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Long Term Consumption of red Bull and Alcohol can Affect Rat Skeletal Muscle Metabolism

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SUMMARY. In the past few years, Red Bull rapidly became a popular energy drink and this increase in popularity is the result of targeted advertising. The effects of short term consumption of Red Bull are known and they are beneficial, however the effects of chronic consumption may lead to serious health conditions. The purpose of the study presented in this work is to investigate if long term consumption of Red Bull affects the physiological and functional integrity of the skeletal muscles. The study was conducted on 28 albino male Wistar rats, weighing 182.11±4.7 g, divided into four groups. The Control (C) group received a standard diet and tap water. The Red Bull/Ethanol (RB+E) group were orally administrated 1.5 ml/100 g b.wt. of Red Bull and 0.6 ml/100 g b.wt. of ethanol daily, for 30 days. The same concentrations were administered to the groups that received the individual drinks. In the last six days of the experiment the animals were tested for physical performance using a weight-loaded forced swim test. After 30 days of treatment, immediately after exhaustion, the animals were killed under anesthesia and samples from the gastrocnemius muscle were harvested for biochemical parameters analysis and enzymatic activity assays. A significant decrease in glucose and glycogen concentration was registered in E and RB+E groups. Total protein concentration as well as AST and LDH activities remained unchanged in all groups. According to these results we can say that long term consumption of Red Bull energy drink, especially when combined with alcohol, may lead to significant changes in biochemical parameters strictly related to the carbohydrate metabolism. Red Bull and ethanol did not affect the physiological integrity of the skeletal muscle, although a transition probably occurred from type 2 (glycolytic, fast twitch) to type 1 (oxidative, slow-twitch) muscle fibers.

Keywords: energy drink, Red Bull, skeletal muscle

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Introduction

In the past few years, Red Bull rapidly became a popular energy drink and this increase in popularity is the result of targeted advertising. It is marketed as an enhancer of physical performance and as a stimulant for attention, concentration and the level of alertness ("Company" Red Bull, 2016). This makes it an appealing beverage for teenagers, athletes and people who undertake physical activities for recreational purposes.

One other way individuals choose to consume Red Bull is in combination with alcohol. The main reason for this habit is the sweetening of the alcoholic beverages and a diminished perception of alcohol intoxication (Pennay *et al.*, 2011). As stated in the aforementioned article, Red Bull can mask the signs of alcohol intoxication leading to greater levels of alcohol intake and consequently alcohol poisoning.

The short term effects of Red Bull consumption are beneficial for the organism because of its ergogenic role while engaging in physical activities, as observed in previous studies (Forbes *et al.*, 2007; Verma and Biswas, 2014).

However, the effects of chronic consumption are not yet studied in detail. Results from our past experiments show that these effects are of concern, especially on cardiac and hepatic biochemical parameters and enzymatic activities (Crișan *et al*., 2013; Crișan *et al*., 2014). In the studies performed by Seifert *et al.* (2011) and Rath (2012), a long term consumption of energy drinks has been associated with convulsions, diabetes and changes in behavior and disposition, especially in adolescents and young adults. Other possible effects, observed by Waguih *et al.* (2012) and Pennington *et al.* (2010) are cardiovascular diseases, obesity, insomnia, anxiety, dehydration and difficulty in concentration.

The present study is part of a larger research project targeting the effects of long term consumption of Red Bull and alcohol, in combination and separately, on male Wistar rats. With the majority of studies focusing on the physical performance of human subjects, there is little to no evidence of the effects of Red Bull on rat skeletal muscle. The purpose of this study was to investigate the effects of long term consumption of Red Bull and alcohol, in combination and separately, on the skeletal muscle in physically trained rats.

Materials and methods

All reagents used in this study were of analytical grade and were purchased from Sigma-Aldrich Chemie GmbH, Germany, Nordic Invest S.R.L., Romania and S.C. BioZyme S.R.L, Romania. The Red Bull energy drink was bought from the local market.

The study was conducted on 28 albino male Wistar rats, weighing 182.11±4.7 g, divided into four groups, Control (C), Red Bull (RB), Ethanol (E) and Red Bull/Ethanol (RB+E). All animals received a standard diet (S.C. Siamond Prod. S.R.L., Cluj Napoca, Romania). The C group (n=7) had *ad libitum* access to tap water, RB group (n=7) were orally administrated 1.5 ml/100 g b.wt. of Red Bull daily, for 30 days and EtOH group (n=7) received 0.6 ml/100 g b.wt. of ethanol daily. The same concentrations were administered to the RB+E group (n=7).

In the last six days of the experiment the animals were tested for physical performance using a weight-loaded forced swim test. The rats were forced to swim to exhaustion with a load of 10% of their body weight attached to their tails. Each rat was considered to have reached exhaustion when it remained submerged for longer than 5 seconds. Water temperature varied between 28 and 30^{o} C and none of the animals were affected by hypotermia.

After 30 days of treatment, immediately after exhaustion, the animals were killed by exsanguination under anesthesia. Samples from the gastrocnemius muscle were harvested for the following biochemical analysis: total muscle glucose, glycogen and proteins; enzymatic activities of: ALT, AST, and LDH.

Total glucose concentration was determined with the Somogy-Nelson colorimetric assay (Nelson, 1944). Glycogen concentration was determined using the Montgomery (1957) method modified by Lo *et al*. (1970). Total protein concentration was determined by Bradford (1976) colorimetric assay using the Bradford "ready-to-use" reagent. Reitman and Frankel (1957) photocolorimetric assay was used for the determination of AST and ALT enzymatic activities. The activity of LDH was determined spectrophotometrically by measuring the oxidation rate of NADH (nicotinamide adenin dinucleotide, reduced) at 365 nm (Bergmeyer and Bernt, 1974).

Results were analyzed using the two tailed *t* test and considered statistically significant at $p \leq 0.05$. Multiple comparisons were made (more details are presented in the Figure 1 description).

Results and discussion

The purpose of this study was to investigate the effects of long term consumption of Red Bull and alcohol, in combination and separately, on the skeletal muscle in physically trained rats.

Red Bull and ethanol, administered alone or combined, caused a decrease of skeletal muscle glucose concentration as seen in Figure 1a and Table 1. It has been previously shown that oral administration of niacin, one of the vitamins found in high concentration in Red Bull, or intense physical activity causes a muscle fiber transition from type 2 (glycolytic, fast twitch) to type 1 (oxidative, slow-twitch) (Khan *et al.*, 2013). Type 1 fibers are rich in mitochondria and mainly use fatty acids as an energy

source (Ringseis *et al.*, 2013); at the same time, glucose uptake by the skeletal muscle is inhibited. One explanation for the decrease in glucose concentration could be the result of these changes in muscle fiber phenotype and metabolism.

Xu *et al.* (1996) showed that alcohol has an inhibitory effect on the uptake of glucose by the skeletal muscle. Despite the consistent observation that acute and chronic alcohol intake impairs the *in vivo*-determined insulin-mediated glucose uptake by skeletal muscle cells (Spolarics *et al.*, 1994; Lang *et al.*, 2014; Wan *et al.*, 2005), there is little knowledge about the mechanism by which this happens. In theory, as described by Steiner *et al.* (2015), alcohol can affect insulin signaling at a number of key regulatory steps such as PI3K/AKT signal transduction and/or GLUT4 translocation.

The combined treatment caused a significant decrease in glucose concentration in the RB+E group, which was probably determined by the complementary action of Red Bull and ethanol.

As seen in Figure 1b and Table 1, glycogen concentration decreases significantly after Red Bull and ethanol consumption. As stated previously, type 1 fibers are rich in mitochondria and mainly use fatty acids as an energy source, therefore this type of muscle fibers usually have fewer glycogen deposits (Ringseis *et al.*, 2013). Similarly to the glucose concentration, glycogen deposits in rats treated with Red Bull may have decreased due to the changes in muscle fiber phenotype and metabolism.

A study by Peters *et al.* (1996) demonstrated that ethanol inhibits glycogen accumulation in type 1 fibers during the immediate recovery from high-intensity exercise. Other groups proposed that alcohol inhibits the activation of glycogen synthase, which leads to a low level of glycogen in the skeletal muscle (Xu *et al.*, 1992; Xu *et al.*, 1996). The animals in our study were sacrificed just after exhaustion. This fact, alongside the evidence that intense physical exercise facilitates the transition between muscle fibers, offered by Khan *et al.* (2013), can explain the decrease in glycogen concentration in the E group.

When administered together, the action of Red Bull and ethanol was complementary, determining the depletion of the glycogen deposits in the skeletal muscle (Fig. 1b, Table 1).

Interestingly, although we expected a decrease of the total protein concentration in each of the treatment groups, such an effect was not observed (Figure 2a, Table 1). Both ethanol and some ingredients from Red Bull are known to cause muscle injury (Martin *et al.*, 1985; Campana *et al.*, 2014), hence our expectations. In cases dating from 1989 to the present, patients were hospitalized presenting rhabdomyolysis and other severe affections caused by intoxication with large amounts of caffeine (Wrenn and Oschner, 1989; Chakraborty and Rajeswaran, 2007; Campana *et al.*, 2014), one of the main ingredients of RB.

Table 1.
Effects of Red Bull, ethanol and the combined drink on biochemical parameters and enzymes of the skeletal muscle in phisycally trained rats

Parameter	Control	RB	E	RB+E
Glucose concentration (mg/g tissue)	1,086±0,20	0,83±0,175	0,32 ±0,14 *	0,21 ±0,07 ** *
Glycogen concentration (mg/g tissue)	3,43±0,41	2,06±0,25 *	2,14 ±0,24 *	0,70±0,19 *** ** **
Total protein concentration (mg/g tissue)	46,05±2,73	47,45±1,47	47±2,11	47,86±0,92
LDH activity (μmol pyr/g tissue/min)	0,0133±0,0012	0,0131±0,0005	0,013±0,0013	0,0125±0,0005
AST activity (μg pyruvate/g tissue/ hour)	3269±151	3367±82	3347±84	3357±63
ALT activity (μg pyruvate/g tissue/ hour)	10061±166	9653±307	9570±298	9441±350

RB-Red Bull; E-Ethanol; RB+E-Red Bull+Ethanol; LDH-lactate dehydrogenase; AST-aspartate aminotransferase; ALT-alanine aminotransferase. Multiple comparisons were made: black - *vs* Control group; red - *vs* RB group; blue - vs E group; * p<0.05; ** p<0.01; *** p<0.001.

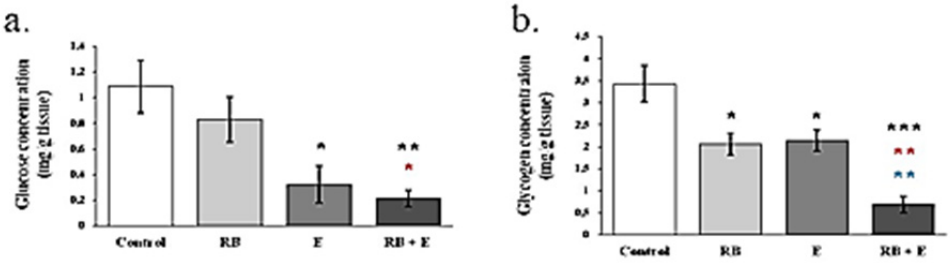

Figure 1. Effects of Red Bull, ethanol and the combined drink on skeletal muscle (a) glucose concentration and (b) glycogen concentration, of physically trained rats. n=7 in each group. The results are expressed as mean ± SE. Multiple comparisons were made: black - *vs* Control group; red - *vs* RB group; blue - vs E group; * p<0.05; ** p<0.01; *** p<0.001.

Although the amount of caffeine contained in the energy drink is high, it is unlikely to cause rhabdomyolysis. Even so, after 30 days of treatment we expected a decrease of the total protein concentration in the skeletal muscle of rats treated

with Red Bull. A plausible explanation can be the membrane stabilizing effect of taurine on the skeletal muscle (Huxtable and Bressler, 1973). The process is not yet fully understood but it is related to the ability of taurine to control the function of ion channels and consequently membrane excitability. Taurine also influences calcium homeostasis and excitation-contraction coupling (De Luca *et al.*, 2015).

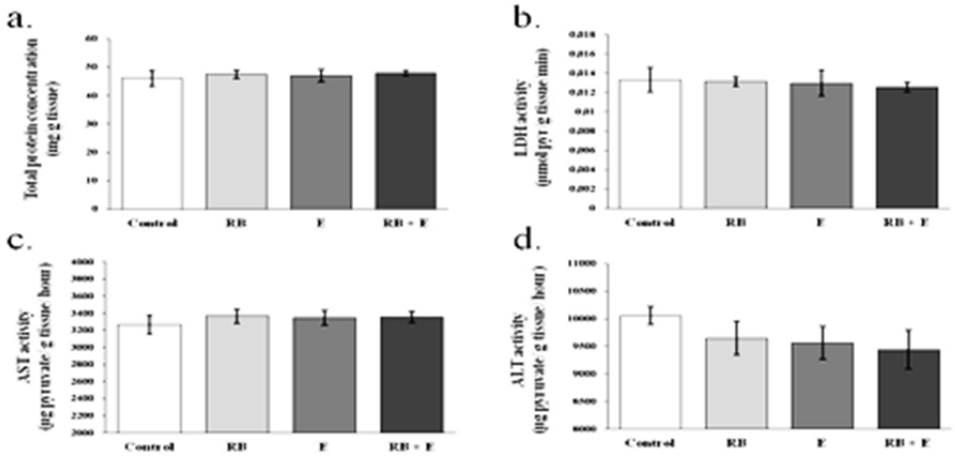

Figure 2. Skeletal muscle (a) total protein concentration; (b) LDH, (c) AST and (d) ALT activity of physically trained rats treated with Red Bull, ethanol and the combined drink. n=7 in each group. The results are expressed as mean ± SE

Considering the beneficial effects of taurine on the skeletal muscle, we assume that the markers for cell integrity will not vary between groups. LDH together with AST and ALT activities are enzymatic markers which, if found in high amount in the blood stream, indicate muscle injury and leaking of the enzymes from the tissue (Janssen *et al.*, 1989).

LDH is also a marker for glycolytic metabolism (Spriet *et al.*, 2000) and the administration of Red Bull and ethanol, separately or combined, did not affect its activity (Figure 2b, Table 1). These results confirm the fact that the skeletal muscle of the rats in all groups has undergone a change regarding the type of muscle fibers and consequently a transition from glycolytic to oxidative metabolism.

Similarly to LDH, AST activity did not suffer any noteworthy changes (Figure 2c, Table 1) and judging by these results we can assume that the physiological integrity of the skeletal muscle was not affected.

Interestingly, although not statistically significant, ALT activity was affected by the treatment and suffered a slight decrease in all groups (Figure 2d, Table 1). The decline in glucose utilization may have led to a low pyruvate concentration in the skeletal muscle and consequently to the decreased activity of ALT (Felig, 1973).

Conclusions

According to our results, we can say that long term consumption of Red Bull energy drink, especially when combined with alcohol, may lead to significant changes in biochemical parameters strictly related to the carbohydrate metabolism. Red Bull and ethanol did not affect the physiological integrity of the skeletal muscle, although a transition probably occurred from type 2 (glycolytic, fast twitch) to type 1 (oxidative, slow-twitch) muscle fibers.

The health conditions that Red Bull can lead to are dose-dependent, which necessitates the proper labeling of the product. This way, customers can make a conscious decision if they choose to consume the energy drink or not.

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