Introduction. The primary consumers of energy drinks were athletes, to combat fatigue, but in reality, thanks to their fast expansion and economic growth, young adults and teenagers represent the new target market. Consumption of energy drinks by both recreational and competitive athletes has increased dramatically in recent years, though they are often unaware of what is being ingested, believing to improve their physical and psychological performance. The literature shows contradictions about the capacity of energy drinks to enhance psychophysical results. In relation to probable adverse effects induced by the irregular consumption of energy drinks, which in several cases are not so clear, we decided to investigate the possible relationship between the intake of energy drinks and the presence of mental and physical stress in young people and athletes.

Methods. Two experimental sessions, separated at least by 1 week, according to a randomized cross-over design, following this protocol were conducted: in the first session a mental and physical stress was conducted without the consumption of energy drinks, the second after energy drinks consumption. BAI (Beck Anxiety Inventory) and BDI (Beck Depression Inventory II) tests have been used to test the mental stress, and a “cycle ergometer test” to test the physical stress.

Results. BAI and BDI tests results showed that before the consumption of energy drinks, subjects are considered in the range of “minimal level of anxiety”, (10 and 60 percentiles) and do not report a level of depression. After the energy drinks consumption, a “mild level of anxiety” has been recorded, and the BDI showed a case with a pathological profile. The physical test recorded a small increase in the maximum heart rate was verified with the intake of an energetic beverage.

Conclusions. The stimulating effect of Energy Drinks EDs on nervous system and cardiovascular system, must be checked and studied in deeper detail, because it may represent a risk for the health of young athletes.
fructose) seems to increase the quantity of energy provided during exercise [8]. Energy drinks, in fact, contain high quantity of carbohydrates (25-30 grams in 240 ml) and this amount does not conformity to the recommended content of 6-8% (6-8 grams in 100 ml) suggested by the International Society of Sport Nutrition (ISSN), the American College of Sports Medicine [9, 10], and the Panel on Dietetic Products, Nutrition and Allergies of European Food Safety Authority (EFSA) [11]. These doubts about health problems arising from the use of energy drinks represent a health problem still to be explored, not only from a physical but also psychological point of view.

Since the initial diffusion of caffeinated drinks, their regulatory aspect has always shown some deficiencies due to the lack of knowledge about the physiological effects produced by the substances used in manufacturing, compromising the level of security in their composition. In fact, exacerbation and increase of anxiety, acutemania, seizures, and mood and behavior problems have been correlated to the use of energy drinks [12]. Furthermore, in young people, high levels of soft drink consumption are related to psychological problems, with emotional symptoms including mental distress, hyperactivity, behavioural problems, and suicidal behaviors [13, 14].

In relation to probable adverse effects induced by the irregular consumption of energy drinks, which in several cases are not so clear, we decided to investigate the possible relationship between the intake of energy drinks and the presence of mental and physical stress in young people and athletes.

**Methods**

**Study population**

Ten healthy young adults, five females and five males, aged 18 to 27 years, were recruited for a mental and physical stress test. The study was carried out in healthy, non-obese, non medicated individuals who were taking any medication affecting cardiovascular or autonomic regulation, non-medicated individuals who were free from any somatic or mental condition. The study criteria were met by 20 individuals, 10 females and 10 males, and the experimental protocol has been described. Only 10 individuals accepted to participate. The sample selected was invited not to take drinks with caffeine (as energy drinks) and substances of abuse in the week before the test, and in the hours before the physical and mental test.

The mean height of the participants was 167.7 ± 1.0 cm, their body weight was 59.8 ± 2.4 kg, and the medium BMI (Body Mass Index) was 21.5. Some participants were trained (subjects with regular physical activity); in particular inside the female group only the participant 1 and 2 were trained. Inside the male group only the number 6 was untrained. Three of participants, inside the untrained group, reported to smoke regularly.

**Study design**

The adherence to the protocol including the mental and physical tests was made by each subject who completed and signed the informed consent. This form was valid for all tests performed. In particular, the informed consent was important for the physical test. In fact, during the exam to raise the heart rate, it is possible to perceive some complications like chest pain or on the electrocardiogram, and this was generally followed by the spontaneous interruption of the exercise. In some cases, it is possible to verify cardiac arrhythmias with early stoppage of the test.

**Psychological and physical tests**

All experiments took place in a quiet, temperature-controlled (22°C) laboratory and started between 08.00 a.m. and 09.00 a.m. Every subject attended 2 separate experimental sessions (each session separated at least by 1 week) according to a randomized cross-over design. The first week, on arrival at the laboratory subjects were asked to sit in a comfortable armchair; subjects were called one at a time to the cardiologist’s surgery, and were given their ECG under physical stress. A baseline recording was made for 20 minutes. Then, a mental arithmetic stress test was performed for 5 minutes: subjects subtracted the number 6 or 7 (chosen at random) continuously from a random 3 or 2 digit number and were instructed to give a written response. Each mental stress task was presented to the subjects on a monitor and comprised 60 unique calculations, with a 5-second interval between each calculation. Immediately after the mental task, subjects were asked to rate their perceived stress using BAI (Beck Anxiety Inventory) [15] and BDI (Beck Depression Inventory II) [16] tests. The BAI and BDI-II tests in young adults were validated [17, 18]. The BAI is a self-report instrument that allows to measure the severity of anxiety symptoms in adults; it was designed to include anxiety symptoms only minimally overlapping with those of a depressive nature. The test consists of 21 items (descriptions of somatic symptoms of anxiety, subjective or related to phobias), to be evaluated on a four-point scale (0 to 3). The clinical study can then continue assessment using DSM (Diagnostic and Statistical Manual of Mental Disorders) criteria to arrive at a specific diagnostic category and plan interventions targeting the underlying cause of the respondent’s anxious symptomatology and/or diagnosis. The BDI-II is a self-report instrument that allows you to assess the severity of depression in adults and adolescents (including a psychiatric diagnosis). The test consists of 21 items and gives a total score and scores on two areas: Somatic-Affective and Cognitive. After the BAI and BDI tests, physical stress was evaluated using a “cycle ergometer test” with the purpose of estimating maximal oxygen consumption (VO2max). It is an instrumental examination which consists in the recording of an electrocardiogram before, during and after the execution of a physical effort, increasing blood pressure and blood flow to the coronary arteries thus...
ensuring the influx of blood to the cardiac muscle. Our performance was executed with a cycle ergometer, regularly calibrated, a particular exercise bike with regulators and electronic controllers of the effort that is made by the patient. In this way it was possible to evaluate the cardiovascular reaction to physical exercise, particularly concentrating on heart rate, pressor response and variation of the ECG to achieve the maximum heart rate (MHR) expected for the age of the subject. The calculation of the MHR was performed by using the Karvonen formula that keeps the difference between the theoretical maximum heart rate (220) and the age of the subject in correlation, ensuring the most accurate method for making the measurements in percentages: prediction of HR_{max} (220 bpm - age). The preparation test included a recommendation for the subject to have a light breakfast, avoiding alcohol consumption, in the hour before the test was administered. Before starting the cycling exercise, all procedures were briefly explained to all the patients. Exercise testing protocols were assisted by supervising laboratory staff. It was recommended that selected protocol be based on the limitations of each individual. The electrode pads of the ECG were placed on the thorax of the patient in order to record a basal electrocardiogram. In this manner it was possible to extrapolate an overview of the cardiovascular profile characterized by the load (Watt), the maximum heart rate (bpm), percentage on the theoretical heart rate, the maximum systolic and diastolic pressure. Analysis is divided into three phases: 1) pre-exercise; 2) exercise; 3) post-exercise. For each period time, number of heartbeats, blood pressure and potency were monitored as collection data. In the first phase, physical effort started at 0 Watts by pedaling on the exercise bike and becomes progressive during the exercise period, through gradual increments (steps) given by resistance from the bicycle ergometer pedals, until reaching the point of highest fatigue. This increase of strength was provided by adding 25 Watts every minute (25W×1) until reaching exhaustion. When the subject was no longer able to stand a further increase, they were told to finish with a final sprint which allowed to measure maximum heart rate. In the final stage, post-exercise, the patient continued to pedal without workload. The collection data included the measurement of blood pressure taken at rest, in both a supine (at the end of the exercise period) and standing position (prior to cycling), necessary to determine the presence of any ECG abnormalities that might interfere with test results. The desired testing endpoint was researched within 8 to 12 minutes of testing. The second week, the same protocol was repeated. On arriving at the laboratory, the test subjects, not wearing blindfolds, ingested either 250 ml of an energy drink containing caffeine (114 mg), taurine (1420 mg), and glucuronolactone (84.2 mg), sucrose, and glucose (39.1 g). Subjects were asked to ingest their drink at a convenient pace over a 4 minute period. After 15/20 minutes, the ECG was carried out again under physical stress, followed by the mental stress test and participants were then asked to rate their perceived stress using BAI and BDI tests. The study was conducted in accordance with the latest version of the Declaration of Helsinki.

**Statistical analysis**

The data were processed using an Excel Workbook (Microsoft Office), and analyzed using XLSTAT Software [19]. The Student t-test was used to analyze possible relationship between the data recorded before and after the Energy drinks consumption, and statistical significance was established at $p<0.05$. The t-Student test was chosen, among other non-parametric tests such as Wilcoxon-Mann-Whitney (WMW), which are frequently applied in medical statistics to compare the outcomes of a treatment, because the WMW has a very high probability of rejection of the null hypothesis. This happens when the means or medians are equal [20, 21].

**Results**

**Psychological Tests**

After a mental task, subjects were asked to rate their perceived stress rate using BAI and BDI tests. **BAI test.** Before the consumption of energy drinks, subjects with a raw score between 0 (10 percentiles) and 7 (60 percentiles) were considered in the range of “minimal level of anxiety”, they were considered on average and do not represent a particular clinical significance. Only two cases presented a raw score between 8 (70 percentiles) and 15 (80 percentiles), meaning that they were in the range showing a “mild level of anxiety”; it is significant that these two cases were women, who usually have higher anxiety levels than men. After drinking the energy drink, scores remained the same or decreased; only one measured 11 (80 percentiles), meaning a “mild level of anxiety” (Fig. 1). **BDI test.** Before drinking energy drinks, the total scores in percentiles were less than 85, were considered on average and without a particular clinical significance: the subjects did not report a level of depression with a clinical significance (Fig. 2). Percentiles related to somatic-affective factors all come under 85, so the subjects did not present significant symptoms of depression. Cognitive factors were all under 85 percent (no clinical significance) except in four cases: three were 90, meaning the subject presents cognitive manifestations bordering on pathological aspects; the other was 97, indicating a particular difficult situation with cognitive symptoms of depression (that only in some cases can be considered serious).

Furthermore, in the test after drinking EDs, the total scores in percentiles were under 85%, so they do not play a clinical significance. For the somatic-affective factors, we found only one case measuring 90% (so the score was higher than before). This case has to be considered bordering on pathological.
The comparison between the BDI-II of somatic-affective sphere, before and after the EDs intake, showed a t-Student value (t(18) = 1.77, p = 0.09) that, even if not statistically significant, represents a medical relevance. Scores for the cognitive factors decreased significantly and only one of the subjects presented values greater than 85 percentiles (the cut-off point) (Fig. 3).

Statistical analysis (t Student), applied to the psychological test, showed a statistically significant by comparing the BDI-II values of the cognitive sphere before and after the intake of EDs (t(18) = 2.1, p = 0.05).

**Physical tests**

The physical test was conducted only with 8 participants because two were ill. The effects of energy drinks appear to be largely on cardiac parameters (Tab. I); in fact in the conditions compared, a small increase in the maximum heart rate was verified with the intake of an energetic beverage. In the post-workout phase, an efficient background is demonstrated by evaluating recovery at the third minute, which is a critical time, of the heart rate. A rapid drop in the heart rate is seen when it drops below 120 bpm, as index of an high vagal tone and therefore associated with good physical performance and health status.

For trained subjects, recovery time is good because is achieved in a short time after exercise, after which the heart rate returns near the baseline; instead, untrained subjects reached maximal heart rate during the exercise period and leading to a slow recovery.

As is possible to appreciate in Table I, our results show the second and fifth female and the last male showed a fast recovery phase, while other participants displayed no great discrepancy between both conditions. For resting subjects, HRR (Hearth Rate Recovery) was lower, meaning that their performance corresponds to an untrained physical state. For each individual, a parallel observation about cardiovascular changes procured with or without ingesting energy drinks is reported below. In the following results, which have been listed progressively, females are shown first, followed by males.
Females.

- Pre-exercise: different heart rate was noted, slightly higher after energy drink ingestion. Exercise: stable condition in both states (with and without energy drink). Post-exercise: weak recovery period, a little stronger after ingesting the energy drink, highlighted by a slow achievement of baseline blood pressure.

- Pre-exercise: a stable setting for both conditions. Exercise: prominent increase of heart rate after ingesting the energy drink, during the lead-up period. Post-exercise: fast recovery for both, but more marked with the energy drink.

- Pre-exercise: equilibrium between the two conditions. Exercise: no divergence, only slight anomalies observed. Post-exercise: very slow recovery.

- Pre-exercise: no differences were observed for both conditions because an identical heart rate was maintained. Exercise: in the first phase we observed an increase in heart rate after the energy drink was ingested, which then remained unchanged. Post-exercise: recovery is moderate.

- Pre-exercise: flattening of the heart rate for both states. Exercise: energy drink ingestion initially produces an increase of the heart rate, compared to when EDs were not consumed. Post-exercise: faster recovery is noted without the intake of energy drinks, through a strong reduction of heart beat noted near the baseline at the third minute.

Males.

- Pre-exercise: a higher heart rate is observed on ingestion of the energy drink. Exercise: a higher heart rate remains constant for the whole period thanks to the energy drink. Post-exercise: a quick drop with energy drink and modest recovery compared to the other state, in which the heart rate is higher. Basically, the overall recovery phase was weak.

- Pre-exercise: a small increase in heart rate is observed without the ingestion of energy drinks. Exercise: the entire phase is characterized by a flattening of heart activity. Post-exercise: in the energy drink state, the recovery phase was faster than in the other condition, because the heart rate decreased, but being

<table>
<thead>
<tr>
<th>Participants</th>
<th>MHB* before EDs (heart beats)</th>
<th>MHB* after EDs (heart beats)</th>
<th>HRR** before EDs (heart beats)</th>
<th>HRR** after EDs (heart beats)</th>
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</thead>
<tbody>
<tr>
<td><strong>Females</strong></td>
<td></td>
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<tr>
<td>1 (trained)</td>
<td>185</td>
<td>187</td>
<td>125</td>
<td>129</td>
</tr>
<tr>
<td>2 (trained)</td>
<td>166</td>
<td>164</td>
<td>107</td>
<td>94</td>
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<tr>
<td>3 (no trained)</td>
<td>185</td>
<td>187</td>
<td>129</td>
<td>142</td>
</tr>
<tr>
<td>4 (no trained)</td>
<td>178</td>
<td>176</td>
<td>109</td>
<td>112</td>
</tr>
<tr>
<td>5 (no trained)</td>
<td>174</td>
<td>174</td>
<td>87</td>
<td>89</td>
</tr>
<tr>
<td><strong>Males</strong></td>
<td></td>
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<tr>
<td>6 (no trained)</td>
<td>176</td>
<td>168</td>
<td>137</td>
<td>129</td>
</tr>
<tr>
<td>7 (no trained)</td>
<td>200</td>
<td>194</td>
<td>144</td>
<td>133</td>
</tr>
<tr>
<td>8 (trained)</td>
<td>174</td>
<td>185</td>
<td>111</td>
<td>115</td>
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* = MHB maximum heart rate; ** = Heart Rate Recovery.
higher compared to the baseline, the entire recovery was sluggish.

- Pre-exercise: phase of equilibrium in which the maximum heart rate is kept stable during both states. Exercise: there was a brief increase of the heart rate in the first time period. Post-exercise: moderate recovery without any changes in heart rate at the third minute.

Discussion

Physical stress. Judging from outcomes obtained in this study, energy drinks are able to provide an additional burst of energy in a short-time period, more noticeable in trained subjects than untrained. For this reason, to achieve benefits from the intake of an energy drink, short and low-intensity activities are most favored. On the basis of the full results obtained from this investigation we can appreciate how energy drinks are able to stimulate the cardiovascular system for a short time after their intake [22], leading to an initial increase in heart rate, which then drops and remains constant the second time around. This has been observed in particular for physically trained subjects; in fact, the beverage provides a larger energy boost in the first instant of exercise because metabolic processes occur in a brief stretch of time. The sports activities most suited to exploiting the effects energy drinks claim to give are short term and high intensity strength activities. In many cases, before an event or during training, serious adverse effects can arise such as restlessness and irritability, an excessive increase of blood pressure and dehydration problems. Medical illnesses, especially underlying heart problems, have to be checked and studied in deeper detail, because their ingestion may exacerbate these conditions.

Psychological stress. Regarding the BAI test, as in a previous study, during our experimental phase we found that the energy drink gives significant improvements in mental performance including choice reaction time, concentration (number cancellation) and memory (immediate recall), which reflected in increased subjective alertness. These consistent and wide-ranging improvements in performance are interpreted as reflecting the effects of the combination of ingredients [23]. However, there are also articles that contradict this thesis [22], so this hypothesis needs to be further investigated with studies and research. Considering BDI test, after taking the energy drinks, anxiety levels decreased or remained unchanged; only in two cases did they increase and this might depend on the tolerance that individuals develop to caffeine. The intake of energy drinks has also caused an increase in somatic-affective factors, decreasing cognitive ones and the perception of depression. Probably this kind of “tolerance” is due to the fact that, nowadays, modern society is accustomed to “fast, excited” states, and a small dose (1 can) of an energy drink no longer affects the psyche of our subjects. The fact that in some cases anxiety and depression decrease after drinking EDs makes us also think that those feelings of mild dysphoria, mood-swings and anxiety were due to not knowing what would happen in the experiment; in fact, in the second phase of the experiment, they returned to normal levels. Energy drinks are known to improve mental performance, for example concentration and memory. Three studies published in two articles by Kennedy and Scholey demonstrate the positive effects of energy drinks on cognitive performance [25, 26]. In our case, anxiety and depression decreased after the ingestion of energy drinks, but they are the most common psychological side effects of energy drink abuse: probably it is an initial effect that will then change; for example, alcohol, cannabis, and other substances people abuse also initially give an illusion of wellness, and when doses are increased, these effects take a turn for the worse (drowsiness, stomach pain, nausea, vomiting etc.) [27, 28]. Precisely this apparently positive effect may lead the user to increase doses of these substances, with the risk of incurring negative effects and posing a risk to health. Our result seems to agree with what is written in literature [25, 26, 29, 30].

Conclusions

Energy drinks are emerging as a public health threat and, increasingly consumed by youth internationally, are often marketed through youth-oriented media and venues [31-33]. Another crucial issue is the communication of health benefits to consumers in order to provide the knowledge necessary for them to make an informed choice. On this question, a strong policy that contrasts the excessive consumption of energy drinks among young people does not yet exist. Furthermore, alterations in mood, such as stress, anxiety and depression, are factors that have to be seen as if they were an alarm bell. The stimulating effect of EDs on the cardiovascular system in relation with physical activity must be checked and studied in deeper detail, because it may represent a risk for the health of young athletes, at times exacerbating congenital heart diseases. In accordance with other pilot studies, the authors aim to verify the results of this pilot study applying the same experimental protocol to a larger group of young people [34, 35].

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Authors’ contributions

FP: conceived and coordinate the study, evaluated the results and wrote the manuscript. IG: coordinated and contributed to the manuscript writing. DE: contributed to the recruitment of the participants, the acquisition of epidemiological data. PP: contributed to the recruitment of the participants, supervision during the physical test. PC: contributed to critically revised the manuscript. LK: contributed to critically revised the manuscript. SS: conceived and contributed to the supervision of the study, evaluated the results.

References


