Energy drinks do not alter aerobic fitness assessment using field tests in healthy adults regardless of physical fitness status

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Abstract:
Purpose: The purpose of this study was to evaluate the effects of energy drink ingestion on the performance of running performance in amateur runners with different levels of physical fitness. Material: Sixty healthy subjects were selected and randomized according to the level of physical fitness (Low: <29.9 ml.kg-1.min-1; Moderate: 30-37.9 ml.kg-1.min-1; and High: > 38 ml.kg-1.min-1). Thereafter, they were further distributed in Placebo (27g glucose) and Energy Drink (27g glucose, 30g sodium, 1000mg taurine, 600mg glucuronsolactone, 80mg caffeine, 50mg inositol, 16mg vitamin B3, 5mg vitamin B5, 1,3mg vitamin B2, 3 mg vitamin B6 and 2.4 mg vitamin B12), resulting in six groups according to physical fitness level such Placebo (P, Low: L, Moderate: M, High: H) and Energy Drink (ED, Low: L, Moderate: M, High: H). The drinks were administered 60 minutes prior to the cooper test. Results: Energy drink ingestion did not elicit performance improvement despite physical fitness level. However, the L group running distance was longer (P:3168 ± 167; ED: 3228 ± 218, meters) than M (P:1962 ± 75; ED: 2035 ± 105, meters) and L (P: 1422 ± 74; ED: 1440 ± 62, meters) (p<0.01). The same result was found following the use of the equation for calculating oxygen consumption (L group P: 20±1.4; BE: 23±1.4; ml.kg-1.min-1; M group P: 35±1.0; BE: 34±0.9 ml.kg-1.min-1; and H group P: 54±3.7; ED: 60±4.8 ml.kg-1.min-1). Conclusion: Data from the present study demonstrated that the use of energy drinks does not enhance performance of amateur runners regardless of the level of physical fitness. Keywords: sports supplements, caffeine, taurine, exercise performance, running

Introduction
During the last two decades, nutritional and performance enhancement companies introduced into the consumer market drinks that contain caffeine, taurine and carbohydrates (collectively known as energy drinks). The popularity of these drinks increased, and both athletes, and individuals involved in recreational sports, started to use the drinks to benefit from the reported stimulating effects and potential performance enhancement (Wiklund et al., 2009; Ivy et al., 2009). This fact was enhanced by studies that demonstrate that energy drinks can improve cycling performance when the athletes consume the drink 40 minutes prior to a 1h cycling trial (Ivy et al., 2009). Other study (Ragsdale et al., 2009) suggest that there is a relationship between the effects of energy drinks) the caffeine and taurine, more specifically) and improvements in blood pressure profiles during stressful situations. Further to this, there has been no negative associations found between cardiovascular abnormalities and energy drinks.

One of the most important constituent parts of energy drinks is caffeine. This is a component that acts like a central nervous system stimulator that promotes sympathetic adrenal-medullar system activation (Lane, 1983). The symptoms associated with excess caffeine consumption include, insomnia, gastrointestinal upset, tremors, tachycardia, psychomotor agitation, among others in accordance with the guidelines of the American
Psychiatric Association (1994). Some studies have shown that athletes who ingest caffeine prior to exercise can improve performance (Cooper, 1968). However, other studies show that the continuous use of caffeine may cause dependence, and increase anxiety when compared with subjects who are nondependent (Bernstein et al., 2002). There is little information concerning the correct use and dosage of caffeine in athletes and recreational sports people. The relationship with the ergogenic effects and health status when using caffeine deserves more attention (Burke, 2008).

In addition to caffeine, energy drinks also include taurine. Taurine is the major free intracellular amino acid that can produce toxic and oxidant substances in the body (Chesney, 1985). Its effect on the sympathetic nervous system is to decrease function by the modulation of cyclic nucleotide (Ragsdale et al., 2009). However, taurine apparently influences calcium mobilization in cardiac muscle (Passantes- Morales & Ordóñez, 1982). Therefore, taurine affects cholesterol by increasing CYP7A1 mRNA levels in the liver (Yokogoshi et al., 1999). Taurine consumption is also related with increases in alertness and concentration. This may be of benefit during endurance performance by changing/influencing perception of effort (Mandel et al.; 1995). Further to this, it is plausible that taurine can decrease the risk of coronary heart disease (Wójcik et al., 2010). Many studies have shown that fatigue during exercises appears to be associated with glycogen depletion in the working muscles (Tsintzas & Williams, 1998).

The use of carbohydrate supplements show improvement in human performance when compared with water and placebo (Ivy et al., 2009). This has resulted in an increase in the use of beverages that contain carbohydrates during competition (Tsintzas & Williams, 1998; Hermansen & Saltin, 1967). Some studies show that carbohydrate ingestion increases blood glucose levels compared with non-ingested controls (Millard-Stafford et al., 1992). However, in endurance runners the effect of exogenous carbohydrate intakes appears to not affect performance during the submaximal states of running exercise (Tsintzas & Williams, 1998).

The effects of energetic drinks on running performance are still unclear. Therefore, the aim of this study was to assess the effects of energy drink consumption on aerobic performance assessed by a field test performance in healthy adults. In addition, the studies were conducted to analyze any differences in performance based on physical fitness levels.

**Material and methods**

**Experimental designer**

Sixty volunteer’s amateur male runners (20-45 years old) were recruited from the Public Park running club in São Paulo city, Brazil. All the participants had health checks, and medical evaluation performed by a physician, and all completed a questionnaire regarding medical history. All testing protocols were performed in accordance with the ethical standards of the Helsinki Declaration (1964, amended in 1975 and 1983) and the study was approved by the Research Ethics Committee of Nove de Julho University (164.497). Exclusion criteria were as follows: the symptomatic cardiorespiratory disease or cardiac alterations noted during the Bruce protocol.

One week before of cooper test all subjects were submitted to aerobic power test was performed by all subjects, on a treadmill according to the Bruce protocol to estimate the aerobic power. Increments in both speed and grade in 3-minute intervals was chosen to determine cardiovascular abnormalities and oxygen consumption values to randomize the subjects according to physical status. A one-minute warm-up at 10 mph without treadmill inclination preceded the Bruce treadmill protocol, and all subjects were fully familiarized to testing procedures prior to data collection. Velocity and inclination were adjusted gradually as described previously (Higgins et al., 2018). The subjective level of exertion was assessed using the Borg 6–20 scale, and the exercise test was terminated if subjects showed symptoms of exhaustion or were disqualified according to previously described criteria (Vacanti & Sarpi, 2004). The test was considered satisfactory if the participant reached the age-graded maximal recommended heart rate without stoppage. The VO₂ max (maximal oxygen consumption) was calculated as proposed by the American College of Sports Medicine (2005).

Following the Bruce treadmill protocol the subjects were blinded and randomized into Placebo (P) and Energetic drink (D) The VO₂ max values calculated were utilized to distribute randomly the subjects into six groups (10 subjects per group) according to physical status. The groups comprised of: Lower (< 29.9 mL·kg⁻¹·min⁻¹), Medium (30 to 37.9 mL·kg⁻¹·min⁻¹) and higher (> 38 mL·kg⁻¹·min⁻¹) as previously described by American Heart Association, (1972).

**Energetic and placebo drinks solutions**

Using a double blind method the subjects of D groups received a solution dissolved in 500ml water containing 27g glucose, 30g sodium, 1000mg taurine, 600mg glucuronolactone, 80mg caffeine, 50 mg inositol, 16mg vitamin B3, 5 mg vitamin B5, 1.3 mg vitamin B2, 1.3 mg vitamin B6 and 2.4 mg vitamin B12. Groups P received a placebo solution containing 27g of glucose also dissolved in 500ml of water. All solutions were the same color, flavor and consistency, and were administered for all groups 60 minutes before the field test run in accordance with Dall’agnol and Souza (2009).
Measurements

Environmental parameters

The air pollution and humidity, temperature, pressure, wind fore and visibility were evaluated such environmental parameters on the day of field test run according to boletin by Secretaria Estadual do Meio Ambiente do Estado de São Paulo (www.ambiente.sp.gov.br).

Body composition and anthropometry

Body composition and anthropometric parameters were evaluated and conformed to previous methodologies used by our group (Serra et al., 2009). Height (m) and weight (kg) were measured to calculate body mass index (BMI = weight / height²). Body fat percentage was derived using skin fold thicknesses as previously described (Serra et al., 2009).

12-minute walk/run test (Cooper test)

The 12-minute walk/run test (Cooper, 1968) involved running and/or walking as far as possible in 12 minutes. The distance covered was then recorded and VO₂max was estimated by equation: VO₂max = distance – 504 / 45. Tests were executed outdoors on the field at the athletic center at São Paulo.

Prior to, immediately at the end, and after one, two and three minutes post walk/run test heart rate and blood pressure was assessed using the Polar-heart rate monitors (S150; Sao Paulo, Brazil) and a manual sphygmomanometer to measure systolic (SBP) and diastolic blood pressure (DBP). Blood pressure was measured in the left arm by the same physician throughout all protocol sessions in a seated position. Mean blood pressure (MBP) was calculated according to previously study (Moran et al., 1995), which considers changes in the diastolic and systolic periods caused by exercise tachycardia. The subjective level of exertion Borg 6–20 scale (Borg, 1982), was used to estimate fatigue and exercise intensity following the field run test.

Statistical Analyses

Data (means ± SD) were analyzed using the Prism software (version 4.0, San Diego, CA, USA). Two-way ANOVA for repeated-measures analysis was used to examine differences within and between groups over time, and the Tukey post hoc test were used when appropriate. The significance level was set at P < 0.05.

Results

Following the Bruce treadmill protocol, sixteen voluntaries were excluded from the study due to cardiovascular abnormalities. Therefore, our data sets are based on fifty-five subjects, randomized in lower (P: 34 ± 5; D: 33 ± 6, years old); medium (P:31 ± 9; D: 31 ± 8, years old) and higher (P:35 ± 7; D: 34 ± 7, years old) according to physical activity status. No statistical differences were found for age between groups.

Table 1. Biometrics parameters.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Lower</th>
<th>Medium</th>
<th>Higher</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass (kg)</td>
<td>Placebo Drink</td>
<td>Placebo Drink</td>
<td>Placebo Drink</td>
</tr>
<tr>
<td></td>
<td>78 ± 2²</td>
<td>76 ± 2²</td>
<td>75 ± 2²</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>174 ± 1²</td>
<td>175 ± 1²</td>
<td>173 ± 1²</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26 ± 4</td>
<td>25 ± 4</td>
<td>26 ± 4</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>28 ± 1²</td>
<td>28 ± 1²</td>
<td>21 ± 1²</td>
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<td>Fat mass (kg)</td>
<td>22 ± 1²</td>
<td>22 ± 2²</td>
<td>16 ± 1²</td>
</tr>
<tr>
<td>Lean mass (kg)</td>
<td>56 ± 1²</td>
<td>55 ± 2²</td>
<td>60 ± 1²</td>
</tr>
</tbody>
</table>

Values are presented as mean ± standard deviation. Different letters represent statistical differences (P < 0.05) by ANOVA-Tukey test.

Anthropometric parameters are shown in table 1. No differences were found between corresponding groups according to physical activity status. However, the lower status group presented higher (p< 0.01) fat mass compared to medium and higher status groups. Similar results were observed for BMI and body fat percentage, the values of the higher physical status group were statistically different (p< 0.01) from medium and lower status group which was smaller (p< 0.05) than medium group. The values for lean mass of the lower status groups were smaller (p< 0.001) than other groups. However, only the energy drink group in the higher status group differed (p< 0.05) from medium status groups that were similar to placebo in the higher status group. No differences were found in weight and height between groups.

Table 2. Environmental parameters at the day of endurance field test.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Lower</th>
<th>Medium</th>
<th>Higher</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air pollution</td>
<td>Placebo Drink</td>
<td>Placebo Drink</td>
<td>Placebo Drink</td>
</tr>
<tr>
<td></td>
<td>34 ± 3²</td>
<td>35 ± 3²</td>
<td>29 ± 2²</td>
</tr>
<tr>
<td>Air humidity</td>
<td>79 ± 1²</td>
<td>78 ± 1²</td>
<td>80 ± 1²</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>23 ± 1²</td>
<td>23 ± 1²</td>
<td>20 ± 1²</td>
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<tr>
<td>Pressure (hPa)</td>
<td>1026 ± 1²</td>
<td>1026 ± 1²</td>
<td>1026 ± 1²</td>
</tr>
<tr>
<td>Wind force (nos)</td>
<td>7 ± 0.4²</td>
<td>7 ± 0.4²</td>
<td>7 ± 0.4²</td>
</tr>
<tr>
<td>Visibility</td>
<td>8839 ± 387²</td>
<td>8848 ± 313²</td>
<td>8605 ± 408²</td>
</tr>
</tbody>
</table>
Values are presented as mean ± standard deviation. Different letters represent statistical differences statistical difference ($p<0.05$) between groups.

No differences were found in environmental parameters on the day of the field test in all groups, outlined in table 2.

Figure 1. Physical capacity at placebo (empty column) and energetic drink (filled column) groups according to physical status. Panel A: distance of run field test. Panel B: maximum consumption of oxygen. Panel B maximum consumption of oxygen indexed to weight body. Values are mean ± SEM. Different letters represent statistical difference ($P < 0.05$) by two-way ANOVA-Tukey test.

As shown in figure 1A there were no statistically intra group differences. However, the higher group (P: 3168 ± 167; ED: 3228 ± 218, meters) running distance was longer ($p<0.001$) than medium (P: 1962 ± 75; ED: 2035 ± 105, meters) and lower (P: 1422 ± 74; ED: 1440 ± 62, meters) ($p<0.01$). As shown in Figure 1B, there were no differences between groups regarding physical status to maximum consumption of oxygen. However the values of the lower group (P: 20 ± 1.4; ED: 23 ± 1.4, ml/kg/g/min) were smaller ($p<0.001$) compared to the medium groups (P: 35 ± 1.0; ED: 34 ± 0.9, ml/kg/g/min), which were also smaller in relation higher groups (P: 54 ± 3.7; ED: 60 ± 4.8, ml/kg/g/min).

Figure 2. Heart rate (Panel A); systolic blood pressure (Panel B); diastolic blood pressure and mean arterial pressure (Panel C) obtained from groups according to physical status. Lower placebo (○); lower drink (●); medium placebo (△); medium drink (▲); higher placebo (□); higher drink (■). Values are mean ± SEM. * indicate statistical differences ($p<0.05$) from medium and high groups.
At figure 2 outlines the hemodynamic parameters, after, immediately post and after one, two and three minutes by recovery following the endurance test. There were no differences found in all blood pressure parameters between groups (Panel B, C and D).

However, during the heart rate recovery period (Panel A) the higher groups (1 minute: P:123 ± 2; D: 120 ± 2, bpm; 2 minute: P: 99 ± 3.4; D: 97 ± 4.4, bpm; 3 minute: P: 76 ± 1.7; D: 76 ± 1.9, bpm) presented bradycardia after exercise, and was more evident (p<0.001) than medium (1 minute: P:144 ± 2.5; D: 144 ± 3.3, bpm; 2 minute: P: 123 ± 1.5; D: 122 ± 2.2, bpm; 3 minute: P:82 ± 1.6; D: 83 ± 1.5, bpm) and lower (1 minute: P:176 ± 2.5; D: 172 ± 2.6, bpm; 2 minute: P: 163 ± 1.8; D: 161 ± 2.2 bpm; 3 minute: P:116 ± 1.6; D: 115 ± 2.1, bpm). No differences were found on heart rate in rest and immediately post exercise.

Figure 3. Perceived exertion (Panel A) and pain (Panel B) obtained from groups according to physical status. Lower placebo (O); lower drink (●); medium placebo (△); medium drink (▲); higher placebo (□); higher drink (■). Values are mean ± SEM. * indicate statistical differences (p< 0.05) from medium and high groups.

No differences were found in perceived exertion immediately and post test between groups, however, the recovery period in the higher (1 minute: P:12 ± 0.2; D: 12 ± 0.3; 2 minute: P: 10 ± 0.2; D: 10 ± 0.3; 3 minute: P:7 ± 0.2; D: 7 ± 0.3) and medium (1 minute: P: 13 ± 0.2; D: 14 ± 0.2; 2 minute: P: 11 ± 0.2; D: 12 ± 0.1; 3 minute: P: 10 ± 0.1; D: 10 ± 0.1) groups were more evident than the lower groups (1 minute: P: 12 ± 0.2; D: 12 ± 0.3; 2 minute: P: 10 ± 0.2; D: 10 ± 0.3; 3 minute: P: 7 ± 0.2; D: 7 ± 0.3).

Discussion

Although the popularity of the use of energy drinks has increased in recent decades (Higgins et al., 2018), the potential of its consumption for physical performance enhancement in amateur runners is poorly understood. The main findings of the present study suggest energy drink consumption before running elicited no effects on physical performance, hemodynamic parameters and perceived exertion and pain. Similar results were found by Candow et al. (Candow et al., 2009), evaluating the effects of energy drink ingestion in 17 university-level amateur runners using the same dosage as used in our study.

One of the hypotheses for this result may have been related to the low dosage used for some of the ingredients. For example, we can immediately cite the concentration of caffeine that was 80mg which represents less than 1.05 mg / kg when considering the average weight of the participants who received the energy drink. This value is lower than the values used in other studies, with values ranging from 3 to 6 mg (Graham & Spriet, 1995; Silva-Cavalcante et al., 2013, who found improvements in the performance of cyclists (Graham & Spriet, 1995; Silva-Cavalcante, 2013, when the drink was consumed 60 minutes before exercise. Additionally, studies (Candow et al., 2009; Schubert & Azevedo, 2013), using doses below 3mg.kg did not find significant differences in performance. It seems therefore that higher doses may exert a significant influence on the performance of endurance activities.

Another substance contained in the energy drink is taurine. This substance is associated with an improvement in endurance performance, both in animal models (Yatabe et. al., 2003; Sajid et al., 2017) and in human studies (Warnock et al., 2017; Lim et al., 2018). However, ingested dosage, such as caffeine, seems to exert a decisive influence on performance improvement. Studies suggested that improvements in physical performance can occur when 2 to 6 g of taurine is ingested (Baum & Weis, 2001; Zhang et al., 2004). Dosages lower than 2g, as used in the present study, do not seem to exert a significant influence on performance (Rutherford & Stellingwerff, 2010). The amount of glucose present in our supplementation protocol (26g) has also been shown to be below that necessary for improvements in endurance performance (Candow et al., 2009; Higgins & Higgins, 2010). Supplementation of much higher doses of carbohydrate (i.e 60 to 70 g) have been
suggested for improvements in endurance performance (Jeukendru & Moseley, 2005). Finally, the multivitamin present in the energy drink did not positively influence the runners’ performance. This result was expected, since prior studies reported no association between B vitamin supplementation and improvements in endurance performance (Higgins & Higgins, 2010; Doherty & Smith, 2005). It is worth noting that, even when using an energy drink containing ~2 times the concentration of caffeine, taurine and carbohydrate (160 mg, 2 g, and 57 g, respectively) when compared to those used in present study, also did not report an improvement in exercise endurance performance in trained male cyclists performing a 25-mile simulated race (Ivy et al.; 2009). The test used in the study (Cooper test), although recognized as an indirect endurance measurement field test, the time taken to perform the test is lower than those of studies reporting significant improvements in exercise performance, which were superior to 20 minutes (Campbell et al., 2013; Nelson & Dengel, 2014).

When we refer to the influence of the energy drink on hemodynamic parameters, the literature is still contradictory in relation to outcome measures (Lovallo et al., 1991; Nelson & Dengel 2014; Wiklund et al., 2009) demonstrated that the consumption of 250 ml of Red Bull® in adults does not change heart rate. Yamakoshi et al., (2013) demonstrated that the consumption of 80 mg of caffeine, administered by drivers in prolonged monotony, caused significant changes in mean arterial pressure and cardiac output. Lovallo et al., (1991) stated that amounts between 200 and 250 mg may cause significant changes in cardiovascular parameters such as SBP and DBP, however, dosages below these values may not be sufficient for significant differences. However, the results of these previous studies refer to rest or low-intensity exercise conditions (Nelson & Dengel, 2014; Lovallo et al.; 1991; Yamakoshi et al., 2013; Wiklund et al., 2009). To our knowledge, little is known about the influence of energy drinks on hemodynamic parameters during or immediately after physical activity, so our results are the first to analyze the influence of the energy drink on hemodynamic parameters in runners.

In addition, the results of this study in runners corroborate to other studies reporting no effect of energy drink consumption and subjective perception of effort during cycling (Ivy et al.; 2009; Candow et al.; 2009; Nelson & Dengel, 2014). Our study evaluated the effects of the energy drink after the exercise, curiously we did not find any influence of the energy drink, however, the level of physical fitness interfered in the response. The reason for this result is probably due to the greater physical exhaustion these individuals present in relation to the more conditioned groups (Borg, 1982).

Although there were no significant differences in the perception of pain in our study, others (Ragsdale et al.; 2009) found discordant results. Ragsdale et al. (Ragsdale et al.; 2009) demonstrated that the consumption of energy drinks increases the tolerance to pain. Thought the use of energetic drink to increment physical perform is not successful strategy, some important considerations should be address in this study. The indirect aerobic test used to classify the aerobic fitness as well as Cooper test may be not sensible to identify alteration after drink intake, in this way new studies using direct test to do a better sample distributed and longer field run test should be performed.

Conclusion
The results of this study indicated that energy drink ingestion prior to a field-based running test did not resulted in increased performance in amateur runners of different physical fitness status. Additionally, no differences on heart rate recovery, blood pressure, perceived exertion and pain were observed in energy drink condition compared to placebo. Therefore, an energy drink containing 80 mg of caffeine, 1 g of taurine, 27 g of carbohydrate, and B vitamin complex 60 minutes prior to a running test did not favorably influence physical performance, hemodynamic properties, nor perceptual responses to exercise in healthy subjects.

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