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Tissue Carnitine Accretion and Fat Metabolism in Rats Supplemented with Carnitine, Choline and Caffeine Regardless of Exercise

Nobuko Hongu and Dileep S. Sachan

The objective of this study was to determine if the combination of carnitine, choline and caffeine supplementation would alter the concentrations of carnitine in various rats tissues (i.e., liver, heart, kidneys, brain, and testes) with or without exercise. Male, 7 week old Sprague-Dawley rats were given free access to a nonpurified diet with or without supplementation of carnitine, choline and caffeine at concentration of 5, 11.5 and 0.1 g kg^{-1} diet, respectively for 4 weeks. One half of each dietary group was assigned to exercise on a treadmill for 3 weeks. Results showed that the concentration of nonesterified and total carnitine was higher in serum, urine and all tissues of the supplemented rats. Exercise further promoted this effect in the liver and kidney. There was variable effect of supplement and exercise on the other fractions of carnitine in tissues except that short-chain acylcarnitines were consistently higher in the in skeletal and cardiac muscles and reflected in the serum and urine of the supplemented animals. Serum aspartate aminotransferase (AST) and alanine amino transferase (ALT), liver proteins and DNA were not adversely altered by the supplement. In conclusion, the oral feeding of carnitine, choline and caffeine (ccc) supplement to rats promotes carnitine influx in all tissues and changes in acylcarnitines of skeletal and cardiac muscles indicating enhanced fatty acid oxidation, which is supported by the changes in serum and urinary acylcarnitine profiles and loss of adipose tissue mass.

Key words: Carnitine, choline, caffeine, tissue, exercise, rats

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Introduction

Carnitine is an endogenous quaternary amine that is synthesized from the essential amino acids, lysine and methionine (Cox and Hoppel, 1973). It is present in all mammalian tissues, with higher concentrations in heart, skeletal muscle and lower concentrations in blood plasma, liver and kidney (Bieber, 1988). Carnitine is essential in the oxidation of fatty acids, serving as a "carrier" of activated fatty acids, fatty acyl-CoA esters, across the mitochondrial inner membrane to the site of β-oxidation. Carnitine is also necessary for maintaining intra mitochondrial acylCoA/CoA ratio by accepting acyl moieties from acyl CoA (Bieber *et al.*, 1982). A positive outcome of this process is the removal of toxic acyl-CoAs generated from the catabolism of branched chain amino acids (Bieber, 1988).

Carnitine is present in tissues as free or nonesterified carnitine (NEC) or as esterified carnitine (acylcarnitine). Many pathophysiological conditions affects serum and tissue carnitine concentrations and their excretion in urine. Exercise training has been reported to increased NEC and short-chain acylcarnitine or acid soluble acylcarntine (ASAC) in skeletal muscle and heart of swim-trained female rats (Lennon and Mance, 1986). There was an increase in NEC and in skeletal muscle and long-chain acylcarnitine or acid insoluble acylcarnitine (AIAC) in plasma and liver of treadmill trained male rats (Negrao *et al.*, 1987). Experiments in humans indicate increased carnitine concentrations in serum and skeletal muscle of trained individuals (Lennon and Mance, 1986; Arenas *et al.*, 1991). A number of studies have shown to increased carnitine concentrations in different tissues as a result of carnitine supplementation and/or exercise, however, the magnitude of the changes have been highly variable (Paulson *et al.*, 1984; Negrao *et al.*, 1987; Simi *et al.*, 1990).

We have reported that choline supplementation promotes tissue carnitine conservation in humans and animals and causes significant accumulation in the skeletal muscle of guinea pigs, where it was associated with leaner body composition, improved exercise performance but no change in R.Q. (Daily and Sachan, 1995; Dodson and Sachan, 1996; Daily *et al.*, 1998). The dosage of choline similar to that given to humans and guinea pigs did not result in carnitine conservation in adult rats (Daily and Sachan, 1995; Daily *et al.*, 1998). However, relatively higher doses of choline in combination with caffeine plus carnitine given to rats for 4 weeks resulted in a significant increase in carnitine status of serum, skeletal muscle and urine (Sachan and Hongu, 2000). A functional consequence of the changes due to supplementation was significant loss of fat pad mass (Hongu and Sachan, 2000), increase in VO_2 max and indices of fat oxidation (Sachan and Hongu, 2000).

This research paper addresses distribution of carnitine, acylcarnitine and acetylcarnitine among the tissues of rats fed diets supplemented with choline, carnitine and caffeine under sedentary or exercising regimen.

Materials and Methods

Animals and treatments: The study was conducted at the University of Tennessee and all animals procedures were approved by the Institutional Animal Care and Use Committee, and were in accordance with the NIH guidelines (Anonymous, 1985). Twenty, 7 week old, male Sprague-Dawley rats (Harlan Sprague-Dawley, Indianapolis, IN), weighing 210-230 g, were individually housed in wire mesh cages in a room with controlled temperature (20-22°C), relative humidity (50%), and light cycle (12-hr light/dark cycle) for the entire 5 weeks study period. The rats were randomly divided into two dietary groups to receive either a nonsupplemented or supplemented diet with caffeine, carnitine, and choline at concentrations of 0.1, 5, and 11.5 g kg^{-1} nonpurified diet, respectively. Food intake and body weight were determined daily. Average food intake (Table 1) was calculated by using the daily food intake throughout the 4 weeks.

After 1 week of dietary treatment, one half of each dietary group (n = 5) were put to exercise on the rodent treadmill (Columbus Instrument Internationals, Inc., Columbus, OH) for 10 min at 15% grade, 6 days per week. The running speed and duration were increased gradually during the experiment period (3 weeks) to maximize at 18 m min^{-1} for 25 min day^{-1}.

Sample collection: In all animals 24 h urine was collected after 3 weeks of the dietary treatment by placing rats in the stainless steel metabolic cages. The urine was collected in the exercised animals after completing last bout of 2 weeks exercise period even though they were kept on the exercise regimen for another week i.e., 24 h prior to being killed. Urine sample was quantified, centrifuged, and an aliquot was stored at -80°C for later analysis. After 4 weeks of dietary treatment and 3 weeks of exercise regimen, the rats were anesthetized with methoxyflurane (Pitman-Moore, Mundelein, IL) and killed by exsanguination after cardiocentesis. The blood samples were immediately centrifuged and serum was stored at -80°C (Hongu and Sachan, 2000). The tissues were removed and immediately frozen in liquid nitrogen and stored at -80°C. Only the heart samples were freeze dried and stored at -80°C. In the exercise groups, blood and tissue were collected 24 hr after the last bout of exercise (Sachan and Hongu, 2000).

Assays: Carnitine concentrations in tissues, serum, and urine were determined by a radio enzymatic assay originally described by Cederblad and Lindstedt (1972) and modified by Sachan *et al.* (1984). All samples were fractionated in perchloric ($PClO_4$) acid to allow determination of NEC, acid soluble carnitine (ASC) and AIAC. The ASAC fraction is calculated by subtracting NEC from the ASC. Total carnitine (TC) refers to the sum of the NEC, ASAC and AIAC. Acetylcarnitines (AC) in all samples were determined according to the method of Pande and Caramancion (1981).

Tissue protein was determined using the Bio-Rad Coomassie dye binding assay (Hercules, CA) with bovine serum albumin as the standard (Bradford, 1976). The concentration of DNA in a liver sample was determined using bisbenzimide dye using a calf thymus DNA standard (Sigma, St. Louis, MO). The DNA-dye complex was measured at 425nm by DyNAQuant 200 fluorometer (Pharmacia Biotech, Piscataway, NJ). The activities of serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined spectrophotometrically using a commercial diagnostic kit (Sigma Diagnostics®, St. Louis, MO).

Statistical analysis: All results are presented as group means ± SEM. Data were analyzed using two-way ANOVA to test the effects of exercise, supplementation and their interaction using SAS (SAS Institute, 1998). The main effects of diet and exercise were tested using specific linear contrasts, as was the interaction. Statistical significance level was set at $P \leq 0.05$.

Results

All the four groups of the rats had normal growth over the 4-week experiment. Food intake was similar in all groups (Table 1). The nonsupplemented rats had the nonpurified diet, Teklad 22/5 (Harlan Teklad, Madison, WI), which contains no caffeine, 30 mg of carnitine and 2.1 g of choline per kg of diet. Therefore, the nonsupplemented rat consumed about 0.7 mg of carnitine and 44.5 mg of choline per day. Compared to the nonsupplemented rats, the supplemented rats consumed approximately 150 times more carnitine and 5 times more choline. Exercise resulted in a significantly lower final body weight (Table 1). On the other hand, total fat pad weight was significantly lowered by both the exercise and supplement.

The concentration of all carnitine fractions including the AC in serum and urine were higher in the supplemented rats with or without exercise (Table 2). Significant supplement and exercise interaction was observed in the serum TC, NEC and ASAC. These carnitine fractions were lower in the exercised nonsupplemented animals but higher in the serum of exercised supplemented rats, however, the differences were not statistically significant. Supplement and exercise resulted in lower liver weight (Table 3).

Table 1: Food intake, body weight and total fat pad weight of rats with or without supplement and exercise[1]

	Non-supplement		Supplement		Statistical significance[2]		
Parameters	Non-Exercise	Exercise	Non-Exercise	Exercise	Supplement	Exercise	S x E
Food intake (g d^{-1})	21.8±0.4	21.1±0.4	22.2±0.5	20.7±0.4	NS	NS	NS
Caffeine intake (mg d^{-1})	-----	-----	2.1±0.04	2.07±0.04	-----	-----	-----
Carnitine intake (mg d^{-1})	0.65±0.01	0.67±0.01	105.0 ±2.0	104.0±1.8	0.0001	NS	NS
Choline intake (mg d^{-1})	45.0±0.9	44.0±0.8	242.0 ±4.6	238.0±4.1	0.0001	NS	NS
Body weight (g)	377.0±5.9	349.0±8.8	365.0±9.2	332.0±6.5	NS	0.0012	NS
Fat pad weight[3] (g)	12.4±1.6	9.3±2.6	10.1±1.8	7.0±1.4	0.0140	0.0019	NS

[1]Values are mean±SEM, n = 5. Rats were fed diet without supplemented or supplemented with caffeine, carnitine, and choline at concentrations of 0,1, 5, and 11.5 g kg^{-1} diet, respectively. The energy distribution of diet: 22% protein, 5% fat, and 73% carbohydrate. The diet provides 16.7 KJ g^{-1} and 3.8% fiber. [2]P values from two-way ANOVA, using the factors supplementation and exercise. S X E, supplement and exercise interaction. NS, not significant, P > 0.05. [3]Total fat pad is the sum of epididymal, inguinal and perirenal fat pad weights.

Table 2: Serum and urinary carnitine profiles of rats with or without supplement and exercise

	Non-supplement		Supplement		Statistical significance[1]		
Parameters	Non-Exercise	Exercise	Non-Exercise	Exercise	Supplement	Exercise	S x E
Serum TC (μmol L^{-1})	82.1±1.6	66.5±0.9	106.9±0.9	17.6±4.9	0.0001	NS	0.0003
Serum NEC (μmol L^{-1})	64.8±2.3	53.7±1.4	85.6±2.0	95.0±3.5	0.0001	NS	0.0007
Serum ASAC (μmol L^{-1})	14.4±1.7	10.2±1.7	16.1±1.1	18.3±1.2	0.0035	NS	0.0380
Serum AIAC (μmol L^{-1})	3.0±0.2	2.4±0.3	5.2±0.3	4.3±0.5	0.0001	NS	NS
Serum AC (nmol L^{-1})	42.7±1.1	34.3±0.7	71.9±5.7	73.7±4.7	0.0001	NS	NS
Urine TC (μmol d^{-1})	1.0±0.3	0.9±0.1	100.4±7.9	105.6±8.7	0.0001	NS	NS
Urine NEC (μmol d^{-1})	0.6±0.2	0.5±0.0	85.2±7.2	87.5±9.2	0.0001	NS	NS
Urine ASAC (μmol L^{-1})	0.4±0.1	0.4±0.0	13.6±3.2	16.4±2.2	0.0001	NS	NS
Urine AIAC (μmol L^{-1})	0.02±0.00	0.03±0.01	1.6±0.1	1.7±0.1	0.0001	NS	NS
Urine AC (μmol L^{-1})	0.7±0.1	0.6±0.03	13.4±1.1	14.4±1.2	0.0001	NS	NS

Values are means±SEM, n = 5.[1]P values from two-way ANOVA, using the factors, supplementation and exercise
S X E; supplement and exercise interaction. NS: not significant, P > 0.05
TC, Total carnitine; AIAC, Acid-insoluble acylcarnitine; ASAC, Acid-soluble acylcarnitine; NEC, Nonesterified carnitine; AC, Acetylcarnitin

Table 3: Serum enzymes, liver weight, protein, DNA and carnitine profiles of rats with or without supplement and exercise

	Non-supplement		Supplement		Statistical significance[1]		
Parameters	Non-Exercise	Exercise	Non-Exercise	Exercise	Supplement	Exercise	S x E
Serum AST (U L^{-1})	40.0±4.7	31.9±1.9	42.4±8.6	29.0±1.1	NS	NS	NS
Serum ALT (U L^{-1})	35.7±1.5	26.8±3.4	27.7±2.0	22.0±2.1	0.0158	0.0071	NS
Weight of liver[2] (g)	13.6±0.5	12.2±0.1	12.5±0.4	11.2±0.4	0.0123	0.0030	NS
Liver protein (mg g^{-1})	109.7±8.8	145.6±7.9	128.2±8.4	126.9±14.5	NS	NS	NS
Liver DNA (mg g^{-1})	0.66±0.02	0.99±0.04	0.85±0.06	0.84±0.08	NS	0.0085	0.0051
Liver TC (nmol g^{-1})	290.2±16.3	316.7±14.2	356.3±30.4	452.2±30.0	0.0006	0.0205	NS
Liver NEC (nmol g^{-1})	256.0±9.0	265.2±15.4	309.2±26.5	383.9±21.0	0.0004	0.0431	NS
Liver ASAC (nmol g^{-1})	28.0±8.5	46.7±12.5	39.3±14.2	59.3±8.1	NS	NS	NS
Liver AIAC (nmol g^{-1})	6.2±0.9	4.9±0.7	7.9±0.9	9.0±1.1	0.0054	NS	NS
Liver AC (nmol g^{-1})	10.2±2.4	21.4±2.6	11.4±1.6	25.6±6.8	NS	0.0050	NS

[1]Values are means±SEM, n = 5. P values from two-way ANOVA, using the factors, supplementation and exercise. S X E; supplement and exercise interaction. [2]g: gram of wet weight of tissue. NS: not significant, P > 0.05.AST, Aspartate aminotransferase; ALT, Alanine aminotransferase; TC, Total carnitine; NEC, Nonesterified carnitine; ASAC, Acid-soluble acylcarnitine; AIAC, Acid-insoluble acylcarnitine; AC, Acetylcarnitine

Table 4: Skeletal and cardiac muscle carnitine profiles of rats with or without supplement and exercise

	Non-supplement		Supplement		Statistical significance[1]		
Parameters (nmol g^{-1})	Non-Exercise	Exercise	Non-Exercise	Exercise	Supplement	Exercise	S x E
Skeletal muscle TC [2]	1024.8±35.4	965.8±59.6	1269.6±26.2	1282.0±56.5	0.0001	NS	NS
Skeletal muscle NEC	576.4±32.2	541.8±33.7	636.8±31.4	727.0±49.8	0.0001	NS	NS
Skeletal muscle ASAC	270.0±29.8	295.4±24.5	533.0±30.6	419.2±24.0	0.0001	NS	0.0218
Skeletal muscle AIAC	164.4±16.5	138.0±6.3	109.8±8.7	147.8±5.6	NS	NS	0.0165
Skeletal muscle AC	244.7±14.9	204.4±19.9	275.6±15.6	254.3±22.1	NS	NS	NS
Cardiac muscle TC [3]	1725.6±128	1465.9±100	2101.5±28.9	2573.1±172	0.0001	NS	0.0075
Cardiac muscle NEC	1236.2±73.2	1113.8±68.4	1590.4±21.6	1930.0±168	0.0001	NS	0.0318
Cardiac muscle ASAC	453.8±70.9	286.6±59.1	475.5±45.3	578.5±48.0	0.0200	NS	0.0350
Cardiac muscle AIAC	35.6±8.1	65.5±11.0	43.3±8.2	64.6±12.9	NS	NS	NS
Cardiac muscle AC	216.6±50.1	265.3±52.3	207.8±25.5	399.1±63.2	NS	0.0245	NS

[1]Values are means±SEM, n = 5. P values from two-way ANOVA, using the factors, supplementation and exercise. S X E; supplement and exercise interaction. [2]g: gram of wet weight of tissue. NS: not significant, P > 0.05. [3]g: gram of freeze-dried weight.

However, no significant difference was found when liver weight was expressed as a percentage of the body wight (data not shown). Therefore, the liver weight may be simply reflecting the body weight, which was significantly affected only by exercise. Total protein in liver was not affected by either supplement or exercise. DNA concentration in liver was significantly higher in the exercise group, and there was significant interaction between supplement and exercise, but the supplement alone had no significant effect on hepatic DNA concentration. Although no differences were observed in AST activities, supplement and exercise resulted in significantly lower ALT activity (Table 3). The concentration of TC, NEC and AIAC in the liver were markedly higher in the supplemented rats (Table 3). There was no significant difference in the ASAC of the groups, even though the ASAC was 53% higher in the supplemented and exercised animals compared to the nonsupplemented and nonexercised group. Acetylcarnitine in liver was higher in the exercised rats with or without supplement. Both exercise groups had twice as much AC concentration compared to their nonexercised counterparts. Supplementation resulted in significantly higher concentration of

Table 5: Testes and brain carnitine profiles of rats with or without supplement and exercise

	Non-supplement		Supplement		Statistical significance[1]		
Parameters (nmol g^{-1})	Non-Exercise	Exercise	Non-Exercise	Exercise	Supplement	Exercise	S x E
Testes TC[2]	185.5±4.2	184.7±6.9	204.9±14.7	235.8±6.6	0.0012	NS	NS
Testes NEC	137.5±4.1	134.9±2.5	158.8±12.3	188.7±7.0	0.0001	NS	0.0442
Testes ASAC	45.4±2.7	47.5±7.7	43.9±4.8	43.7±5.7	NS	NS	NS
Testes AIAC	2.6±0.6	2.2±0.7	2.2±0.4	3.4±0.3	NS	NS	NS
Testes AC	32.7±1.1	30.0±2.0	47.2±3.4	46.3±1.7	0.0001	NS	NS
Brain TC	110.5±6.1	103.4±2.6	160.5±3.5	165.8±9.7	0.0001	NS	NS
Brain NEC	88.0±4.8	79.1±2.2	135.9±1.0	141.8±9.5	0.0001	NS	NS
Brain ASAC	16.7±4.6	17.6±5.2	18.1±2.4	15.9±3.1	NS	NS	NS
Brain AIAC	5.8±1.3	6.7±0.9	6.5 ±0.6	8.1±0.8	NS	NS	NS
Brain AC	14.9±2.2	17.8±3.0	17.7±2.1	19.8±1.6	NS	NS	NS

[1]Values are means±SEM, n = 5. P values from two-way ANOVA, using the factors, supplementation and exercise. S X E; supplement and exercise interaction.[2]g: gram of wet weight of tissue. NS: not significant, P > 0.05.

Table 6: Kidney carnitine profiles of rats with or without supplement and exercise

	Non-supplement		Supplement		Statistical significance[1]		
Parameters (nmol g^{-1})	Non-Exercise	Exercise	Non-Exercise	Exercise	Supplement	Exercise	S x E
Kidney TC [2]	874.1±52.1	873.1±36.9	1307.1±62.4	1607.0± 19.8	0.0001	0.0036	0.0034
Kidney NEC	743.8±48.2	732.8±32.3	1172.1±47.6	1454.2± 26.8	0.0001	0.0071	0.0041
Kidney ASAC	119.1±13.4	130.0±8.5	126.5±22.8	139.8±17.8	NS	NS	NS
Kidney AIAC	11.2±1.4	10.2±1.7	8.5±0.8	13.0±1.7	NS	NS	NS
Kidney AC	90.7±2.3	88.3±6.2	122.2±2.3	126.1±3.9	0.0001	NS	NS

[1]Values are means±SEM, n = 5. P values from two-way ANOVA, using the factors, supplementation and exercise. S X E; supplement and exercise interaction. [2]g: gram of wet weight of tissue. NS: not significant, P > 0.05.

TC, NEC and ASAC in the skeletal and cardiac muscles (Table 4). In the skeletal muscle, supplement and exercise interaction was significant for the ASAC and AIAC. Exercise without supplement produced slightly lower concentration of AIAC, but exercise with supplement had the opposite effect. In the cardiac muscle, supplement caused significantly higher concentration of TC, NEC and ASAC but there was no significant supplement and exercise interaction. The AIAC in cardiac muscle of the exercised animals were increase 48-84%, however, these were statistically non significant. In contrast to skeletal muscle, in the cardiac muscle, AC concentration was significantly higher in the exercise groups particularly in the supplemented groups, where it was almost doubled. The decrease in the concentration of ASAC in the cardiac muscle of exercised rats without supplement was proportional to the increase in the concentration of AC, which amounted to more than 90 % of the entire ASAC.

Supplement resulted in a significantly higher concentration of TC and NEC in the testes, brain (Table 5) and kidney (Table 6) and AC in the testes (Table 5) and kidney (Table 6). There were no significant group differences in ASAC and AIAC of the testes, brain or kidney (Table 5 and 6).

Discussion

In our earlier reports it was shown that a combination of choline, carnitine and caffeine (CCC) supplementation produced carnitine conservation and fat oxidation in intact rats (Hongu and Sachan, 2000; Sachan and Hongu, 2000). Now we report that the CCC supplementation produced positive carnitine balance in all tissues examined (Table 3-6) and the dosages did not have toxic effect (Table 3). Further the supplement increased tissue content of short-chain and/or acetyl carnitine which are considered to be the biochemical markers of fatty acid mobilization and oxidation (Bremer, 1997).

In this study exogenous CCC were simultaneously fed in the diet unlike any other study reported in the literature outside of our laboratory. Most of the carnitine was concentrated in skeletal muscle of these rats very much like reported by other investigators suggesting normal transport and redistribution of the supplemented carnitine (Rebouche, 1977; Paulson *et al.*, 1984). The difference was that the concentrations of carnitine were consistently higher in muscles of the supplemented rats (Table 4). The muscle carnitine pool exists in free and acylated forms through a rather complex equilibrium with the carnitine pool of serum, liver, kidney and others. It has been suggested that carnitine fractions of the body compartments change in size because of the increased esterification of the muscle free carnitine (Lennon *et al.*, 1983). The muscle and other tissue carnitine pools remained at a high state of repletion (Table 3-6) in the supplemented animals in spite of the significant losses in the urine (Table 2) of these animals. The size of acylcarnitine pool within the muscle tissue must depends on its metabolic activity and supply of fatty acids to this tissue. The supplemented animals put to exercise regimen showed significant interactive effect on size of the acylcarnitine (ASAC, AIAC) pool under the conditions of our study (Table 4). The presence of caffeine in the supplement promoted lipolysis and therefore, supply of fatty acids.

The AC pool was increased in serum, urine and kidney of the supplemented animals and in the liver and heart of the exercised animals (Tables 2-4 and 6). The sources of acetyl moiety can be pyruvate and/or fatty acids depending on the fed or unfed state (Harris *et al.*, 1987; Bremer, 1997; Sachan and Hongu, 2000). As the rate of acetylCoA production exceeds the rate of its utilization, the carnitine acetyl transferase facilitates transfer of acetyl moiety of acetylCoA to carnitine (Bieber *et al.*, 1982; Harris *et al.*, 1987). This process can be augmented by enhanced lipolysis induced by hormones or chemicals, supply of carnitine, and energy demands. These conditions were present in our supplemented exercised animals. It is apparent that the supplement also promotes formation of ASAC and AIAC in tissue specific manner. These metabolites appear in serum and are excreted in urine, a process termed "fatty acid dumping" (Sachan and Hongu, 2000). None of these animals were starved and only half of them were exercised and yet there was an increase in the pool size of acylcarnitine in all animals. These observations suggested that there are undefined points of control of fat metabolism that can be manipulated by dietary means.

The supplement enlarged pools of total and acylcarnitine in liver similar to that seen in livers of trained female rats (Lennon and Mance, 1986) and streptozotocin-diabetic rats (Fogle and Bieber, 1979). Exercise also enlarged carnitine pools particularly the AC pools of liver (Table 3). An increase in the cardiac TC pool of exercised rats has been reported (Ciman *et al.*, 1979; Lennon and Mance, 1986), however, in the heart of our animals this was not the case unless they were supplemented with CCC (Table 4). This is perhaps because of the differences in the type and intensity of exercise regimen (Lennon and Mance, 1986) or the strain of animals (Ciman *et al.*, 1979). The supplement prevented a fall in the size of cardiac NEC pool in spite of the enlargement of

acylcarnitine (ASAC) pool (Table 4), which is contrary to the fall in NEC pool observed in starved and diabetic rat hearts (Fogle and Bieber, 1979; Bremer, 1997). The enlargement of cardiac AC pool by exercise especially in the supplemented group suggested up-regulation of fatty acid oxidation in this tissue. This and possible modulating of carnitine carrier proteins of cardiac myocytes (Cantrell and Borum, 1982) by the supplement remains to be investigated. In any case the supplement may be of benefit to ischemic and aged hearts, where reduced carnitine concentrations have been observed (Shug *et al.*, 1978; Costell and Grisolia, 1993). Carnitine is important for reproductive system in males, where it has been shown that oral carnitine supplementation improved total number of spermatozoa per ejaculate and sperm motility in men (Costa *et al.*, 1994; Vitali *et al.*, 1995). The supplement significantly enlarged the TC, NEC and AC pools in the testes (without epididymis) of the supplemented rats but the exercise had no effect on testicular carnitine fraction (Table 5). The improved reproductive functions in the CCC supplemented males is entirely speculative at this time.

Role of carnitine in brain function has been vigorously investigated because carnitine is found in brain (Bresolin *et al.*, 1982; Shug *et al.*, 1982), it crosses blood-brain barrier (Mroczkowska *et al.*, 1996; 2000) and has a novel carnitine transport protein in cerebral cortex neurons (Wawrzenczyk *et al.*, 2001). There is some evidence that carnitine may plays a different roles in central nervous system, where the fatty acid oxidation is relatively lower than in the peripheral tissues (Warshaw and Terry, 1976). Clinical studies have shown that carnitine and acetylcarnitine are effective in slowing down the progression of mental deterioration in Alzheimer's disease (Forloni *et al.*, 1994). The mechanisms of the therapeutic properties are unknown except the postulate that it provides activated acetyl moiety for the synthesis of acetylcholine (Rebouche, 1992; Nalecz and Nalecz, 1996). Addition of carnitine and choline together has been shown to promote acetylcholine synthesis in rat cerebral cortex cells (Wawrzenczyk *et al.*, 1995). The supplement augmented only brain TC and NEC pools in our study, where acylcarnitines were essentially unchanged (Table 5). Relative concentrations of ASAC plus AIAC in all groups were about 15-20 % of TC which is in accord with 10% reported by in the supplemented animals (Wawrzenczyk *et al.*, 1995). Kidney participates in the biosynthesis, acylation and excretion of carnitine (Carter and Frenkel, 1979; Guder and Wagner, 1990). The CCC supplement enlarged the pool of NEC and AC (Table 6) similar to the streptozotocin-diabetic rats kidney (Fogle and Bieber, 1979). Kidney TC and NEC pools were also enlarged not only by the supplement but also by the exercise regimen and there was significant supplement and exercise interaction on these carnitine species. Such an effect on kidney is not produced by choline alone in rats (Rein *et al.*, 1997) or guinea pigs (Daily *et al.*, 1998). As a matter of fact choline supplementation decreased carnitine concentration in the kidneys and urine of rats and guinea pigs (Daily and Sachan, 1995; Rein *et al.*, 1997; Daily *et al.*, 1998), and urine of humans (Daily and Sachan, 1995; Dodson and Sachan 1996). It is unlikely that 5 fold higher dosage of choline (Table 1) in this study compared to the earlier reports (Daily and Sachan, 1995; Rein *et al.*, 1997; Daily *et al.*, 1998) would have caused this shift. Perhaps it is the caffeine of the supplement mixture, CCC that contributes to the change in the carnitine profile of kidney. There is no carnitine data on caffeine supplemented animals, however, there are a few reports where a close relative of caffeine, theophylline has been studied (Al-Jafari *et al.*, 1996; Alhomida, 1998). In these studies, there was a significant increase in NEC, ASAC, AIAC and TC in kidney (Alhomida, 1998), and in the heart and skeletal muscle (Al-Jafari *et al.*, 1996) of the adult male rats given theophylline. There is little explanation of the data except that it indicates enhanced transport and oxidation of fatty acids, however, no clear mechanism has been proposed. Of course, there can be other functions of carnitine in tissues such as protection against lipid peroxidation (Dayanandan *et al.*, 2001) and the like.

In conclusion, found that oral supplementation of carnitine, choline and caffeine did not influence dietary intake and produced normal growth of rats, but decreased the fat pad weight. The combination of supplementation increased the total carnitine concentrations in all tissues, serum and urine.

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