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ORIGINAL RESEARCH

# Effects of Acute Caffeinated Coffee Consumption on Energy Utilization Related to Glucose and Lipid Oxidation from Short Submaximal Treadmill Exercise in Sedentary Men

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**Abstract**

**Objective:** Aim of this study was to evaluate the short term effect of coffee drinking on energy utilization in sedentary men.

**Methods:** This study was performed in healthy sedentary men, who were randomized into three groups, control (n = 6), decaffeinated (n = 10), and caffeine (n = 10). The caffeine dose in coffee was rechecked and calculated for individual volunteers at 5 mg/kg. Baseline before drinking, complete blood count (CBC), glucose, antioxidant capacity, lipid peroxide, and caffeine in blood was evaluated. After drinking coffee for 1 hr, the submaximal exercise test with a modified Bruce protocol was carried out, and the $VO_2$ and RER were analyzed individually at 80% maximal heart rate, then the blood was repeat evaluated.

**Results:** Three groups showed a nonsignificant difference in CBC results and physical characteristics. The caffeine group showed significant changes in all parameters; higher $VO_2$ levels, ($P = 0.037$) and lower RER ($P = 0.047$), when compared to the baseline. Furthermore, the glucose level after exercise test increased significantly ($P = 0.033$) as well as lipid peroxide levels ($P = 0.005$), whereas antioxidant capacity did not change significantly ($P = 0.759$), when compared to the before exercise testing. In addition, the blood caffeine level also increased only in the caffeine group ($P = 0.008$).

**Conclusion:** Short consumption of caffeinated coffee (5 mg/kg of caffeine), improves energy utilization and relates to glucose derivation and lipid oxidation.

**Keywords:** caffeine, glucose, lipid, $VO_2$, RER, antioxidant

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## Background

At present, caffeine is a widely used compound in general population and athletics with ergogenic and alertness effects, which is not currently considered a doping substance.[1] Caffeine is contained in various foods and drinks, such as coffee, tea, cola, chocolate, and some energy drinks.[1,2] Previous evidence has shown that absorbance of caffeine in plasma gives a maximal peak of between 30–90 min after supplementation and a half-life of 5 h before excretion through urine as free caffeine (1%–3%).[3] Caffeine has an ergogenic effect stimulates lypolysis to release free fatty acid through inhibiting phosphodiesterease and enhancing cyclic AMP.[4] Furthermore, it spares the body's limited carbohydrate store.[5] Thus, it can be concluded that caffeine mobilizes free fatty acids from adipose tissue to the muscle, which activates the energy production system, and spare glycogen also extends exercise time.[5] Many studies have shown the benefits of caffeine on increased physical performance in athletes,[6–8] for example, its activity on the ergogenic effect acts directly on skeletal muscle and important parts of the brain or spinal cord, which may enhance good performance during endurance exercise.[6] Laurent's study showed that supplementation at 6 mg/kg of caffeine increased plasma beta-endorphin following two hours of cycling at 65% $VO_2$ peak.[7] Previous study has shown that acute supplementation of caffeine at 5 mg/kg of body weight, in runners (aged; 25.4 ± 6.9 yr) in a hot and humid climate, helped significantly to increase running time to exhaustion, but $VO_{2max}$, heart rate, core body temperature, and rate of perceived exertion (RPE) did not change.[8] Updated evidence by Franco and colleague [9] showed that caffeine supplementation at 5 mg/kg of body weight for 7 days reduced the percentage of fat, when compared to creatine supplementation rates. It is suggested also that low to high doses of caffeine (approximately 3–6 mg/kg) enhance the performance in trained athletes, but a high dose (>9 mg/kg) does not.[10] In addition, caffeine is claimed to be an antioxidant agent by inhibiting LDL oxidation in vitro,[11,12] but no evidence shows this activity in humans. In this study, experimental design was set up with a short time after supplement of caffeine at 5 mg/kg of body weight for 1 hr and comparing the changing of energy utilization, glucose release antioxidant status, and lipid oxidation before and after exercise test because of its maximal dose is absorbed in plasma in approximately 30–90 min, and the half-life is about 5 h.[13]

## Methods

All programs were approved by the Human Ethics Committee (Declaration of Helsinki) (2001) at the Faculty of Associated Medical Sciences, Chiang Mai University, Thailand. All volunteers were 20–24 years old, and non-smoking and non-athletic healthy males. Individual health status was interviewed for some illness, such as hospital admitted, drug administrated, or medical treated, and must not took any drugs, or nutrient supplements for 6 months before. Their body mass index (BMI) was within normal range (18.5–24.9 kg/m$^2$), according to the WHO and International Obesity Task Force. All subjects were selected by simple randomized sampling into three groups; Caffeine (n = 10), Decaffeinated (n = 10), and Control (n = 6).

## Protocol Study

Body weight was evaluated in order to calculate the level of caffeine supplementation. Caffeine in coffee powder (MOCCONA INSTANT, Expressor, Thailand) was measured accurately by high performance chromatography (HPLC) by comparison to the standard caffeine (Sigma St. Louis, USA). On the day of experiment, all volunteers could not consume coffee for at least 1.5 h before the blood sample was taken and the protocol could start. Energy utilization; $VO_2$ and RER at 80% maximal heart rate (MHR), was evaluated twice; the first test for baseline and the second for supplemented caffeinated coffee, decaffeinated coffee (Instant freeze dried decaffeinated coffee; Expressor, Thailand) or no supplement (control). For biochemistry laboratories, glucose, lipid oxidation, caffeine, and total antioxidant capacity were analyzed before and after supplementation. Caffeine in coffee was supplemented at 5 mg/kg of body weight in each subject of the caffeinated group. $VO_2$ and RER were analyzed by gas analysis using the Gas-analysis Breath-by-Breath Technique.

## Energy Utilization Measurement

Direct oxygen consumption ($VO_2$) and respiratory exchange ratio (RER) were parameters analyzed automatically with a CPX Ultima Gax exchange from MedGraphics® (St.Paul, Minnesota, USA). Subjects were

asked to wear a mouthpiece held with a mask connected to a pneumatic sensor that could detect bi-directional differential pressure, and the heart rate was monitored at the middle finger. Each breath passing through the system was controlled with a nose clip. Submaximal treadmill exercise with a modified Bruce protocol was started automatically using GE's CASE® Exercise Testing System (USA). Warm up started with running on the treadmill at 0.8 mph, on a 0% slope for 2 min, and the program progressed every three minutes with changing speed and slope. Directed $VO_2$ and RER were analysed automatically by the Breeze-Suit Software program. When the heart rate was 80% MHR, exercise was stopped, and the $VO_2$ (mL/kg/min) and RER results were shown after calm down by walking on the treadmill for 3 minutes. The criteria, precautions, and time to stop exercise followed the guideline of the American College of Sport Medicine (ACSM 2004),[14] for example; target heart rate at 80% MHR, complaint of strong dyspnea (Borge Scale) or physical signs such as muscle pain, cramp, headaches, heart palpitations, heartburn, blurred vision, or severe dyspnea.

## Biochemical Parameters

Lipid peroxidation was measured by the TBARs method, which followed Leelarungrayub's protocol.[15] Two hundred and fifty microliters of plasma were mixed with 750 µl of ortho-phosphoric acid (2.5%, v:v) and vortexed. Then, 500 µl of Thiobarbituric acid (TBA) (0.2 mol/L) in Tris solution (0.14 mol/L) was added. After incubation in a water bath (90 °C) for 30 min, all samples were cooled and centrifuged at 10,000 rpm for 3 min. A clear pink color of supernatant was read with a spectrophotometer at 532 nm. The yield of MDA in the sample was calculated by comparing with the absorbance of standard Tetramethoxypropane (TMP) (Sigma) (0–50 µmol/L).

Total antioxidant capacity (TAC) of fresh plasma was assayed with ABTs cation radical decolorization.[16] Stock ABTs cation radical was produced by mixing ABTs (14 mmol/L) and potassium persulfate (14 mmol/L) together, and leaving in the dark overnight. Working ABTs cation radical was diluted in de-ionized water until absorbance was shown between 0.68 and 0.74 at 734 nm, before adding plasma. Ten µl of plasma were added to 990 µl of working ABTs cation radical and gently alternated inversely by shaking nine times before adding in the spectrophotometer. Decreased absorbance was recorded continuously every minute for three minutes and finally calculated to ΔA/min. The TAC of the plasma was calculated by comparing with the ΔA/min of standard Trolox (Sigma) (0–10 mmol/L).

Glucose was analyzed with a commercial chemical kit at the Clinical Medical Technology Central Laboratory, Chiang Mai University, Thailand.

Caffeine in coffee and plasma were analyzed with HPLC.[17] Each 200 µl plasma sample was added to 200 µl of 0.8 M perchloric acid.[18] The mixed solution was vortexed for 10 s and centrifuged at 6,000 rpm for 5 min. The 20 µl of supernatant was injected into the chromatrographic system and the peak height was identified with a Phenomenex C18 analytical column (Waters Milipore Inc, Milford. MA) (1.5 cm × 4.6 mm) together with a C18 guard column. An isocratic mobile phase was run, with a mixed solution of methanol (HPLC grade, Labscan, Thailand) and water (70:30 *v/v*), and a flow rate set at 1 ml/min. The specific retention time of caffeine was identified at 254 nm.

## Statistic Analysis

Normal distribution of glucose, anti-oxidant capacity, lipid peroxide, $VO_2$, RER, and caffeine levels in the blood was tested with a Kolmogorov-Smirnov test. One-way ANOVA was statistical analyzed between three groups either before or after the submaximal treadmill exercise test, and a Least Significant Difference (LSD) was used for comparisons within the groups. All data between pre- and post-consumption of coffee were analyzed by an non-parametric paired t-test (Wilcoxon Sign Rank Test) in an SPSS program (Version 10). Raw data represented the mean ± SD. A $P < 0.05$ was considered significant.

## Results

Characteristics of the subjects are presented in Table 1, in which there is no statistical difference between age, weight, height, or body mass index (BMI). In the control, decaffeinated, and caffeine group, the CBC results showed values within the reference ranges.

From the HPLC evaluation the caffeine in Moccona caffeinated instance coffee, the standard caffeine showed a specific peak and retention time at 3.52 ± 0.11 min and the results showed

**Table 1.** Characteristics of sedentary men in the control, decaffeinated and caffeine group.

| | Control (n = 6) | Decaffeinated (n = 10) | Caffeine (n = 10) |
|---|---|---|---|
| Aged (years) | 20.17 ± 0.98 (19–21) | 20.3 ± 0.48 (20–21) | 20.5 ± 0.53 (20–21) |
| Weight (kg) | 67.08 ± 10.70 (52–80) | 64.75 ± 12.13 (50–83) | 62.94 ± 10.38 (50–81) |
| Height (m) | 1.75 ± 0.03 (1.67–1.77) | 1.70 ± 0.06 (1.60–1.80) | 1.73 ± 0.05 (1.68–1.85) |
| BMI ($kg \cdot m^{-2}$) | 23.06 ± 3.60 (18.64–28.03) | 22.27 ± 3.56 (17.92–29.76) | 22.84 ± 2.65 (17.71–28.75) |

the yield of caffeine in coffee at one gram was 167.8 ± 1.25 milligram, whereas no caffeine peak showed from the decaffeinated coffee.

From the results all data either before or after exercist test showed normal distribution, then comparison between three groups with one-way ANOVA and LSD had been followed. In this study, energy utilization was determined with directed $VO_2$ (mL/kg/min) at heart rate was 80% MHR from a Breath-by-Breath analysis following the modified Bruce protocol on treadmill. In Table 2, baseline or the first test of all groups showed no significant difference in $VO_2$, except in the decaffeinated group (control = 27.3 ± 1.89, decaffeinated = 32.52 ± 5.19, and caffeine = 28.07 ± 4.45 mL/kg/min), which also differed in the second test (control = 29.68 ± 3.90, decaffeinated = 34.51 ± 6.34, and caffeine = 32.41 ± 5.17 mL/kg/min). When comparing between the first and second tests in each group, the $VO_2$ level increased (32.41 ± 5.17 mL/kg/min) significantly from baseline (28.07 ± 4.45 mL/kg/min) in only the caffeine group ($P = 0.037$). Whereas, no statistical difference was seen in the control ($P = 0.115$) or decaffeinated group ($P = 0.214$).

The respiratory exchange ratio (RER) is shown in Table 2. There was no statistical difference either in the first (control = 1.09 ± 0.07, decaffeinated = 1.07 ± 0.10, caffeine = 1.06 ± 0.08, $P = 0.758$) or the second test (control = 1.03 ± 0.06, decaffeinated = 1.02 ± 0.05, caffeine = 0.99 ± 0.06, $P = 0.464$). However, while a statistical difference was found between the first and second tests of the caffeine group ($P = 0.047$), a paired t-test showed no change in the control ($P = 0.077$) or decaffeinated ($P = 0.201$) group.

From the blood biochemistry results (Table 3), the glucose level of the first test showed no significant difference between groups (control = 79.33 ± 7.99, decaffeinated = 89.10 ± 9.39, caffeine = 84.50 ± 5.83 mg/dL, $P = 0.073$ from one-way ANOVA test), whereas a significant difference existed in the second test between groups (control = 76.66 ± 7.33, decaffeinated = 88.00 ± 7.33, and caffeine = 88.30 ± 4.87, $P = 0.003$ from one-way ANOVA test). When comparing between the first and second tests in each group, results showed that the glucose level increased significantly in the caffeine group ($P = 0.033$), but no difference in the control ($P = 0.225$) or decaffeinated ($P = 0.540$) group according to the paired t-test.

**Table 2.** Energy utilization; $VO_2$ and RER among the control, decaffeinated and caffeine group.

| | Control (n = 6) | | Decaffeinated (n = 10) | | Caffeine (n = 10) | |
|---|---|---|---|---|---|---|
| | first test | second test | first test | second test | first test | second test |
| $VO_2$ (mL/kg/min) | 27.3 ± 1.89 (29.28–25.50) | 29.68 ± 3.90 (33.77–24.34) | 32.52 ± 5.19[a] (36.24–23.06) | 34.51 ± 6.34 (38.04–25.26) | 28.07 ± 4.45 (31.25–17.90) | 32.41 ± 5.17[c] (36.11–24.56) |
| RER | 1.09 ± 0.07 (1.05–1.24) | 1.03 ± 0.06 (0.93–1.11) | 1.07 ± 0.10 (0.95–1.25) | 1.02 ± 0.05 (0.96–1.13) | 1.06 ± 0.08 (0.91–1.18) | 0.99 ± 0.06[c] (0.86–1.13) |

**Notes:** [a]$P < 0.05$, compared to other groups at pre-drinking with an LSD in the one-way ANOVA test; [b]$P < 0.05$, compared to other groups at post-drinking with an LSD in the one-way ANOVA test; [c]$P < 0.05$ (Wilcoxon Signed Ranks Test), compared to the pre-drinking group.

**Table 3.** Blood biochemistry parameters; glucose, total antioxidant capacity (TAC), malondialdehyde (MDA), and caffeine among the control, decaffeinated and caffeine group.

| | Control (n = 6) | | Decaffeinated (n = 10) | | Caffeine (n = 10) | |
|---|---|---|---|---|---|---|
| | Before | After | Before | After | Before | After |
| Glucose (mg/dL) | 79.33 ± 7.99 (66–86) | 76.66 ± 7.33[b] (65–85) | 89.10 ± 9.39 (72–102) | 88.00 ± 7.33 (78–98) | 84.50 ± 5.83 (76–92) | 88.30 ± 4.87[c] (81–95) |
| TAC (mmol Trolox/L) | 0.84 ± 0.59 (0.78–0.92) | 0.80 ± 0.05 (0.74–0.89) | 0.98 ± 0.14 (0.75–1.16) | 1.05 ± 0.24 (0.80–1.62) | 1.00 ± 0.22 (0.59–1.27) | 0.99 ± 0.22 (0.54–1.62) |
| MDA (µmol/L) | 5.64 ± 2.04 (2.66–8.85) | 5.11 ± 1.86 (2.89–8.09) | 8.86 ± 3.91 (3.55–14.66) | 6.34 ± 1.86 (3.55–9.70) | 6.87 ± 1.86 (3.22–18.65) | 11.77 ± 8.45[b,c] (4.20–31.11) |
| Caffeine (mg/mL) | 2.83 ± 6.9 (0–17) | 0.00 ± 0.00 (0–0) | 6.10 ± 10.07 (0–27) | 1.3 ± 4.11[c] (0–13) | 5.9 ± 5.44 (1 –17) | 18.7 ± 9.6[b, c] (1 –36) |

**Notes:** [a]$P < 0.05$, compared to other groups at pre-drinking with an LSD in the one-way ANOVA test; [b]$P < 0.05$, compared to other groups at post-drinking with an LSD in the one-way ANOVA test; [c]$P < 0.05$, (Wilcoxon Signed Ranks Test), compared to the pre-drinking group.

Total antioxidant capacity (TAC) showed no significant difference in either the first (control = 0.84 ± 0.59, decaffeinated = 0.98 ± 0.14, caffeine = 1.00 ± 0.22 mmol Trolox/L, $P = 0.199$) or second test (control = 0.80 ± 0.05, decaffeinated = 1.05 ± 0.24, and caffeine = 0.99 ± 0.22 mmol Trolox/L, $P = 0.100$). When comparing between the first and second test in each group, there was no statistical difference ($p_{control} = 0.140$, $p_{decaffeine} = 0.153$, $p_{caffeine} = 0.759$).

In lipid peroxide (Malondialdehyde), there was no statistical difference in the first test between groups (control = 5.64 ± 2.04, decaffeinated = 8.86 ± 3.91, and caffeine = 6.87 ± 1.86 µmol/L, $P = 0.259$), but the MDA levels in the second test were significantly different between groups (control = 5.11 ± 1.86, decaffeinated = 6.34 ± 1.86, and caffeine = 11.77 ± 8.45 µmol/L, $P = 0.042$). The LSD statistical tool showed a higher level of MDA in the caffeine group when compared to the control ($P = 0.028$) and decaffeinated group ($P = 0.037$). When comparing the statistical difference between before and after exercise in each groups, the result showed a higher level of MDA in the caffeine ($P = 0.005$) group, but the control and decaffeinated group showed no statistical difference ($P = 0.500$ and $P = 0.074$).

Finally, the results of blood caffeine in the first test showed no statistical difference between groups (control = 2.83 ± 6.9, 6.10 ± 10.07, 5.9 ± 5.44 mg/mL, $P = 0.691$), but the caffeine group showed a significantly higher level (18.7 ± 9.6 mg/mL) than the control (0.00 ± 0.00 mg/mL) and decaffeinated (1.3 ± 4.11 mg/mL) group ($P = 0.000$) in the second test. When comparing between the first and second tests within groups, the control group showed no changes ($P = 0.317$), as in the decaffeinated group ($P = 0.066$), but the caffeine group showed a significantly increased level ($P = 0.008$).

## Discussion

There have been two studies of caffeine supplementation at 5 mg/mL in healthy men that was tested by the Wingate test,[19–21] and results showed no effect on physical performance. However, a report by Glaister showed a significant increase in sprint time.[22] Whereas, some studies of athletes showed a significant improvement in power output and endurance,[23] after supplementation. No reports showed a consistency of results, especially in healthy or non-athletic men. Furthermore, no evidence of part oxygen utilization or respiratory exchange ratio (RER) was shown from short term caffeine supplementation at 5 mg/kg of body weight.

Previous evidence reported that a source of energy utilization is changed after caffeine supplementation from alternating carbohydrate to lipid by increasing plasma free fatty acid (FFA), muscle uptake, and then fat oxidation, which leads to intramuscular glycogen spared through the Randle effect.[4] Previous evidence showed increases of nonesterified fatty acids and glucose availability on metabolism from moderate or intense dynamic exercise for at least 15–60 min.[24] The results from this study showed that the blood glucose level increased significantly in the second test when compared to the first one. In addition, MDA from lipid peroxidation is the most common marker used to present the nonenzymatic oxidation in case of more mono- or polyunsaturated fatty acids in blood circulation. Previous

report showed that mobilization of the free fatty acid from adipose tissue was related to the intensity of exercise between 25% to 65% of $VO_{2\ max}$.[25] The results showed significant increase in MDA in the caffeine group from the first to second test. Thus, these results were similar and confirmed previous evidence. This study was similar to a previous one by Sprient and colleagues,[26] who showed that the supplementation of caffeine at 5 mg/kg of body weight increased significantly in intramuscular fat oxidation during leg ergometer cycling.

Physical performance and source of energy utilization were studied by measuring $VO_2$ and RER. The respiratory exchange ratio (RER) is a common parameter for energy source during exercise, as it can be calculated from the ratio of $VCO_2$ and $VO_2$ and breath-by-breath analysis. A ratio of more than 1.0 indicates anaerobic metabolism, and a normal RER ratio should be in the range of 0.82–0.85. Source utilization for exercise is delivered from carbohydrate, fat, or protein, and the presents with a RER ratio such as 1.0 (100% carbohydrate), 0.7 (% 100 fat), and 0.8 (100% protein), therefore shifting the RER at less than 1.0 represents the fat or protein was more metabolized as well as carbohydrate. This study showed similar results of changes in both $VO_2$ and RER in the caffeine group by a significant increase of $VO_2$ level between the first and second test and significantly reduced RER from the first to second test, whereas, no significant difference was shown in the control and decaffeinated group. This result was similar to that in a previous study which researched 10 sedentary men by supplementing coffee with 3 g of caffeine, and results showed increasing $VO_2$ values of high-intensity effort from a 1,500 meter treadmill test.[27] Although this study did not determine the running time to exhaustion, a previous one showed that caffeine supplementation at 5 mg/mL increased the running time to exhaustion compared to runners on a placebo.[8] In the study of Grossman, caffeine supplementation increased exercise time to exhaustion regardless of whether it was in a hydrous or anhydrous form, and in the range of 3–7 mg/kg of body weight it provided an average increase in performance of 24% over placebo.[28]

In addition, caffeine is claimed to be an antioxidant compound[12] that scavenges free radicals and oxidation on lipid membranes in vitro. This study evaluated the total antioxidant capacity in blood before and after the submaximal exercise test. The results showed a non-significant difference within the three groups.

This study rechecked the level of caffeine in all groups and results confirmed that caffeine supplementation increased the caffeine level when compared to the control and decaffeinated group. Previous to this study period, blood caffeine in all groups could be detected from regular daily supplement, as there are many plants, vegetables and some beverages like tea and milk chocolate that also contain caffeine.[2] However, in the control and decaffeinated group, the caffeine level was reduced or absent after exercise, which was opposite to the caffeine group.

Many studies have explained the mechanisms of caffeine in physical performance, such as activation of calcium release from the sarcoplasmic reticulum and effects on muscle contraction and across the membranes of nerves and muscle cells that have more powerful effects on steps other than metabolism[29] and also primary action on the central nervous system.[30] In order to improve endurance performance, another possible caffeine mechanism is releasing beta-endorphin after caffeine supplement at 6 mg/kg of body weight during two hours cycling at 65% $VO_2$ max.[7] Current evidence suggests that the combined supplement between caffeine and creatine will improve physical performance better than caffeine on its own. For example, the study of Lee and coworker (2011) showed the significant benefit of creatine loading before caffeine supplementation in a peak power test on a sprinter.[31]

## Conclusion

It can be concluded that a moderate dose of caffeine is a well described ergogenic aid in various commercial products, as coffee powder shows enhancing effects on physical performance by increased $VO_2$ consumption and altered source of energy by releasing glucose and lipid oxidation in blood circulation. Although this study was performed in a small group of sedentary men, we found some interesting in practical useful on supplement or nutrition for improve exercise capacity or physical performance in sedentary subjects. But it also needs more confirmation on the dose and type of exercise that may have influence on the results.

## Authors' Contributions

LD was responsible for the study designs, overseeing data collection, biochemical work, statistical analysis, and preparation of the manuscript. MS was responsible for data collection/and assistance with laboratory work, and SC was rechecked and all data.

## Disclosures

Author(s) have provided signed confirmations to the publisher of their compliance with all applicable legal and ethical obligations in respect to declaration of conflicts of interest, funding, authorship and contributorship, and compliance with ethical requirements in respect to treatment of human and animal test subjects. If this article contains identifiable human subject(s) author(s) were required to supply signed patient consent prior to publication. Author(s) have confirmed that the published article is unique and not under consideration nor published by any other publication and that they have consent to reproduce any copyrighted material. The peer reviewers declared no conflicts of interest.

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