

Blood Dopamine Level Enhanced by Caffeine in Men after Treadmill Running

Jeong-Beom Lee¹, Hye-Jin Lee¹, Seung-Jea Lee², Tae-Wook Kim^{1*}

¹Department of Physiology, College of Medicine, Soonchunhyang University, Dongnam-Gu, Cheonan, Korea, ²Department of Medical Sciences, Soonchunhyang University, Asan-Si, Korea

Abstract

The aim of this study was to investigate the plasma dopamine and serum serotonin levels in humans with and without caffeine (CAFF) ingestion during treadmill running exercise. Thirty male volunteers participated in the randomized experiment involving two groups: CON ($n = 15$, 200 mL of tap water) versus CAFF ($n = 15$, 3 mg/kg CAFF and 200 mL tap water). After treadmill running, the dopamine level was significantly increased in the CAFF group ($P < 0.01$) and was significantly higher than in the CON group ($P < 0.01$). Serotonin was significantly increased in both groups after treadmill running ($P < 0.05$). However, serotonin levels showed no significant statistical difference between the groups. Prolactin and cortisol were significantly increased in both groups after treadmill running ($P < 0.01$). However, there was no significant statistical difference between groups. β -endorphin level was significantly increased in the CAFF group at after treadmill running ($P < 0.01$) and was significantly higher than in CON after treadmill running ($P < 0.01$). In conclusion, 3 mg/kg CAFF ingestion before treadmill running stimulated dopamine release without inhibiting serotonin, which may reduce central fatigue.

Keywords: Caffeine, dopamine, prolactin, serotonin, β -endorphin

INTRODUCTION

Physical exercise has been shown to improve psychological and cognitive functioning in humans. Among these effects, the secretion of neurotransmitters, especially monoamines, has been linked to exercise-induced neuronal adaptation. Serotonin and dopamine are the major monoamine neurotransmitters modulated by exercise.^[1]

Acute and exhaustive endurance exercises increase the synthesis, concentration, and metabolism of serotonin in several brain regions. Therefore, serotonin may play a role in central fatigue after prolonged and exhaustive exercise.^[2] For this reason, the central fatigue hypothesis suggests that increased concentrations of serotonin in the brain impairs central nervous system functioning during prolonged exercise, resulting in deterioration of exercise performance.^[3]

Although the role of serotonin in central fatigue has been well documented, it is likely that other neurotransmitters such

as dopamine influence fatigue. Increased concentrations of dopamine in specific areas of the brain inhibit the synthesis of serotonin during exercise and delay fatigue.^[4]

Animal studies have suggested that caffeine (CAFF) administration increased dopamine release.^[5-7] CAFF also potentially reduces serotonin levels during exercise.^[8] Increased dopamine levels improve endurance exercise performance,^[9] whereas reduced dopamine levels in the brain decrease the run time to exhaustion.^[10]

Although substantial evidence suggests that exercise and CAFF influence dopamine and serotonin levels, these results were identified separately and have been investigated using animals *in vivo*.^[5-10] Nucleus accumbens, which is associated with reward and reinforcing processes,^[11] has been suggested as a major region involved in depression.^[12] This brain region is densely innervated by dopamine, serotonin, and

***Address for correspondence:** Dr. Tae-Wook Kim,

Department of Physiology, College of Medicine, Soonchunhyang University,
31 Suncheonhyang 6-Gil, Dongnam-Gu, Cheonan 31151, Korea.
E-mail: ktwcau@gmail.com

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β -endorphin-containing terminals.^[13] Therefore, this study investigated the effect of CAFF on dopamine and serotonin levels after treadmill running by humans.

MATERIALS AND METHODS

Subjects

Thirty male individuals participated in the study (age, 22.2 ± 3.12 years; height, 176.2 ± 3.12 cm, weight, 68.9 ± 4.16 kg; muscle mass, 34.7 ± 1.65 kg; body surface area, 1.84 ± 0.07 m²; body mass index, 22.2 ± 1.11). None of the individuals ingested CAFF habitually. Inclusion criteria were as follows: absence of side effects from CAFF, absence of health problems, and no smoking history. Each subject provided written informed consent to participate in the study after being thoroughly acquainted with the purpose and experimental procedures as well as any potential risks. Also, no suspected symptoms of hypersensitivity reactions such as a wheal-flare reaction and respiratory distress. All procedures complied with the 2013 Declaration of Helsinki of the World Medical Association and rules of Soonchunhyang University Research Committee. Individuals fasted for 6 h and consumed ~ 5 –7 mL/kg of tap water 4 h before participation on the day of the test. They were instructed to refrain from alcohol consumption and medications 24 h before the test.

Measurement and experimental procedure

The study followed a double-blinded randomized design. The tests were conducted to compare the dopamine and serotonin levels between healthy males who either ingested CAFF habitually or who did not run regularly on a treadmill. The tests were performed in a climate chamber, and the environmental conditions were maintained at $25.0^\circ\text{C} \pm 0.5^\circ\text{C}$, $60.0\% \pm 3.0\%$ relative humidity, and 1 m/s air velocity. We conducted this experiment between 2 and 5 pm to control for the influence of body temperature circadian rhythm. After the individuals arrived in the laboratory, the specific gravity of urine was tested with a urine strip (Uriscan, Seoul, Korea) to confirm hydration equilibrium, and blood samples were also obtained. Individuals were randomized using a random-number table blocked in groups of two without stratification: Group A ($n = 15$) was supplied 200 mL of tap water (CON) and Group B ($n = 15$) was administered 3 mg/kg CAFF and 200 mL of tap water (CAFF). All individuals sat in a chair in a relaxed posture for 60 min. Later, a 75% VO_2 max treadmill running exercise was conducted for 40 min with no drinking. Blood was sampled at 60 min before treadmill running (Pre-EX) and immediately after 40 min treadmill exercise (Post-EX). Figure 1 shows the experiment protocol.

Caffeine dose

CAFF levels in blood plasma peak about 40–60 min after ingestion.^[14] Therefore, treadmill running was initiated 60 min after CAFF ingestion. The CAFF used in this study was 99.9% pure CAFF powder produced by Scientific Fitness (Oakmont, PA, USA) and ingested as a capsule.

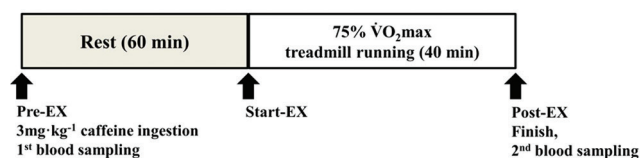


Figure 1: Protocol of experiments. Pre-EX, at -60 min before Start-EX; Start-EX, at start of treadmill running; Post-EX, immediately after 40-min treadmill running.

Blood analysis

Blood samples were collected at Pre-EX and Post-EX. Blood samples were collected in serum separator tube from the antecubital vein and left at room temperature for 15–30 min to enable clotting, after which the samples were centrifuged at 3000 rpm ($2000 \times g$) and 4°C for 10 min. Serum was then harvested and stored at -80°C until analysis. Serotonin level was determined via high pressure liquid chromatography (HPLC) using the Alliance Waters 465 HPLC (Waters, USA) in combination with a Serotonin Kit (Recipe, Munich, Germany). The prolactin level was determined using Prolactin II (Roche, Germany) by Modular Analytics E (Roche, Germany). The cortisol level was determined using CORTISOL RIACT (AMP, Germany) using a γ -counter COBRA 5010 QUANTUM (PACKARD, USA). The β -endorphin level was determined using Human Endorphin-beta ELISA Kit (USCNK, China) with a Microplate Reader VERSA Max (Molecular Device, USA). Blood samples were collected in EDTA tubes from the antecubital vein. The samples were centrifuged at 3000 rpm ($2000 \times g$) and 4°C for 10 min. Plasma was harvested and stored at -80°C until analysis. The dopamine level was determined using the HPLC method (Plasma Catecholamine Kit, Bio-Rad, Germany) in combination with the Acclaim HPLC (Bio-Rad, USA).

Statistical analysis

Descriptive statistics are expressed as mean $\pm$ standard deviation using commercially available computer software (SPSS for Windows, version 21.0; SPSS Inc., Chicago, IL, USA). Wilcoxon signed rank test and Mann–Whitney U-test were used to examine the differences between and within groups. Significant differences were assumed at $P < 0.05$.

RESULTS

Dopamine level

Post-EX dopamine levels in the CAFF group increased significantly (12.03 ± 6.21 vs. 17.10 ± 6.31 pg/mL, $P < 0.01$), and significant differences were found in the two groups ($P < 0.01$) [Figure 2].

Serotonin level

Post-EX serotonin levels increased significantly in both groups compared with the Pre-EX levels (CON, 106.28 ± 22.76 vs. 132.36 ± 37.23 ng/mL, $P < 0.05$; CAFF, 97.70 ± 22.15 vs. 119.40 ± 33.94 ng/mL, $P < 0.05$) [Figure 3]. However, the

serotonin levels showed no significant differences between the CON and CAFF groups.

Prolactin level

Post-EX prolactin levels increased significantly in both groups compared with the Pre-EX levels (CON, 8.34 ± 1.50 vs. 16.63 ± 4.58 ng/mL, $P < 0.01$; CAFF, 9.40 ± 2.63 vs. 19.54 ± 4.47 ng/mL, $P < 0.01$) [Figure 4]. However, the prolactin level showed no significant differences between the CON and CAFF groups.

Cortisol level

Post-EX cortisol levels increased significantly in both groups compared with the Pre-EX levels (CON, 7.04 ± 0.96 vs. 12.40 ± 4.65 μ g/dL, $P < 0.05$; CAFF, 6.12 ± 2.19 vs. 12.75 ± 2.50

μ g/dL, $P < 0.05$) [Figure 5]. However, the cortisol level was not significantly different between the CON and CAFF groups.

Beta-endorphin level

Post-EX β -endorphin levels increased significantly in CAFF compared with the Pre-EX levels (1.67 ± 0.48 vs. 2.46 ± 0.65 pg/mL, $P < 0.01$), and the post-EX levels were significantly higher in the CAFF than in the CON group ($P < 0.01$) [Figure 6].

DISCUSSION

This is the first study that investigated the plasma levels of dopamine and serum serotonin after treadmill running exercise following CAFF consumption in humans. We confirmed that exposure to 3 mg/kg CAFF had a significant effect on the levels

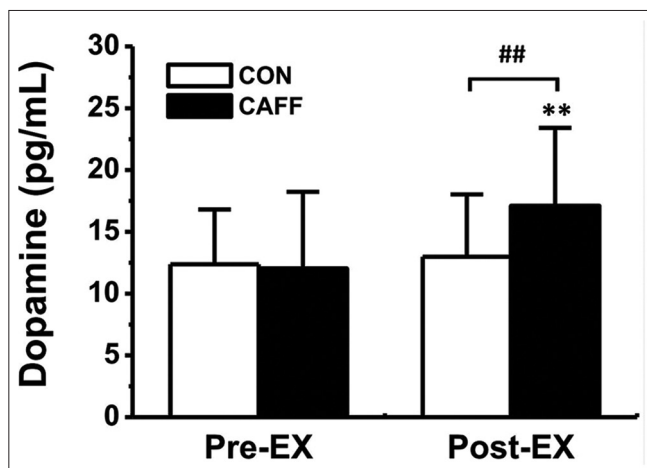


Figure 2: Plasma dopamine concentrations at Pre-EX and Post-EX. Values are means $\pm$ SD. Significant difference, CON versus CAFF, $##P < 0.01$. Significant difference, Pre-EX versus Post-EX, $**P < 0.01$. CON, no caffeine group; CAFF, 3 mg $\cdot$ kg $^{-1}$ caffeine ingestion group; Pre-EX, at -60 min before treadmill running time; Post-EX, immediately after treadmill running exercise at 75% VO_2 max for 40 min.

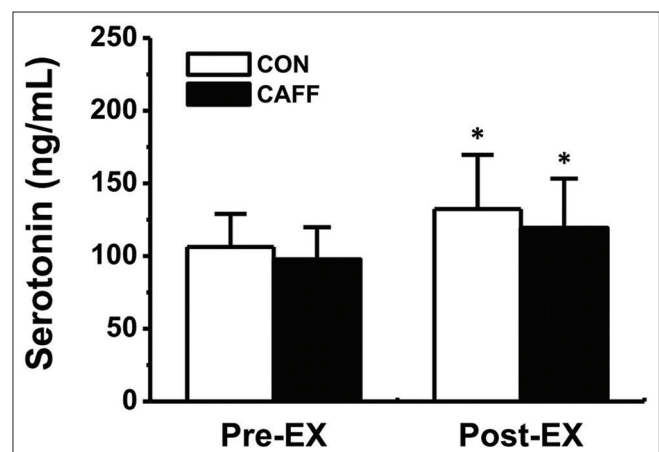


Figure 3: Serum serotonin concentrations at Pre-EX and Post-EX. Values are means $\pm$ standard deviation. Significant difference, Pre-EX versus Post-EX, $*P < 0.05$. CON, no caffeine group; CAFF, 3 mg $\cdot$ kg $^{-1}$ caffeine ingestion group; Pre-EX, at -60 min before treadmill running time; Post-EX, immediately after treadmill running at 75% VO_2 max for 40 min.

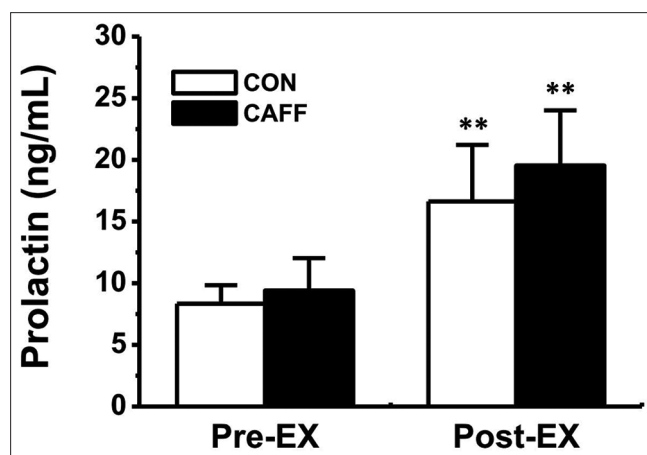


Figure 4: Serum prolactin concentrations at Pre-EX and Post-EX. Values are means $\pm$ standard deviation. Significant difference, Pre-EX versus Post-EX, $**P < 0.01$. CON, no caffeine group; CAFF, 3 mg $\cdot$ kg $^{-1}$ caffeine ingestion group; Pre-EX, at -60 min before treadmill running time; Post-EX, immediately after treadmill running at 75% VO_2 max for 40 min.

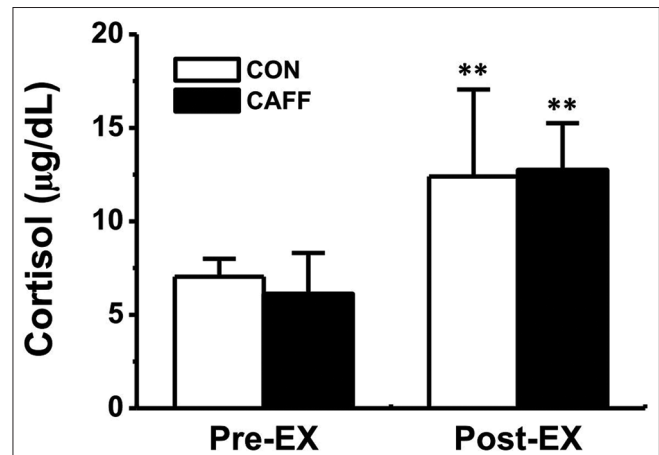


Figure 5: Serum cortisol concentrations at Pre-EX and Post-EX. Values are means $\pm$ standard deviation. Significant difference, Pre-EX versus Post-EX, $**P < 0.01$. CON, no caffeine group; CAFF, 3 mg $\cdot$ kg $^{-1}$ caffeine ingestion group; Pre-EX, at -60 min before treadmill running time; Post-EX, immediately after treadmill running at 75% VO_2 max for 40 min.

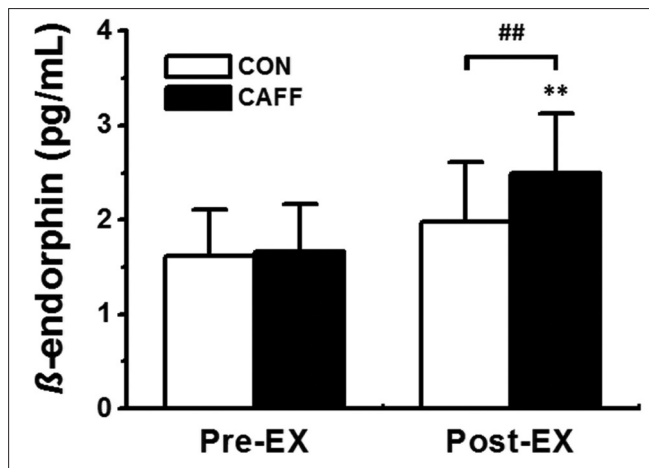


Figure 6: Serum β -endorphin concentrations at Pre-EX and Post-EX. Values are means $\pm$ SD. Significant difference, CON versus CAFF, $##P < 0.01$. Significant difference, Pre-EX versus Post-EX, $**P < 0.01$. CON, no caffeine group; CAFF, $3 \text{ mg} \cdot \text{kg}^{-1}$ caffeine ingestion group; Pre-EX, at -60 min before treadmill running time; Post-EX, immediately after treadmill running at $75\% \text{ VO}_2 \text{ max}$ for 40 min.

of dopamine but not serotonin after 40 min of $75\% \text{ VO}_2 \text{ max}$ treadmill exercise.

In the central nervous system, dopamine mediates reward mechanisms and control of limb movement.^[15] Adenosine inhibits the release of most brain excitatory neurotransmitters, particularly dopamine,^[6] and may reduce dopamine synthesis.^[16] Decreases in dopamine have been linked to central fatigue during exercise.^[17] CAFF is a potent adenosine antagonist.^[18] It has been shown to neutralize most of the inhibitory effects of adenosine following neuroexcitation.^[19]

Multiple studies support the view that CAFF boosts motor activity, for the most part, by inhibiting the indirect pathway via blockade of the adenosine A_{2A} receptors and thereby complementing the effect of dopamine binding to the dopamine receptors D_2 .^[20] Blocking adenosine receptors by CAFF inhibits the effects of adenosine and delays fatigue.

As a result, it decreases the central fatigue by weakening the increase in serotonin/dopamine levels. Previous studies suggested that CAFF delayed the onset of fatigue via a similar mechanism blocking adenosine receptors.^[21] Therefore, the ability of CAFF to block adenosine receptors and alter the release of dopamine is an important ergogenic effect of CAFF.

We found increased plasma levels of dopamine after the treadmill running exercise following CAFF ingestion. Previous studies demonstrated that CAFF significantly increased the release of extracellular dopamine. In addition, the administration of CAFF improved performance during endurance exercise.^[22] These findings support the hypothesis that dopamine release in the brain is correlated with the ergogenic effect of CAFF. We measured prolactin because other studies reported that dopamine was the most important prolactin-inhibiting factor.^[23]

A decrease in prolactin concentration increased the dopamine levels. The circulating levels of prolactin have often been used as an indirect marker for central serotonin activity. The present study showed that prolactin was significantly increased in both groups after treadmill exercise without any significant differences between CON and CAFF groups. The core temperature may stimulate prolactin secretion.^[24] CAFF increased the core temperature during exercise.^[25] Therefore, presumably, the rate of increase in core temperature by CAFF may have abrogated the inhibitory effect of prolactin in the present study.

The levels of serotonin also play a role in many physiological processes. The original central fatigue hypothesis emphasized the importance of serotonin in the fatigue process. Consequently, increased serotonin concentrations may cause both chronic fatigue and poor performance. However, serotonin is not exclusively responsible for the onset of central fatigue, an observation that does not confirm the central fatigue hypothesis.^[26] In search of potential mechanisms underlying fatigue, in addition to the serotonin neurotransmitter system, the possible interaction between different neurotransmitter systems results in fatigue.^[17] The present study demonstrated a significant increase in serotonin in both CON and CAFF after treadmill exercise, without any significant differences between the two groups. Enterochromaffin cells of the gastrointestinal tract also produce serotonin. Our results do not show a possible role of the peripheral action of CAFF.

However, cortisol concentration has also been suggested as a peripheral marker of serotonin activity, and cortisol is the precursor of β -endorphin. Despite the lack of evidence, it is suggested that serotonin acts extensively in the hypothalamus resulting in the release of endorphins. Increase in serotonin neurotransmission facilitates the release of β -endorphin in brain.^[27] Therefore, we have analyzed cortisol and β -endorphin levels. We found a trend toward higher cortisol levels in CAFF than in CON group after treadmill running intervention, with no significant differences between the two groups. Therefore, exposure to acute CAFF did not significantly influence serotonin concentration.

However, β -endorphin was significantly higher in CAFF than in CON group after treadmill running intervention. CAFF intake resulted in the release of β -endorphin by the anterior pituitary. Furthermore, CAFF lowers the threshold for β -endorphin.^[28] The present study further confirms this result, as CAFF induced a significant increase in β -endorphin concentration; however, the mechanisms responsible for the increase in β -endorphin are unclear.

According to results reported previously, intake of a low dose of CAFF did not induce any changes in neurotransmitter release in the brain, but a high dose increased brain dopamine release. It has been reported that a high dose of CAFF has a more significant and beneficial effect on alertness during prolonged wakefulness than low or moderate doses.^[29] A previous study suggested that exposure to 3 mg/kg CAFF

did not induce any changes in extracellular dopamine and serotonin release, whereas exposure to 10 mg/kg CAFF caused a significant increase in extracellular dopamine release at rest in rats.^[22] Moreover, exposure to 3 and 6 mg/kg CAFF did not alter exercise endurance in individuals exposed to chronic intake.^[30] These results indicate that high doses of CAFF have a significant and beneficial effect on extracellular dopamine and serotonin release at rest.

However, our data indicated that CAFF combined with exercise affected dopamine release. Moreover, we observed increased dopamine during treadmill exercise after ingesting 3 mg/kg CAFF in humans. In temperate environments, a CAFF intake of 3–13 mg/kg enhanced performance in endurance exercise. Lower doses can be as effective as higher doses during exercise without any negative consequences.^[31]

The release of dopamine and serotonin depends on exercise intensity. Different exercise intensities may induce different degrees of feedback in the hypothalamus–pituitary gland–adrenal gland axis.^[1] Mandatory treadmill exercise and voluntary wheel running are the two most common types of exercise involving different exercise intensities. Treadmill exercise is more effective than wheel-running exercise in enhancing muscle aerobic capacity and in increasing serum corticosterone level.^[32]

Therefore, our results may be attributed to different exercise intensity and variation in the coffee ingestion habits of daily subjects. Although we cannot rule out the role of dopamine in the onset of centrally mediated fatigue during treadmill exercise following CAFF ingestion, the results of the present study demonstrate that acute ingestion of 3 mg/kg CAFF mediates the release of dopamine in humans during exercise.

CONCLUSION

In conclusion, exposure to 3 mg/kg CAFF had a significant effect on the levels of dopamine. Our data suggest that ingesting 3 mg/kg of CAFF 1 h before 75% VO₂ max treadmill running increases dopamine, without any effect on serotonin inhibition.

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Conflicts of interest

There are no conflicts of interest.

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