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 inhibition 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\textsuperscript{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>
<thead>
<tr>
<th>Variable</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testosterone, total</td>
<td>487.33 ± 36.40 ng/dl</td>
</tr>
<tr>
<td>DHEAs</td>
<td>396.25 ± 28.38 IU/L</td>
</tr>
<tr>
<td>Insulin</td>
<td>9.17 ± 0.79 µU/ml</td>
</tr>
<tr>
<td>Thyroxine, free</td>
<td>15.87 ± 1.00 pmol/L</td>
</tr>
<tr>
<td>Blood lactate</td>
<td>1.68 ± 0.25 mmol/L</td>
</tr>
</tbody>
</table>

**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 trial 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 DHEA's 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 Thyroxin 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.

<table>
<thead>
<tr>
<th>Time of rep (s)</th>
<th>1 rep</th>
<th>2 rep</th>
<th>3 rep</th>
<th>4 rep</th>
<th>5 rep</th>
<th>6 rep</th>
<th>7 rep</th>
</tr>
</thead>
<tbody>
<tr>
<td>SD-CAF</td>
<td>3.724 ± 0.121</td>
<td>3.854 ± 0.116</td>
<td>3.969 ±0.116</td>
<td>4.090 ± 0.137</td>
<td>4.118 ± 0.124*</td>
<td>4.240 ± 0.173*</td>
<td>4.331 ± 0.210*</td>
</tr>
<tr>
<td>SD</td>
<td>3.720 ± 0.125</td>
<td>3.851 ± 0.115</td>
<td>3.980 ±0.105</td>
<td>4.097 ± 0.073</td>
<td>4.305 ± 0.135#</td>
<td>4.479 ± 0.195#</td>
<td>4.610 ± 0.197#</td>
</tr>
<tr>
<td>PLA</td>
<td>3.724 ± 0.119</td>
<td>3.855 ± 0.120</td>
<td>3.996 ±0.142</td>
<td>4.128 ± 0.099</td>
<td>4.482 ± 0.145#</td>
<td>4.663 ± 0.173</td>
<td>4.816 ± 0.171</td>
</tr>
</tbody>
</table>

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\textsuperscript{34} and increased muscular power\textsuperscript{35}. Huang et al. (2004) \textsuperscript{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\textsuperscript{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\textsuperscript{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\textsuperscript{14, 37-39}, it is the primary potential ingredient founded in the coffee and energy drink\textsuperscript{21} and thus in sports drink. Therefore, caffeine and sports drink containing carbohydrates together have positive effect on central nervous system\textsuperscript{15}.

Moreover, it has been reported that caffeine stimulates the secretion of stress (cortisol) hormone\textsuperscript{30}, catecholamines\textsuperscript{40}, and dopamine\textsuperscript{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) \textsuperscript{41} found that caffeine-intake coffee, but not decaffeinated coffee, significantly increased total testosterone in men. However, another trial\textsuperscript{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\textsuperscript{41}.

Caffeine also decreases 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\textsuperscript{34}. 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) \textsuperscript{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). 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\textsuperscript{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 Scholoy et al.’s (2014) \textsuperscript{40} 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\textsuperscript{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) \textsuperscript{49}. In another study, Schneiker et al. (2006) \textsuperscript{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) \textsuperscript{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) \textsuperscript{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% VO\(_{2}\)peak). In addition to its effect on physical performance, caffeine can increase lipolysis and lipid oxidation which increases free fatty acids levels in the blood\(^{[56-59]}\). 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.

### Acknowledgements
The authors thank Dr. Nabeel Mushasha for statistical analysis of the manuscript. D.S.R revised the language of the manuscript.

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

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