3 g/kg diet) over and above normal dietary levels (1.85 g/kg chow) enhanced tissue carnitine accretion, decreased percent body fat, and increased the percent of protein without significantly affecting live BW or RER. Increased concentrations of ASAC in muscle tissue is an indicator of fatty acid oxidation; however, the magnitude was small and not reflected in an increase in plasma and urinary ASAC. On the other hand, increased fatty acid oxidation due to starvation was of sufficient magnitude to be reflected in plasma and urinary ASAC of NS and ChS animals. The altered body composition, and possibly longer exercise endurance time, are important consequences of choline–carnitine interactions and deserve further characterization in humans.

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