



Effect of Caffeine Contained in Sports Drink on Hormones Producing Energy Following Sprint Test Performance in Male Soccer Players

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ABSTRACT

This study investigated the effect of caffeine contained in sports drink on hormones producing energy and sprint test performance in male soccer players. Twelve participants (25.97 ± 2.70 y) performed the test under three conditions (one week apart): caffeine with sports drink (SD-CAF), sports drink (SD), and placebo (PLA). Using a double-blind, placebo-controlled, randomized, crossover protocol, participants performed SD-CAF trial (5 mg/kg of caffeine contained in 300 ml of sports drink 30 minutes prior to sprinting test (7×30 m), SD trial (solely 300 ml of sports drink 30 minutes prior to sprinting test), or placebo. Blood analysis indicated significantly higher level of free thyroxine in SD-CAF (21.450 ± 3.048) compared to SD (18.742 ± 1.151) and PLA (16.983 ± 1.783). Similar findings existed regarding insulin ($P < 0.05$). However, DHEAs was significantly higher in PLA compared to SD-CAF and SD. No statistical differences were observed between trials in testosterone and blood lactate ($P > 0.05$). No significant differences were observed between trials in first-fourth repetitions ($P > 0.05$). Time of fifth-seventh repetitions were significantly lower in SD-CAF compared to SD and PLA ($P < 0.05$), and were significantly lower in SD than that in PLA ($P < 0.05$). The time of 7th repetition was (4.331 ± 0.210 , 4.610 ± 0.197 , 4.816 ± 0.171 s for SD-CAF, SD, and PLA, respectively; $P < 0.05$). In conclusion, caffeine interferes hormones that are responsible for producing energy which in turn have a positive effect on repeated sprint bouts.

Keywords: Caffeine, energy hormones, sports drink, thyroxine, testosterone.

INTRODUCTION

Soccer players run roughly 10-12 km during a 90-minute game^[1]. However, players may be predisposed to fatigue not because of prolonged time spent in a game, but due to repeated sprints^[2] where the glycogen-lactic acid transferring system is the main source for sprinting^[3]. Players have practiced hard training to ameliorate milliseconds in short distances^[4] and during a game, the players' energy expenditure may exceed 1500 Kcal^[5]. Nourishment interventions appear to be beneficial to store and increase energy^[6].

Isotonic sports drink which contains carbohydrate, minerals, and electrolytes^[7, 8], has become commonly used by athletes to delay the onset of fatigue^[9-11]. Sports drinks are consumed pre-exercise to store calories and during exercise to replenish energy^[11]. Lee et al. (2011)^[9] reported that ingestion of sports drink (equivalent to 150 % of sweat lost) improved capacity of endurance (1.14 ± 0.22 h) as compared to only water (0.85 ± 0.27 h) and placebo (0.92 ± 0.25 h). Therefore, variant sport drinks are nowadays more popular and importantly enriched with caffeine^[6].

Caffeine (1,3,6-trimethylxanthine) is an alkaloid^[12,13] that has been used as the most frequently pharmaceutical product consumed by athletes to enhance physical and cognition performances^[14-16]. Caffeine is rapidly absorbed through gastrointestinal tract^[12-14, 17, 18] and reaches its steady state plasma concentration within 30-60 minutes^[13, 17, 19-21]. Furthermore, caffeine easily cross blood-brain barrier due to its moderate hydrophilicity and lipophilicity^[13, 22, 23]. This explains partially that caffeine is central nervous system (CNS) stimulant which delays fatigue^[18]. It is important to notice that the enhancement of performance may occur when combining caffeine with carbohydrate sports drink rather than carbohydrate sports drink intake alone^[6]. Stevenson and colleagues found that the ingestion of sports drink contained caffeine (16 mg/100 ml) before and during golf game improves putting performance.

The main mechanism that explains caffeine's ergogenic effect is the inhabitation of adenosine receptors^[12, 13, 24], namely A1 and A2a^[17, 19]. Actually, caffeine acts on ryanodine receptors that stimulate sarcoplasmic reticulum to release Ca⁺²^[25], increase acetylcholine^[17, 26] and induce excitation contraction coupling^[27]. Caffeine can change other neurotransmitters into the synaptic cleft^[26] such as dopamine and serotonin^[17, 19, 25, 26]. There is also evidence that caffeine could increase secretion of hormones including glucocorticoids, insulin^[28], and catecholamins^[25, 29, 30]. Lee, Lin & Ching. (2011)^[25] examined the effect of ingestion of 6 mg/kg of caffeine with 20 g creatine on the time to exhaustion, but no statistical differences in adrenaline and noradrenaline levels were detected either after taking caffeine with creatine, or taking placebo.

To our knowledge, no previous research investigated the effects of caffeine in sports drink on energy hormones, namely thyroxine (T4) despite its importance on body metabolic rate (BMR). T4 is the main regulator of biochemical reactions, and it has been considered to elevates BMR from 50 % to 100 %. Consequently, the primary aim of the present study was to investigate the effect of caffeine contained in sports drink on the concentration of energy hormones including T4, testosterone, dehydroepiandrosterone sulfate (DHEAs) and insulin. The second aim was to determine the effect of caffeine contained in sports drink on sprint test performance. We postulated that caffeine may stimulate the secretion of hormones producing energy which in turn improves physical performance.

MATERIALS AND METHODS

Participants

Twelve soccer players from professional Jordanian teams were chosen to be participants in the current study. The mean ± SD for the participants' age was (25.97±2.70 years), height: (180.25±2.26 cm), weight: (76.64±3.46 kg), and BMI: (23.61±0.86 kg/m²). Written consent was signed by each participant. Before being accepted to enroll in the trial, all players were asked about their health issues and the use of any ergogenic aids. The players maintained regular training which occurred routinely in the stadium, but were asked to abstain from intense exercise for 48 hours prior to each trial. These trials were approved by the Faculty's Health Research Ethics Committee at Yarmouk University.

A randomized, double-blind, placebo-controlled, crossover design was used. The study consisted of three consequential trials: sports drink containing caffeine (SD-CAF) trial, sports drink (SD) trial, and placebo (flavor syrup) trial. The separation period was one week between each two consequent trials. All trials were beginning in the early morning (9,00 a.m) to maintain the circadian cadence. Two days prior to commencement of the trials, blood samples were collected from antecubital vein at 8:30 am after an overnight fasting (roughly 10 hours). The blood samples were taken to determine the baseline measurements of biochemical parameters. The baseline measurements of biochemical parameters results are illustrated in Table 1.

Table 1. Baseline measurements of biochemical parameters. Data are Mean ± SD

Variable	Value
Testosterone, total	487.33 ± 36.40 ng/dl
DHEAs	396.25 ± 28.38 IU/L
Insulin	9.17 ± 0.79 μU/ml
Thyroxine, free	15.87 ± 1.00 pmol/L
Blood lactate	1.68 ± 0.25 mmol/L

Procedure

When the participants arrived the stadium 30 minutes prior to beginning the trial, they consumed 300 ml of either; sports drink (OSHEE), sports drink containing 5 mg/kg of caffeine or the same amount of placebo. After that, the participants performed their routinely warm-up exercises for 20 minutes. Subsequently, the participants started the sprint test (7 × 30 m) and the sprinting time was recorded at the end of each repetition. Blood samples were taken from each participant immediately at the end of sprint test (following the seventh repetition).

Sprint test

A 30-meter sprint test was used to specifically examine fatigue induced by the repetition of the sprints. Each participant performed seven 30-meter sprint tests. Participants were asked to take 30-second rest between each two consequent repetitions to allow for recovery. The sprint time was measured using an (Infrared Control System, Clock Counter, 63501IR, USA).

Blood sample analysis

The blood samples were centrifuged for analysis of biochemical assays. Testosterone total was stored in plane tube, centrifuged at 5000 RPM/5 min and analyzed using (Cobas 6000, (Roche) German). Free T4 was stored in plane tube, centrifuged at 5000 RPM/5 min and analyzed using (Elecsys, 2010, Switzerland). Insulin was frozen at -20°C, centrifuged at 5000 RPM/5 min and performed by (Cobas 6000, (Roche) German). DHEAs was stored in plane tube, centrifuged at 5000 RPM/5 min and analyzed using (Cobas 6000, Roche, German). Integral 400 (Switzerland) was used to examine blood lactate.

Statistical analysis

A repeated measures analysis of variance (ANOVA) with a Greenhouse-Geisser correction were used to determine possible differences between trials in biochemical responses and sprinting time during each repetition. When a significant *F* rate was achieved, post-hoc tests using the Benferroni correction was used for pairwise comparisons using adjusted means. Data are presented as mean ± SD. *P* value < 0.05 was considered to be significant. Statistical Package of social science software (SPSS) version 18.0 was used for all analysis.

RESULTS

Biochemical tests results

Figures 1-5 below showed the results of the biochemical parameters examined in the trials. It was found, that caffeine elicited a significant increment in free T4 concentration in the SD-CAF trail than SD and PLA trials (*P* < 0.05) (Figure 1). Additionally, free T4 was significantly higher in SD than PLA (*P* = 0.026). There was a slight insignificant increment in testosterone concentration in SD-CAF (900.83 ± 94.85 ng/dl) comparing to the other two trials: SD and PLA (837.92 ± 107.90 ng/dl, and 825.83 ± 121.27 ng/dl, respectively) with *P* value = 0.160 (Figure 2). Serum DHEAs concentration in the PLA trial was significantly higher than that in the SD-CAF and SD (*P* = 0.002 and *P* = 0.006, respectively) (Figure 3), however, no statistical difference was observed in DHEAs level between SD-CAF and SD (*P* = 1.000). Regarding serum insulin, there were comparable increments in insulin concentrations among SD-CAF and SD participants (*P* = 0.477), however, both trials' levels were significantly higher than PLA (*P* < 0.05), (Figure 4). No statistical differences were observed in blood lactate concentrations between trials (*P* = 0.126) (Figure 5).

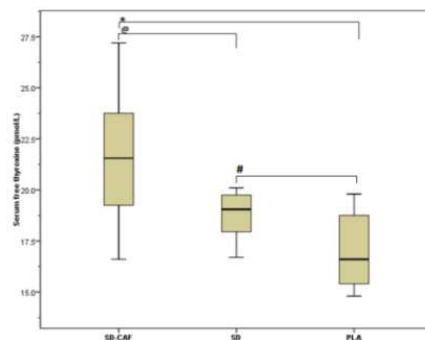


Figure 1. Free Thyroxine concentration in SD-CAF, SD, and PLA trials. *Significantly different to PLA (*P* < 0.05). #Significant difference between SD and PLA (*P* < 0.05). @Significantly different to SD (*P* < 0.05).

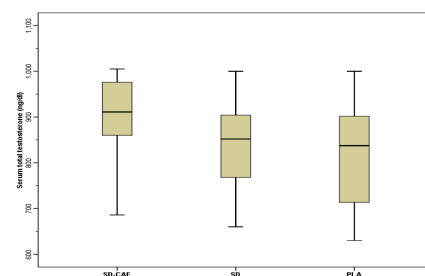


Figure 2. Serum total testosterone concentration in SD-CAF, SD, and PLA trials. The serum total testosterone is not significantly different between trials (*P* > 0.05).

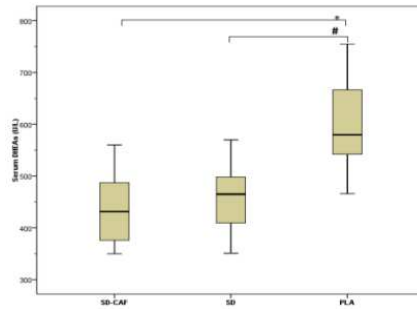


Figure 3. Serum dehydroepiandrosterone-sulfate (DHEAs) concentration in SD-CAF, SD, and PLA trials. *Significantly different to SD-CAF (P < 0.05). # Significantly different to SD (P < 0.05).

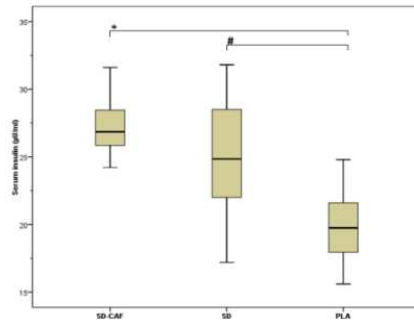


Figure 4. Serum insulin concentration in SD-CAF, SD, and PLA trials. * Significantly different to PLA (P < 0.05). #Significantly different to SD and PLA (P < 0.05).

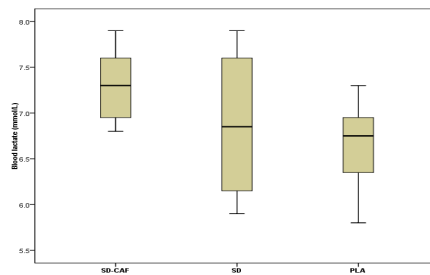


Figure 5. Blood lactate concentration in SD-CAF, SD, and PLA trials. The blood lactate is not significantly different between trials (P > 0.05).

Sprint test performance results

All participants completed the seven repetitions of the sprint test in each trial. The time spent for each repetition was measured, the results are shown in Table 2. There was a significant decrease in the time needed to complete the 5th, 6th and 7th repetitions in the SD-CAF trial (P < 0.05) compared to SD and PLA trials. However, there were no significant differences between trials in the first four repetitions with F values range (0.403-4.056).

Table 2. Time of repetition in the three trials during sprint test.

Time of rep (s)	1 rep	2 rep	3 rep	4 rep	5 rep	6 rep	7 rep
SD-CAF	3.724 ± 0.121	3.854 ± 0.116	3.969 ± 0.116	4.050 ± 0.137	4.118 ± 0.124*	4.240 ± 0.173*	4.331 ± 0.210*
SD	3.720 ± 0.125	3.851 ± 0.115	3.980 ± 0.105	4.097 ± 0.075	4.305 ± 0.135#	4.479 ± 0.195#	4.610 ± 0.197#
PLA	3.724 ± 0.119	3.855 ± 0.120	3.996 ± 0.142	4.128 ± 0.099	4.482 ± 0.145	4.663 ± 0.173	4.816 ± 0.171

SD-CAF refers to sports drink containing caffeine trial. SD refers to sports drink trial. PLA means placebo. rep= repetition. *Significantly different from SD and PLA (P < 0.05). #Significantly different from PLA (P < 0.05). Values are mean ± standard deviation

DISCUSSION

Biochemical responses

This study aimed to investigate the effect of caffeine contained in sports drink on hormones producing energy following sprint test performance in male soccer players. There was a significant increase in free T4 among SD-CAF players compared to SD and PLA. This result can be explained by the role of T4 in increased BMR^[31-33] which

is elevated 50%-100% of BMR^[34] and increased muscular power^[35]. Huang et al. (2004)^[36] found that thyroid hormone, specifically free T4, increased 1 hour after incremental treadmill exercise protocol but still within normal range (25.5 ± 0.6 pmol/l) in adult healthy military recruits. Their result seems to concur with our finding regarding the SD-CAF (21.453 ± 0.480 pmol/l) although Huang and colleagues^[36] did not use caffeine intervention. Therefore, further research about free T4 concentration after consumption of caffeine is needed.

In addition, there was a significant increase in insulin hormone in SD than that in PLA. This result can be attributable to carbohydrates inside sports drink that induced elevation in blood glucose level and consequent released of insulin. Glucose is the primary resource of biochemical reactions for the brain. In addition, insulin hormone decreases satiety and increases energy expenditure^[3, 34]. This seems to be a potential explanation for the role of sports drink (Carbohydrates alone) to decrease sprint time in 5th–7th repetitions compared to placebo. Furthermore, SD-CAF trial showed a lower sprint time in 5th – 7th repetitions compared to SD and PLA. Despite that caffeine can spare glycogen manipulation^[14, 37-39], it is the primary potential ingredient founded in the coffee and energy drinks^[21] and thus in sports drink. Therefore, caffeine and sports drink containing carbohydrates together have positive effect on central nervous system^[6].

Moreover, it has been reported that caffeine stimulates the secretion of stress (cortisol) hormone^[30], catecholamines^[40], and dopamine^[13]. These hormones facilitate energy to perform hard activity. Little data has been published on coffee consumption and sex hormones in men. Testosterone was more increased in SD-CAF trial than that in SD and PLA, but without significant differences. Wedick et al. (2012)^[41] found that caffeinated coffee, but not decaffeinated coffee, significantly increased total testosterone in men. However, another trial^[42] found that consumption of two cups of instant coffee had no acute effect on testosterone or estradiol concentrations after 30 minutes. Another study suggested that ingestion of caffeine via chewing gum, delayed fatigue during repeated, high-intensity sprint associated with testosterone elevation^[43].

Caffeine also decrease levels of the steroid hormone, dehydroepiandrosterone, (DHEA). This was explained by the inverse relationship between cortisol and DHEAs which are secreted by adrenal gland: high levels of stress and elevated cortisol are associated with lower levels of DHEAs^[44]. It is known that in any case, high levels of stress, caffeine consumption and increased levels of cortisol are associated with lower levels of DHEAs.

The present study showed no significant differences in blood lactate concentration in all trials. This result concurs, but without significant, with study of Lee, Lin & Cheng, (2011)^[25]. In their study, blood lactate was increased significantly at post-test in creatine+caffeine (11.9 ± 2.3 mmol/L) compared to CRE+PLA (10.8 ± 1.8 mmol/L) and Base (9.7 ± 1.9 mmol/L) ($P < 0.05$). However, the test in that study concluded incremental exercise protocol on cycling ergometer until exhaustion and the supplements were creatine plus caffeine. Some researches demonstrated that caffeine increases blood lactate concentration^[45].

Sprint test performance

It was observed that time needed to complete the last three repetitions (5th -7th) was significantly less in SD trial than PLA trial. This indicates that sport drink could boost energy during physical performance. In the present study, sport drinks contained 10.7g CARBS/100 ml. This result seems to support Scholey et al.'s (2014)^[46] findings that 25-50 g of glucose are sufficient to improve the physical performance. Additionally, it is well known that both fat and carbohydrate are the main fuels utilized during exercise. Each of intensity and duration of exercise determine which fuel acts as the primary source of energy. As high intensity exercises mainly depend on carbohydrate energy supply^[47, 48], SD participants had more stamina and attention to perform the sprint test faster than other trials' participants.

Importantly, the time of the last three repetitions (5th-7th) spent by SD-CAF trial players were significantly lower compared to SD and PLA players. This finding support previous research in which caffeine improved cycling time performance (19.7 ± 3.3 min) compared to placebo (20.5 ± 3.5 min) (Miller et al. 2014)^[49]. In another study, Schneiker et al. (2006)^[50] found that ingestion of 6 mg/kg of caffeine supplement by 10 recreationally soccer and hockey players improved total sprint work of intermittent–sprint test by 8.5% in the first half and 7.6% in the second compared to placebo. Similar results were showed in swimming as Collomp et al. (1991)^[51] found that intake of 4.3 mg/kg of caffeine increased swimming velocity in two 100 m freestyle swimming dash in trained compared to untrained swimmers. Furthermore, Lee, Lin, & Cheng (2011)^[25] showed that the time to exhaustion was significantly longer in creatine+caffeine trial (1087.2 ± 123.9 s) compared to each of creatine+placebo trial (1040.3 ± 96.1 s) and baseline trial (no ergogenic aid; 1009.2 ± 86.0 s). Additionally, improvement in sprinting performance especially during the last three repetitions in SD-CAF trial can be attributable to high energy amount resulted from caffeine liberated from muscle fibers that sustained the ability to run in higher speed compared to the other two trials.

Caffeine could stimulate the excretion of β -endorphins^[12], which increases during exercise and has been considered to be responsible for pain relief^[52]. Thus, the combination of caffeine and exercise can increase the concentration of plasma β -endorphins. This could be the explanation for the beneficial effects of caffeine in reduction of pain sensation^[9, 53, 54] during sprint test. Laurent *et al.* (2000)^[55] found that ingestion of 6 mg/kg of caffeine caused increased concentration of β -endorphin after cycling (2 hours at 65% VO₂peak). In addition to its effect on physical performance, caffeine can increase lipolysis and lipid oxidation which increases free fatty acids levels in the blood^[9, 12, 14, 28, 37, 56-59]. Thus, there is evidence that caffeine increases not only energy expenditure^[28] but induces lipid mobilization and fat oxidation^[16, 60].

CONCLUSION

In summary, caffeine contained in sports drink had a positive effect on sprint test (7 x 30 m) performance by soccer players. This suggests that ingest 5 mg/kg caffeine with 300 ml of sport drink (OSHEE) 30 min prior to sprint test enhance repetitions time by an increment of the secretion of hormone producing energy including T4, testosterone, and insulin.

Key points

- Ingestion of caffeine contained in sports drink enhances physical performance rather than sports drink alone.
- Ingestion of caffeine (5 g/kg) 30 minutes prior to exercise improves high intensity sprint bouts performance (7 x 30 m).
- Ingestion of caffeine before exercise stimulates increased secretion of thyroxine hormone as well as testosterone,

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Declaration of Conflicting Interest

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

REFERENCES

- [1] Koundourakis NE, Androulakis NE., Malliaraki N, Margioris AN. Vitamin D and exercise performance in professional soccer players. *PLOS ONE*. 2014; 9(7): 1-6
- [2] Rampinini E, Sassi A, Morelli A, Mazzoni S, Fanchini M, Coutts AJ. Repeated-sprint ability in professional and amateur soccer players. *Appl. Physiol. Nutr. Metab.* 2009; 34: 1048-1054
- [3] Guyton AC, Hall JE. *Textbook of Medical Physiology*, 11th ed. ELSEVIER SAUNDERS Publisher; 2006
- [4] Haugen T, Tonnessen E, Oksenholt O, Haugen FL, Paulsen G, Enoksen E, Seiler S. Sprint conditioning of junior soccer players: Effects of training intensity and technique supervision. *PLOS ONE*. 2015; 1-13
- [5] Nikolaidis PT, Theodoropoulou E. Relationship between nutrition knowledge and physical fitness in semiprofessional soccer players. *Scientifica*. 2014; 1-5
- [6] Stevenson EJ, Hayes PR, Allison SJ. The effect of a carbohydrate-caffeine sports drink on simulated golf performance. *Appl. Physiol. Nutr. Metab.* 2009; 34: 681-688
- [7] Shirreffs SM, Armstrong LE, Chevront SN. Fluid and electrolyte needs preparation and recovery from training and competition. *Journal of Sports Sciences*. 2004; 22: 57-63
- [8] Xiang N, Wethington H, Onufrak S, Belay B. Characteristics of US health care providers who counsel adolescence on sports and energy drink consumption. *International Journal of Pediatrics*. 2014; 1-10
- [9] Lee JKW, Nio AQX, Ang WH, Law LL, Lim CL. Effects of ingestion a sports drink during exercise and recovery on subsequent endurance capacity. *European Journal of Sport Science*. 2011; 11(2): 77-86
- [10] Montain SJ, Sawka MN, Latzka WA, Valeri CR. Thermal and cardiovascular strain from hypohydration: Influence of exercise intensity. *International Journal of Sports Medicine*. 1998; 19: 87-91
- [11] Yunusa I, Ahmed IM, Adegbusi HS, Abdulkader RS, Huzafa U, Kabara HT. (2013). Sports/energy drinks consumption among young athletes in kano, Nigeria. *Bayero Journal of Pure and Applied Sciences*. 2013; 6(2): 1-5
- [12] Goldstein ER, Ziegenfuss T, Kalman D, Kreider R, Campell B, Wilborn C, *et al.* Internaotional society of sports nutrition position stand: Caffeine and performance. *Journal of the International Society of Sports Nutrition*. 2010; 7(5): 1-15
- [13] Nawrot P, Jordan S, Eastwood J, Rotstein J, Hugenholtz A, Feeley M. Effects of caffeine on human health. *Food Additives and Contaminants*. 2003; 20(1): 1-30

- [14] Campbell B, Wilborn C, Bounty PL, Taylor L, Nelson MT, Greenwood M, et al. (2013). International society of sports nutrition position stand: energy drinks.
- [15] Duncan MJ, Tallis J, Wright SL, Eyre ELJ, Bryant E, Langdon D. The effect of acute caffeine ingestion on coincidence anticipation timing in younger and older adults. *Nutritional Neuroscience*. 2014; 17(5): 234-238
- [16] Graham TE. Caffeine and exercise: metabolism, endurance & performance. *Sports Medicine*. 2001; 31(11): 785-807
- [17] Camfield DA, Stough C, Farrimond J, Scholey AB. Acute effects of tea constituents L-theanine, caffeine, and epigallocatechin gallate on cognitive function and mood: a systematic review and meta-analysis. *Nutrition Review*. 2014; 72(8): 507-522
- [18] Gonzalez AM, Hoffman JR, Wells AJ, Mangine GY, Townsend JR, Jajtner AR, et al. Effects of time-releasing caffeine containing supplement on metabolic rate, glycerol concentration and performance. *Journal of Sports Science and Medicine*. 2015; 14: 322-332
- [19] Dixit A, Goyal A, Thawan R, Vaney N. Effect of caffeine on information processing: Evidence from stroop task. *Indian Journal of Psychological Medicine*. 2012; 34(3): 218-222
- [20] Phillips GC. In Johnson DL, Mair SD *Clinical sports medicine*. MOSBY Publisher; 2006. pp 35-46
- [21] Johnson LA, Foster LD, McDowell LJ. Energy drinks: review of performance benefits, health concerns, and use by military personnel. *Military Medicine*. 2014; 179(4): 375-380
- [22] Fredholm BB, Battig K, Holmen J, Nehlig A, Zvartau EE. Action of caffeine in the brain with special reference to factors that contribute to its widespread use. *Pharmacol Rev*. 1999; 51: 83-133
- [23] McCall AL, Millington WR, Wurtman RJ. Blood-brain barrier transport of caffeine: Dose-related restriction of adenosine transport. *Life Sci*. 1982; 31: 2709-2715
- [24] Dodd FL, Kennedy DO, Riby LM, Haskell-Ramsay CF. A double-blind, placebo-controlled study evaluating the effects of caffeine and L-theanine both alone and in combination on cerebral blood flow, cognition and mood. *Psychopharmacology*. 2015; 232: 2563-2576
- [25] Lee CL, Lin JC, Cheng CF. Effect of creatine plus caffeine supplements on time to exhaustion during an incremental maximum exercise. *European Journal of Sport Science*. 2011; 1-9, iFirst article
- [26] Pilli R, MUR N, Pingali UR, Takallapally RKR. Study of cardiovascular effects of caffeine in healthy human subjects, with special reference to pulse wave velocity using photoplethysmography. *International Journal of Nutrition, Pharmacology, Neurological Diseases*. 2012; 2(3): 243-250
- [27] Souissi M, Abdelmalek S, Chtourou H, Boussita A, Hakim A, Sahnoun Z. Effects of time-of-day and caffeine ingestion on mood states, simple reaction time, and short-term maximal performance in elite judoists. *Biological Rhythm Research*. 2013; 44(6): 897-907
- [28] Shearer J, Graham TE. Performance effects and metabolic consequences of caffeine and caffeinated energy drink consumption on glucose disposal. *Nutrition Reviews*. 2014; 72(S1): 121-136
- [29] Gimba, CE, Abechi SE, Abbas NS, Gerald IU. Evaluation of caffeine, aspartame and sugar contents in energy drinks. *Journal of Chemical and Pharmaceutical Research*. 2014; 6(8): 39-43
- [30] Lovallo WR, Whitset, TL, Al'Absi M, Sung BH, Vincent AS, Wilson MF. Caffeine stimulation of cortisol secretion across the waking hours in relation to caffeine intake levels. *Psychosomatic Medicine*. 2005; 67(5): 734-739
- [31] Acheson KJ, Zahorska-Markiewicz B, Pittet P, Anantharaman, K, Jequier E. Caffeine and coffee: Their influence in metabolic rate and substrate utilization in normal weight and obese individuals. *The American Journal of Clinical Nutrition*. 1980; 33(5): 989-997
- [32] Dulloo AG, Geissler CA, Horton T, Collins A, Miller DS. Normal caffeine consumption: Influence on thermogenesis and daily energy expenditure in lean and postobese human volunteers. *The American Journal of Clinical Nutrition*. 1989; 49(1): 44-50
- [33] Koot P, Deurenberg P. Comparison of changes in energy expenditure and body temperature after caffeine consumption. *Annals of Nutrition and Metabolism*. 1995; 39(3): 135-142
- [34] Tortora GJ, Derrickson B. *Principles of anatomy and physiology*. 11th ed. WILEY Publisher; 2009
- [35] Wazaify M, Bdair A, Al-Hadidi K, Scott J. Doping in gymnasiums in amman: The other side of prescription and nonprescription drug abuse. *Substance Use & Misuse*. 2014; 49:1296-1302
- [36] Huang W, Yu M, Lee M, Cheng C, Yang S, Chin H, Wu S. Effect of treadmill exercise on circulating thyroid hormone on measurements. *Medical Principles and Practice*. 2004; 13: 15-19
- [37] Graham TE, Hebbert E, Sathasnam P. Metabolic and exercise endurance effects of coffee and caffeine ingestion. *J Appl Physiol*. 1998; 85: 883-889
- [38] Kovacs EM, Stegen J, Brouns F. Effects of caffeinated drinks on substrate metabolism, caffeine excretion, and performance. *J Appl Physiol*. 1998; 85: 709-715
- [39] McLellan TM, Bell DG. The impact of prior coffee consumption on the subsequent ergogenic effect of anhydrous caffeine. *Int J Sport Nutr Exerc Metab*. 2004; 14: 698-708
- [40] Benowitz NL. Clinical pharmacology of caffeine. *Annual Review of Medicine*. 1990; 41: 277-288

- [41] Wedick NM. The effects of caffeinated and decaffeinated coffee on sex hormone-binding globulin and endogenous sex hormone levels: a randomized controlled trial", *Nutrition Journal*. 2012; 11, 12-20
- [42] Phillips GB. The variability of the serum estradiol level in men: effect of stress (college examinations), cigarette smoking, and coffee drinking on the serum sex hormone and other hormone levels. *Steroids*; 1992; 57:135–141.
- [43] Paton CD, Lowe T, Irvine A. Caffeinated chewing gum increases repeated sprint performance and augments increases in testosterone in competitive cyclists. *Eur J Appl Physiol*. 2010; 110(6):1243-50.
- [44] Wolkowitz OM, Epel ES, Reus VI. Stress hormone-related psychopathology: pathophysiological and treatment implications. *World Journal of Biological Psychiatry*. 2001; 2(3): 115-43
- [45] Leonie FJ, Mettler S, Perret C. Influence of caffeine and sodium citrate ingestion on 1,500-m exercise performance in elite wheelchair athletes: Apilot study. *Int J Sport Nutr Exerc Metab*. 2014; 24(3): 296-304
- [46] Scholey A, Savage K, O'Neil BV, Owen L, Stough C, Priestley C. Effects of two doses of glucose and a caffeine-glucose combination on cognitive performance and mood during multi-tasking. *Hum. Psychopharmacol Clin Exp*. 2014; 20: 434-445
- [47] Coggan AR, Coyle EF. Carbohydrate ingestion during prolonged exercise: Effects on metabolism and performance. *Exerc Sports Sci Rev*. 1991; 19: 1-40
- [48] Coyle EF, Montain SJ. Carbohydrate and fluid ingestion during exercise: are there trade-offs. *Med Sci Sports Exerc*. 1992; 24: 671-678
- [49] Miller B, O'Connor H, Orr R, Ruell P, Cheng HL, Chow CM. Combined caffeine and carbohydrate ingestion: effects on nocturnal sleep and exercise performance in athletes. *Eur J Appl Physiol*. 2014; 114: 2529-2537
- [50] Schneider KT, Bishop B, Dawson B, Hackett LP. Effect of caffeine on prolonged intermittent-sprint ability in team sport athletes. *Med Sci Sports Exerc*. 2006; 38: 578-585
- [51] Collomp K, Ahmaidi S, Audran M, Chanal JL, Prefaut C. Effects of caffeine ingestion on performance and anaerobic metabolism during the wingate test. *Int J of Sports Med*. 1991; 12: 439-443
- [52] Grossman A, Sutton JR. Endorphins: What are they? How are they measured? What is their role in exercise?. *Med Sci Sports Exerc*. 1985; 17: 74-81
- [53] Harvanko AM, Derbyshire KL, Schreiber LRN, Grant JE. The effect of self-regulated caffeine use on cognition in young adults. *Hum. Psychopharmacol Clin Exp*. 2015; 30: 123-130
- [54] Smith AP, Nutt DJ. Effects of upper respiratory tract illnesses, ibuprofen and caffeine on reaction time and alertness. *Psychopharmacology*. 2014; 231: 1963-1974
- [55] Laurent D, Schneider KE, Prusaczyk WK, Franklin C, Vogel SM, Krssak M, Petersen KF, Goforth HW, Shulman GI. Effects of caffeine on muscle glycogen utilization and the neuroendocrine axis during exercise. *J Cline Endocrinol Metab*. 2000; 85: 2170-2175
- [56] Bonati M, Latini R, Galletti F, Young JF, Tognoni G, Gerattinin S. Caffeine disposition after oral doses. *Clin Pharmacol Ther*. 1982; 32: 98-106
- [57] Davoodi SH, Hajimiresmaiel SJ, Ajami M, Mohsen-Bandpi A, Ayatollahi SA, et al. Caffeine treatment prevented from weight regain after calorie shifting diet induced weight loss. *Iranian Journal of Pharmaceutical Research*. 2014; 12(2): 707-718
- [58] Heckman, MA, Weil, J, Gonzalez de Mejia E. Caffeine (1,3,7-trimethyl-xanthine) in foods: a comprehensive review on consumption, functionality, safety, and regularity matters. *J Food Sci*. 2010; 75: R77-87
- [59] Ivy JL, Costill DL, Fink WJ, Lower RW. Influence of caffeine and carbohydrate feedings on endurance performance. *Med Sci Sports Exerc*. 1979; 11: 6-11
- [60] Gliottoni RC, Molt RW. Effect of caffeine on leg-muscle pain during intense cycling exercise: possible role of anxiety sensitivity. *Int Jour of Sprt Nut and Exerc Metab*. 2008; 18(2): 103-115