Effects of Carnitine Coingested Caffeine on Carnitine Metabolism and Endurance Capacity in Athletes

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Summary The purpose of this study was to examine whether caffeine (CAF), carnitine (CAR), or CAF+CAR mixture administration affects exercise endurance time via carnitine metabolism. Water (CON), CAF, CAR, or CAF+CAR mixture was administered to five male rugby athletes participating in this study by a randomized double-blind fashion who were made to ride a cycle ergometer for exercise. The CAF effect on exercise endurance time was small, but the CAR trial significantly increased the exercise endurance time compared with CON trial; a further CAP+CAR mixture trial had greater effects on the exercise endurance time than those of a CON, CAF, or CAR trial. A CAR or CAF+CAR mixed trial increased urinary nonesterified carnitine (NEC) and total carnitine (TCAR), but no changes were observed in acid-soluble acylcarnitine (ASAC) and acid-insoluble acylcarnitine (AIAC) excretion. A CAR or CAF+CAR mixed trial resulted in higher levels of plasma NEC, ASAC, and TCAR fractions than the CON and CAF trials did on exhaustion time. Total cholesterol, triglyceride, and free fatty acid in blood were significantly increased at exhaustion time, but they were not affected in the CAF or the CAR trial. These results suggest that carnitine ingestion could promote fat oxidation, resulting in higher endurance performance in athletes, and especially these ergogenic effects of carnitine coingested with caffeine may be greater than those of carnitine alone.

Key Words carnitine, caffeine, exercise, endurance performance, fat oxidation

Most human skeletal muscle uses stored glycogen for the production of energy at the first stage of exercise (1). During prolonged exercise, it is also required that free fatty acid is used to generate energy for working muscular activity. Therefore it has been reported that fat metabolism is stimulated and muscle glycogen is spared during the active exercise periods to prolong exercise endurance time and to delay fatigue (2, 3).

Carnitine (CAR) is an essential factor that plays an important role in the transportation of long-chain fatty acids into the mitochondria matrix during the process of fat oxidation. CAR is synthesized from the essential amino acids, lysine and methionine. Ascorbate, vitamin B6, and iron are also required for the synthesis of CAR. Therefore it is believed that the source of substrates for CAR biosynthesis is mainly by food consumed (4). Especially, since muscle tissues have no CAR biosynthesis ability and CAR transported into the muscle is drained during the long-term exercise period, CAR required for the supply of fatty acids into muscle mitochondria must be provided mainly by exogeneous intake (5, 6). It has been reported that endogenous CAR synthesized in the body is insufficient for fat metabolism during active exercise (7). Therefore many attempts have been made to increase the exercise endurance time and exercise ability by supplementing the needed CAR from exogenous sources (8–14).

We have studied lipolytic food component effects on endurance performance in rats and in human athletes. The lipolytic food means that the food stimulates lipolysis and/or fat oxidation at rest or during exercise in humans. We have studied and surveyed the lipolytic foods that are most likely useful for athletes, such as caffeine (15, 16), capsaicin (17–19), (-)-hydroxycitrate (20, 21), and fructose (22).

It has been also reported that caffeine (CAF) inhibits phosphodiesterase, an enzyme-degrading cAMP, consequently increasing cAMP concentration in the adipose tissues and adrenal gland. The increased cAMP concentration caused the stimulation of catecholamine secretion from the adrenal medulla and consequently caused the stimulation of stored fat mobilization (14). Therefore it has been suggested that CAF can induce an increase in free fatty acids in blood and spare glycogen in liver and muscle (23–25). However, the effects of CAF...
or CAR trial on fat metabolism during long-term exercise are in controversy, and a clear conclusion remains to be addressed.

In the present study we addressed the effects of separate and mixed administration of CAF and CAR on endurance exercise capacity and CAR metabolism in athletes.

MATERIALS AND METHODS

Subjects. Five healthy rugby players participated in this experiment. They understood and agreed to the purpose of the study and gave written consent to participate. They had no disease and had not used drugs for at least 1 y. Even though they were not habitual caffeine consumers, we educated them to never take drinks containing caffeine. The subjects were trained for at least 5 y and had an average maximal oxygen uptake (VO2max) of 53.2 mL/kg/min. VO2max was measured by using an indirect calorimetric technique during an incremental exercise bout, as previously reported (15). Subject characteristics are shown in Table 1. The experiment was approved in accordance with the Helsinki Declaration of 1975.

Subjects arrived at the laboratory 150 min before endurance exercise. They were given 640 kcal of formal diet (Table 2) 120 min before exercise. They were then offered experimental drinks including caffeine (CAF; 5 mg/kg in 250 ml), carnitine (CAR; 15 g/250 ml), caffeine+carnitine (CAF+CAR; 5 mg/kg of caffeine and 15 g of carnitine in 250 ml), or water (250 ml) 1 h after the meal. After the diet, rest-expired gas was measured for 5 min.

The International Olympic Committee (IOC) agreed that caffeine ingestion is illegal if urinary caffeine is over 12 mg/L. Jackman et al. (26) reported that 6 mg/kg of caffeine ingestion before exercise does not exceed the IOC’s limitation. From this point of view, we hypothesized that 6 mg/kg of caffeine ingestion would be the maximum amount in humans. Therefore 5 mg/kg of caffeine were selected in this experiment.

Experimental procedure. A randomized double-blind fashion was used to choose the different administration substances for subjects and examiners. The intervals between each trial were at least 1 wk. A bicycle ergometer (Mornak, Sweden) was used to carry an exercise load of this experiment on 60% and 80% of VO2max. The exhaustion time was determined by deciding the point when the subject cannot continue pedaling at 50 rpm. The exercise endurance time was measured by counting the time from starting the experiment to exhaustion time. Blood samples were taken from the subjects 1 h before exercise (just before supplement administration), 1 min before exercise (1 h postadministration), and during exhaustion time shown in Fig. 1.

They did stretching exercises for 2–3 min after 5 min of resting metabolic rate (RMR) measurement at 10 min before exercise. And a 3-way catheter was inserted in the antecubital vein before exercise for blood collection every 10 min of exercise for 40 min and at 45 min. They exercised on the cycle ergometer at 60% VO2max for 45 min, and exercise intensity was then increased at
Expired gas during exercise was collected in a Douglas bag (Fukuda Sangyo, Tokyo, Japan) every 2 min at the first period of exercise at 60% VO2max and 1 min at 80% of VO2max. The experimental procedures are shown in Fig. 1.

Blood was collected in heparinized tubes and centrifuged to separate the plasma. Urinary samples during 24h were collected on the day before the experiment and on the experiment day to determine CAR excretion. Thymol was used as a preservative for urine, and plasma and urine were stored at −20°C until analyzed. Biochemical parameters were analyzed according to established procedures, total cholesterol (TC) was analyzed with a commercial kit based on the enzymatic method (Youngdong Pharmaceutical Co., Korea), and urinary creatinine was determined by the alkaline-picrate method (27). Triglyceride (TG) was analyzed with a commercial kit based on the Trinder method (Youngdong Pharmaceutical Co.). Free fatty acid (FFA) was analyzed with an Auto Blood Chemistry Analyzer (Hitachi 7150, Japan) based on the enzymatic method. Nonesterified carnitine (NEC), all acid-soluble acylcarnitine (ASC), and acid-insoluble acylcarnitine (AIAC) in blood and urine were determined by the radioenzymatic procedure of Cederblad and Lindstedt (27), as modified by Sachan et al. (28). Acid-soluble acylcarnitine (ASAC) determined the difference between the NEC and the ASC fractions, and the sum of the NEC, ASAC, and AIAC fractions was called total carnitine (TCAR). Urinary CAR excretion was expressed in µmol/g creatinine.

Statistics. Data are described as mean and standard error (M±SE). To compare the differences between groups, the one-way ANOVA repeated measure method was used (29). The p<0.05 was obtained if a statistically significant difference was found between the groups.

RESULTS

Endurance capacity

A CAR trial significantly increased endurance exercise time compared with a CON or CAF trial (Fig. 2). Furthermore, a CAF+CAR mixture trial had a greater effect on endurance capacity than CAR alone, as shown in Fig. 2.

Blood carnitine levels

The effects of an administration supplement (CON, CAF, CAR, and CAF+CAR mixture) on plasma CAR levels are shown in Table 3. In the rest condition, all CAR levels were the same between the various administration groups. Plasma CAR levels at 1 h after administration in CON and CAF trials did not differ from the before levels. However, the CAF+CAR mixture trial showed higher plasma NEC, ASAC, and TCAR concentrations after administration at 1 h. Plasma CAR levels after exercise (at exhaustion time) were not different between CON and CAF trials, but CAR and CAF+CAR mixture trials significantly increased blood NEC, ASAC, and TCAR levels. The CAF+CAR mixture trial increased blood NEC, ASAC, and TCAR levels after 1 h; the CAR trial increased only plasma ASAC, but the CAF trial did not. Acute exercise increased plasma CAR concentrations significantly in control and in all supplement groups. Therefore blood CAR levels excluding AIAC in CAR and CAF+CAR trials were higher than in CON and CAF trials, which means carnitine administration increased blood CAR fractions.

Carnitine excretion in urine

The results of urinary CAR excretion during 24 h are presented in Fig. 3. The variation under rest conditions was small in comparison to the variation among subjects under exercise conditions. Even though the excretion of all fractions of CAR was higher in control subjects under the exercise conditions than under the nonexercise conditions, there was no statistical significance between them. Therefore exercise did not affect CAR excretion. A CAR or CAF+CAR mixture trial has significantly higher NEC and TCAR than among subjects who were in rest, a CON trial, or a CAF trial. However, urinary excretions of ASAC and AIAC were not statistically different between the groups.

The effects of acute exercise and various trial substances on plasma TC, TG, and FFA levels are shown in Table 4. The TC level at 1 h after administration in a CAR trial was lower than in a CAF+CAR trial. The TG level was unchanged in all groups. However, FFA concentrations at 1 h after the exercise in CAR and CAF+CAR trials were significantly lower than in the CON and CAF trials.

DISCUSSION

In this study, we asked two meaningful questions. The first, whether a single bout of CAF or CAR coingested with caffeine (CAF+CAR) trial affect exercise endurance time. The second, whether CAR metabolism might be modulated to change time and blood lipid profiles by different trial substances and/or prolonged...
The effects of various supplementations and exercise on plasma carnitine levels.

<table>
<thead>
<tr>
<th>Group</th>
<th>Carnitine type</th>
<th>Before (mmol/L)</th>
<th>After 1 h (mmol/L)</th>
<th>After exercise (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CON</td>
<td>NEC</td>
<td>34.36±6.40</td>
<td>35.96±3.62</td>
<td>28.44±5.47</td>
</tr>
<tr>
<td></td>
<td>ASAC</td>
<td>5.28±3.19</td>
<td>6.46±4.94</td>
<td>14.62±2.95</td>
</tr>
<tr>
<td></td>
<td>AIAC</td>
<td>4.06±1.17</td>
<td>3.92±0.85</td>
<td>6.74±3.38</td>
</tr>
<tr>
<td></td>
<td>TCAR</td>
<td>43.7±6.41</td>
<td>46.34±5.84</td>
<td>49.8±7.73</td>
</tr>
<tr>
<td>CAF</td>
<td>NEC</td>
<td>34.96±6.92</td>
<td>33.64±6.18</td>
<td>28.48±3.65</td>
</tr>
<tr>
<td></td>
<td>ASAC</td>
<td>4.92±3.47</td>
<td>5.82±2.95</td>
<td>16.04±1.94</td>
</tr>
<tr>
<td></td>
<td>AIAC</td>
<td>3.04±0.53</td>
<td>3.28±0.70</td>
<td>5.68±3.23</td>
</tr>
<tr>
<td></td>
<td>TCAR</td>
<td>42.92±7.34</td>
<td>42.74±7.06</td>
<td>50.22±5.01</td>
</tr>
<tr>
<td>CAR</td>
<td>NEC</td>
<td>33.64±3.75</td>
<td>41.74±6.52</td>
<td>70.52±10.15</td>
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<tr>
<td></td>
<td>ASAC</td>
<td>5.48±3.14</td>
<td>14.56±5.80</td>
<td>50.16±10.85</td>
</tr>
<tr>
<td></td>
<td>AIAC</td>
<td>3.36±0.43</td>
<td>3.02±0.89</td>
<td>6.78±1.19</td>
</tr>
<tr>
<td></td>
<td>TCAR</td>
<td>42.48±5.03</td>
<td>59.32±11.63</td>
<td>127.44±21.05</td>
</tr>
<tr>
<td>CAF+CAR</td>
<td>NEC</td>
<td>33.82±4.67</td>
<td>44.82±2.79</td>
<td>69.86±11.52</td>
</tr>
<tr>
<td></td>
<td>ASAC</td>
<td>4.98±2.95</td>
<td>16.08±5.46</td>
<td>65.46±29.16</td>
</tr>
<tr>
<td></td>
<td>AIAC</td>
<td>1.96±1.0</td>
<td>1.96±1.0</td>
<td>6.66±4.12</td>
</tr>
<tr>
<td></td>
<td>TCAR</td>
<td>40.76±6.06</td>
<td>64.16±4.29</td>
<td>142.06±41.79</td>
</tr>
</tbody>
</table>

Values are mean±SD. The values with different superscripts within the same row are significantly different between the time course (p<0.05). CON, control; CAF, caffeine; CAR, carnitine; NEC, nonesterified carnitine; ASAC, acid-soluble acylcarnitine; AIAC, acid-insoluble acylcarnitine; TCAR, total carnitine.

Fig. 3. Carnitine excretion in urine. Values are M±SE. NEC, nonesterified carnitine; ASAC, acid-soluble acylcarnitine; AIAC, acid-insoluble acylcarnitine; TCAR, total carnitine. The difference superscripts are significantly different between the experimental groups (p<0.05).

The effects of CAF and CAR ingestion on fat metabolism have been tested by using animals and human models. Most previous studies have been done with human subjects whose diets have had high-fat and high-protein (30). Korean athletes participating as subjects in this study were good models because the Korean diet has a relatively low CAR content compared with non-Asian diets, and Koreans do not consume many drinks containing CAF (31). The effects of CAR ingestion on exercise-related parameters are controversial, and no clear conclusion has yet been reached. For example, it has been reported that a CAR supplement (2 g/d) for 28 d on athletes resulted in enhancing exercise performance by increasing the utilization of fat and glycogen sparingly on muscle (32). Three grams of CAR injection before a 40 min exercise showed decreasing respiratory quotient (RQ) and increasing fat oxidation (33). However, Oyono-Enguelle et al. (34) and Vukovich et al. (35) provided data showing that endogenous CAR is sufficient for fat metabolism in a normal person, and CAR is not a rate-limiting substance for fat metabolism if CoA is sufficient.

Carlin et al. (36) and Soop et al. (37) insisted that a CAR trial did not affect energy metabolism during exercise because CAR is released to the blood after being acylated in the liver. CAF administration on fat metabolism is also controversial (38–42). It has been generally accepted that the effects of CAF administration during exercise are different, depending on various factors including exercise intensity, methods of administration, and state of training, and CAF administration had a glycogen-sparing effect by increasing blood FFA in a long exercise (more than 30 min). The physiological availability of CAR orally administrated is generally very low, and it is different for every person. Previous studies have shown that 2 g of CAR a day resulted in about 13% physiological availability (43), and a peak of CAR concentration in blood was shown after 3 h postadministration (44, 45). However, 15 g of CAR showed that high blood CAR levels were reached within 30 min, remaining at that level for 3 h (46).

In the present study, a CAR trial (15 g/d) resulted in increasing exercise endurance time. A CAF+CAR mixture trial showed much longer exercise endurance time
Table 4. The effects of various supplementations and exercise on plasma lipid levels.

<table>
<thead>
<tr>
<th>Group</th>
<th>Lipid</th>
<th>Before</th>
<th>After 1 h</th>
<th>After exercise</th>
</tr>
</thead>
<tbody>
<tr>
<td>CON</td>
<td>TC (mg/100 mL)</td>
<td>134 ± 13</td>
<td>149 ± 9(^{12})</td>
<td>156 ± 8</td>
</tr>
<tr>
<td></td>
<td>TG (mg/100 mL)</td>
<td>46.5 ± 4.5(^{a})</td>
<td>48.3 ± 2.7(^{a})</td>
<td>64.7 ± 5.0(^{b})</td>
</tr>
<tr>
<td></td>
<td>FFA ((\mu)Eq/L)</td>
<td>272 ± 146</td>
<td>495 ± 128(^{b})</td>
<td>778 ± 281(^{c,1})</td>
</tr>
<tr>
<td>CAF</td>
<td>TC (mg/100 mL)</td>
<td>127 ± 9</td>
<td>157 ± 23(^{12})</td>
<td>149 ± 13</td>
</tr>
<tr>
<td></td>
<td>TG (mg/100 mL)</td>
<td>46.0 ± 6.8(^{a})</td>
<td>40.4 ± 8.8(^{a})</td>
<td>72.2 ± 9.2(^{b})</td>
</tr>
<tr>
<td></td>
<td>FFA ((\mu)Eq/L)</td>
<td>374 ± 234(^{a})</td>
<td>630 ± 308(^{a})</td>
<td>806 ± 32(^{7b,1})</td>
</tr>
<tr>
<td>CAR</td>
<td>TC (mg/100 mL)</td>
<td>122 ± 9</td>
<td>129 ± 29(^{1})</td>
<td>152 ± 23</td>
</tr>
<tr>
<td></td>
<td>TG (mg/100 mL)</td>
<td>55.9 ± 10.7(^{a})</td>
<td>50.14 ± 8.2(^{a})</td>
<td>85.3 ± 10.3(^{b})</td>
</tr>
<tr>
<td></td>
<td>FFA ((\mu)Eq/L)</td>
<td>350 ± 302(^{a})</td>
<td>477 ± 235(^{a})</td>
<td>731 ± 33(^{7b,1})</td>
</tr>
<tr>
<td>CAF+CAR</td>
<td>TC (mg/100 mL)</td>
<td>138 ± 15(^{a})</td>
<td>163 ± 10(^{6,2})</td>
<td>198 ± 22(^{b})</td>
</tr>
<tr>
<td></td>
<td>TG (mg/100 mL)</td>
<td>59.4 ± 6.0(^{a})</td>
<td>61.5 ± 7.4(^{a})</td>
<td>90.7 ± 12.3(^{b})</td>
</tr>
<tr>
<td></td>
<td>FFA ((\mu)Eq/L)</td>
<td>233 ± 151(^{a})</td>
<td>584 ± 258(^{b})</td>
<td>602 ± 126(^{b,2})</td>
</tr>
</tbody>
</table>

Values are mean±SD. The values with the different superscripts within the same row are significantly different between the time course (\(p<0.05\)). The values with different superscript numbers between the groups mean significant differences (\(p<0.05\)). CON, control; CAF, caffeine; CAR, carnitine; TC, total cholesterol; TG, triglyceride; FFA, free fatty acid.

compared with a CON trial. However, a CAF trial alone did not affect exercise endurance time. Vecchiet et al. (47) have reported that pretreatment L-CAR (2 g) increased work capacity with increasing VO\(_{2}\)max, but without affecting RQ. They discussed two possible mechanisms for their results: 1) The administration of CAR during exercise might reduce acyl-CoA accumulation, which has adverse effects on energy production; 2) The administration of CAR might promote an increase of glucose utilization by buffering the intramitochondrial acetyl CoA/CoA ratio. In this study, a CAP+CAR mixture trial showed decreased R0, but no change in VO\(_{2}\)max (data not shown). Previous studies have shown that exercise increased plasma acylcarnitine, which is mostly acetylcarnitine (10). Our results also show that acute exercise is associated with a significant increase in plasma ASAC (Table 3). Plasma CAR levels during exercise might be dependent on exercise intensity and the administration substances. Lennon et al. (48) have shown that plasma NEC levels decreased significantly after exercise.

On the other hand, ASAC increased in moderately trained subjects. Our study also showed that NEC levels slightly decreased and that ASAC levels increased with a CON or CAF trial. This alternation of CAR levels was explained by the enhanced oxidation of both pyruvate and fatty acids in muscles during prolonged exercise (49). Plasma CAR concentrations at exhaustion time were significantly increased in CAR-supplemented groups. Only CAF trial failed to affect plasma CAR levels, but a CAF+CAR mixture trial 1 h postadministration showed higher plasma TCAR levels than those of the other conditions.

Increased urinary CAR excretion has been shown to occur in subjects on exercise days, compared with subjects on nonexercise control by other investigators (50). Urinary CAR excretion was not changed by a CAF trial compared with CON. Any fractional CAR excretions were not significantly different between the day of exercise CON and the nonexercise day in this study (Fig. 3). Although a CAR or a CAF+CAR trial increased the urinary excretion of NEC, the TCAR, ASAC, and AIAC levels did not change as a result of a CAR trial. The excretion of urinary CAR was responsible for maintaining blood CAR concentrations. Therefore the urinary excretion of ASAC and AIAC is one factor to keep high blood ASAC and AIAC levels in our study.

Exercise has been associated with variable effects on blood lipid profile. Therefore we conducted the study to see whether acute exercise and/or a CAF, CAR, or CAF+CAR mixture trial affects blood TC, TG, and FFA levels. Aerobic exercise increases HDL-cholesterol with moderate exercise in obese women (51) and men (52), but other studies (53, 54) have shown little or no effect on TC, TG, and LDL cholesterol. In our study, acute exercise increased TC, TG, and FFA levels, but a CAF and a CAR trial did not affect them (Table 4). However, a CAF+CAR mixture trial at 1 h postadministration showed significantly higher plasma FFA concentration in comparison with the rest condition. And the level of a CAF+CAR trial at 1 h after exercise then was significantly lower than that of a CAR trial. We therefore could carefully suggest that a CAF+CAR mixture trial would promote fatty acid oxidation from plasma FFA during exercise. It has been reported that the ability of muscle to oxidize fat has been thought to be limited by CAR palmitoyl transferase activity to transport fatty acid across the mitochondrial membrane during prolonged exercise (55). Our results showed that FFA levels under all administration conditions were higher during exhaustion time in comparison with a rest situation. Although CAF or CAR alone had no effect on plasma FFA levels, their exhaustion time was longer than in a CON or CAF trial. Another important form of fat for oxidation by muscle during exercise is intramuscular TG (56).
Plasma TG is a potential source of energy for muscle and is important for the recovery of intramuscular TG during long periods of exercise. Thus plasma TG showed higher levels under acute exercise conditions in our study. These levels may be affected by the administration of CAR and mixed CAF+CAR. However, our data did not show their effects, perhaps because we collected blood samples at times other than exhaustion time.

It has been reported that the effects of CAF or CAR on fat metabolism are different. From the previous data, we find that CAF can induce an increase of FFA in blood and spare glycogen in the liver and muscle (57), and CAR can increase fat utilization by the transport of long-chain fatty acids into the mitochondrial matrix for β-oxidation (4). CAR administration might increase FFA in the blood, but fatty acid transport to mitochondria needs to CAR. Therefore CAR appears to be a rate-limiting substance to maintain prolonged exercise in this case as shown the data that exercise endurance time positively related to plasma TCAR levels (Table 3). To summarize, this study showed that carnitine ingestion could promote fat oxidation and endurance performance in athletes, and especially these ergogenic effects of carnitine coingested with caffeine more than a single bout of carnitine.

Acknowledgments

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REFERENCES