ORIGINAL SCIENTIFIC PAPER

Caffeine content in energy drinks: deviation of declared from analytical value

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Abstract

Convenient caffeine sources include energy drinks that are increasing in popularity among recreational and professional athletes, military personnel, young adults, and other consumers seeking ''energy boosters''. Since energy drinks are a relatively concentrated source of caffeine, there is a risk of missing individually targeted caffeine intake or risk of inadvertently high intake with adverse effects. Therefore, the accuracy of declared caffeine content of energy drinks is highly important, especially from a consumer point. The purpose of this study was to determine caffeine content of 15 beverages available on the Croatian market and compare the analytical with the declared value. Caffeine was determined by high performance liquid chromatography technique with photodiode-array detection (HPLC-PDA). In 5 of total 15 products, a higher caffeine content was determined in average of 3.01 % than the one declared, resulting with increased intake of 4.75 mg caffeine/serving. In another 10 analysed products the content of caffeine was lower in average of 5.33 % (4.79 mg caffeine/serving) in compare to the one declared. Therefore, only with accurate information about the caffeine content in energy drinks may consumers and clinicians be assured of safe usage.

Keywords: *caffeine, energy drinks, food label, HPLC, methylxanthines*

Introduction

An energy drink (ED) is usually defined as a beverage containing multiple ingredients which service, with expressed intent, one or more facets of perceived neurological and/or psychophysiological efficiency'' (Caffeine Informer, 2014). The very beginnings of EDs production were in Japan in about 1963. In Europe, the first ED was presented in 1987 by Dietrich Mateschitz in Austria under the commercial name Red Bull, while its wide sales began in Germany and England few years later, in 1994, because, of the time needed for the legislative regulation (Red Bull, 2017). Today, this is a multibillion-dollar industry with strong market growth and several major producers (Sepkowitz, 2012). Many of them are present on the Croatian market: Red Bull, Monster, Burn, Rodeo, Mad Bat, Crazy Wolf, Shark, Hell Red, MTV Up, Multipower, Black Energy, etc.

There are several reasons why consumers seek EDs and they include enhancing energy availability, increasing daily energy expenditure, decreasing fatigue, decreasing the sense of effort associated with physical activity, enhancing physical performance, increasing motor performance, enhancing cognitive performance, increasing alertness, wakefulness, and feelings of "energy," decreasing mental fatigue, quicker reactions, increasing the accuracy of reactions, enhancing the ability to concentrate and focus attention, enhancing short-term memory, increasing the ability to solve problems requiring reasoning, increasing the ability to make correct decisions, enhancing cognitive functioning capabilities and neuromuscular coordination (Reissig et al, 2009; Glade, 2010). EDs provide convenient acute caffeine dose, and it was proven that habitual caffeine intake does not blunt the ergogenicity of an acute intake (Gonçalves et al, 2017). There are even indications that caffeine elicits anabolic effect (Moore et al, 2017).

Therefore, target groups include: adolescents, athletes, students and military personnel (Scholey and Kennedy, 2004; Malinauskas et al, 2007; Attila and Cakir, 2011; Ishak et al, 2012; Reid et al, 2015; Rosenbloom, 2014; Lara et al, 2014; Attipoe et al, 2016; Garcia et al, 2017). The prevalence of adolescent and students consuming EDs is in a range from 30 to 50 % (Malinauskas et al, 2007; Seifert et al, 2011). They often consume EDs with alcohol and they are unaware of the potential risk of it, which is prescribed as not recommended. About 6 % of young men in the United States report consuming an ED on a daily basis. The increasing popularity of EDs consumption is also recorded among military personnel and in recent survey of US overseas troops, 45% of them reported daily use of EDs (Centers for Disease Control and Prevention, 2012).

Great popularity and widespread consumption of those drinks are result of the large doses of metabolic stimulants in their composition such as caffeine, carbohydrates, taurine, glucuronlactone, and B vitamins (Attila and Cakir, 2011). They may also contain some additives such as mineral substances and some herbal extracts such as guarana, yerba mate, akai, ginseng and *Ginkgo biloba* (Miller, 2008). Caffeine is one of the main ingredients of EDs, with the chemical name of 1,3,7-trimethylxanthine, naturally occurring alkaloid (Wang et al, 2008). When it enters the organism it is divided into three molecules; paraxanthine (84 %), theobromine (12 %) and theophylline (4 %), with specific neurological effects. Its activity begins 15-30 minutes after consumption, and lasts for 5-6 hours (for individuals up to 10 hours). 75 % of caffeine is excreted from the body as urine (Berthou et al, 1991; Miners and Birkett, 1996). However, consuming excessive daily doses of

caffeine has a number of negative effects on the human body such as: anxiety, irritability, dizziness, gastrointestinal problems, dehydration, decreased bone mineralization, insomnia and sleep disturbances. Also, EDs with caffeine may cause arrhythmia, tachycardia, seizures and hallucinations. There is a higher risk of hyperactivity and inattention for the consumers of said beverages (Reissig et al, 2009; Seifert et al, 2011; Velazquez et al, 2012; US Department of Health and Human Services, 2012; Rosenbloom, 2014; Schwarz et al, 2015).

The content of caffeine in EDs is in the range of 50-505 mg caffeine/serving; 2.5-35.7 mg caffeine/oz which is higher compared to other caffeinated beverages such as a 12-ounce cola (range 34-54 mg; 2.9-4.5 mg caffeine/oz) or a 6-ounce cup of coffee (range 77-150 mg; 12.8-25 mg caffeine/oz) (Reissig et al, 2009).

Recent studies have found that the concentration of caffeine labelled on EDs is not always the same as its actual concentration, and the deviation is also higher than the legally prescribed one. Unfortunately, studies reporting distribution of total caffeine intake among food groups or specific products usually do not capture EDs (Frary et al, 2005). There are some recent estimations of caffeine intake through EDs, for children it has increased significantly from none in 1999-2000, to 0.24 mg/day in 2003-2004, and 2.3 mg/day in 2011-2012, and for adults also a significant rise is witnessed from zero in 1999-2000 to 0,31 mg/day in 2003-2004, and 2.9 mg/day in 2011-2012 (Drewnowski and Rehm, 2016). Nowadays, neither uniform criteria nor labels for EDs in the world or the EU exist, but manufacturers have agreed that the packaging of EDs must contain nutrition labels, total caffeine content, and several cautionary statements like; regarding daily intake and sensitive tip of population (EFSA Panel on Dietetic Products, Nutrition and Allergies, 2015).

Therefore, the purpose of this study was to determine the actual proportion of caffeine in EDs available on the Croatian market and to compare with those listed on labels, moreover, to warn of the eventual existence of irregularities in the writing of declarations and their potential bad impact on human health. The actual proportion of caffeine in this study was determined in 15 different beverages by HPLC-PDA.

Materials and Methods

Material

To capture the representative range of EDs, dietary supplements and refreshing drink with caffeine and guarana, available on the national market, product range of major supermarkets, major web shops for athletes, and sports nutrition stores was considered. The final list consisted of 15 beverages (Table 1).

Table 1. *Nutrition composition of evaluated EDs*

Samples	Product category	Serving size (mL)	Calories (kcal/100mL)	Carbohydrates (g/100 mL)	Caffeine (mg/100 mL)	L -Carnitine (mg/100 mL)	Taurine (g/100 mL)	Other
1	drink, dietary supplement with sweeteners	25	33	5.9	800	/	/	C, Thiamine, Pantothenic acid, B_6
2	drink, dietary supplement with sweeteners	60	0	0	133.3	/	2275	C, B_6, Folic acid, B_{12}
3	refreshing drink with sweeteners, contains high caffeine content	500	19	5	30	/	/	/
4	refreshing drink with caffeine and guarana	700	0	0	21.3	57.1	/	/
5	energy drink	250	46	11	32	/	400	Niacin, Pantothenic acid, B_6, B_{12}
6	energy drink	250	45	10	32	/	/	Riboflavin, Niacin, Pantothenic acid, B_6, B_{12}
7	energy drink	500	45	11	32	4	400	Riboflavin, Niacin, B_6, B_{12}
8	energy drink	250	47	11.3	32	/	400	Niacin, Pantothenic acid, B_6, B_{12}
9	energy drink	250	62	15	32	/	400	Niacin, Pantothenic acid, B_6, B_{12}
10	energy drink	250	45	11	32	/	400	Niacin, Pantothenic acid, B_6, B_{12}

Samples	Product category	Serving size (mL)	Calories (kcal/100mL)	Carbohydrates (g/100 mL)	Caffeine (mg/100 mL)	L -Carnitine (mg/100 mL)	Taurine (g/100 mL)	Other
11	energy drink	250	44	11	32	/	/	Niacin, Pantothenic acid, B_6, B_{12}
12	energy drink	250	49	11	32	/	400	Niacin, Pantothenic acid, B_6, B_{12}
13	energy drink	250	44	10.1	30	/	/	B_6, B_{12}
14	energy drink	250	47.4	11.1	32	/	400	Niacin, Pantothenic acid, B_6, B_{12}
15	energy drink	250	46	10.9	38.4	/	/	Riboflavin, Niacin, Pantothenic acid, B_6, B_{12}

Determination of caffeine content in EDs by high performance liquid chromatography (HPLC)

Sample preparation

EDs were degassed in an ultrasonic bath for 15 minutes and then diluted with water at a ratio of 1:10 (v/v), with the exception of sample 1 diluted 20 times due to the high caffeine content, as labelled. The chromatographic system was injected with 10 µL of the filtered (cellulose acetate microfilter, pore-size 0.45 µm) sample.

Chemicals

All the chemicals used were of high analytical (eg.) or HPLC purity; redistilled water, AQUA pro injection (Zagreb, Croatia), O-phosphoric acid, Kemika (Zagreb, Croatia) and methanol, Mallinckrodt Baker B.V. (Deventer, Netherlands).

Procedure

HPLC analysis was conducted using Agilent 1200 Series instrument coupled with PDA („Photo Diode Array“) detection (Agilent Technologies, USA). Analysis of individual compounds was performed by reverse phase chromatography on the ACE Excel SuperC18 (250 x 4.6 mm, 5 µm) column (non-polar stationary phase) based on the polarity differences of individual components by elution with polar solvents (Belščak-Cvitanović et al, 2012). For HPLC analysis a binary mobile phase was used, consisting of 0.1 % *o*-phosphoric acid in water (A) and 0.1 % *o*-phosphoric acid in methanol (B), and a gradient elution as follows: 0 min – 10 % B, 30 min – 100 % B, equilibration time 5 min (total analysis time was 35 min). The flow rate was set to 1 mL/min; the column temperature was 25 °C; while the effluent was monitored at 278 nm (photodiode array detection). All samples for HPLC analyses were prepared in parallel, as well as analysis runs.

Results and discussion

In this paper caffeine content in 11 products from the category of EDs, 2 products from the category of dietary supplements and 2 products from the category of refreshing drinks, were analysed. Results (Table 2) showed that among 15 products, a higher caffeine content than the one declared was found in 5 of them, the range of deviations was from 0.74 % to 9.14 %, in other 10 analysed products the content of caffeine was lower than one declared, in a range from 0.40 % to 19.48 %.

Table 2. *Difference between laboratory-determined and labelled caffeine content (mg/serving) in EDs samples*

Samples	Caffeine content determined by HPLC method (mg/serving ± SD)	Caffeine label claim (mg/serving)	Difference between determined and labelled caffeine content (mg)	% relative to the analytical value
1	218.27 ± 9.66	200	18.27	+9.14
2	81.64 ± 0.77	79.98	1.66	+2.08
3	151.12 ± 0.29	150	1.12	+0.74
4	142.62 ± 0.00	149.1	6.48	-4.35
5	76.35 ± 0.42	80	3.65	-4.56
6	79.68 ± 0.06	80	0.32	-0.40
7	155.30 ± 0.36	160	4.70	-2.94
8	81.33 ± 0.06	80	1.33	+1.66
9	76.18 ± 0.16	80	3.82	-4.78
10	78.04 ± 0.08	80	1.96	-2.45
11	75.44 ± 0.08	80	4.56	-5.70
12	74.10 ± 0.06	80	5.90	-7.38
13	74.06 ± 0.22	75	0.94	-1.25
14	64.42 ± 0.06	80	15.58	-19.48
15	97.38 ± 0.06	96	1.38	+1.44

The increasing popularity of consuming EDs among different populations leads to larger public, media and scientific expert's interest. Although the effect of caffeine has been studied in many scientific papers, comparison of caffeine content in EDs with the product labelled value in scientific literature has not been studied frequently so far. The results of this study can be compared with the recent study of Attipoe et al (2016) where deviations of caffeine content in EDs were also within 15 %, but this is still not in accordance with FDA (Food and Drug Administration) guidelines that indicate 100 % of the amount or concentration of ingredients that are specifically added to the product with permitted miscible analytical mistakes, whereas naturally occurring dietary ingredients must be present at 80 % of the declared value. Also, above-mentioned research (Attipoe et al, 2016) suggest potential consumers of EDs, to make the best choice to take the drink with a label that quantifies the actual amount of caffeine, given that products without the caffeine content listed may contain more than 300 mg. However, recent data from this and Attipoe et al (2016) studies were more favourable than results of Cohen et al (2013) where 45 % of caffeine-containing dietary supplements that quantified caffeine on their label had within ±10 % of the amounts on the label, whereas 25 % varied widely from the laboratory-determined levels; 30 % listed caffeine as an ingredient without quantifying the amount on the label. The findings in this study are comparable to those of Inácio, de Oliveira et al (2016) where the main conclusion is discrepancy in the amount of caffeine claimed in the dietary supplements studied. Among the nine studied caffeine dietary supplements commonly consumed by soccer players, eight of them showed a significant difference in caffeine content than that claimed on their respective labels, four were shown to have caffeine content below stated amount (29.1-253.6 mg/serving), and four above stated amount (91.4-631.6 mg/serving). Another study by Andrews et al (2007) also found that most (89 %) of the tested beverages had caffeine within ±20 % of amounts indicated on labels.

To reach the potential lethal dose of 3 g of caffeine, person should ingest at least 12 highly-caffeine EDs in just a few hours (Sepkowitz, 2012). According to a conducted survey the one of the most vulnerable population group consuming this type of drink is youth (such as college students, and athletes through sponsorships with extreme sports, such as BMX racing, wakeboarding, mountain biking, surfing, snowboarding, and downhill skiing, as well as partnerships with NASCAR, monster truck rallies, and music concerts), where 54 % of respondents consuming EDs are 18-25 years old and 24 % are 25-30 years old. 55 % of them (34 % women and 21 % men) mix alcoholic beverages with EDs regularly, so the mentioned dose of 12 drinks as a potential lethal dose is not high, moreover, it is worrying. Similar data were also found in the study by Breda et al (2014). According to the EFSA (EFSA Panel on Dietetic Products, Nutrition and Allergies, 2015) consolidated data for 16 EU countries, 68 % of adolescents (10-18 years of age), 30 % of adults and 18 % of children (under 10 years) consume EDs. Furthermore, a large group of everyday consumers of EDs are athletes. It is important for them to know the content of caffeine in EDs because the International Olympic Committee treats caffeine as a conditional drug, in fact it limits the urine findings to the contestants. The 12 mg/L concentration is acceptable, but all the above is treated as doping. About 8 cups of strong coffee can "urge" urine results to as much as 1000 mg/L (Maughan et al, 2004; World Anti-Doping Agency, 2017). The EFSA Panel considers unborn children to be the most vulnerable group for adverse effects of caffeine among the general population, considering the reduced maternal clearance and prolonged half-life during pregnancy, and the foetus's exposure to maternal caffeine plasma levels (EFSA Panel on Dietetic Products, Nutrition and Allergies, 2015).

Today, millions of people consume EDs, thinking they will have more energy, be more alert or concentrated, build up their stamina, but are not aware of the amount of caffeine that they are ingesting. Consequently, a random overdose may occur (Avci et al, 2013). It is important to emphasize that EDs are not the only source of caffeine; some of the most common are coffee, tea, chocolate and cocoa (National collegiate athletic association, 2013) and it is important to consider other EDs ingredients beside caffeine, since taurine can also affect erogenicity (Souza et al, 2016). Available studies do not provide sufficient information to conclude on whether consumption of *p*-synephrine, or of other substances found in EDs than caffeine, modifies the acute effects of caffeine on cardiovascular and neurological system (EFSA Panel on Dietetic Products, Nutrition and Allergies, 2015). In EFSA panel study (2015) it was concluded that 100 mg of caffeine (about 1.4 mg/kg BW for a 70 kg adult) may increase sleep latency and reduce sleep duration in some individuals, particularly when consumed close to bedtime.

In the research of Grasser et al (2014) consuming the Red Bull results in an elevation in blood pressure and diminished cerebral blood flow velocity relative to the reference water sample, and they emphasize that other components of Red Bull (sweet taste, calorie content, sugars, caffeine, taurine and gluconolactone) can also, in their own rights or through interactions with each other, be contributing to these differential hemodynamic effects. Furthermore, the EFSA Panel notes that caffeine consumption increases blood pressure and decrease myocardial blood flow in actually all adult population subgroups tested, regardless of baseline blood pressure, regular caffeine consumption/time of caffeine withdrawal, age, sex or hormonal status. The effect was observed at single doses of caffeine ranging from 80 to 300 mg. Blood pressure generally increases 30 minutes after caffeine consumption, reaches a peak after 60–90 minutes and returns to baseline after about two to four hours, which is consistent with the pharmacokinetics of caffeine.

Earlier studies on the consumption of EDs and the monitoring of unwanted symptoms of their increased consumption confirmed that arrhythmia is listed as the most common cause of caffeine-related death (Sepkowitz, 2012), also high caffeine intake can produce troubling side effects such as gastrointestinal distress, nervousness, mental confusion, inability to focus, disturbed sleeping (Spriet, 2014; Richards and Smith, 2016), tachycardia, severe hypertension, arrhythmia and even death (Kinugawa et al, 2011; Jabbar and Mark, 2013; Van der Hoeven et al, 2014; Eichner, 2014).

The difference in the intake of caffeine by consuming coffee and EDs is significant. Niseteo et al (2012) found that a Turkish/Greek coffee brews contains 1942.38 mg caffeine/L, Instant coffee brew 4716.28 mg caffeine/L and espresso brew 977.12 mg caffeine/L. The caffeine content of regular coffee

brews ranged from 6.98 to 33.69 mg/g of ground coffee. Coffee is generally consumed slowly and sipped while EDs are consumed in a short period. EDs are often high in sugar, while even sweetened coffee will contain less of it. Monster ED has 54 g of sugar, which corresponds to 13.5 teaspoons (Caffeine Informer, 2014). Coffee is an all-natural beverage, on the contrary EDs are often full of sweeteners, dyes and preservatives. Also, it is important to emphasize that adults drink coffee, and children commonly do not (Mitchell et al, 2014).

It is necessary to read declaration for ''safe'' caffeine consumption and avoid drinking beverages that do not cite caffeine and sugar content (McCusker et al, 2006). EFSA issued a statement in 2009 that 400 mg of caffeine match a ''safe'' dose and does not cause concern to consumers, and higher doses are regarded as harmful (Ivy et al, 2009). Legislative regulations, apart from the general provision of the Food Act (NN 81/2013) to which foods must be health correct, do not exist for EDs in Croatia. According to the Ordinance on general declaration or labelling of food (NN 114/2004), the caffeine content > 150 mg/L must be labelled with the indication ''High caffeine content'' and in brackets must be expressed the data on the amount of caffeine in mg/100 mL, also in accordance with the Ordinance on amendments to the ordinance on dietary supplements (NN 41/2013) the biggest daily dose of caffeine is 350 mg, and must be labelled with the indication ''Not recommended for children, pregnant women or nursing mothers''.

Summing up the above data of this research, it is necessary to conduct more research that can lead to the actions of regulatory agencies. Currently, the FDA limits caffeine content only in cola-type beverages to 0.2 % or 71 mg/12 oz; they considered banning caffeine from soft drinks but accepted the soft drinks maker argument that caffeine was a flavour enhancer (Reissig et al, 2009). However, disclosing the caffeine content is not required on beverages marketed as dietary supplements, but is required on products marketed as food if caffeine is added to the food (vs. naturally occurring) (US Food and Drug Administration, 2015). In research of Attipoe et al (2016) this fact was considered as the reason why over half of the beverages they tested had supplement facts label, and the remaining had nutrition facts label. France and Denmark have banned the sale of these kinds of EDs and the governments of Sweden and Canada published their concerns about a limited safety data on the caffeinated EDs (Howland et al, 2010). Labels of caffeine-containing products should provide caffeine content so individuals can make informed decisions (Lieberman et al, 2012).

Conclusions

Results of this study confirm previous reports of mismatch between analytical and labelled caffeine content of EDs. However, the average deviation from analytical caffeine content of EDs was smaller in comparison with previous reports. When expressed as amount expected in a serving size, the deviation was less than 0.5 mg/kg of body weight for an average person (70-75 kg), therefore, the practical implications of the deviation are not large.

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