Total plasma fatty acid responses to maximal incremental exercise after caffeine ingestion

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Abstract

Aerobic exercise is associated with changes in plasma fatty acid profile, whereas caffeine is known to exert action on lipolysis. However, the majority of this previous work has studied fatty acids as a single entity and not on an individual fatty acid basis, despite their differing roles and functions. The aim of this study was to assess the effect of 5 mg/kg body mass caffeine on maximal exercise and total plasma fatty acids profile. In a crossover, double-blinded, randomized placebo-controlled trial involving 20 untrained men who were not customary caffeine consumers, performance and ventilatory responses during an incremental maximal aerobic exercise protocol, along with plasma total fatty acid profile (by gas chromatography), were assessed. Exercise time to exhaustion \( (p < 0.01) \), maximum power output \( (p < 0.05) \) and maximum oxygen consumption increased \( (p < 0.05) \) significantly after caffeine ingestion. After exercise with caffeine, saturated fatty acids as a proportion of total fatty acids increased \( (p < 0.05) \), while decreases in trans-oleic acid \( (p < 0.05) \), arachidic acid \( (p < 0.05) \) and delta 9.2 desaturation index \( (p < 0.05) \) were found. The ingestion of caffeine before maximal incremental exercise produces an ergogenic effect, with the combination of caffeine and improved exercise performance resulting in greater relative fat oxidation and post-exercise differences in individual plasma free fatty acid proportions.

Keywords: Endurance; Ergogenic aid; Lipolysis; Metabolism; Trimethylxanthine

Introduction

There is a strong connection between the free fatty acid profile and health status. Greater concentrations of saturated fatty acids are associated with potential deleterious health outcomes (e.g., hypertension or obesity), whereas unsaturated fatty acids may play a cardiometabolic protective role. Aerobic exercise, with its reliance on fatty acids for energy production, induces alterations in fatty acid profile towards improved health outcomes. Certain substances are also forwarded as conferring beneficial modifications to the fatty acid profile, with one such substance being caffeine, a trimethylxanthine commonly found in our diet and in some medications. Caffeine action and its influence on fat metabolism have been well studied, with several studies showing that this alkaloid can enhance fatty acid mobilization from adipose tissue stores to mitochondria for oxidation during physical activity. However, in most studies, fatty acids are studied as a single entity as opposed to analyzing them according to type, despite differing individual roles and functions. The limited work that has analyzed changes in the profile of individual plasma fatty acids following exercise and caffeine ingestion.
suggests that the combination of these conditions elicits a more potent influence on lipolysis and changes in individual plasma fatty acid concentrations than either stimulus alone.

Alterations to the total plasma fatty acids profile may have potential health implications, with the unsaturated-to-saturated fatty acids ratio associated with cardiovascular disease risk. Caffeine alone can act as a pro-oxidant, increasing catecholamine production, and catecholamine metabolic inactivation can augment lipid peroxidation, converting unsaturated fatty acids to saturated small-to-medium-chain fatty acids. However, little is known about modifications in individual fatty acid profiles under the combined conditions of caffeine and exercise.

Therefore, the aim of this study was to assess the effect of caffeine ingestion on the performance of an incremental exercise test and the associated changes in the plasma free fatty acid profile. The outcomes generate new knowledge about the interaction of caffeine on fat metabolism during maximal exercise performance and, in particular, its influence on individual plasma fatty acids with differing roles and functions.

Materials and methods

Participants

Participants were 20 men who reported no routine caffeine consumption and did not exercise regularly. Participant characteristics were: age 20.9 ± 1.3 years, height 175.3 ± 6.1 cm, body mass (BM) 71.0 ± 5.5 kg, and body fat 10.1 ± 1.1%. Values were calculated using anthropometric techniques. Criteria of <1 hour of physical exercise per week for the past 6 months and no endurance training history were used to establish the “untrained status” of the participants.

Participants did not receive any form of nutritional supplementation before or during the study other than the caffeine used for the trials. During the week of testing, diet was controlled and fixed at 2000–2500 kcal/day, comprising ~60% carbohydrate, ~25% fat and ~15% protein. The last meal (breakfast) before the trials was consumed 3 hours before exercise, and it was standardized for all participants, containing 1.5 g carbohydrate per kilogram BM, while maintaining the same relative composition of macronutrients as outlined above.

Participants were informed of the procedures of this study and gave written consent.

Experimental design

The experimental protocol was a crossover, double-blinded, randomized design in which all participants performed incremental maximal effort cycling under two conditions: placebo and caffeine. Placebo capsules were empty. Caffeine capsules were prepared using pure caffeine (Sigma, Germany) relative to participant BM at a concentration of 5 mg/kg BM. Participants ingested capsules (with water) at 60 minutes before the trials.

The two cycling trials, placebo or caffeine, were performed in the morning and separated by 3 days to provide sufficient recovery. Exercise trials consisted of an incremental maximal test on a cycle ergometer (Ergo-metrics 900, Ergo-line, Germany). The starting intensity was 100 W, increasing by 50 W every 2 minutes until 300 W, when intensity was increased by 25 W every 2 minutes until exhaustion.

Measurements

Expired gases, ventilation and heart rate were measured during exercise using a Medical Graphics gas analyzer (MGC, model no. 762014-102, USA) and a heart rate monitor (Polar® S 720, Finland) with interface (Polar® Advantage interface).

Blood samples were obtained from the antecubital vein in the supine position before placebo or caffeine ingestion, and immediately after exercise for assessment of plasma caffeine concentration and plasma free fatty acids profile. Plasma was obtained by centrifugation and was stored at −20 °C until analysis. Plasma samples were analyzed using gas chromatographic techniques to determine total fatty acids profile: saturated C12:0, C14:0, C16:0, C18:0, C20:0; mono-unsaturated C16:1, C18:1, C24:1; and polyunsaturated C18:2.6, C18:3.6, C18:3.3, C20:3, C20:4, C20:5.3, C20:5.3, C22:6.

For biochemical analyses, blood samples were centrifuged for 10 minutes at 3000 rpm, and plasma was separated. Two milliliters of methanol/benzene (4:1) was added to 500 μL plasma with 200 μg internal patron (C:17) diluted. Using a magnetic stirrer, 200 μL acetyl chloride was added slowly. Test tubes were sealed and heated at 100 °C for 1 hour. Following this, tubes were cooled with water, and 5 mL K2CO3 (6%) was added before centrifugation for 5 minutes at 6000 rpm. From the benzenic extract obtained after centrifugation, 2 μL were taken for injection into a gas chromatograph (HP-5890 Series II, USA) coupled to a mass detector.

The chromatograph and detector conditions for the total plasma fatty acids profile assays were as follows: carrier gas He N-50, flow-rate 1 mL/min, split 6 mL/min, split ratio 27:1, temperatures of 250 °C at the detector and 300 °C at the injector pressure of 62.05 kPa at 185 °C, and solvent delay 7.5 minutes. The column was a SUPELCO model Omegawax (Sigma-Aldrich, USA) 320 of 30 m length × 0.32 mm internal diameter × 0.25 μm film thickness. The oven temperature program was: initial, 185 °C for 15 minutes; first ramp, 3 °C/min to 190 °C, 15 minutes; second ramp, 3 °C/min to 245 °C, 30 minutes. The detector emission current was 70 eV, and the mass range was from 100 to 700 (m/z).

Fatty acid identification was performed by comparing their retention times with spectra contained in spectra galleries. The internal patron used for fatty acid quantification was heptadecanoic acid, due to it being well placed in the chromatogram and not interfering with other sample peaks. Plasma fatty acid profile was expressed as a percentage. After obtaining total plasma fatty acids, delta desaturase indexes were calculated values as follows: delta 9.1: C16:1/C16:0, delta 9.2: C18:1/C18:0 and delta 5: C20:4/C20:3.
Values represent mean ± standard deviation; \( n = 20 \). Significant difference between placebo versus caffeine trial at \(* p < 0.05\) and \(** p < 0.01\). CAF = caffeine; HR = heart rate; RER = respiratory exchange ratio; RR = respiratory rate; \( \text{VCO}_2 \) = carbon dioxide consumption; VE = minute ventilation; \( \text{VO}_2 \) = oxygen consumption.

Plasma caffeine concentrations were determined to verify that no caffeine ingestion took place before placebo or caffeine trials and to assess concentrations after the caffeine trial. Plasma caffeine was measured by high-performance liquid chromatography (HPLC; Spectra SERIES P100/UV 100. UK).\(^{14}\)

The experiments carried out in this study were governed by the norms of investigation in the Declaration of Helsinki. The Regional Government Ethics Committee provided permission for the undertaking of this experiment.

**Statistics**

SPSS for Windows version 16.0 was used for statistical analysis. The Kolmogorov–Smirnov test for normality showed data to be not normally distributed, thus, the nonparametric Wilcoxon test for paired (dependent) samples was used. A value of \( p < 0.05 \) was used to determine statistical significance.

**Results**

Peak values for performance and ventilatory parameters for exercise trials under placebo and caffeine conditions are shown in Table 1. Participants in the caffeine trial cycled longer, achieved greater peak power output, heart rate and relative oxygen consumption than in the placebo trial, whereas the respiratory exchange ratio was lower.

Analysis of plasma caffeine concentration (via HPLC) showed no presence of caffeine before or after placebo trials or before caffeine ingestion, whereas mean plasma caffeine concentration after the caffeine trial was \( 7.45 \pm 4.13 \) \( \mu \text{g/mL} \).

Table 2 describes the proportion (%) of the different groups of plasma fatty acids before and after the incremental maximal trials under placebo and caffeine conditions. In the caffeine trial, only plasma saturated fatty acids as a proportion of total plasma fatty acids exhibited a significantly greater change from the initial to final proportion \( ( p < 0.05) \) when compared to changes in the placebo trial, with an increase in proportion observed. For the other fatty acid groups shown in Table 2, there were no differences in the proportional changes from pre- to post-exercise between placebo and caffeine conditions.

Table 3 shows the proportions of individual plasma fatty acids before and after exercise trials. Under caffeine conditions, the decrease in the arachidic acid (C20:0) proportion following exercise was the only saturated fatty acid to change to a significant extent compared with that in the placebo trial \( (p < 0.05) \). However, stearic acid (C18:0) did demonstrate a trend towards a greater increase \( (p = 0.09) \) from its initial to final proportion than that observed in the placebo trial. For plasma monounsaturated fatty acids, trans-oleic acid (C18:1t) was the only fatty acid whose change from its pre- to post-exercise proportion was significantly different to that observed in the placebo trial, with a decrease observed in the caffeine trial \( (p < 0.05) \). There were no plasma polyunsaturated fatty acid proportions that differed significantly from their initial to final proportions as a result of maximal exercise when comparing placebo to caffeine changes.

Table 4 shows lipid desaturation indexes before and after maximal exercise in the placebo and caffeine trials. Lipid desaturation indexes refer to the activity of desaturase enzymes responsible for fatty acid desaturation. The calculated delta 9.2 index decreased to a greater extent in the caffeine versus placebo trial \( (p < 0.05) \), with no significant changes observed in other desaturase indexes following maximal exercise.

**Discussion**

In this placebo-controlled experiment involving incremental exercise to volitional fatigue, the ingestion of 5 mg/kg BM caffeine resulted in an improvement in exercise performance, as demonstrated by a significantly greater time to exhaustion,
maximal power output and maximal $\text{O}_2$ consumption. This improved exercise performance in the presence of elevated plasma caffeine concentrations was associated with an increase in relative fat oxidation as well as the novel finding of post-exercise differences in individual free fatty acid proportions when compared with the placebo condition.

In this study, we took the novel approach of assessing plasma caffeine concentrations of each participant immediately after each exercise bout, rather than simply assuming that the ingestion of exogenous caffeine had increased circulating plasma caffeine concentrations. The post-exercise plasma caffeine concentrations of 7.45 ± 4.13 $\mu$g/mL in the caffeine trials substantiated the efficacy of the dosage, whereas the absence of caffeine in plasma was verified in the placebo trials.

The increases observed in time to exhaustion, maximal power output and maximal oxygen consumption following caffeine ingestion are consistent with other studies. This ergogenic effect of caffeine was associated with an increase in relative fat oxidation, as reflected by lower respiratory exchange ratio values, that may reflect a lesser reliance on glycogen and a subsequent lower production of glycolytic metabolites (e.g., lactate). These outcomes can ultimately lead to a greater time to exhaustion. However, changes in subjective effort perception associated with caffeine have been identified, thus, adenosine receptor antagonism with caffeine and the associated attenuation of pain and increased arousal may also have contributed to the observed improvement in exercise performance.

Saturated fatty acids as a proportion of the total plasma fatty acid profile increased following exercise in the caffeine trial, and this might reflect their greater mobilization for oxidation during exercise. Alternatively, it might be hypothesized that this is due to an increase in lipid peroxidation and the consequent change from unsaturated to saturated fatty acids, but our data showed no significant decreases in plasma unsaturated fatty acids. Moreover, under the same maximal exercise conditions as the present study, increases in oxidative stress have not been observed with caffeine ingestion. Therefore, it is likely that the increase in the plasma saturated fatty acids proportion is due in part to their greater mobilization for use as a source of energy during maximal aerobic exercise. Further work investigating caffeine and its influence on the plasma free fatty acid profile could be performed at submaximal exercise intensities at which fat oxidation nears maximal rates because, at these intensities, changes in plasma fatty acids might be more marked.

This increase in the plasma saturated fatty acids proportion following maximal exercise in the caffeine trial also appeared

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**Table 3**

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<th></th>
<th>Placebo trials</th>
<th>Caffeine trials</th>
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<tr>
<td></td>
<td>Initial (%)</td>
<td>Final (%)</td>
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<td></td>
<td>Initial (%)</td>
<td>Final (%)</td>
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<tr>
<td>C12:0</td>
<td>0.73 ± 0.77</td>
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<td>C14:0</td>
<td>1.02 ± 0.76</td>
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<td>C16:0</td>
<td>20.11 ± 3.44</td>
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<td>C18:0</td>
<td>8.08 ± 1.30</td>
<td>8.46 ± 1.97</td>
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<td>C16:1</td>
<td>0.27 ± 0.28</td>
<td>0.40 ± 0.31</td>
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<tr>
<td>C18:1t</td>
<td>1.61 ± 0.85</td>
<td>1.70 ± 1.19</td>
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<tr>
<td>C18:1c</td>
<td>19.23 ± 5.95</td>
<td>17.78 ± 5.47</td>
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<tr>
<td>C18:1t</td>
<td>1.52 ± 0.66</td>
<td>2.84 ± 4.47</td>
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<td>C20:4</td>
<td>0.95 ± 0.33</td>
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<td>32.61 ± 3.76</td>
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<tr>
<td>C20:3.3</td>
<td>0.29 ± 0.21</td>
<td>0.36 ± 0.20</td>
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<tr>
<td>C20:3.3</td>
<td>0.20 ± 0.18</td>
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<td>C20:3</td>
<td>1.94 ± 0.94</td>
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<tr>
<td>C20:4</td>
<td>7.61 ± 2.6</td>
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<td>C20:5.3</td>
<td>0.86 ± 0.6</td>
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<td>C22:5.3</td>
<td>1.01 ± 0.45</td>
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<td>C22:6</td>
<td>1.89 ± 1.09</td>
<td>2.29 ± 1.08</td>
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Values represent mean ± standard deviation; $n = 20$. *Significant difference ($p < 0.05$) for % change (from initial to final) observed in the placebo trial versus that observed in the caffeine trial.

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**Table 4**

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<tbody>
<tr>
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<td>Initial (%)</td>
<td>Final (%)</td>
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<tr>
<td></td>
<td>Initial (%)</td>
<td>Final (%)</td>
</tr>
<tr>
<td>Delta 9.1</td>
<td>0.08 ± 0.25</td>
<td>0.09 ± 0.44</td>
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<tr>
<td>Delta 9.2</td>
<td>2.57 ± 1.08</td>
<td>2.44 ± 1.05</td>
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<tr>
<td>Delta 5</td>
<td>3.92 ± 1.77</td>
<td>4.19 ± 1.36</td>
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Values represent mean ± standard deviation; $n = 20$. *Significant difference ($p < 0.05$) for % change (from initial to final) observed in the placebo trial versus that observed in the caffeine trial.
to be a consequence of a proportional increase \( (p = 0.09) \) in stearic acid \((C18:0)\) and a decrease \( (p < 0.05) \) in the mono-unsaturated fatty acid, trans-oleic fatty acid \((C18:1t)\). These proportional changes in the two individual fatty acids result in a decrease in the calculated lipid desaturation index, delta 9.2, suggesting that caffeine inhibits delta desaturase enzymatic activity. However, with delta desaturase activity here being estimated through desaturation index calculations, direct measures of the activity of this enzyme is required to verify the proposed inhibition. Inhibition of delta-desaturase enzymatic activity could act to prevent decreases in stearic acid, thereby helping to maintain availability of saturated fatty acids as a substrate for exercise. It should also be noted that the decrease in trans-oleic fatty acid \((C18:1t)\) confers a potential health benefit and provides a potential avenue through which exercise with caffeine reduces the risk of cardiovascular heart disease.\(^4\)

It is important to bear in mind that any changes observed post-exercise in the free fatty acids profile in the caffeine trial cannot be considered the result of caffeine alone. In the caffeine trial, exercise performance was enhanced and this may have also contributed to the observed changes in circulating substrate immediately post-exercise. For example, the proposed increases in saturated fatty acid mobilization and skeletal muscle use (as suggested by lower respiratory exchange ratio values) of free fatty acids such as arachidic acid and trans-oleic fatty acid may be the consequence of the greater energy demands of a greater power output and exercise time in the caffeine trial rather than solely the influence of caffeine on substrate metabolism.

In conclusion, our data show that the ingestion of 5 mg/kg BM caffeine at 60 minutes before incremental maximal exercise provides an ergogenic benefit to untrained individuals. Moreover, the interaction of this caffeine dosage and an enhanced exercise performance induces significant changes in the plasma total fatty acid profile, with specific changes in individual fatty acid proportions within the total fatty acid spectrum.

References