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EVALUATION OF THE EFFECT OF CONSUMING AN ENERGY DRINK ON THE CONCENTRATION OF GLUCOSE AND TRIACYLGLYCEROLS AND ON FATTY TISSUE DEPOSITION. A MODEL STUDY

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ABSTRACT

Background. The animal model study was aimed at evaluating the effect of diet composition and energy drink intake on body weight, accumulation and distribution of deposited fatty tissue, and concentrations of glucose and triacylglycerols in blood plasma.
Material and methods. The experiment was carried out on 30 male rats. The animals were sorted into three groups, fed on group I – standard feed, groups II and III – modified feed, in which part of whole wheat and corn grains were isocalorically substituted with wheat flour and saccharose. Animals from groups I and II were receiving settled tap water for drinking, whereas these from group III were administered 3 ml of an energy drink, and then were provided drinking water.
Results. In analysing the results obtained it was stated that the addition of the energy drink to diet affected diminished body weight gains of the animals (per energy unit in the diet) as compared to the group of animals fed modified diet. The animals receiving the energy drink were additionally characterised by a lower content of peri-intestinal and intramuscular fatty tissue, whereas were found to deposit significantly higher amounts of peri-cardiac fatty tissue. Samples of blood plasma of these animals were found to contain a significantly higher concentration of glucose, compared to those of the animals fed modified diet. In turn, the concentration of triacylglycerols was comparable in all groups of animals.
Conclusions. The analysis of results achieved enabled concluding that the addition of energy drink to diet was significantly modifying the rate and tendency of metabolic changes, which was manifested in: increased glucose concentration in blood plasma, diminished body weight gains of the animals and deposition of pericardial fat.

Key words: rat, energetic drinks, carbohydrate-lipid metabolism

INTRODUCTION

A changing pace of life, omnipresent haste and the necessity of being available 24-h a day, make that an increasing number of persons are reaching for a group of drinks available on the market for a few years and having a different character than the traditional beverages. These include, among others, energy drinks. The first drink of this type was Red Bull. It was introduced onto the market in 1987 in Austria and ten years later in the USA. In Poland, the first drinks of this type were introduced onto the market in 1995 and were in majority imported [Wierzejska et al. 2002]. Nowadays, most of the new assortments of those drinks appearing on the

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Polish market originate from local production, and the market of energy drinks is the most dynamically developing market of alcohol-free beverages [Drożdż 2008].

According to nutritional guidelines the term "energy drinks" refers to dietetic food products in which the main source of energy are carbohydrates, whose energy value is not lesser than 80 kJ/100 ml (19 kcal/100 ml), and which contain one or more of the following compounds: caffeine, guarana, inositol, glucuronolactone or taurine. However, the content of those compounds in 100 ml of a drink may be diversified and depends on the producer.

Energy drinks are products designed for occasional consumption by persons with intensified psychophysical strain. Producers declare that these products are supposed to increase physical fitness of the body under conditions of effort, including: long-term arduous work, stress, and need of greater concentration. Unlike traditional beverages, they should not be treated as carriers of water, meaning as thirst-quenching beverages, but as functional food products.

Literature data provide different values of their intake and frequency of consumption depending on the population studied. Those drinks are consumed mainly by young men, in the quantity of 3-8 cans a week. According to some surveys, 34% of respondents at the age of 18-24 years declare regular consumption of those drinks [O'Brien et al. 2008].

An overview of literature indicates that results of investigations addressing the impact of energy drinks on the body are inexplicit. Some of the studies show that they were evoking a moderate increase of physical endurance and intellectual abilities and that they improved well-being [Alford et al. 2001, Scholey and Kennedy 2004], whilst other investigations do not confirm such a positive effect of those drinks on the body [Smith and Rogers 2002]. Attention should, however, be paid to the fact that those studies were conducted on a small group of respondents and referred to a short period of time, immediately after single consumption of such a drink. Despite a strong biological effect of compounds present in energy drinks, the effect of their long-term consumption on the body has as yet never been determined.

Therefore the objective of this study was to evaluate, in a model study, the impact of energy drink consumption on the carbohydrate-lipid metabolism.

MATERIAL AND METHODS

The experiment, after approval of the Local Ethical Committee in Szczecin (Approval no. 3/2006), was carried out in the vivarium of the Department of Human Nutritional Physiology, on 30 SPRD-strain male rats aged 5-7 months, of initial body weight 361 ±25.6 g.

Following a week long conditioning in the vivarium environment (temperature 21-22°C, atmospheric humidity 55-60%, 12 h/12 h light/dark cycle), the animals were randomised and sorted into three equinumerous groups of equal body weight, fed *ad libitum* on pelleted feeds composed of the same components, besides those differentiating, produced by the Feeds and Concentrates Plant in Kcynia, Poland, after having implemented the procedure 5.14.5. "Cleaning of machines and devices". Group I was fed standard feed (Labofeed H), while groups II and III received modified feed, in which 83.5% of wheat was substituted with wheat flour (type "500"), and 50% of corn grain was substituted with saccharose. The percentage of the remaining components was unchanged (Table 1). All diets were based on the balanced modification of the AIN-93 diet formulation [Reeves et al. 1993]. Changes of feed components was designed to reflect the changes taking place today in the composition of diets, which contains more simple sugars and refined carbohydrates.

In order to establish the chemical composition of the feeds, basic chemical assays were carried out (Table 2).

For drinking, animals from group I and II were provided pure, settled tap water. Whereas animals from group III were receiving, in the period of intensified activity, 3 ml of energy drink. The amount of administered drink was calculated taking into account its consumption among the people, expressed per kilogram of body weight. Having drunk the drink, the animals were re-drunk with pure, settled tap water. The basic composition of the drink was given in Table 3.

The experiment lasted for six weeks, the amounts of feed consumed by the animals were recorded daily, whereas once a week the animals were weighed. After 6-week experiment, the animals were anaesthetized. Blood was sampled from their heart to tubes with anticoagulant. Peri-cardial and peri-intestinal fat was

Table 1. Component composition of feeds used in the experiment

Component	Basic feed, %	Modified feed, %
Wheat	36.4	6
Corn grain	20	10
Wheat bran	20	20
Dry whey	3	3
Fodder salt[1]	0.3	0.3
Soya-bean grain	17	17
Fodder chalk[2]	1.5	1.5
Phosphate 2-CA[3]	0.8	0.8
Premix LRM[4]	1	1
Wheat flour	–	30.4
Saccharose	–	10

[1]Mainly NaCl.
[2]Mainly $CaCO_3$.
[3]$CaHPO_4$.
[4]Vitamin-mineral composition used in animals feeds.

Table 2. Chemical composition of feeds used in the experiment

Component	Basic feed	Modified feed
Total protein, %	18.1	17.9
Crude fat, %	2.10	2.19
Carbohydrates, %	65.8	66.2
Dry matter, %	92.1	91.6
Total ash, %	6.08	5.69
Brutto energy		
kcal·g^{-1}	3.95	3.95
kJ·g^{-1}	16.6	16.6
Metabolic energy		
kcal·g^{-1}	3.54	3.54
kJ·g^{-1}	14.8	14.8

Table 3. Declared energy value and composition of an energy drink given to animals

Component	Amount in 100 ml of drink
Energy, kJ	192
kcal	45
Carbohydrates, g	11.3
including saccharose and glucose, g	10.7
Vitamin B_6, mg	2
Niacin, mg	8
Pantotenic acid, mg	2
Vitamin B_{12}, µg	2
Taurine, mg	400
Glucuronolactone, mg	240
Caffeine, mg	32

dissected out immediately after sacrificing the rats, and weighed.

Blood plasma obtained after clot centrifugation was assayed for the concentration of glucose and triacylglycerols with the colorimetric method using reagents by Biosystems company on a Metertech spectrophotometer. Intramuscular fat was obtained from the thigh muscles (*triceps femoris*). The samples were used to determine the percentage of crude fat, with the Soxhlet technique in Soxtec HT6 apparatus (Foss Tecator).

The resulting data were tested for normality of distribution and processed statistically by means of the Statistica software package, using the Duncan test at the significance level $\alpha = 0.05$ [Statsoft 2009].

RESULTS AND DISCUSSION

The analysis of the results achieved demonstrated that despite isocaloricity of feed mixtures administered to the animals and their similar intake, the animals from group III were characterised by a higher energy intake expressed per 100 g of body mass than the animals from the group fed the modified feed mixture (Table 4). It resulted from the administration of energy drink which was also a carrier of energy (45 kcal/100 ml).

Table 4. Effect of diet type and energy drink consumption on feed and energy consumption in rats ($\bar{x}$ ±SD, n = 30)

Trait	Group I (a)	Group II (b)	Group III (c)	Significance of differences
Feed consumption g/100 g body weight	269 ±7.2	259 ±12.9	270 ±11.9	–
Energy consumption kcal/100 g body weight	945 ±24.6	910 ±45.3	1009 ±40.2	a-c*, b-c**

Statistically significant difference: *p ≤ 0.05; **p ≤ 0.01.

Table 5. Effect of diet type and energy drink consumption on body weight gain and amount and localization of fatty tissue in rats ($\bar{x}$ ±SD, n = 30)

Trait	Group I (a)	Group II (b)	Group III (c)	Significance of differences
Body weight gain, g	66.9 ±10.9	81.7 ±18.6	61.0 ± 3.5	a-b*, b-c*
Body weight gain g/100 kcal	1.64 ±0.192	2.02 ±0.210	1.42 ±0.198	a-b*, a- c*, b-c**
Peri-intestinal fat g/100 g body weight	1.43 ±0.318	1.55 ±0.334	1.27 ±0.212	–
Peri-intestinal fat g/100 kcal	0.151 ±0.033	0.165 ±0.035	0.125 ±0.021	a-c*, b-c*
Peri-cardial fat mg/100 g body weight	63.7 ±10.5	47.9 ±14.2	89.4 ±13.0	a-b*, a-c**, b-c**
Peri-cardial fat g/100 kcal	6.73 ±1.11	5.27 ±1.56	8.84 ±1.28	a-b*, a-c*, b-c**
Intramuscular fat, %	6.21 ±0.48	6.58 ±0.62	5.89 ±0.51	a-c*, b-c*

Statistically significant difference: *p ≤ 0.05; **p ≤ 0.01.

In analysing the results achieved it was stated that the feed mixture in which whole cereal grains were substituted with wheat flour and saccharose facilitated increased body weight gains of the animals expressed per unit of consumed energy, without affecting the content of peri-intestinal and intramuscular fatty tissue, and was reducing the peri-cardial fatty tissue (Table 5).

In contrast, the addition of the energy drink to a diet affected decreased body weight gains of the animals (per unit of consumed energy), compared to the animals fed the modified feed mixture. The animals administered the energy drink were additionally characterised by a lower content of peri-intestinal and intramuscular fatty tissue, and by a higher adiposity of peri-cardial tissues compared to the animals fed the modified diet (Table 5).

The analysis of experimental results showed a significant effect of both diet composition and energy drink consumption on the concentration of glucose in blood plasma of the animals examined (Table 6), i.e. a higher glucose level was determined in blood plasma of the animals fed the modified feed mixture than in those receiving the basal diet. The animals that were systematically consuming the energy drink were characterised by a statistically significantly higher concentration of glucose in plasma than the animals fed the modified feed mixture. In contrast, no significant effect of diet composition and energy drink consumption

Table 6. Effect of diet type and energy drink consumption on plasma glucose and triacylglycerols concentration in rats ($\bar{x}$ ±SD, n = 30)

Trait	Group I (a)	Group II (b)	Group III (c)	Significance of differences
Glucose, mmol·l^{-1}	4.44 ±0.59	5.01 ±0.26	5.77 ±0.38	a-b*, a-c**, b-c**
Triacylglycerols, mmol·l^{-1}	0.66 ±0.12	0.63 ±0.11	0.71 ±0.14	–

Statistically significant difference: *p ≤ 0.05; **p ≤ 0.01.

was observed in respect of triacylglycerols concentration in blood plasma of the animals under study.

A correlation between the frequency of energy drinks consumption and body mass was demonstrated by Bajerska et al. [2009]. In their survey, the adolescents characterised by excessive body mass were more frequently reaching for energy drinks that contain saccharose and contribute to increased energy intake. Also in our study, the energy intake was higher in the rats that were administered the energy drink, yet no increase in body weight gains intensity was observed in their case. On the contrary, it was found that the systematic consumption of the energy drink by the animals was decreasing the rate of body weight gains compared to that observed in the animals fed the modified feed mixture. It seems that excessive body mass of the adolescents surveyed by Bajerska et al. [2009] was due to many complex reasons. Those respondents (having excessive body mass and reaching more frequently for energy drinks) were also characterised by a number of improper eating habits which could – to a great extent – contribute to the development of their overweight and obesity.

The metabolic effects of energy drinks are attributed to caffeine, saccharose and B-group vitamins occurring in those products. Investigations conducted by other authors and referring to the impact of those components on the carbohydrate-lipid metabolism are not explicit. Rush et al. [2006] found out that the administration of a caffeine-saccharose solution to young women resulted in enhanced oxidation of carbohydrates in the body, and in diminished oxidation of lipids, which could suggest the lipogenic effect triggered by caffeine. In turn Greer et al. [2001] demonstrated increased insulin resistance and decreased glucose absorption by skeletal muscles as well as restricted oxidation of carbohydrates manifested by an increased plasma level of glucose under the influence of the administered caffeine. It is, however, difficult to compare results achieved owing to differences in the applied doses of caffeine and time span in which those changes were monitored. Rush et al. [2006] applied a low dose of caffeine (1.3 mg/kg b.w.), and the metabolic effects were determined after 30 min since drink consumption, whereas the dose applied by Greer et al. [2001] was remarkably higher (5 mg/kg b.w.), and changes in metabolism were monitored for 3 hours. In our experiment, the animals were administered caffeine at a dose of 1.28 mg/kg b.w. every day for six weeks. The study demonstrated a higher concentration of glucose in blood plasma of the animals consuming caffeine originating from the energy drink. Glucose concentration was determined in fasted animals, and caffeine was ingested systematically till the day preceding blood sampling. The results achieved are, hence, an effect of permanent metabolic changes that do not result from the direct impact of caffeine present in the blood circulation.

The animals consuming the energy drink were additionally characterised by different than in the other animals site of fatty tissue deposition. A decrease was observed in fat content of muscles, whereas an increase was noted in peri-cardial fatty tissue deposition. Those changes could also be attributed to caffeine which intensifies metabolism and increases energy consumption. Fatty acids, originating from blood and from hydrolysis of triacylglycerols accumulated inside myocytes, are becoming a predominating source of energy at long-term physical effort with a low intensity [Dyck et al. 1997, Jeukendrup 2002]. Their deficiency may, therefore, diminish body capability for the re-synthesis of ATP and physical effort tolerance. In contrast, their lower content should increase glucose tolerance and reduce insulin resistance [Bonen

et al. 2007, Kusminski et al. 2009]. However, the reported experiment demonstrated an increased concentration of glucose in blood plasma of the animals consuming the energy drink and characterised by a lower fat content in muscles, which may indicate developing insulin resistance.

In the conducted experiment, the animals receiving the energy drink were characterised by increased deposition of peri-cardial fat. Such a localization of adipose tissue in humans may pose diagnostic difficulties, for it has no significant effect on body mass increase and does not result in increased body sizes, e.g. waist or chest circumference, and in live subjects its presence may only be detected with the use of advanced research techniques. No studies have been found in available literature that would be devoted to the effect of an energy drink, or its individual components, on deposition of peri-cardial fatty tissue. A few works however, for instance a study by Acheson et al. [2004], have demonstrated that caffeine may trigger re-distribution of fatty tissue, intensifying lipolysis (i.e. degradation of triacylglycerols accumulated in adipocytes to glycerol and free fatty acids) and re-esterification of fatty acids to a great extent than β-oxidation (i.e. hydrolysis of free fatty acids for energy production). Under physiological conditions, β-oxidation is a resultant of lipolysis, conducted in order to produce free fatty acids introduced into the process of β-oxidation. A research conducted by Acheson et al. [2004] demonstrated that the administration of caffeine increased twice the turnover of free fatty acids, however only 24% of liberated fatty acids were β-oxidised and the other 76% were again re-esterified and accumulated.

The peri-cardial fatty tissue acts as a system buffering an excessive concentration of fatty acids. The excess of fatty acids released after lipolytic processes, appearing in the circulation, may disturb the cardiac performance cycle and therefore is accumulated in adipocytes of the peri-cardial fatty tissue [Marchington et al. 1989, Corradi et al. 2004]. From this point of view, its presence exerts a positive effect on cardiac performance. It has, however, been demonstrated that it produces numerous inflammatory mediators that display a significantly higher expression of inflammatory chemokines and cytokines compared to the subcutaneous fatty tissue [Zhang et al. 2003]. Result of a research by McTernan et al. [2002] indicate that the local distribution of fatty tissue plays a significant role in the development of an unfavourable metabolic profile and increases cardiovascular risk. A question why in the conducted study the fatty tissue was accumulated in the pericardiac area still remains open.

The metabolic effects observed after caffeine administration result from its influence on the secretion of hormones, including among others adrenaline and cortisol [Keijzers et al. 2002, Leblanc et al. 1995]. They are referred to as "stress hormones", and their metabolic effects are aimed at providing energy sources necessary to survive the stress. Both adrenaline and cortisol enhance lipolysis and affect an increase in plasma concentration of glucose. They additionally enhance gluconeogenesis and reduce peripheral glucose consumption by inhibiting the activity of key glycolytic enzymes as a result of increasing the concentration of fatty acids in blood. A typical trait of their action is increased consumption of fatty acids instead of glucose for energy production. However, the action of glucocorticoids results in the increase in the total fat content of the body and in the changes in fatty tissue distribution which accumulates in the upper parts of the body [Milewicz 1995]. In addition, caffeine reduces tissues' susceptibility to insulin and impairs glucose metabolism [Keijzers et al. 2002, MacKenzie et al. 2007, Moisey et al. 2008], which in the reported experiment could be manifested in the increased concentration of glucose in blood of the animals administered the caffeine-containing energy drink.

The energy drinks provided to animals contained also B-group vitamins that are indispensable co-factors of enzymatic reactions linked with energy production and carbohydrate metabolism. Friedrich and Sadowska [2005] were observing higher body weight gains and lower deposition of perio-organ fatty tissue in the animals supplemented with B-group vitamins. In the conducted experiment, the animals that were receiving additional doses of B-group vitamins, originating from the energy drink, were characterised by lower body weight gains and lower deposition of peri-intestinal fatty tissue, presumably as an effect of the intensified rate of metabolic transformation under the influence of caffeine present in the drink. The observed enhancement of metabolism might occur only upon providing additional doses of B-group vitamins that participate in the carbohydrate-lipid metabolism.

It seems that taurine present in the drink had no impact on systemic metabolism, because its metabolic effects are manifested mainly during intensified physical exercise [Kulasek et al. 2004].

CONCLUSIONS

The analysis of the results achieved demonstrated that the systematic consumption of an energy drink caused:

1. Diminished body weight gains at simultaneously increased energy consumption, which suggests the increasing rate of catabolic transformations in the investigated animals.

2. Diminished deposition of peri-intestinal fat and increased accumulation of peri-cardial fatty tissue which may be a source of chemokines and cytokins with pro-inflammatory properties.

3. Increased concentration of glucose in blood plasma resulting, most likely, from metabolic changes leading to the enhancement of lipolysis and development of insulin resistance.

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OCENA WPŁYWU SPOŻYWANIA NAPOJU ENERGETYZUJĄCEGO NA STĘŻENIE GLUKOZY I TRIACYLOGLICEROLI ORAZ ODKŁADANIE TKANKI TŁUSZCZOWEJ. BADANIA MODELOWE

STRESZCZENIE

Wstęp. Celem badań była ocena, na modelu zwierzęcym, wpływu składu diety i spożywania napoju energetyzującego na masę ciała, gromadzenie i lokalizację gromadzonej tkanki tłuszczowej oraz stężenie glukozy i triacylogliceroli w osoczu krwi.

Materiał i metody. Badania przeprowadzono na 30 samcach szczura. Zwierzęta podzielono na trzy grupy, które żywiono: mieszanką podstawową – grupa I, mieszanką zmodyfikowaną, w której część pełnych ziaren zastąpiono mąką pszenną i sacharozą – grupy II i III. Do picia zwierzęta grupy I i II otrzymywały odstaną wodę wodociągową. Grupa III otrzymywała 3 ml napoju energetyzującego, a następnie zwierzęta dopajano wodą.

Wyniki. Analizując uzyskane wyniki, stwierdzono, że dodatek do diety napoju energetyzującego wpływał na zmniejszenie przyrostów masy ciała badanych zwierząt (w przeliczeniu na jednostkę energii w diecie) w porównaniu z pozostałymi grupami. Zwierzęta pojone napojem energetyzującym charakteryzowały się mniejszą ilością okołojelitowej i śródmięśniowej tkanki tłuszczowej, a gromadziły istotnie większe ilości okołosercowej tkanki tłuszczowej. W osoczu krwi tych zwierząt stwierdzono większe stężenie glukozy w porównaniu z oznaczonym u zwierząt w pozostałych grupach. Stężenie triacylogliceroli było porównywalne we wszystkich grupach zwierząt.

Wnioski. Analiza uzyskanych wyników pozwoliła na stwierdzenie, że dodatek do diety napoju energetyzującego istotnie modyfikował tempo i kierunek przemian metabolicznych, co manifestowało się: wzrostem stężenia glukozy w surowicy krwi, zmniejszonymi przyrostami masy ciała badanych zwierząt oraz gromadzeniem tłuszczu okołosercowego.

Słowa kluczowe: szczur, napoje energetyzujące, metabolizm węglowodanowo-lipidowy

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