Acute Caffeine Consumption Prior to Aerobic Exercise Does Not Influence Substrate Utilization in Recreationally Trained Males and Females

Original Research

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Abstract

Introduction: This study aimed to examine if acute caffeine consumption influenced substrate utilization between recreationally trained males and females during submaximal aerobic exercise.

Methods: Implementing a counter-balanced, crossover design study, 14 recreationally trained males (n = 7) and females (n = 7) consumed either 4 mg/kg of caffeine in 8 oz of water (CAFF) 60 min prior to aerobic exercise at varying submaximal intensities or the solitary consumption of 8 oz of water (CON). Substrate utilization was assessed via indirect calorimetry by measuring the respiratory exchange ratio.

Results: There were no significant main effects for substrate utilization between sexes for the CAFF (p = .265) or CON (p = .253) trial. There were also no significant main effects for independent sex analysis between the two conditions (males, p = .917; females, p = .869).

Conclusions: This study suggests no significant difference in substrate utilization between sexes when consuming caffeine. Although previous literature has indicated caffeine has the potential to increase fat utilization during moderate-intensity aerobic exercise, the current results revealed that caffeine also had no impact on substrate utilization when independently analyzing males and females.

Key Words: sex-specific, fat, carbohydrate

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Introduction

The influence of intensity on substrate utilization during exercise has always been a pertinent topic within the peer-reviewed literature.[1,2] As exercise intensity rises, there is a curvilinear increase in carbohydrate utilization and a curvilinear decrease in fat utilization to yield adenosine triphosphate (ATP), providing energy to stimulate skeletal muscle contraction.[1] The gradual changes in substrate utilization across an intensity spectrum may be influenced by training status, type of training performed, dietary patterns, dietary supplements, and sex.[3,4,5,6,7] One area of consideration is the sex-specific utilization of carbohydrates and fats at various exercise intensities.

The current body of literature has shown that females may exhibit an increase in fat utilization at higher exercise intensities when compared to males.[3,8] Data from a large cohort of males and females (n = 300) showed that males reported a maximal fat oxidation rate at ~45% of maximal oxygen uptake (VO_{2max}), while females reported a maximal fat oxidation rate at

~52% VO_2max (8). Chenevière and colleagues also reported similar findings indicating that females exhibited increased fat utilization rates from 35% up to 85% VO_2max compared to males.[9] Possible mechanisms contributing to this difference in substrate utilization include but are not limited to, a greater percentage of type I muscle fibers in females, greater activation of adrenergic receptors, increased adiposity mass, and increased estradiol-ß-17 secretion and concentration.[3,8,10] However, further research is warranted to examine these possible mechanisms and their roles in substrate utilization.

In addition to the sex-specific influence on substrate utilization, previous studies have shown that the acute consumption of caffeine at rest and prior to exercise may have an effect on substrate utilization.[4,11] Specifically, caffeine consumption may elicit an increase in fat utilization rates. However, data appears to be inconclusive and depends on a multitude of factors.[4,12] Acute consumption of caffeine has been shown to augment the release of catecholamines, specifically epinephrine (EPI), which may aid in increasing the rate of lipolysis.[13] This can increase circulating non-esterified fatty acid concentration and promote a potential increase in fat oxidation.[13] EPI stimulates lipolysis in both males and females by binding to beta-adrenergic receptors located on adipocytes.[14] However, adipocytes have both α- and ß-adrenergic receptors located on the cell membrane.[15] The α-adrenergic receptors have been proposed to elicit an antilipolytic effect, while the ß-adrenergic receptors may enhance lipolysis.[17] It has been theorized that EPI binding capacity to ß-receptors is enhanced in females compared to males.[14,16] Whereas in males, higher EPI secretion may elicit a greater antilipolytic effect by binding to α-receptors.[14] Therefore, although speculative in theory, females may possess the ability to augment fat utilization during exercise following the consumption of caffeine. Currently, there is a paucity of data comparing the efficacy of acute caffeine consumption on substrate utilization between sexes. The primary purpose of this study was to examine if acute caffeine consumption prior to aerobic exercise influences substrate utilization between males and females.

Scientific Methods

Participants

This study was approved by the local college's Institutional Review Board for the use of human participants, and each participant's written informed consent was obtained prior to any testing procedures. All testing procedures were administered in accordance with the ethical standards of the Helsinki Declaration. A total of 14 recreationally trained individuals of similar aerobic fitness participated in this study (females $n = 7$; males $n = 7$; Table 1).

Table 1. Participant characteristics.

	Females	**Males**	***P-v*alue**
Age (yrs.)	20 ± 1.2	22.9 ± 4.3	.026*
Height (in)	66.6 ± 4.4	70.9 ± 1.6	.002*
Weight (kg)	63.1 ± 9.5	88.2 ± 11.1	< .001*
Bodyfat (%)	22.7 ± 7.9	17.4 ± 4.1	.035*
Caffeine Dose (mg)	252.5 ± 38.3	352.8 ± 44.4	< .001*
VO_2peak (ml/kg/min)	46.3 ± 4.4	47.7 ± 6.6	.525

All data are presented as means ± standard deviations; $p < 0.05$; * denotes significant differences between groups.

Participants were deemed eligible to participate in this study if they were between the ages of 18 and 45 years old, were considered physically active[17], had no known cardiovascular or metabolic disease, were currently not smoking, or had quit smoking in the last six months, were not pregnant or attempting to become pregnant, and reported no musculoskeletal injuries that may enable them to perform exercise.

Females who were not taking any form of contraceptive performed both experimental trials 2-to-8 days after the onset of menses in attempts to control for any hormonal fluctuations in fat oxidation rates during exercise.[18] Females taking oral contraceptives were able to participate in this study. Each female's contraceptive information was documented and noted during the initial laboratory visit. Females using oral contraceptives performed their experimental trials during the placebo/withdrawal phase of the prescribed medication.

Participants were asked to report to the Human Performance Laboratory in a well-rested, hydrated state and be at least 3 to 4-h postprandial, as well as having abstained from caffeine the day of testing. Each participant was also instructed to eat a meal with similar macronutrient and liquid content 4-h before each experimental trial. All testing procedures, risks, and benefits were explained to each participant before data collection began.

Protocol

Aerobic Capacity Testing (Visit 1): Participants were required to arrive in a well-hydrated state and to have avoided any physical activity prior to testing. Upon arrival at the laboratory, participants provided consent and were able to ask any

questions about the research protocol at this time. Following consent, basic anthropometric data was collected. Participants' height (in) and body mass (kg) were measured using a stadiometer and a balance beam scale (Health-O-Meter Professional, Pelstar, Bridgeview, IL, USA). Percent body fat was estimated using a bioelectrical impedance device (InBody 770, Cerritos, CA, USA). Participants were required to provide an estimate of the average caffeine (mg) consumed per day. Females were also required to provide information regarding contraceptive use. This data was utilized to schedule female visits during the experimental trials to control for the menstrual cycle's influence on substrate utilization.[11] This information was not required prior to aerobic capacity testing because the menstrual cycle has been shown to not influence peak oxygen uptake (ml/kg/min).[19] Participants were then required to perform a peak oxygen consumption test on a motorized treadmill (Lifetime Fitness, Chanhassen, MN). A heart rate monitor (Polar Inc., Port Washington, New York, USA) was fit around the participant's chest at the level of the xiphoid process to monitor heart rate (HR) during the test. Participants were connected to an indirect calorimeter (ParvoMedics TrueOne 2400, Sandy, UT) via a mouthpiece and hose to record oxygen consumption (VO_2 ml/kg/min), carbon dioxide production (VCO_2 ml/kg/min) and respiratory exchange ratio (RER) every two-minutes during the test. The indirect calorimeter was calibrated in accordance with the manufacturing guidelines before each participant performed a peak oxygen test (VO_2peak). The participants began walking on a treadmill at 3.5 mph at a 3% grade. The treadmill grade increased by 3% every 2 minutes until it reached 15%. After the grade reached 15%, the speed increased by 0.5mph every 2 minutes until volitional fatigue. The highest VO_2 (ml/kg/min) value recorded during the test was considered VO_2peak (ml/kg/min).

Experimental Trials (Visits 2 & 3): Participants were required to perform two separate experimental trials. This study implemented a randomized, counter-balanced, crossover-design study. The randomization of the trials was scheduled via a coin flip for the first participant. Prior to the experimental trials, all participants were verbally instructed to refrain from caffeine consumption the day of testing, be 3 to 4 hours postprandial, perform no exercise or strenuous physical activity, be well-hydrated, and adhere to the same dietary intakes leading up to both of the experimental trials. This was verbally instructed by one of the researchers following visit one and experimental trial 1. Upon arrival at the laboratory, participants were fitted with a heart rate monitor and connected to the indirect calorimeter, following the same procedures as stated in visit 1. Participants performed an incremental treadmill test (ITT) to measure and record substrate utilization via RER at various exercise intensities. For one of the experimental trials, the participants were required to consume 4 mg/kg of caffeine 60 minutes prior to the ITT (CAFF). The caffeine administered was in powder form (Nutricost, Utah, USA) and mixed with 8 oz of water. Prior to the second experimental trial, the participants consumed only 8 oz of water and served as the control trial (CON). The ITT began with participants walking on a motorized treadmill at 3.3 mph with a 2.5% grade. Every 4 minutes, the grade increased by 2.5%. The last stage of the ITT was 3.3 mph, with a grade of 11.5%. The total duration of the ITT was 20 minutes. During the ITT, HR, RER, VO_2 (ml/kg/min), and VCO_2 (ml/kg/min) were measured and recorded.

Statistical Analysis

A one-way analysis of variance (ANOVA) was analyzed to compare age, anthropometrics, aerobic capacity (VO_2peak), and absolute caffeine intake during the experimental trials between sexes. A between-subjects repeated measures ANOVA was utilized to compare the main effects of substrate utilization between the control trial and the caffeine trial. In addition, a between-subjects repeated measures ANOVA was utilized to compare the main effects of sex and substrate utilization in the control trial and the caffeine trial. When appropriate, univariate post-hoc follow-ups were analyzed to identify significant differences and a 95% confidence interval for real change. All data was presented as mean ± SD unless stated otherwise. Statistical significance was set a priori at a $p \leq 0.05$, and all data was analyzed using the statistical package for social sciences (SPSS, v. 28, IBM Corporation, Armonk, NY).

Results

This study recruited 22 recreationally trained males and females to participate. However, due to scheduling constraints and athletic obligations, only 14 individuals completed all three trials (7 males and 7 females).

Table 1. Participant Characteristics

	Females	Males	*p*-Value
Age (yrs.)	20 ± 1.2	22.9 ± 4.3	.026*
Height (in)	66.6 ± 4.4	70.9 ± 1.6	.002*
Weight (kg)	63.1 ± 9.5	88.2 ± 11.1	< .001*
Bodyfat (%)	22.7 ± 7.9	17.4 ± 4.1	.035*
Caffeine Dose (mg)	252.5 ± 38.3	352.8 ± 44.4	< .001*
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All data are presented as means ± standard deviations; $p < 0.05$; * denotes significant differences between groups

Sex comparison analysis*:* Figure 1 displays the differences in RER between males and females during the CON trial. When comparing RER between males and females during CON, there were no significant main effects between the sexes (p = .253). When comparing RER values between males and females (Figure 2) during the CAFF trial, there were also no significant main effects during the ITT (p = .265).

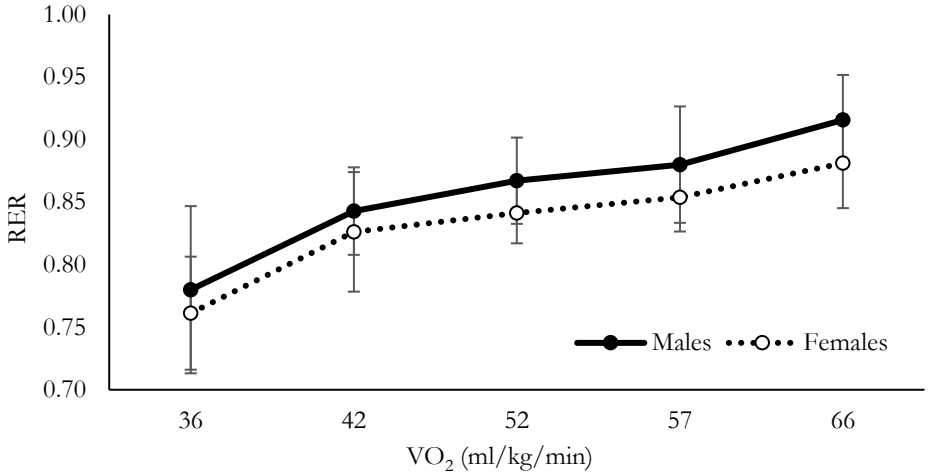

Figure 1. Substrate utilization between males and females during the CON trial. All data is reported as means ± SD.

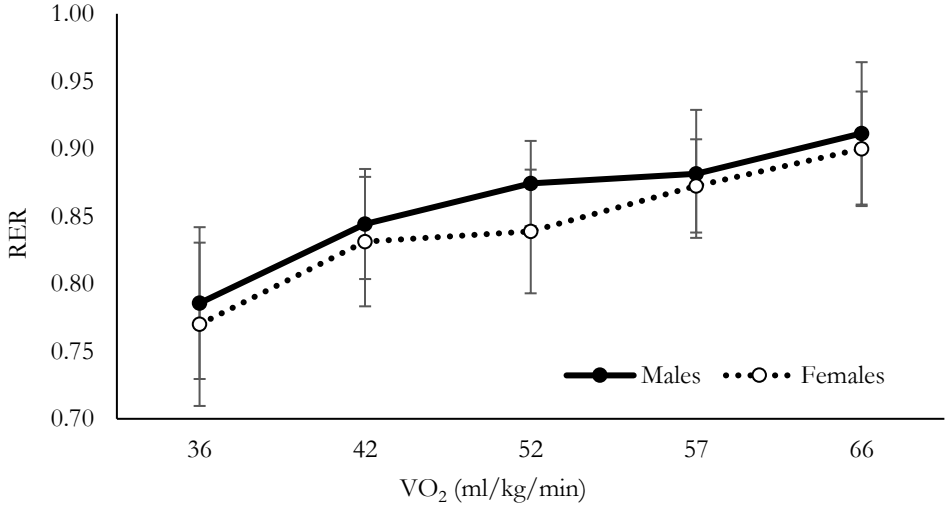

Figure 2. Substrate utilization between males and females during the CAFF trial. All data is reported as means ± SD.

*Independent sex analysis: T*here were no significant between-subject main effects for RER in females when comparing the CON and CAFF trials (p = .869). Similarly to the female data, males displayed no significant between-subjects main effects for RER when comparing the CON and CAFF trials (p = .917).

Discussion

There is a sizable quantity of peer-reviewed literature examining the influence of acute caffeine consumption on substrate utilization during aerobic exercise.[4,5,12,20,21] Previous literature has indicated that acute caffeine consumption prior to aerobic exercise performed at a submaximal intensity may augment fat oxidation.[20,21] However, there are many factors that may contribute to this potential alteration in substrate utilization during exercise.[5,12] Moreover, there appears to be a paucity of data addressing the potential sex differences that may potentially influence substrate utilization following acute caffeine consumption during aerobic exercise. Therefore, this study aimed to analyze the influence of acute caffeine consumption on substrate utilization during various aerobic exercise intensities. The most pertinent finding from this study revealed that 4 mg/kg of caffeine prior to aerobic exercise did not influence substrate utilization in either males or females.

The acute consumption of caffeine at rest and prior to a bout of aerobic exercise has been reported to increase circulating levels of catecholamines.[5,22,23] EPI is one of the primary catecholamines released from the adrenal medulla following acute caffeine consumption. As previously discussed, EPI has been theorized to have an augmented binding affinity to the ß-receptors on adipose tissue in females compared to males, therefore potentially amplifying lipolysis and, thus, fat oxidation.[14,16] Although the potential mechanism for increasing fat oxidation in females is plausible, the current study did not observe any significant differences within or between males and females in substrate utilization when 4 mg/kg of caffeine was consumed 60 minutes prior to submaximal aerobic exercise. As previously alluded to, there are many physiological differences between males and females that elicit a dissimilarity in substrate utilization during exercise. Previous literature has indicated the primary contributing factors, such as hormonal differences, skeletal muscle fiber distribution, intramuscular triglyceride storage, body composition, and the effect of catecholamines on substrate utilization.[3] One important contributing factor that may have influenced the acute effect of caffeine on substrate utilization during exercise is training status.[3,24] This study recruited recreationally trained males and females. Both males and females recorded a VO_2peak of 47.7 ml/kg/min and 46.3 ml/kg/min, respectively. According to the American College of Sports Medicine, the participants in this study were aerobically conditioned.[17] It is noteworthy to discuss these findings due to the influence of training status on substrate utilization. As an individual undergoes regular aerobic exercise, specific oxidative adaptations from exercise are constructed to improve oxygen utilization and, more pertinent to the topic, lipid metabolism.[1,25] These adaptations may increase fat utilization to produce ATP at submaximal intensities.[26,27] The adaptations produced may also influence the ability of caffeine to augment fat utilization during submaximal intensities. A systematic review and meta-analysis by Collado-Mateo et al. indicated that recreational-trained individuals did not measure an increase in fat oxidation during exercise following the consumption of a wide range of caffeine. However, individuals who were considered sedentary or untrained did report an increase in fat-oxidation rates when caffeine was consumed prior to aerobic exercise.[4] The acute consumption of 4 mg/kg of caffeine did not influence any significant shift in substrate utilization in the male or female participants in the current study, possibly due to their training status.

In addition, results indicated there were no significant differences between males and females when no caffeine was consumed prior to submaximal aerobic exercise (Figure 1). The current body of literature indicates that females typically elicit an increase in fat oxidation rates during submaximal aerobic exercise intensities when compared to males.[8] Upon examining Figure 1, the data displays a slightly augmented increase in fat utilization for females across the intensity spectrum during the control trial when compared to males. However, it failed to reach significance at any of the exercise intensities. Although speculative in nature, one potential cause for the lack of discrepancy between males and females could have been attributed to body composition. Body composition is also cited as a contributing factor to substrate differences between males and females during exercise.[3] Females with a higher percentage of body fat may have increased lipolysis during exercise, which may aid in increasing fat oxidation rates.[3,8] On average, college-aged females typically have a higher relative percentage of body fat than males.[28] One contributing factor to sex-specific differences in body composition is attributed to their higher amount of essential fat. Females typically have around 12% essential body fat, while males have around 3%.[29] As shown in Table 1, the females in the current study measured a body fat value of 17.4%. Although there is no universal acceptance or range of what is considered low or high for body fat percentage in females, it has been reported that this level of body fat in this female sample is considered "good" or "lean".[29] Although theoretical, the lower amounts of body fat measured in this female population could have the potential to limit fat oxidation rates when compared to males.

While these findings may be novel and contribute to the current body of literature on caffeine and sex differences during exercise, they are not without limitations. Our participants were required to be 3-4 hours post-prandial instead of the more commonly reported 10-12 hour fast prior to testing. The macronutrient distribution of the meal consumed on the day of testing may have also contributed to changes in substrate utilization during exercise.[30] However, the researchers verbally instructed the participants to eat the same meals and consume their meals at the same time prior to both of the experimental trials. Due to equipment and funding limitations, another potential constraint to the study was the inability to measure hormone levels in the female participants. The fluctuations in hormone values may have contributed to changes in substrate utilization during the time of testing.

Conclusions

The primary findings from this study illustrated that acute caffeine consumption prior to submaximal aerobic exercise does not influence substrate utilization in recreationally trained males or females. This study demonstrates that individuals who are considered more aerobically trained may not elicit an increase in fat utilization during exercise following the acute consumption of caffeine.

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References

1. Brooks GA, Mercier J. Balance of carbohydrate and lipid utilization during exercise: the "crossover" concept. *J Appl Physiol.* 1994; 76(6): 2253-61. doi: 10.1152/jappl.1994.76.6.2253
2. Hargreaves M, Spriet L. Exercise metabolism: fuels for the fire. *Cold Spring Harb Perspect Med.* 2018; 8(8). Doi: 10.1101/cshperspect.a029744
3. Cano A, Ventura L, Martinez G, Cugusi L, Caria M, Deriu F, Manca A. Analysis of sex-based differences in energy substrate utilization during moderate-intensity aerobic exercise. *Euro J Appl Physiol.* 2022; 122: 29-70. Doi: 10.1007/s00421-021-04802-5
4. Collado-Mateo D, Lavín-Pérez AM, Merellano-Navarro E, Del Coso J. Effect of acute caffeine intake on the fat oxidation rate during exercise: a systematic review and meta-analysis. *Nutrients* 2020; 12: 3606. Doi: 10.3390/nu12123603
5. Guest NS, VanDusseldorp TA, Nelson MT, et al. International society of sports nutrition position stand: caffeine and exercise performance. *J Inter Society Sport Nutr.* 2021;18:1. Doi: 10.1186/s12970-020-00383-4
6. Purdom T, Kravitz L, Dokladny K, Mermier C. Understanding the factors that effect maximal fat oxidation. *J Int Soc Sports Nutr.* 15: 3, 2018. Doi: https://doi.org/10.1186/s12970-018-0207-1
7. Staudacher HM, Carey AL, Cummings NK, Hawley JA, Burke LM. Short-term high-fat diet alters substrate utilization during exercise but not glucose tolerance in highly trained athletes. *Int J Sport Nutr Exerc Metab.* 11(3): 273-86, 2001. Doi: 10.1123/ijsnem.11.3.273
8. Venables MC, Achten J, Jeukendrup AE. Determinants of fat oxidation during exercise in healthy men and women: a cross-sectional study. *J Appl Physiol.* 98: 160-167, 2005. Doi: 10.1152/japplphysiol.00662.2003
9. Cheneviére X, Borrani F, Sangsue D, Gojanovic B, Malatesta D. Gender differences in whole-body fat oxidation kinetics during exercise. *Appl Physiol Nutr Metab.* 2011; 36(1): 88-95. Doi: 10.1139/H10-086
10. Willett HN, Koltun KJ, Hackney AC. Influence of menstrual cycle estradiol-ß-17 fluctuations on energy substrate utilization-oxidation during aerobic, endurance exercise. *Int J Environ Res Public Health.* 18(13): 7209, 2021. Doi: 10.3390/ijerph18137209
11. Barcelos RP, Lima FD, Carvalho NR, Bresciani G, Royes LFF. Caffeine effects on systemic metabolism, oxidative-inflammatory pathways, and exercise performance. *Nutr Research* 2020; 80, 1-17. Doi: 10.1016/j.nutres.2020.05.005
12. Conger SA, Tuthill LM, Millard-Stafford ML. Does caffeine increase fat metabolism? A systematic review and meta-analysis. *Int J Sport Nutr Exerc Metab.* 2022; 33(2): 112-120. Doi: 10.1123/ijsnem.2022-0131
13. McLellan TM, Caldwell JA, Lieberman HR. A review of caffeine's effect on cognitive, physical and occupational performance. *Neurosci Biobehav Rev.* 2016; 71: 294-312. Doi: 10.1016/j.neubiorev.2016.09.001
14. Schmidt SL, Bessesen DH, Stotz S, Pellor III FF, Miller BF, Horton TJ. Adrenergic control of lipolysis in women compared with men. *J Appl Physiol.* 117(9): 1008-1019, 2014. Doi: 10.1152/japplphysiol.00003.2014
15. Lafontan M, Langin D. Lipolysis and lipid mobilization in human adipose tissue. *Prog Lipid Res.* 2009; 48: 275-297. Doi: 10.1016/j.plipres.2009.05.001
16. Isacco L, Thivel D, Pereira B, Duclos M, Boisseau N. Maximal fat oxidation but not aerobic capacity is affected by oral contraceptive use in young healthy women. *Eur J Appl Physiol.* 2015; 115: 937-945. Doi: 10.1007/s00421-014-3075-7
17. Axtell RS, Bement MH, Berry R, et al. ACSM's guidelines for exercise testing and prescription, tenth edition. *Wolters Kluwer* 2017.
18. Deemer SE, Castleberry TJ, Irvine C, et al. Pilot study: an acute bout of high intensity interval exercise increase 12.5 h GH secretion. *Physiol Rep.* 2018; 6(2): e13563. Doi: 10.14814/phy2.13563
19. Janse de Jonge XAK. Effects of the menstrual cycle on exercise performance. *Sports Med.* 2003; 33: 833-851. Doi: 10.2165/00007256-200333110-00004
20. Ruiz-Moreno C, Gutiérrez-Hellín J, Amaro-Gahete FJ, et al. Caffeine increases whole-body fat oxidation during 1 h of cycling at fatmax. *Euro J Nutr.* 60: 2077-2085, 2021. Doi: 10.1007/s00394-020-02393-z
21. Varillas-Delgado D, Aguilar-Navarro M, Muñoz A, López-Samanés A, et al. Effect of 3 and 6 mg/kg of caffeine on fat oxidation during exercise in healthy active females. *Biol Sport.* 40(3): 827-834, 2023. Doi: 10.5114/biolsport.2023.121321

22. Papadelis C, Kourtidou-Papadeli C, Vlachogiannis E, et al. Effects of mental workload and caffeine on catecholamines and blood pressure compared to performance variations. *Brain Cogn.* 51(1): 143-54, 2003. Doi: 10.1016/s0278-2626(02)00530-4
23. Van Soeren MH, Graham TE. Effect of caffeine on metabolism, exercise endurance, and catecholamine responses after withdrawal. *J Appl Physiol.* 85(4): 1493-501, 1998. Doi: 10.1152/jappl.1998.85.4.1493
24. González-Haro C, Galilea PA, González-de-Suso JM, Drobnic F and Escanero JF. Maximal lipidic power in high competitive level triathletes and cyclist. *Br J Sport Med.* 2007; 41(1): 23-28. Doi: 10.1136/bjsm.2006.029603
25. Mika A, Macaluso F, Barone R, Di Felice V, Sledzinski T. Effect of exercise on fatty acid metabolism and adipokine secretion in adipose tissue. *Front Physiol.* 10, 2019. Doi: 10.3389/fphys.2019.00026
26. Muscella A, Stefáno E, Lunetti P, Capobianco L, Marsigliante S. The regulation of fat metabolism during aerobic exercise. *Biomolecules* 10(12): 1699, 2020. Doi: 10.3390/biom10121699
27. Tunstall RJ, Mehan KA, Wadley GD, et al. Exercise training increases lipid metabolism gene expression in human skeletal muscle. *Am J Physiol Endrocrinol Metab.* 283(1): E66-72, 2002. Doi: 10.1152/ajpendo.00475.2001
28. Deliens T, Deforche B, Chapelle L, Clarys P. Changes in weight and body composition across five years at university: A prospective observational study. *PLoS ONE* 2019; 14(11). Doi: 10.1371/journal.pone.0225187
29. Jeukendrup A, Gleeson M. *Sport Nutrition*, 3rd ed. Human Kinetic, 2019.
30. Gregory S, Wood R, Matthews T, VanLangen D, Sawyer J, Headley S. Substrate utilization is influenced by acute dietary carbohydrate intake in active, healthy females. *J Sports Sci Med.* 2011; 10(1): 59-65.