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Increases in VO_2max and metabolic markers of fat oxidation by caffeine, carnitine, and choline supplementation in rats

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We have previously shown that the combination of caffeine, carnitine, and choline supplementation decreased body fat and serum leptin concentration in rats and was attributed to increased fat utilization for energy. As a result, it was hypothesized that the supplements may augment exercise performance including physiological and biochemical indexes. Twenty 7-week-old male Sprague-Dawley rats were given free access to a nonpurified diet with or without supplementation of caffeine, carnitine, and choline at concentrations of 0.1, 5, and 11.5 g/kg diet, respectively. One half of each dietary group was exercised on a motor-driven treadmill for 3 weeks and maximal aerobic power (VO_2max) was determined on the 18th day of exercise. Rats were killed 24-hr postexercise, and blood, regional fat pads, and skeletal muscle were collected. The VO_2max was increased ($P < 0.05$) in the supplemented/exercised group; however, the respiratory quotient (RQ) was not affected. Postexercised concentrations of serum triglycerides were decreased but β-hydroxybutyrate, acylcarnitine, and acetylcarnitine were increased in the supplemented animals. The changes in serum metabolites were complemented by the changes in the muscle and urinary metabolites. The magnitude of increase in urinary acylcarnitines (34–45-fold) is a unique effect of this combination of supplements. Cumulative evidence indicates enhanced β-oxidation of fatty acids without a change in the RQ because acetyl units were excreted in urine as acetylcarnitine and not oxidized to carbon dioxide. For this phenomenon, we propose the term "fatty acid dumping." We conclude that supplementation with caffeine, carnitine, and choline augments exercise performance and promotes fatty acid oxidation as well as disposal in urine. (J. Nutr. Biochem. 11:521–526, 2000)

Keywords: caffeine; carnitine; choline; fat oxidation; VO_2max; exercise; rats

Introduction

Professional and nonprofessional athletes seek nutritional supplements that will enhance exercise performance. These substances theoretically improve exercise capacity by enhancing lipid oxidation and slowing rates of muscle glycogen depletion,[1] therefore reducing fatigue.[2] Substantial research has been done on the use of a single substance (or nutrient) to enhance endurance performance, but only a few studies have been carried out to determine favorable effects of a combination of ergogenic substances on metabolic processes.[3,4] It is well known that athletes supplement their diets not only with single but also with multiple nutritional factors.[5] Therefore, a study using a combination of three popular dietary supplements (caffeine, carnitine, and choline) was envisioned, where the parameters of energy substrate utilization and exercise could be determined.

It has been suggested that caffeine enhances lipolysis and fat oxidation and reduces glycogen breakdown.[6,7] However, the results of studies on the ergogenic effects of caffeine are equivocal. Carnitine is essential for translocation of long-chain fatty acids into mitochondrial matrix as well as for the shuttling of acyl groups out of the mitochondria.[8] Clinical studies have shown that carnitine supplementation improves

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Table 1 Food intake, body weight, and fat pad weight of rats with or without supplementation and exercise

Parameters	Nonsupplement		Supplement		Statistical significance*		
	Nonexercise	Exercise	Nonexercise	Exercise	Supplement	Exercise	S × E
Food intake (g/day)	21.8 ± 0.4	21.1 ± 0.4	22.2 ± 0.5	20.7 ± 0.4	n.s.	n.s.	n.s.
Caffeine intake (mg/day)	—	—	2.1 ± 0.04	2.1 ± 0.04	—	—	—
Carnitine intake (mg/day)	0.7 ± 0.01	0.7 ± 0.01	105 ± 2.0	104 ± 1.8	0.0001	n.s.	n.s.
Choline intake (mg/day)	45 ± 0.9	44 ± 0.8	242 ± 4.6	238 ± 4.1	0.0001	n.s.	n.s.
Body weight (g)	377 ± 5.9	349 ± 8.8	365 ± 9.2	332 ± 6.5	n.s.	0.0012	n.s.
Fat pad weight† (g)	12.4 ± 1.6	9.3 ± 2.6	10.1 ± 1.8	7.0 ± 1.4	0.0140	0.0019	n.s.

Values are mean ± SEM, $n = 5$. Rats were fed diet without supplements or supplemented with caffeine, carnitine, and choline at concentrations of 0.1, 5, and 11.5 g/kg diet, respectively. The energy distribution of diet: 22% protein, 5% fat, and 73% carbohydrate. The diet provides 16.7 KJ/g and 3.8% fiber.

*P-values from two-way analysis of variance using the factors supplementation and exercise.

†Total fat pad weight is the sum of epididymal, inguinal, and perirenal fat pad weights.

S × E–supplement and exercise interaction. n.s.–not significant, $P > 0.05$.

muscle function and exercise capability in peripheral arterial disease and hemodialysis patients.[9] Yet, it is not clear whether carnitine supplementation benefits normal healthy individuals. Choline supplementation has been shown to enhance acetylcholine synthesis.[10] A decline in plasma and urinary choline concentrations has been observed in athletes after running a marathon.[11,12] This has lead to the hypothesis that choline supplementation may prevent decrement in physical performance. However, the mechanism by which choline could affect exercise performance has not been clearly established.

Recently, nutrient–nutrient interactions between choline and carnitine have been reported in healthy humans and animals.[13–17] Choline supplementation promotes tissue carnitine conservation, especially in skeletal muscle, which may have positive functional consequences in terms of exercise performance.[14,15] However, choline-supplemented exercised guinea pigs showed no change in respiratory quotient (RQ) even though their carcass fat was significantly reduced.[15] Choline dosage similar to that given to humans and guinea pigs when administered to adult rats did not produce carnitine conservation.[14,16] Relatively higher doses of choline in combination with caffeine plus carnitine given to rats for 4 weeks significantly reduced their body fat and serum leptin concentration.[17] We report now the effects of the combination of caffeine, carnitine, and choline supplementation on RQ, maximal aerobic power (VO_2max), and relevant metabolic and biochemical markers of fatty acid oxidation.

Methods and materials

Animals and treatment

The experimental protocol was approved by the University of Tennessee Institutional Review Board. Twenty 7-week-old male Sprague-Dawley rats (Harlan Sprague-Dawley, Indianapolis, IN USA), 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). 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 nonpurified diet, respectively. Food intake and body weight were determined daily. Average food intakes (*Table 1*) were calculated by using the daily food intakes throughout the experimental period.

After 1 week of dietary treatment, one half of each dietary group ($n = 5$) were put to exercise on the rodent treadmill (Columbus Instrument International, Inc., Columbus, OH USA) for 10 min at 15% grade, 6 days per week. The running speed and duration were gradually increased during the experiment to maximize at 18 m/min for 25 min/day. Expired gasses were continuously analyzed using an Ametek S-3A oxygen analyzer and a CD-3A carbon dioxide analyzer (Ametek, Inc., Paoli, PA USA). The expired gases were monitored to calculate RQ. The gas exchange on the first day of exercise period was used to calculate resting RQ. On the 18th day of exercise, the VO_2max was determined according to the method described by Bedford et al.[18] The exhaustion RQ was determined at the time an animal stopped running and the running time was recorded as the exhaustion time (*Table 2*). A tail-vein blood sample was taken at exhaustion for lactate determination.

Analytical methods

At the end of the experimental period, rats were anesthetized with methoxyflurane (Pitman-Moore, Mundelein, IL USA) and killed by exsanguination after cardiocentesis. In the exercised rats, blood (cardiac) and tissue collections were done 24 hr after the last bout of exercise. The blood samples were immediately centrifuged and serum was stored at −80°C. Following blood collection, regional fat pads and skeletal muscle (gastrocnemius) samples were collected, frozen in liquid nitrogen, and stored at −80°C. The samples were assayed for the concentrations of lactate,[19] triglycerides,[20] free fatty acids,[21] and carnitine fractions[22,23] according to the established procedures. Glucose and β-hydroxybutyrate were determined by using Sigma kits (Sigma, St. Louis, MO USA).

Statistical analysis

Data are reported as means ± SEM ($n = 5$). The differences between exercise groups at exhaustion were tested with Student's *t*-test (*Table 2*). Two-way analysis of variance was used to determine effects of exercise, supplementation, and their interaction on serum and skeletal muscle metabolites and urinary carnitine excretion. Statistics were performed using SAS.[24] A value of $P < 0.05$ was considered to be significant.

Table 2 Effects of caffeine, carnitine, and choline supplementation on RQ, VO_2max, exhaustion time, and blood lactate concentrations at exhaustion in exercised rats

Parameters	Nonsupplement		Supplement	
	Nonexercise	Exercise	Nonexercise	Exercise
RQ				
At rest	0.879 ± 0.02	0.875 ± 0.02	0.866 ± 0.02	0.847 ± 0.01
At exhaustion	—	0.920 ± 0.02	—	0.906 ± 0.03
VO_2max (ml/kg/min)	—	47.80 ± 1.12	—	52.13 ± 1.28*
Exhaustion time (min)	—	32.26 ± 9.3	—	54.72 ± 15.8
Lactate at exhaustion (mmol/L)	—	2.66 ± 1.46	—	1.89 ± 0.53

Values are mean ± SEM, $n = 5$. Rats were fed diet without supplements or supplemented with caffeine, carnitine, and choline at concentrations of 0.1, 5, and 11.5 g/kg diet, respectively. The energy distribution of diet: 22% protein, 5% fat, and 73% carbohydrate. The diet provides a 16.7 KJ/g and 3.8% fiber.
RQ–respiratory quotient. VO_2max–maximal aerobic power.
*$P < 0.05$.

Results

There were no significant differences in food intakes of the groups (*Table 1*). Nonsupplemented rats consumed no caffeine, but consumed about 0.7 mg of carnitine and 44.5 mg of choline per day because the nonpurified diet, Teklad 22/5, contained 30 mg of carnitine and 2.1 g of choline per kg of diet. Final body weights of the exercised rats were significantly lower than nonexercised rats and, therefore, exercise was the main effector of body weight. On the other hand, a significant decrease in fat pad weights was brought about by exercise as well as by the supplement. Although the duration and intensity of exercise administered were identical between the exercise groups with or without supplement, the mean total body fat was 25% lower in supplemented than in nonsupplemented rats (*Table 1*).

No significant differences were observed in the mean RQ values of the groups at rest and at exhaustion between the two exercised groups (*Table 2*). The VO_2max of the supplemented group was significantly higher than that of the nonsupplemented group, which is indicative of the enhanced exercise capacity of the supplemented rats. Although statistically not significant, the 70% longer time to exhaustion and 29% lower blood lactate concentration in the supplemented rats are physiological changes too large to be easily ignored.

One day after the last bout of exercise, cardiac blood and tissues were collected. Triglycerides were significantly lower in serum (*Table 3*) but significantly higher in skeletal muscle (*Table 4*) of both supplemented groups. There were no significant differences in serum glucose and free fatty acids. Serum β-hydroxybutyrate was affected by exercise, even though it was determined in the blood samples collected 24 hr after exercise periods (*Table 3*). All fractions of serum carnitine were significantly higher in the supplement groups with or without exercise (*Table 3*). Serum carnitine fractions of the nonsupplemented exercise (NSE) group were 20–29% lower compared with the nonsupplemented nonexercise (NSNE) group. There was significant interactive effect of supplement and exercise on total carnitine (TC), nonesterified carnitine (NEC), and acid-soluble acylcarnitine (ASAC) in serum.

The supplementation resulted in significantly higher concentration of triglycerides, TC, NEC, and ASAC in skeletal muscle (*Table 4*). TC was about 18% lower in the

Table 3 Effects of caffeine, carnitine, and choline on serum metabolites at rest in rats with or without supplement and exercise

Metabolites	Nonsupplement		Supplement		Statistical significance*		
	Nonexercise	Exercise	Nonexercise	Exercise	Supplement	Exercise	S × E
Glucose (mmol/L)	9.3 ± 0.57	9.1 ± 0.19	9.2 ± 0.36	7.9 ± 0.28	n.s.	n.s.	n.s.
Triglycerides (mmol/L)	0.8 ± 0.04	0.7 ± 0.09	0.4 ± 0.06	0.5 ± 0.04	0.0003	n.s.	n.s.
β-Hydroxybutyrate (mg/dL)	0.6 ± 0.15	1.6 ± 0.32	1.3 ± 0.38	2.2 ± 0.44	n.s.	0.0153	n.s.
Free fatty acids (mmol/L)	282 ± 30.9	290 ± 38.9	284 ± 29.4	195 ± 24.4	n.s.	n.s.	n.s.
Total carnitine (μmol/L)	82.1 ± 1.6	66.5 ± 0.9	106.9 ± 0.9	117.6 ± 4.9	0.0001	n.s.	0.0003
NEC (μmol/L)	64.8 ± 2.3	53.7 ± 1.4	85.6 ± 2.0	95.0 ± 3.5	0.0001	n.s.	0.0007
ASAC (μmol/L)	14.4 ± 1.7	10.2 ± 1.7	16.1 ± 1.1	18.3 ± 1.2	0.0035	n.s.	0.0380
AIAC (μmol/L)	3.0 ± 0.2	2.4 ± 0.3	5.2 ± 0.3	4.3 ± 0.5	0.0001	n.s.	n.s.
AC (nmol/L)	42.7 ± 1.1	34.3 ± 0.7	71.9 ± 5.7	73.7 ± 4.7	0.0001	n.s.	n.s.

Values are means ± SEM, $n = 5$. Rats were fed diet without supplements or supplemented with caffeine, carnitine, and choline at concentrations of 0.1, 5, and 11.5 g/kg diet, respectively. The energy distribution of diet: 22% protein, 5% fat, and 73% carbohydrate. The diet provides 16.7 KJ/g and 3.8% fiber.
AIAC–acid-insoluble acylcarnitine. ASAC–acid-soluble acylcarnitine. NEC–nonesterified carnitine. AC–acetylcarnitine. S × E–supplement and exercise interaction. n.s.–not significant, $P > 0.05$.
*P-values from two-way analysis of variance using the factors supplementation and exercise.

Table 4 Effects of caffeine, carnitine, and choline on skeletal muscle metabolites in rats with or wtihout supplement and exercise

Metabolites (units/g wet tissue)	Nonsupplement		Supplement		Statistical significance*		
	Nonexercise	Exercise	Nonexercsie	Exercise	Supplement	Exercise	S × E
Triclycerides (μmol)	32.1 ± 2.7	34.1 ± 4.7	61.2 ± 5.2	62.2 ± 6.2	0.0001	n.s.	n.s.
Free fatty acids (mmol)	2.1 ± 0.1	2.8 ± 0.3	2.3 ± 0.1	2.8 ± 0.1	n.s.	0.0050	n.s.
Total lipid (mg)	13.6 ± 0.8	20.2 ± 1.2	11.2 ± 1.2	19.1 ± 2.1	n.s.	0.0001	n.s.
Total carnitine (nmol)	1024.8 ± 35.4	965.8 ± 59.6	1269.6 ± 26.2	1282.0 ± 56.5	0.0001	n.s.	n.s.
NEC (nmol)	576.4 ± 32.2	541.8 ± 33.7	636.8 ± 31.4	727.0 ± 49.8	0.0001	n.s.	n.s.
ASAC (nmol)	270.0 ± 29.8	295.4 ± 24.5	533.0 ± 30.6	419.2 ± 24.0	0.0001	n.s.	0.0218
AIAC (nmol)	164.4 ± 16.5	138.0 ± 6.3	109.8 ± 8.7	147.8 ± 5.6	n.s.	n.s.	0.0165
AC (nmol)	244.7 ± 14.9	204.4 ± 19.9	275.6 ± 15.6	254.3 ± 22.1	n.s.	n.s.	n.s.

Values are means ± SEM, $n = 5$. Rats were fed diet without supplements or supplemented with caffeine, carnitine, and choline at concentrations of 0.1, 5, and 11.5 g/kg diet, respectively. The energy distribution of diet: 22% protein, 5% fat, and 73% carbohydrate. The diet provides 16.7 KJ/g and 3.8% fiber.
AIAC–acid-insoluble acylcarnitine. ASAC–acid-soluble acylcarnitine. NEC–nonesterified carnitine. AC–acetylcarnitine. S × E–supplement and exercise interaction. n.s.–not significant, $P > 0.05$.
*P-values from two-way analysis of variance using the factors supplementation and exercise.

NSE group compared with NSNE group; however, its concentration remained identical in both supplemented groups with or without exercise. The muscle free fatty acids and total lipid were significantly higher in the exercised rats with or without supplements (*Table 4*).

Urinary excretion of TC and all fractions of carnitine were significantly higher in the supplemented groups (*Table 5*). There was a 34–45-fold increase in ASAC and a 56–80-fold increase in acid-insoluble acylcarnitine (AIAC) in the urine of the supplemented rats. There were no differences between the nonexercised and exercised rats. The majority (88–98%) of the urinary ASAC was acetylcarnitine (AC) in the supplemented groups.

Discussion

The known biochemical and physiological effects of caffeine, carnitine, and choline formed the basis of our hypothesis that coadministration of these agents should augment exercise endurance and provide energy from fat oxidation in muscle. The conceptual model was that caffeine will promote fatty acid release from adipose tissue, choline will readily allow entry of carnitine into skeletal muscle cells, and carnitine will enhance translocation of fatty acids (as acylcarnitine) into the mitochondrial matrix of muscle. As a result, there should be a shift in the indices of exercise and fat oxidation. Measurements of VO_2max, RQ, enzyme activities, and substrate or metabolite concentrations were chosen as endpoints in exercise studies to assess aspects of physical performance. VO_2max provides an assessment of maximal exercise capacity[25] and is an accepted index of the functional limit of the cardiovascular system.[26] It is commonly used to evaluate the effect of the ergogenic aids including nutritional supplements.[27,28] RQ is a convenient and general guide to the relative rates of glucose and fat oxidation during steady-state exercise below the lactate threshold.[29]

The results of this study (*Table 2*) show that the combination of supplements augmented exercise performance, as indicated by 8% increased VO_2max, 70% longer time to exhaustion, and 29% lower blood lactate concentration. These results are consistent with those reported by Marconi et al.[28] who found a significant increase in the VO_2max after carnitine loading (4 g per day over a period of 2 weeks) of competitive athletes engaged in training programs. However, the results of other studies in humans supplemented with carnitine are contradictory.[30,31] Some of the contradiction may be related to variations in carnitine

Table 5 Effects of caffeine, carnitine, and choline on urinary carnitine excretion in rats with or without supplement and exercise

Carnitine (μmol/day)	Nonsupplement		Supplement		Statistical significance*		
	Nonexercise	Exercise	Nonexercise	Exercise	Supplement	Exercise	S × E
Total	1.0 ± 0.3	0.9 ± 0.1	100.4 ± 7.9	105.6 ± 8.7	0.0001	n.s.	n.s.
NEC	0.6 ± 0.2	0.5 ± 0.0	85.2 ± 7.2	87.5 ± 9.2	0.0001	n.s.	n.s.
ASAC	0.4 ± 0.1	0.4 ± 0.0	13.6 ± 3.2	16.4 ± 2.2	0.0001	n.s.	n.s.
AIAC	0.02 ± 0.00	0.03 ± 0.01	1.6 ± 0.1	1.7 ± 0.1	0.0001	n.s.	n.s.
AC	0.7 ± 0.2	0.6 ± 0.1	13.4 ± 2.5	14.4 ± 2.8	0.0001	n.s.	n.s.

Values are means ± SEM, $n = 5$. Rats were fed diet without supplements or supplemented with caffeine, carnitine, and choline at concentrations of 0.1, 5, and 11.5 g/kg diet, respectively. The energy distribution of diet: 22% protein, 5% fat, and 73% carbohydrate. The diet provides 16.7 KJ/g and 3.8% fiber.
AIAC–acid-insoluble acylcarnitine. ASAC–acid-soluble acylcarnitine. NEC–nonesterified carnitine. AC–acetylcarnitine. S × E–supplement and exercise interaction. n.s.–not siginificant, $P > 0.05$.
*P-values from two way analysis of variance using the factors supplementation and exercise.

loading. For example we gave carnitine at the rate of 105 mg/day for 4 weeks, which was 150-fold higher than that of the nonsupplemented animals and 5–10-fold greater than the dosage used in humans (2–4 g/day). Further, the polypharmacy nature of the supplement (combination of choline, carnitine, and caffeine) may lend a unique advantage to the system not present in other studies.

The RQ range (0.84–0.87) of the animals (*Table 2*) suggests that energy substrates were both fat and carbohydrates (50:50), according to the published reports.[32] The RQ was not altered by the supplements in our study; similar results have been reported by Marconi et al.[28] This means that fat was not the major energy substrate under these conditions, which is difficult to reconcile with the significant loss of body fat both in supplemented as well as in supplemented exercised (SE) animals.[17] We have argued that the loss of body fat was due to fat being used as the energy substrate, as feed composition and intakes were not different among the groups (*Table 1*). Therefore, markers of fatty acid oxidation other than RQ need closer examination.

The lower serum lactate concentration in SE compared to NSE animals (*Table 2*) is suggestive of lesser glucose catabolism and more fatty acid oxidation to provide energy for exercise.[33] A more direct evidence of fatty acid oxidation is seen in the higher β-hydroxybutyric acid (>40%), and lower triglycerides (40%) and free fatty acids (31%) in the serum of the SE group compared to the NSE group (*Table 3*). Additional evidence is presented by the changes in short-chain acylcarnitine (ASAC) and AC, both being markers of fatty acid oxidation.[8] The serum ASAC and AC are nearly doubled in the supplemented animals (*Table 3*). A decrease in ASAC (29%) and AC (20%) in the exercised animals without supplement (NSE vs. NSNE) suggests limited production of these metabolites due to lack of supplement. The lower concentrations of ASAC and AC in the NSE group could not be explained by complete oxidation of acetyl groups because RQ was not decreased (*Table 2*).

The changes in the markers of fatty acid oxidation in serum were complimentary to the changes in skeletal muscle where supplementation increased accretion of triglyceride and carnitine, particularly the ASACs, which are indicators of fatty acid oxidation (*Table 4*). The ASACs were increased about 1.4-fold by the supplement and by exercise in the gastrocnemius muscle, which is consistent with the changes in serum and urine, and supports the argument that the supplement promoted fatty acid oxidation.

So why there was no decrease in the RQ of the supplemented animals if fatty acid oxidation was enhanced? To reconcile the lack of concert between the RQ and biochemical markers of fat oxidation, we postulate the "fatty acid dumping" hypothesis with the following rationale. The activation and subsequent oxidation (via β-oxidation) of fatty acids produces acylCoAs of various chain lengths, including acetylCoA.[34] As the ratio of acetylCoA/CoA rises in muscle mitochondria after exercise,[35,36] the acetyl moiety is readily transferred to carnitine[37,38] in the presence of readily available carnitine in the muscle, promoted by choline in the supplement, as reported elsewhere.[14,15,17] The AC is not a substrate for condensing enzyme, therefore it can not enter the tricarboxylic acid cycle, and must exit mitochondria.[8] The cytosolic AC enters blood and is filtered out in urine. This is supported by the rise in AC concentrations by 2-fold in serum (*Table 3*) and 25-fold in urine (*Table 5*) but not in the muscle (*Table 4*) of the supplemented animals. The AC did not accumulate in the muscle, as it was allowed to exit into serum and urine where it makes up 88–98% of ASAC (*Table 5*). A similar scenario holds true for the fatty acids of various chain lengths, particularly the short-chain fatty acids (ASAC). The rise of acylcarnitine content of skeletal muscle (2-fold), serum (2-fold), and urine (35–45-fold) of the supplemented animals (*Tables 3–5*) is consistent with our hypothesis. Thus, a significant amount of fatty acid carbons do not get oxidized to CO_2 and the RQ remains unchanged. Instead, the fatty acid carbons are loaded on to carnitine and dumped in urine as acylcarnitines.

Could this be an effect of caffeine or carnitine alone? Caffeine promotes release of fatty acids from adipose tissue, which does not necessarily mean that it enhances fatty acid oxidation. There is paucity of clean biochemical data resulting in a great deal of controversy about the promotion of fat oxidation by caffeine alone.[39,40] With regard to carnitine alone, there is reasonable evidence that it promotes fatty acid oxidation in intact animals; however, the magnitude of the effect is small and controversial. Negrao et al.[41] found no significant increase in plasma ASAC of rats given i.p. carnitine for 8 weeks. In one of our studies,[42] we fed a 0.5% carnitine-supplemented diet to rats for 5,10, 20, 30, and 40 days and found no significant increase in serum ASAC at any of these time points; however, there was about a 2-fold increase in serum ASAC and AIAC after 40 days of treatment. In another study,[43] when rats were fed diets supplemented with various doses of carnitine for 10 days, there was about a 2- and 10-fold increase in the ASAC concentrations of blood and urine, respectively. So, carnitine alone brings about respectable increases in urinary acylcarnitines (10-fold) but nowhere near the 34–45-fold increase in ASAC and 56–80-fold increase in AIAC seen in the current study (*Table 5*). The magnitude of AC loss by the combination of carnitine, caffeine, and choline compared to that by carnitine alone is a very important point. A sustained loss of fatty acid carbons, even in smaller amounts, are analogues to a dripping water faucet. Thus, we call this phenomenon "fatty acid dumping." The shift in the normal direction of flow of fatty acid carbons must perturb the metabolic pathways and modulate normal homeostasis and regulation of energy substrate utilization.

In conclusion, the combination of caffeine, carnitine, and choline supplements increases aerobic work capacity by enhancing fatty acid oxidation in the skeletal muscle. The simultaneous presence of caffeine, carnitine, and choline is the key for promotion of facilitated availability and transport of fat to the site of oxidation as well as disposal of the end products of β-oxidation of fatty acids, i.e., acetyl groups. The loss of fatty acids as acylcarnitine or AC leaves RQ unchanged, even though fatty acids are oxidized but not to CO_2, as conventionally expected.

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