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RESEARCH ARTICLE

Effect of Caffeine on the Metabolic Responses of Lipolysis and Activated Sweat Gland Density in Human During Physical Activity

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Abstract This study measured caffeine-induced changes in activated sweat gland density (ASGD) and fat oxidation using a randomized crossover design with 10 healthy volunteers given caffeine (Caffe-I, 3 mg/kg ingested 30 min before experiment) and non-caffeine (No-Caff). Subjects were 173.0±3.2 cm in height, 72.5±4.3 kg in weight, and 21.0±2.5 years in age. All experiments were performed in an automated climate chamber (24.0±0.5°C, relative humidity 50±3%, air velocity less than 1 m/sec) between 2-5 p.m. The ASGD on the chest, upper arm, upper back, and lower back were measured (after 30 min running at 60% VO_{2max}), and blood samples were taken (at 40 min before, immediately before and after 30 min running). Activated sweat gland density levels were higher in Caffe-I (Chest $p<0.05$ and U-Back $p<0.01$) and free fatty acids (FFA) were higher in Caffe-I compared to No-Caff immediately before ($p<0.05$) and after running ($p<0.01$). In summary, caffeine increases ASGD and FFA by stimulating the sympathetic nervous system and increasing of lipolysis.

Keywords: caffeine, activated sweat gland density (ASGD), free fatty acid, lipolysis, running

Introduction

Health-threatening obesity is a global health problem. Obesity results from a static imbalance of intake and expenditure of energy. Caffeine is a natural herbal ingredient that increases energy expenditure but is not energetic alone (1). Caffeine intake accelerates the central nervous system (CNS) and facilitates lipolysis and thermogenic actions (2). Caffeine also increases metabolic fat oxidation and thermogenesis of the body (3-5). When body heat exceeds the set point of hypothalamus, sympathetic nerves are activated to cause reactions such as sweating, vasodilatation, or hyperpnea. Sweating from eccrine sweat glands is the most important reaction in reducing body heat (6). The preoptic area and anterior hypothalamus regulate sweat gland activation, as does acetylcholine (ACh) secreted from neuroglandular terminals of sympathetic nerves (7).

Likewise, as the CNS and peripheral effectors control acclimation, sweating includes activation of all the central and peripheral nerves (8). Human eccrine sweat glands are stimulated by pharmacological actions of postganglionic sympathetic sudomotor fibers and cholinergic muscarine (9) and ACh, a neurotransmitter secreted from cholinergic sudomotor nerve, activates muscarinic receptors in eccrine sweat glands (10). Caffeine is a strong antagonist of adenosine A_1 and A_{2A} receptors and increases cholinergic activation. Caffeine also inhibits phosphodiesterases, which hydrolyze cyclic adenosine monophosphate (cAMP) into AMP, increasing cAMP levels and sympathetic nervous system (SNS) activities and lipolysis (11). Thus, this study measured the caffeine-induced changes in lipolysis and activated sweat gland density (ASGD), as well as changes in whole body sweat loss volume (WBSL-V) and glycolysis.

Materials and Methods

Subjects All experimental protocols were approved by the University of Soonchunhyang Research Committee and obtaining written informed consent to participate.

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Subjects of this study were 10 male students of Soonchunhyang University trained with daily aerobic exercises. They were 21.0±2.5 years old, height 173.0±3.2 cm, weight 72.5±4.3 kg, and VO_{2max} 54.3±3.5 mL/kg/min. No subject ingested caffeine habitually. Inclusion criteria include no side effects of caffeine, no health trouble, and no smoking. Before the experiment, subjects were briefed about the purpose and importance of the study and possible risks. The experiment was performed with informed consent according to the 1975 Helsinki Declaration.

Setting of physical loading and testing All the experiments were conducted in a climate chamber at 24.0±0.5°C, 50±3% relative humidity, and less than 1 m/sec air velocity. To set precise exercise intensity, a physical loading test was conducted 1 week before the experiment for all subjects. In the physical loading test, the VO_{2max} of each individual was measured and blood pressure and pulse were measured at 5 min intervals with an automatic blood pressure/pulsation monitor (Model 412; Quinton, Bothell, WA, USA). VO_{2max} was obtained by applying the Bruce protocol with a treadmill (Medtrack SR 60; Quinton) and a metabolic test system (COSMED: Quark Pulmonary Function Testing Lung Volumes Module 2 ergo, Rome, Italy). The physical loading test was terminated by subject declaration. Immediately after the test, 60% VO_{2max} was calculated.

Measuring procedure The test used a random, crossover design. Tests were performed twice at the same time (2-5 pm) at a 1 week interval following the ingestion of caffeine. Measurements were set at Pre 40 (point of ingesting caffeine, 40 min before the physical loading), Point 0 (40 min after ingesting caffeine, starting the physical loading), and Post 30 (30 min after the physical loading). Subjects were randomly divided into 2 groups: Group A was given just 200 mL water (No-Caff), but Group B was given 3 mg/kg caffeine with 200 mL water (Caff-I). Subjects were weighed while standing straight with bare feet, and waist size was measured. At Point 0, each subject ran for 30 min on a treadmill at 60% VO_{2max}, with no drinking. Activated sweat gland density (ASGD) in 4 areas and weight and waist measurements were performed at Post 30. The next week, Group A was given 3 mg/kg caffeine and 200 mL water, but Group B was given just 200 mL water and tested again in the same way. Subjects did not have caffeine 48 hr before the measurement, and restrained intense physical activities 24 hr before the measurement. Subjects wore shorts and were bare-chested during the study. Subjects recorded all activities performed, as well as foods and beverages ingested, in the previous 48 hr, and transportation means to the measurement site before the measurement. Subjects were encouraged to be consistent in these areas until the crossover was complete.

Caffeine Caffeine levels in blood plasma peak about 40-60 min after ingestion (12), so measurements were started 40 min after the caffeine intake. The caffeine used in this study was 99.9% pure caffeine power produced by SciFit (Scientific Fitness, Oakmont, PA, USA) and ingested as a capsule.

Activated sweat gland density ASGD was measured with starch-iodide paper at Post 30. To measure, strips of starch-iodide paper were attached to the chest, upper arm (U-Arm), upper back (U-Back), and lower back (L-Back), and then scrubbed to get blue-black colored marks. To obtain average ASGD (count/cm^2), 3 sectors of 0.5×0.5 cm were marked on the paper strip and the number of sweat glands was counted (7). Counting was performed by a single experienced researcher who finished a sector in 10-15 sec. Average ASGD (count/cm^2)=Sum of 3 sectors/3×4.

Waist size and whole body sweat loss volume Before measuring waist size, a subject was standing upright while breathing lightly. In this posture, waist size was measured with a tapeline held horizontally at the middle part between lowermost part of rib and uppermost part of iliac crest. Care was taken not to press the skin. Waist size was measured in 0.1 cm by a single experienced researcher. Measurements were made at 2 times: Pre 40 and Post 30. Because weight loss after one-off physical activity is caused by sweating and measured body weight to obtain WBSL-V.

Blood analysis To analyze cortisol, 5 mL of venous blood was taken with a SS tube and left at room temperature for 30 min for clotting. After that, blood was centrifuged for 10 min at 3,000×g. Separated serum was analyzed with the RIA method (Cobra 5010, Quantum γ-counter; Packard, Meriden, CT, USA). Free fatty acids (FFA) were also analyzed using an automated analyzer (Hitachi 7180; Hitachi, Tokyo, Japan) with serum. To analyze glucose, 5 mL of venous blood was taken with a SS tube and centrifuged for 10 min at 3,000×g. Separated serum was measured with an automated analyzer (ADVIA 1650; Bayer, Tokyo, Japan).

Statistical processing The SPSS statistical package for Windows (ver. 15.0) was used for statistical analysis and generation of mean and standard deviation (mean±SD). The paired *t*-test was used to compare variables in each group. For the comparison between groups, independent sample *t*-test was used. The level of significance was set at $p<0.05$.

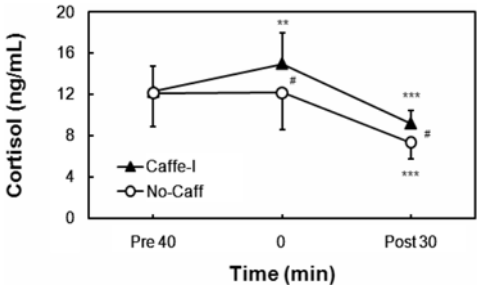

Fig. 1. Mean (±SD) cortisol levels at 3 points. Caffe-I (caffeine intake), No-Caff (non-caffeine), Pre 40 (point of ingesting caffeine), Point 0 (immediately before running), and Post 30 (30 min after running). $^{\#}p<0.05$ (Caffe-I vs No-Caff) and $^{**}p<0.01$, $^{***}p<0.001$ (compared with previous level in each group)

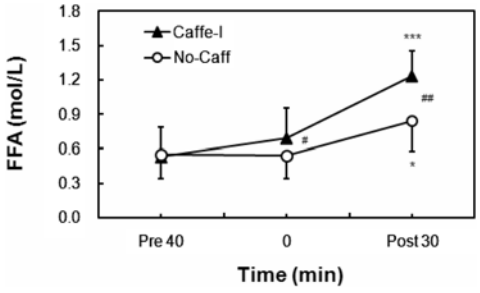

Fig. 2. Mean (±SD) free fatty acids levels at 3 point. Caffe-I (caffeine intake), No-Caff (non-caffeine), Pre 40 (point of ingesting caffeine), Point 0 (immediately before running), and Post 30 (30 min after running). $^{\#}p<0.05$, $^{\#\#}p<0.01$ (Caffe-I vs No-Caff) and $^{*}p<0.05$, $^{***}p<0.001$ (compared with previous level in each group)

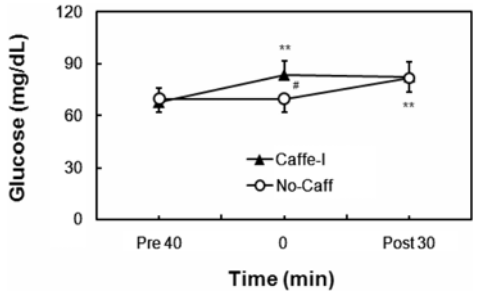

Fig. 3. Mean (±SD) glucose levels at 3 points. Caffe-I (caffeine intake), No-Caff (non-caffeine), Pre 40 (point of ingesting caffeine), Point 0 (immediately before running), and Post 30 (30 min after running). $^{\#}p<0.05$ (Caffe-I vs No-Caff) and $^{**}p<0.01$ (compared with previous level in each group)

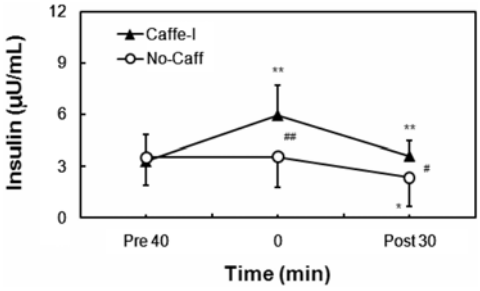

Fig. 4. Mean (±SD) insulin levels at 3 points. Caffe-I (caffeine intake), No-Caff (non-caffeine), Pre 40 (point of ingesting caffeine), Point 0 (immediately before running), and Post 30 (30 min after running). $^{\#}p<0.05$, $^{\#\#}p<0.01$ (Caffe-I vs No-Caff) and $^{*}p<0.05$, $^{**}p<0.01$ (compared with previous level in each group)

Results and Discussion

Caffeine increases cortisol levels Cortisol facilitates fat metabolism to increase FFA levels in the blood and use fat as an energy source (13). In sleeping subjects, caffeine increases cortisol levels (14) as it does in mice (15). The Caffe-I group showed significantly higher cortisol levels during Pre 40-Point 0, cortisol levels decreased from Point 0 to Post 30, though Caffe-I was still significantly higher which should facilitate lipolysis and increase blood FFA levels (Fig. 1).

Caffeine facilitated lipolysis during physical activity Caffeine increases fatty acid levels in plasma (16,17) by accelerating lipolysis downstream of blocking adenosine A_1 receptors on adipocytes (18,19). Thus, caffeine increases FFA levels in the blood by increasing cortisol release and blocking adenosine A_1 receptors to facilitate lipolysis. This study used lower doses than previous studies [6 (16) and 9 mg/kg (17)], which may explain the non-significant increase in FFA levels. After physical exercise, blood FFA levels in Caffe-I were significantly higher than No-Caff (Fig. 2). Physical activities of medium intensity may increase lipolysis and fatty acid mobilization (20,21) to meet the increased energy demand, with rates up to 5 fold higher than when resting (22,23). Here, caffeine seemed to potentiate the normal increase in blood FFA seen in the No-Caff group.

Caffeine restrains glucose uptake by reducing sensitivity to insulin through antagonism of A_1 receptors (24), although may have different effects during rest (Pre 40-Point 0) and physical activity (Point 0-Post 30) (Fig. 3). Caffeine stimulates sympathetic nerves to increase glucose levels during rest, but antagonizes A_1 receptors to restrain inflow of glucose to active muscles and reduce insulin sensitivity. Caffeine can also cause FFA to serve as an energy source rather than glucose, despite reduced insulin levels (Fig. 4). Caffeine therefore facilitated lipolysis during physical activity to increase FFA utilization as an energy source.

Effect of caffeine on ASGD and WBSL-V Caffeine increased thermogenesis, leading to significant increases in ASGD and WBSL-V. Eccrine sweat glands are controlled by postsynaptic innervation, and caffeine may induce a synergistic increase in ASGD by stimulating SNS (10). Obesity may result from decreasing thermogenesis because of hypoactive sympathetic nerves (25), but here caffeine

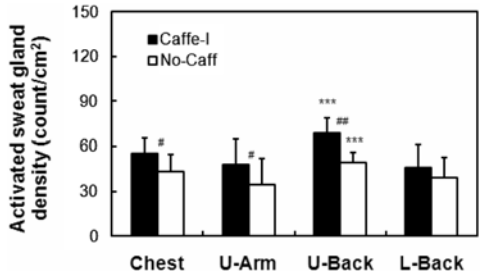

Fig. 5. Activated sweat gland density response. Caffe-I (caffeine intake), No-Caff (non-caffeine). Comparison between Pre 40 (point of ingestion caffeine) and Post 30 (30 min after running), U-Arm (upper arm), U-Back (upper back) and L-Back (low back). $^{\#}p<0.05$, $^{\#\#}p<0.01$ (Caffe-I vs. No-Caff) and $^{***}p<0.001$ (compared with previous level in each group)

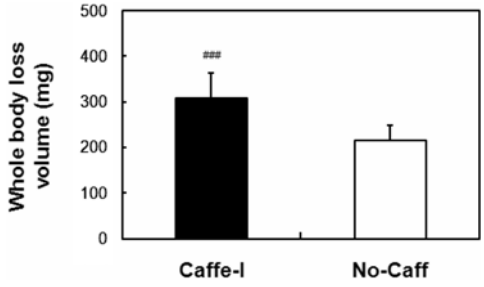

Fig 6. Whole body loss volume. Caffe-I (caffeine intake), No-Caff (non-caffeine). Comparison between Pre 40 (point of ingestion caffeine) and Post 30 (30 min after running). $^{\#\#\#}p<0.001$ (Caffe-I vs. No-Caff)

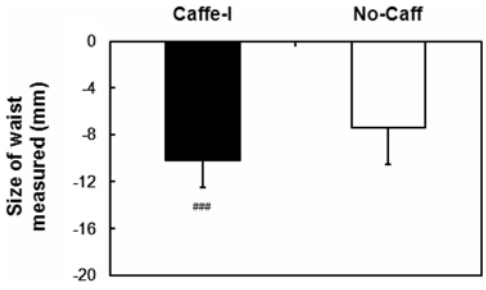

Fig 7. Waist size. Caffe-I (caffeine intake), No-Caff (non-caffeine). Comparison between Pre 40 (point of ingestion caffeine) and Post 30 (30 min after running). $^{\#\#\#}p<0.001$ (Caffe-I vs. No-Caff)

increased sweat gland density by activating sympathetic nerves (Fig. 5), leading to higher energy expenditure through increased sweating. Caffeine also reduced waist size via loss of total body water, as measured by WBSL-V in Fig. 6 and 7.

Caffeine may therefore reduce body fat while resting. Caffeine also increased FFA levels and local ASGD to facilitate lipolysis and increase sweat gland density. Thus, a one-off intake of caffeine could activate sympathetic nerves to increase lipolysis and generate an energy substrate, and to increase ASGD, an important estimate of energy expenditure. Thus, caffeine reduces body fat more effectively during physical loading and may have therapeutic potential for obesity treatment. Further work is needed on caffeine activity in reducing body fat, as well as its effects on long, intense physical activity and recuperation.

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