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THE METABOLIC EFFECTS OF ENERGY DRINKS UPON LABORATORY ANIMALS AND ATHLETES

DOCTORAL THESIS

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SUMMARY

THE METABOLIC EFFECTS OF ENERGY DRINKS UPON LABORATORY ANIMALS AND ATHLETES

Introduction

Key words: energy drinks, Red Bull, glucose, rats, sports.

Red Bull is a commercial energy drink allegedly conceived to improve physical and intellectual performance. This beverage was launched in 1987, but in 1992 it was banned in France and other countries, in Europe and South America, due to its negative effects. In 2008, the commercialization of Red Bull restarted in France, as a result of a growing pressure from the European Union (Reissing *et al.,* 2009).

The banning measure or the sales restriction was determined by the death of a few young people, associated with excessive consumption of energy drinks (U.S. Department of Health and Human Services, 2012). In North America, the Red Bull selling started in 1997. Since 2003 to 2008, the Red Bull selling has tripled in North America and East Europe and doubled in Australia and Middle East.

In Western Europe, the selling of energy drinks has not changed. In Turkey, the energy drinks (EDs) with a high level of caffeine are banned to the young people under 18 years of age. In Denmark and Uruguay, EDs are completely banned. In Norway and Sweden, the energy drinks are sold only in chemist's shops. In Canada, the labels must contain warning messages regarding their consumption in combination with alcohol and stating the highest quantity allowed to consumption. (Buxton and Hagan, 2012). **In Romania, there is no restriction**.

The increase of EDs consumption, especially in young people and athletes, led to many investigations concerning the effects of the energy drinks upon the body, as well as the reasons for their consumption. In the last years, an increasing number of articles started to appear, documenting the negative effects determinated by excessive consumption of energy drinks. The most common adverse effects are registered at the level of the nervous, cardiovascular and gastro-intestinal systems.

Most studies show that, on short term, a temperate EDs consumption improves the cognitive and psychomotor capacity. The stimulating properties, according to the producer, are due to the combined action of the components. There have been performed a lot of studies concerning the effects of the EDs consumption, on short term, but mostly focused on isolated components (ingredients) and not on the whole of the energy drink. The effects on long term

were less investigated, and the effects upon skeletal muscle and cardiovascular system are not known.

The purpose of this study was to test the Red Bull effects on short and long term, in both animals and humans, submitted to endurance physical effort (trained and untrained subjects).

The knowledge of the effects caused by the long term consumption of Red Bull is very important because this drink is addressed especially to young people (including athletes), often with lack of judgement and adequate information, and their health can be seriously damaged.

Due to the negative consequences, seldom noticed, but very dangerous for the body, the expansion of the EDs consumption (especially among athletes) and the producer's promises that energy drinks supply the required energy under stress conditions and permanent demands, our purpose was to investigate the effects of this energy drink under various but relevant conditions.

Our study wanted to check the effects of Red Bull upon body and the manner in which the consumption influences certain physiological and morphological parameters, under effort conditions **relevant to sports**. The tissues considered for study were those of blood, liver, skeletal muscle and myocardium.

I THE CURRENT STATE OF KNOWLEDGE REGARDING THE EFFECTS OF ENERGY DRINKS

1.1 Ingredients

The energy drinks contain, in different concentrations, depending on product: caffeine, taurine, sometimes guarana, sugar, synthetic sweeteners and some vitamins from the B group (Fig.1.1.1, Table 1.1.1). Each ingredient, separately taken, has beneficial effects upon human body or even therapeutic effects. The stimulating properties of Red Bull stem from the combined action of its ingredients: caffeine, taurine, carbohydrates (glucose and sucrose), B3 vitamin, glucuronolactone etc. Usually, energy drinks contain high amounts of carbohydrates along with nutrients purposed to improve cognitive and psychomotor capacity.

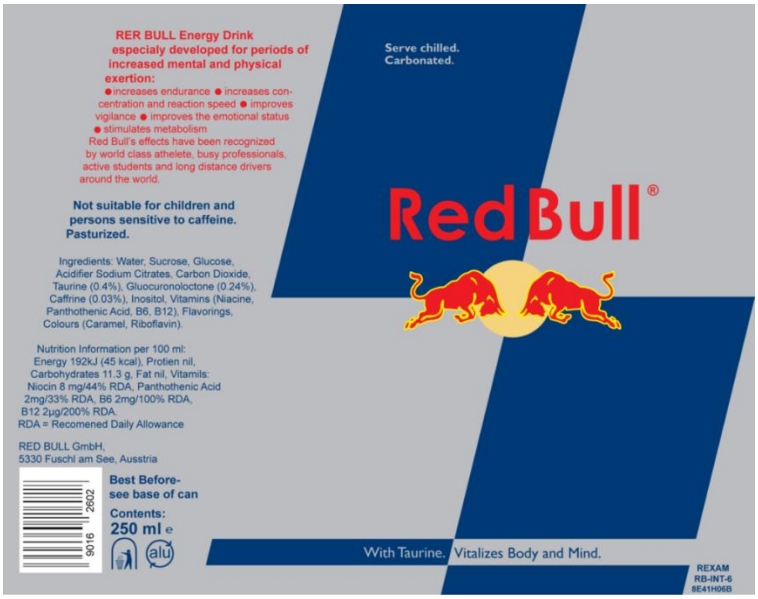

Figure 1.1.1. Red Bull ingredients: water, sucrose, glucose, sodium citrate, caffeine, carbon dioxide, taurine, glucuronolactone, inositol, B vitamins (niacin, pantothenic acid, B6, B12), flavours, dyes (caramel, riboflavin) *(*https://www.media.deviantart.com*)*

Table 1.1.1 The content of Red Bull energy drink. DRD- daily recommended dose (https://www.media.deviantart.com)

Ingredient	Dose
Caffeine	80 mg
Taurine	Unknown
Guarana	Unknown
Sugar	27 g
Sodium	200 mg
B3 vitamin	100% DRD
B6 vitamin	5 mg (250% DRD)
B12 vitamin	80%

Caffeine, the most known psychoactive drug and the most studied substance from the composition of EDs (Zang *et al*., 2014), is found in Red Bull in amount of 80 mg/dose (Fig. 1.1.1), which represents the equivalent of the quantity found in a cup of coffee. In adults, the

intake of up to 400 mg caffeine is considered safe (Clauson *et al.,* 2008). The daily recommended dose in European countries ranges from 280 to 490 mg.. The highest caffeine uptake is in the Scandinavian countries, proportional with the largest coffee consumption in these countries. (Wierzejka and Jarosz, 2012).

Toxicity begins from larger doses than 490 mg, and 10 g could be lethal. (Cannon et al., 2001). Some of the energy drinks contain additional caffeine doses, by adding guarana extract, cola nuts, cocoa, that potentiate coffee effects. (Di Rocco *et al.,* 2011). After IUPAC (International Union of Pure and Applied Chemistry), the caffeine's name is 1,3,7-trimethylxantine.

Caffeine potentiates the muscular contractions by inducing the release of calcium ions from the endoplasmatic reticulum, inhibits a series of phosphodiesterase and enzymes that degrades the glycogen from liver and muscles (Markos and Kavouras, 2005), it is an antagonist of the adenosine by blocking the adenosine receptors, it stimulates the action of the sodium-potassium pump from the plasma membranes (Wolinsky and Driskell, 2004).

Caffeine is a non-selective competitive inhibitor of phosphodiesterase enzymes (Echeverri *et al.,* 2010), which leads to the increase of the adrenergic tonus in the central and peripheral nervous system (Benowitz and colab,. 1982) and it could be an effective therapeutic agent against Alzheimer disease (Arendash and Cao, 2010).

Caffeine activates AMPK (*AMP-activated protein kinase*) (Zheng *et al.,* 2014), a key enzyme that coordinates more signalling pathways implicated in maintaining the energy homeostasis (Viollet *et al.,* 2006). Moreover, it stimulates diuresis and lipolysis (Temple *et al.,* 2009).

From the point of view of improving physical performances, literature data confirm this possibility (Davis and Green, 2009; Lara *et al.,* 2014). It is found that the athletes use the caffeine before their competitions. This fact results from the urine tests at the doping examinations (Del Coso *et al.*, 2012).

The chronic and excessive consume leads to cardiac ischemia and the toxicity at the level of the central nervous system can range from irritability to lethargy and coma. At the start of some caffeine intoxications symptoms of muscular convulsions, fasciculation, clonus, hallucinations, even brain edema, and severe brain blood pressure were described (Mrvos *et al.,* 1989; Dietrich and Mortensen, 1990).

Taurine is implicated in the regulation of a wide range of biological functions. It is an aminoethylsulphonic acid found in biliary secretion (Yamori *et al.,* 2010). In the hepatocit, it is mixed with cholic acids and their result is tauro-cholic acid. The tauro-cholic salts, especially the sodium salts, are later secreted in the bile (Guyton and Hall, 2006). These salts act like a detergent, decreasing the surface tension of the large fat particles, favouring the formation of fine particles, i.e., their emulsification. Taurine is synthesised from methionine and cysteine in the presence of pyridoxine (Birdsall, 1998).

In animal body, it is found like a free molecule and it is not incorporated into the muscle proteins. Often, it is called an amino acid, even it lacks a carboxyl group. Until recently, taurine was considered a non-essential nutrient, but the last studies show that taurine is essential in the visual information process, (Warburton *et al.,* 2001; Zhang *et al.,* 2003) and in the heart activity (Chowdhury *et al.,* 2016).

It is found in large amounts in the skeletal muscle, especially in the slow fibres, increasing the performance of physical exercises (Ripps and Shen, 2012).

Daily recommended input ranges from 20 mg to 400 mg, depending on diet (Finnegan, 2003; Clauson *et al.,* 2008). The taurine content in energy drinks can range from 400 mg to 3000 mg/dose.

In humans, the adverse effects induced by large doses of taurine are controversial. On a short period of time, there are no reports, until now, of adverse effects of taurine administration (Clauson *et al.,* 2008), but it is known that taurine is found in the supplements consumed by athletes. In Europe, after the death of some athletes which consumed large amounts of taurine, certain countries banned the commercialization of energy drinks with a high level of taurine (Babu *et al.,* 2008).

Niacin (B3 vitamin) exerts its role in the body as a coenzyme, under the form of NAD^+ (nicotinamid-adenin-dinucleotid) and $NADP^+$ (nicotinamid-adenin-dinucleotid phosphate) and thereby participates in the decrease of the oxidative stress (Hamound *et al.,* 2013). Moreover, the enzymes depending on $NAD^+/NADP^+$ are involved in redox reactions, in energy generation, cholesterol metabolism, fatty acid oxidation, glucose degradation, pentose phosphate pathway, synthesis and degradation of the amino acids, synthesis of the glucocorticoids hormones and sexual hormones. It can be synthesised in body from tryptophan.

Niacin has been used as a cholesterol lowering agent for more than 50 years (Digby *et*

al., 2012; Song and FitzGerald, 2013; Dunbar and Goel, 2016). However some of the studies show that the lowering effects of niacin upon cholesterol are seen only after 12 weeks of treatment (Parwaresch *et al.*, 1978).

Niacin has positive effects upon all cardiovascular functions and upon atherosclerosis evolution (Julius, 2015). It is used to modify relevant lipid disorders: LDL high concentration, non-HDL cholesterol, high level of triglycerides and lipoproteins, HDL low concentration (Goldberg *et al.*, 2000; Barter *et al.*, 2007; Julius, 2015; Ito *et al.*, 2015).

Besides the healthy effects upon the lipid profile, niacin administration in large doses evidenced serious changes in gene expression of most tissues (Khan *et al.*, 2014). Therefore, there are specific studies in the literature which show that the niacin administration in large doses induces the change of muscle fibres from type II to type I and increases the number of type-I fibers in skeletal muscles of obese rats and pigs (Monin *et al.*, 1987; Fernandez et al., 1994).

In the last time, certain studies have shown that niacin rises the blood concentration of the cholesterol and homocysteine (Dworzanski *et al.*, 2011).

In the Red Bull energy drink, the niacin quantity is larger than the daily recommended dose.

2. Effects of the consumption of energy drinks on animals

There are few studies concerning the energy drink consumption in animals. Furthermore, there are no studies on the acute effects of these drinks on animals. Most of the experiments focus only on the effects determined by their main ingredients, caffeine and taurine.

It has been demonstrated that chronic administration of energy drinks is associated with blood biochemical changes as well as changes in the activity of the hepatic enzymes. For instance, the total cholesterol concentration, triglycerides, high density lipoproteins (HDL), low density lipoproteins (LDL), as well as glucose concentration increase after the consumption of energy drinks. Moreover, the transaminase activity (ALT, AST) in blood increases in both rats and rabbits after chronic administration of energy drinks (Akande *et al.*, 2011; Ebuehi *et al.*, 2011; Khayyat *et al.*, 2014).

At the structural level, the induced changes by the energy drink (Red Bull) is noticed for both erythrocytes and blood peripheral cells. Thus, the erythrocytes become hypochromatic and

display poikilocytosis, anisocytosis, vacuolysis and fragmentation. Neutrophils have irregular shapes, with hiperchromatic, bilobated and pycnotic nuclei. Some of the cytoplasmtic areas present degenerations (Sinha *et al.,* 2006; Khayyat *et al.,* 2014). Therefore, the energy drinks have a devastating effect upon blood figurative elements in male rats (Khayyat *et al.,* 2014).

3. Effects of the consumption of energy drinks on humans

In the last years, the concern associated with potential risks determined by the energy drink consumption has increased very much. Meantime, the producers promote the energy drinks and claim to be safe and adequate for consumption, despite the studies that demonstrated the opposite.

As distinct from other categories of drinks, the sale of energy drinks and other products with a high level of caffeine continues to increase. The real problem is that the sale of this kind of products is often addressed to young people under 18 years of age.

The negative effects of the energy drink consumption are observed through a series of physiological changes (increase of heart beat frequency and blood pressure) and behaviour (anxiety, aggressively) (Jackson *et al.,* 2013). The most vulnerable are the teenagers, in which chronic energy drink consumption is followed by behavioural and cognitive problems (anger, agitation, dependence on other substances, decrease of concentration and memory capacity) (Van Battenburg-Eddes *et al.,* 2013).

The positive effects of the energy drink consumption are mentioned in very few studies. These effects are linked, for example, with effort perception, muscular pain in the lower limbs and optimal use of energy provisions during the sub maximal effort in cycling (Duncan and Hankey, 2013). Other beneficial consequences of the energy drink consumption result from the improvement of the physical and mental performance, including the increase of the memory and concentration capacity (Alford *et al.,* 2001; Scholey and Kennedy, 2004). Some of the authors show that acute administration of the energy drinks determines the increase of right and left ventricle function (Menci *et al.,* 2013).

On short term, the negative effect of the energy drinks are evidenced through an increase of the heart beat frequency and of the systolic and diastolic blood pressure (Elitok *et al.,* 2015; Marczinski *et al.,* 2014; Grasser *et al.,* 2014; Grasser *et al.,* 2015).

3.2 Effects of the consumption of energy drinks in athletes

The nutrition for optimising the training and the performances of athletes evokes a great attention; therefore it has generated a large data base about the ergogenic value of some food supplements. However, for most of the products used by the athletes, there is not enough scientific evidence that they are safe and efficient for increasing the physical performances. For this reason, both the athletes and the trainers need an appropriate information from a scientific point of view regarding the supplements' ergogenic potential (Porrini and Del Bo, 2016).

Some of the studies confirm a performance increase of the endurance exercises. The effect is due to the caffeine high level and/or the carbohydrates from the energy drinks. Other studies, but not many, show a positive effect of muscular power increase. Finally, it is suggested that the increase of the neuromuscular performances is not assigned only to caffeine but to all of the energy drinks' ingredients.

5. Thesis objectives

The present work is an equally basic and applied study, with biomedical and nutritional implications, focussing particularly on the energetic of effort.

Due to the expansion of the energy drinks consumption, especially among young people, accompanied by the producer's promises that they would provide the energy needed under stress conditions and continuous strain, we decided to check the Red Bull effects upon the animal and human body and the way in which the consumption influences certain biochemical, physiological, morphological and ultrastructural parameters. The selected tissues were blood, hepatic tissue, skeletal and cardiac muscular tissues.

This choice was not random. There is a very tight metabolic bond between the four marked tissues. Thus, the hepatic tissue was chosen due to its metabolic functions and the bond it has with other studied tissues. The liver has an important role in maintaining the plasma glucose normal concentration; some of the reactions of the lipid metabolism take place only in the liver; almost all of the blood plasma proteins (90%) are synthesised here, with the exception of some gamma-globulins. Being a distension organ, large amount of blood can be stored in its blood vessels. The regular blood volume of the liver represents almost 10% of the whole blood. When the pressure from the right atrium increases, the blood pressure from the liver has a

retrograde increase. In this way, the liver extends and 0,5-1 l of blood is additionally stored in the hepatic veins and sinusoids. This happens especially in heart failure accompanied by peripheral congestion. Therefore, the liver contributes to the constant maintenance of the blood volume, accumulating blood when there is excess and providing additional blood when the circulator blood volume is reduced (Guyton and Hall, 2006).

The relationship between the liver and skeletal muscle, known as the ”Cori cycle”, is intermediated by blood. In this cycle, the resulted lactate from the glycogen degradation in skeletal muscle is carried through blood to liver, where it is reconverted to glucose. From all of the glucose predecessors, lactate is very important because it is the final product of the glycolysis in the skeletal muscle.

The metabolic bond between the heart muscle and the liver is achieved, among others, by the blood lipoproteins. Their role is to carry out lipid components between liver and other organs. For instance, some of them carry out phospholipids and cholesterol from liver to peripheral tissues and back (Guyton and Hall, 2006).

The metabolic relationship between myocardium and skeletal muscle is assured by the glucose and fatty acid metabolization. Both tissues preferentially metabolise unesterified fatty acids under rest conditions. They provide 60-70% of the heart energy needed under physiological conditions. Only 15-20% of the heart needed energy is obtained from glucose metabolization. Free fatty acids provide 2/3 of the energy needed for an efficient functioning of the skeletal muscle under rest conditions and moderate effort. The remainder is provided by sugars. In exhausting and short term efforts (anaerobic metabolism), the muscle activity is maintained by glucose. In sustained efforts of moderate intensity, the muscular activity is maintained by the oxidative catabolization of the fatty acids at the mitochondrial level.

The effects of the energy drink consumption upon liver, independent of duration (chronic or acute) and quantity were little studied, only a few studies being available in the literature which certify the effects of the energy drinks upon this tissue (Akande and Banjoko, 2011; Vivekanandarajah *et al.,* 2011; Ebuehi *et al.,* 2011; Khayyat *et al.,* 2012).

The effects of the energy drink consumption upon skeletal muscle and myocardium were not studied at all. In the literature, there are only a few reports referring to cardiovascular symptoms in humans after excessive energy drink consumption (Lattavo *et al.,* 2007; Yew and Laczek, 2007; Shannon *et al.,* 2007; Babu *et al.,* 2008; Clauson *et al.,* 2008; Miller *et al.,* 2008;

Reissing *et al.,* 2008).

Since the physical activity potentiates the metabolic bonds among the four tissues, and the earliest effects of the physical effort are observed at their level, our research group from the Animal Physiology Laboratory of the Biology and Geology School (Babes-Bolyai University of Cluj-Napoca) decided to study, for the first time, the chronic effects of Red Bull in animals and the acute ones in both trained (athletes) and untrained people.

Our motivation was completed by the fact that athletes and young people are important targets for the sale of energy drinks. Unfortunately, the athletes' studies were limited at blood samples; we could not take tissue biopsies. The human researches respected the Law 206/2004 prescriptions, concerning the good conduct in scientific research, technological development and innovation, as well as the Helsinki Declaration for research that involves humans subjects (http://sites.jamanetwork.com/declaration-ofhelsinki/ index.html, 1964).

In the last time, the association between energy drinks and alcohol has become more popular among young people. At the same time with the popularity increase, more and more negative reports started to appear in the scientific and medical world. (Seifert *et al.,* 2011; Cheng *et al.,* 2015; Larsen *et al.,* 2015; Peacock *et al.,* 2015; Halubcikova *et al*., 2016; Magnezi *et al*., 2016; Reid *et al.,* 2017). Therefore, we decided to observe, in rats, what are the chronic effects of the combined alcohol and energy drinks administration upon body. Based on the reasons shown above, we took into consideration the following **objectives:**

1. To study the acute effects of the energy drinks in athletes and untrained young people, under the conditions of physical effort;
2. To measure the blood and tissue biochemical changes induced by energy drinks and/or alcohol administration in trained laboratory animals (Wistar rats);
3. To observe the ultrastructural modifications determined by energy drinks and/or alcohol in trained laboratory animals.

THE PRELIMINARY RESEARCH FOR VERIFYING THE CONSUMPTION OF ENERGY DRINKS BY HUMANS

The hypothesis from which we started from was the identification of the effects of energy drink consumption among young people. Our objective was knowing the frequency of the consumption of energy drinks among young people, athletes and untrained people, but also

the effects of energy drinks on health. We got motivated by the fact that in the past years an increasing number of young people started to consume for both relaxation and a source of energy.

6.4. Conclusions

We can say that the majority of our subjects are energy drinks consumers. On top of that, most of them also combine the energy drinks with alcohol.

III THE PERSONAL RESEARCH CONTRIBUTIONS ON THE METABOLIC EFFECTS OF ENERGY DRINKS

7. Effects of the energy drinks consumption in humans

7.1. Experimental design

Materials and methods

Participants. Thirteen healthy voluntary males between the ages of 20-25 years were selected to participate in this study. They were organized in two groups: trained (T) and untrained (U). The trained volunteers were rugby players of the Rugby Team of the Babes-Bolyai University of Cluj-Napoca. The untrained volunteers were young college students who self-reportedly do not engage in regular demanding physical activity. All participants were informed about the purpose and demands of the study before giving their written consent to participate. All volunteers were self-declared as healthy, with no history of cardiovascular, urinary, digestive or metabolic diseases (determined by questionnaire). The protocol was in accordance with the *Declaration of Helsinki* (http://sites.jamanetwork.com/declaration-ofhelsinki/index.html, 1964) for research on human subjects. Table **7.1.1.** shows the main characteristics of the participants in this study.

Experimental design. All subjects were instructed not to consume food, energy drinks, coffee and alcohol 12 h prior to the onset of the experiment. In the first day, anthropometrical measurements were recorded. Blood samples were drawn and blood pressure and heart rate were measured twice, once before and once after performing physical exercises. Following a warm-up the participants were asked to undergo Astrand Cycle Ergometer Test for 5 minutes. One day later, the protocol was repeated, however after the initial recordings and blood sampling, the subjects ingested one dose of Red Bull. A period of 45 minutes of rest followed, after which the

warm-up and Astrand test were performed.

The moments when samples were collected for biochemical and hemodynamic determinations were noted as follows:

1. RU (**RU**, rest time of untrained subjects);
2. **U**, moment when untrained subjects have done physical exercise for a period of 6 minutes;
3. **URB**, when untrained subjects had done physical exercise for a period of 6 minutes after they have drinking a dose of Red Bull;
4. RT (**RT**, rest time of the trained subjects);
5. **T**, moment when the subjects have done physical exercise for a period of 6 minutes;
6. **TRB**, moment when the subjects have done physical exercise for a period of 6 minutes after they consuming a dose of Red Bull

***Assays*.** Blood was collected from the antecubital forearm vein and processed for biochemical examinations at Medstar Laboratory from Cluj-Napoca, Romania. A Conelab.30i determined glycemia, proteinemia, LDH, AST and ALT activities.

***Data analysis*.** The results are presented as mean ± standard error (SE). The data were analysed for statistical significance using the unpaired Student *t*-test. A value of $p < 0.05$ was considered significant.

Tabel 7.1.1. Comparison of anthropometric data between trained (n=6) and untrained (n=7) subjects

	TRAINED (T)	UNTRAINED (U)
WEIGHT (kg)	102.4±2.7	77.74±4.85
HEIGHT (m)	1.83±0.03	1.83±0.03
BMI (kg/m^2)	30.66±1.06	23.15±1.30
Bf %	19.2±1.6	42.54±1.43
Bm%	41.16±1.02	13.32±1.78

BMI-body mass index; Bm%-percent of body muscle; BF%-percent of body fat. Data are mean±standard error.

7.2. Results and discussions

As expected, physical activity increased the systolic pressure in both groups (Table 7.2.1). Physical activity did not produce significant changes in glucose blood concentration (Table 7.2.1, Fig. 7.2.1 a). However, Red Bull was observed to have determined a significant decrease of glycaemia in trained males. These results are in accordance with the results of Phillips *et al.* (2014). It is possible that the results in this study were influenced by the niacin in

Red Bull, which intensified the use of glucose as a cofactor for the enzymes that are involved in the glycolytic pathway.

Tabel 7.2.1. Effects of exercise and Red Bull on hemodynamic and biochemical blood parameters

Parameter	TRAINED			UNTRAINED		
	RT	T	TRB	RU	U	URB
HR (beats/min)	4.71±4.6	167±7.98	172.28±6.1	68.83±4.49	163±4.37	171.66±6.4
SBP (mmHg)	129±6.37	153.33±83	176.66±9.88	117.14±4.34	142.25±8.5	135±5.45
DBP (mmHg)	77.33±3.07	74.16±2.38	78.33±2.78	72.14±2.14	67.14±1.84	72.14±2.14
Glycaemia (mg/dl)	94.5±1.89	91.5±1.78	73.66±7.4*	85.28±3.12	90.28±5.45	85.57±5.09
Proteinemia (g/dl)	7.19±0.13	7.85±0.09**	7.64±0.15	7.11±0.08	8.01±0.15***	8.01±0.10***
LDH activity (U/l)	165±7.58	187.8±4.39	194.8±7.85	136.28±7.85	157.57±11.50	174.14±5.87
ALT (U/l)	24.8±1.39	26.6±1.50	27.25±1.28	17±2.98	18.85±3.58	20.14±2.91
AST activity (U/l)	21.2±3.30	21.8±3.59	24.2±1.31	18.71±1.56	19.28±2.21	25±5.77

RB-Red Bull; HR-heart rate; SBP-sistolic blood pressure; DBP-diastolic blood pressure; RT-resting trained group; T-trained group after exercises; TRB-trained group after exercises and Red Bull administration; RU-resting untrained group; U-untrained group after exercises; URB-untrained group after exercises and Red Bull administration. The results are expressed as mean±SE.

Glycaemia *p < 0.05 *vs* exercises

Proteinemia **p < 0.01 and ***p 0.001 *vs* resting

Proteinemia increased significantly in both groups after physical activity coupled with Red Bull administration (Table 7.2.1, Fig. 7.2.1 b). The increase of protein concentration may be due to muscle damage that occurs during intense effort and this subject matter requires further investigations.

The activities of LDH and ALT also increased after physical activity. Red Bull intensified this increase in both groups. In the case of LDH activity, the increase may be due to the high amount of caffeine which stimulates LDH activity to sustain the lactate production

(Dias *et al*., 2015).

As depicted in Table 7.2.1, AST activity was not affected by physical activity, but rather increased after Red Bull administration. Similarly, Hazar *et al.* (2011) did not find any difference in the AST value after exercise in their study, which was carried out with professional sportsmen. As with ALT activity, the mechanism behind AST activity increase following Red Bull consumption is unclear.

7.3 Partial conclusions

The results of our study indicate that energy drinks such as Red Bull can affect biochemical blood parameters, as well as blood pressure and heart rate. Furthermore, energy drinks may be a risk factor for the development of cardiovascular diseases. Further research is required, while raising awareness among young people and athletes about the potential acute and chronic effects of energy drink consumption is important.

8. Study of metabolic effects produced by consumption of energy drinks and alcohol in animals

8.1 The experimental protocol

The animals used, male Wistar rats, weighing 183,9±5,05 g, were kept in the animal facility of the Department of Molecular Biology and Biotechnology, Faculty of Biology and Geology, Babes-Bolyai University, under hygienic conditions, constant humidity and temperature, 12/12 h light/dark, receiving a Larsen diet. Every experimental group was constituted from 7 individuals. The animal manipulation was gentle, without causing them stress or pain.

The animals from all **experimental groups** received a standard diet and had free access to food and water. The animals were organized into four groups:

a – **C**, the control group;

b – **RB,** the group which consumed an energy drink (Red Bull) daily, for 4 weeks, 1,5 ml/100 mg body weight, which correspondents to a 250 ml Red Bull dose consumed by an adult human weighing 70 kg;

c – **E,** the group which received ethanol daily, in the drinking water, for 4 weeks, 0,6ml/100 g body weight;

d – **RBE,** the group which consumed the combination of energy drink and ethanol (1,5 ml Red Bull/100g body weight and 0,6 ml ethanol/100g body weight).

In the last 6 days of the treatment, the rats were tested for physical effort endurance, by putting them to swim under special conditions. Thus, their tails were tied with weights, which represented 10% of their body weight, then they were put to swim individually, in a swimming pool, at the room temperature, until they remained submerged for at least 5 seconds (Gitay and Bano, 2013).

In the sixth day, immediately after the effort test was finished, the animals were killed under anesthesia by exsanguination. Besides blood, we also collected pieces of hepatic tissues, skeletal muscle and myocardium. The blood was sampled on preservative agent (heparine) and without preservative agent and kept at 4^0C until use, for serum obtaining. The serum was stored at -80^0C until the biochemical determinations were made. The tissues were homogenised with phosphate buffer, 10% concentration and kept at -80ºC until the moment of use. At the same time, for ultrastructural studies, we also took tissue samples. Fine sections were incorporated in a jelly capsule containing an inclusion medium, which could be an ester resin (Vestopal) or an epoxy resin (Epon 812), to which a polymerizing agent was added. After encapsulation, the polymerization of the medium was completed by introducing the capsules in a laboratory oven at 60^0C, for 48-72 hours.

Table 8.1.1 **Tissues analysed and hematological parameters measured**

Hematological	**Tissues (liver, skeletal and cardiac muscle)**
Biochemical	
glycaemia	glycogen concentration
proteinemia	glucose concentration
Cholesterolemia	totale proteins concentration
LDH activity	cholesterol concentration
ASAT, ALAT activities	LDH
	ASAT, ALAT activities

Statistical data processing

Statistical data processing contains the following stages: calculation of the arithmetic mean (Xm) of the standard deviation (SD or σ), of the standard error of the mean (SE) and of the significance of the differences between means according to the Student t-test. In these

calculations, the aberrant values/observations were eliminated by applying the Chauvenet criterion, i.e., those values were eliminated which deviated from the arithmetic mean by more than the standard deviation size (σ) weighted with an α factor which depends on the number of observations (n), i.e, falling outside the interval $Xm \pm \alpha \cdot \sigma$ (Tarba, 2003).

The comparisons were performed between each treated group and the control group (C) and also between the ethanol (E) group and the double-treated group (RBE) using the unpaired two-tailed Student t-test (Microsoft Office 2003, Excel). The differences which have values of the $p \leq 0,05$ were considered statistically significant, in the following way:

$p \leq 0,05$ significant (*)

$p < 0,01$ distinctive significant (**)

$p < 0,001$ highly significant (***).

6.3. Results and discussions

6.3.1. Metabolic changes induced by the Red Bull consumption in blood and liver

The results of our research show that both the energy drink and alcohol have determined the increase insignificantly of the blood sugar. Similarly, the hepatic glucose concentration increases after the separate treatment with the drinks, statistically significant after ethanol administration. The interesting thing is that when they were both administered, the 2 drinks have determined a decrease of the 2 parameters, statistically significant only in blood (Fig. 8.3.1.1 a, Table 8.3.1.1).

The insignificant increase of the blood sugar after the Red Bull consumption could be the result of dehydration induced by caffeine and taurine, since it is known that they can induced diuresis and natriuresis in rats and humans (Maughan and Griffin, 2003; Reisenhuber *et al.*, 2006; Bigard, 2010).

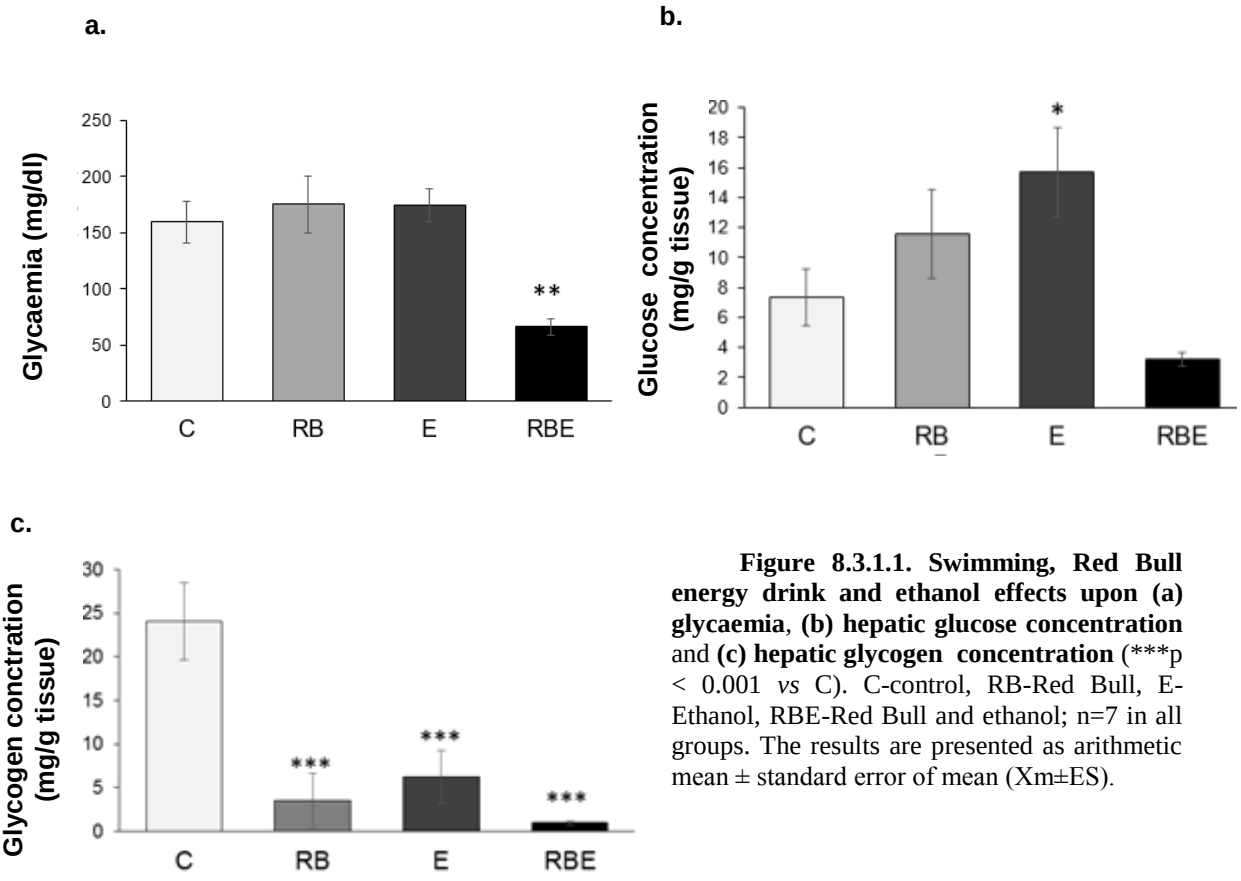

Figure 8.3.1.1. Swimming, Red Bull energy drink and ethanol effects upon (a) glycaemia, **(b) hepatic glucose concentration** and **(c) hepatic glycogen concentration** (***p < 0.001 *vs* C). C-control, RB-Red Bull, E-Ethanol, RBE-Red Bull and ethanol; n=7 in all groups. The results are presented as arithmetic mean ± standard error of mean (Xm±ES).

The marginal increase of the plasma and hepatic glucose concentration might be put on the niacin high content of the Red Bull energy drink (Li *et al.*, 2011; Davidson *et al.*, 2013; Li *et al.*, 2013; Phan *et al.*, 2013; Blond *et al.*, 2014; Chen *et al.*, 2015), though the mechanism is not fully understood.

Ethanol consume increased slightly the blood sugar. The hepatic glucose concentration increased significantly after the ethanol treatment (Fig. 8.3.1.1 a și b, Table 8.3.1.1).

The significant increase by ethanol of the hepatic glucose and the marginal one of the blood glucose can have various explanations. One of them could be represented by dehydration, the same as in the Red Bull case, determined by its diuretic properties (Shirreffs and Maughan, 1997; O'Brien and Lyons, 2000).

However, there are studies which show that chronic alcoholism is associated with insulin

resistance (Patel *et al.*, 1991).

Insulin resistance stimulates the hepatic gluconeogenesis from amino acids, a fact validated in our study by the significant increase of the liver protein concentration (Fig. 8.3.1.3 b; Table 8.3.1.1.), but also by glycaemia increase through hepatic glycogenolysis activation (Dufour *et al.*, 2009; Steiner *et al.*, 2015).

Though the two drinks taken individually do not decrease glucose concentration in either blood or in liver (actually, there is a slight tendency of an insignificant increase), the two substances taken together produce a decrease in liver (Fig. 8.3.1.1 a and b, Table 8.3.1.1). Moreover, the combined treatment with the two substances decreases significantly the blood glucose concentration, as compared to the separate treatment with each of them. It is possible that the ethanol effects are modulated by some of the Red Bull components.

The glycogen concentration decreased significantly in the liver of all treated groups, (Fig. 8.3.1.1 c, Table 8.3.1.1). The significant decrease determined by Red Bull may be due to caffeine. Caffeine determines the adrenaline and noradrenaline release and sensitises the receptors for dopamine through plasma concentration increase of the Ca^{2+} (Cannon *et al.*, 2001; Pohanka and Dobes, 2013). Adrenaline, as it is known, stimulates glycogenolysis through the decrease of glycogen synthesis phosphorylation (Kolves *et al.*, 2015). The effect is strong, producing in a few minutes the release of large amounts of glucose in the blood (Dufour *et al.*, 2009; Steiner *et al.*, 2015). As a result, the blood glucose concentration increases after the treatment with Red Bull (Fig. 8.3.1.1 a and b, Table 8.3.1.1), a fact which might be due to the simulation of hepatic glycogenolysis by caffeine.

Our results demonstrate that chronic ingestion of ethanol has significantly decreased glycogen hepatic concentration (Fig. 8.3.1.1 c, Table 8.3.1.1).

The quoted studies from the literature associate the chronic alcoholism with insulin resistance (Patel *et al.*, 1991; Martin *et al.*, 2004). Insulin resistance emerges, for instance, as a consequence of alcohol chronic consumption, through the inhibition of the hepatic glycogen synthesis. In these conditions, the glycogen phosphorylation activates and catalysis the glycogen split to glucose-l-phosphate. The result is to release the free glucose in circulation, which explains our results relative to the glucose concentration increase in blood and in liver after alcohol chronic consumption. (Fig. 8.3.1.1 a and b, Table 8.3.1.1).

The combined administration of the two drinks (Red Bull and ethanol) significantly

decreased the hepatic glycogen concentration (Fig. 8.3.1.1 c, Table 8.3.1.1), their effects being cumulated.

We found that, at the ultrastructural level, the both drinks produce a series of common modifications (Fig. 8.3.1.9 b; Fig. 8.3.1.10 b; Fig. 8.3.1.11 b). In the cytoplasm, one can observe a lot of mitochondria with a rarefied matrix and thin cristae, part of them associated with RER (rough endoplasmatic reticulum), implicated in protein synthesis. RER is present as ordered parallel profiles, especially around nuclei. These aspects suggest an intensification of the protein synthesis after the treatment and they confirmed the biochemical results.

Partial conclusions

The results obtained in our experiment indicate that the Red Bull treatment deeply affected both the structure and the function of the hepatic tissue, an effect demonstrated by the increase of the serum and hepatic glucose concentration, of the serum cholesterol, as well as the increase of the enzymatic activity of LDH and ASAT from the liver. Our results confirm the literature data concerning the potential of some compounds from Red Bull (caffeine, taurine and niacin) to deeply affect the hepatic tissue (Li *et al.,* 2011; Davidson *et al.,* 2013; Li *et al.,* 2013; Phan *et al.,* 2013; Blond *et al.,* 2014; Chen *et al.,* 2015).

Our study also confirms the detrimental effects of the ethanol upon liver, demonstrated through the stiffening of the membranes, the appearance of a fibrotic net with necrotic areas, followed by a significant increase of the glucose, cholesterol and hepatic protein concentration. Also, the ethanol affected the hepatic enzymatic activity. Our results are similar to those quoted in the literature (Cheng *et al.,* 2001; Kutuk and Basaga, 2007; Ayala *et al.,* 2014).

The novelty of our study consists in the fact that the mixed treatment with the two drinks, Red Bull and ethanol, have synergistic/cumulative effects at both biochemical and ultrastructural level. We do not exclude the possibility that a longer treatment can affect significantly all the parameters involved.

6.3.2. Changes induced by Red Bull consumption in the skeletal muscle

Resistance to effort. The endurance exercises produce metabolic and cardiovascular activity changes.

In our study, the effort resistance increases in all the groups. At the beginning of the test period, the highest resistance to effort was achieved by the animals of the control group. By time, the resistance increases in all groups, but slower in those treated (2, 3 days). Later, the

situation reverses, the highest resistance being achieved in all treated animals (4, 5 days) as compared with the animals in the control group. At the end of the testing period, the situation reverses again, the resistance decreasing in all treated groups below that registered in the control group (Fig. 8.3.2.1; Table 8.3.2.1.).

The transient increase of the effort resistance after Red Bull consumption might be due to the high level of carbohydrates in the energy drink. The subsequent decrease of the effort resistance might be explained by the reduction in the energy storage of the skeletal muscle (Fig. 8.3.2.2 a și b; Tabel 8.3.2.1; Tabel 8.3.2.2) or by the dehydration due to the high caffeine content of Red Bull (Reissehuber *et al.,* 2006; Temple *et al.,* 2009). Caffeine intensifies diuresis and, as a result, Na^{+} and Ca^{2+} are eliminated, they being essential for muscle contraction (Reissenhuber *et al.,* 2006; Emohare and Ratman, 2006).

Similar to the Red Bull case, the physical resistance decrease in ethanol-treated animals might be due to dehydration; it is known that its metabolization has such an effect (El-Sayed *et al.,* 2005; Shirreffs and Maughan, 2006; Vella and Smith, 2010).

The combined treatment with Red Bull and alcohol emphasizes even more the animals resistance decrease, their effects being synergistic.

Table 8.3.2.1. The evolution of the effort resistance of rats (swimming minutes to exhaustion/day))

Day	C	RB	E	RBE
Day 1	7.174±0.726	3.541±0.698**	5.315±1.102	4.962±1.135
Day 2	5.96±0.658	7.004±1.581	5.627±1.142	4.71±1.527
Day 3	9.948±0.866	8.074±1.585	6.894±1.609	6.121±1.97
Day 4	6.975±0.56	12.291±3.241	11.7±1.912*	8.681±1.708
Day 5	17.791±2.025	21.028±3.527	21.267±7.649	18.86±8.109
Day 6	14.701±1.332	14.538±1.599	12.148±1.984	9.667±3.329

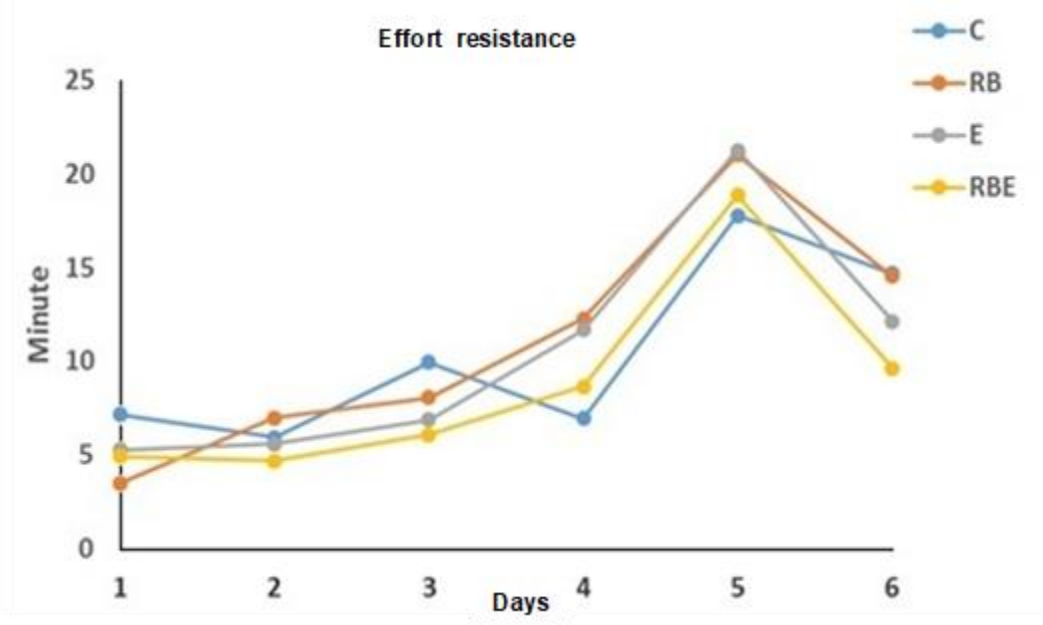

Figura 8.3.2.1. The evolution of the effort resistance of rats treated for 6 days of endurance testing a. C- Control group; RB- Red Bull group; E- Ethanol group and RBE- Red Bull Ethanol group. n=7 in all groups. The resuls are shown as arithmetic mean ± standard error (Xm±ES).

In our experiment, the glucose concentration decreases in all treated groups. This decrease is statistically significant in alcohol consumption groups, i.e., in group E and RBE (Fig. 8.3.2.2 a; Table 8.3.2.2). The glycogen concentration followed the same tendency (Fig. 8.3.2.2 b; Tab. 8.3.2.3). In this case, the change was significant in all treated groups.

Table. 8.3.2.2. The compund effects of swimming, Red Bull consumption and ethanol upon some biochemical parameters from skeletal muscle

	Skeletal muscle			
Parameters	**C**	**RB**	**E**	**RBE**
Glucose (mg/g tissue)	1.26±0.09	0.69±0.19	0.38±0.12**	0.21±0.06***
Glycogen (mg/g tissue)	3.60±0.35	2.19±0.40*	2.27±0.23*	0.87±0.3***
Proteins (mg/g tissue)	101.24±7.46	104.88±3.81	103.77±5.88	106.4±2.54
LDH Activity (µmoles pyruvate/g tissue/min)	0.0137± 0.001	0.0116± 0.0015	0.0130± 0.001	0.0126± 0.0004
ALAT Activity (µg pyruvate/g tissue/hour)	5769.36± 683.63	6632.46± 254.25	7287.22± 651.04	5026.65± 841.57
ASAT Activity(µg pyruvate/g tissue/hour)	3149.13± 151.2	3367.93± 82.06	3347.24± 83.94	3355.16± 64.20

C-Control; RB-Red Bull; E-Ethanol; RBE-Red Bull and Ethanol. Each group contains 7 individuals. The results were expressed as mean± standard error of the mean

Glucose **E****p <0.01**; RBE*****p < 0.001**;** Glycogen **RB***p < 0.05; **E***p < 0.05; **RBE*****p < 0.001; Proteins **RBE***p < 0.05**.**

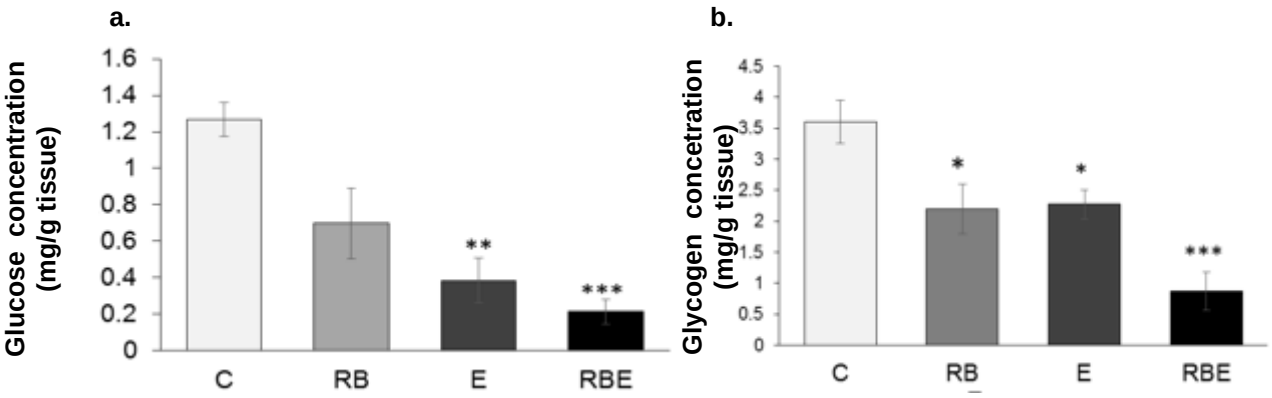

Figure 8.3.2.2. Induced changes by swimming and Red Bull and ethanol consumption upon glucose and glycogen concentration in skeletal muscle. (a) Glucose concentration in skeletal muscle (*p < 0.05 *vs* C; ***p < 0.001 *vs* C), **(b) Glycogen concentration from skeletal muscle** (*p < 0.05 *vs* C; ***p < 0.001 *vs* C). C-control, RB-Red Bull, E-Ethanol, RBE-Red Bull and Ethanol. n=7 in all groups. The results were expressed as mean arithmetic± standard error (Xm±ES).

The decrease of muscle glucose and glycogen concentration after Red Bull consumption (Fig. 8.3.2.2; Table 8.3.2.2) might be due to niacin from the energy drink composition (Li *et al.*, 2010).

In rats, it is known that niacin switches the type-II fibers to type I and increases the number of type-I fibres. As a result of this switching, the oxidative capacity of the skeletal muscle increases (Ringseis *et al.*, 2013). The fiber type of the muscles is associated with insulin sensitivity (Lillioja *et al.*, 1987). The decrease of the glucose and glycogen concentration in the skeletal muscle can be the consequence of the switching of type-II fibers to type I, because these fibers in time consume more glucose. This explanation is just a hypothesis.

The significant decrease of glycogen concentration after energy drink ingestion can be on one side, the result of insulin resistance determined by caffeine, and on the other side due to glucose use from the storage, favouring the serum glucose protection.

The significant decrease of glucose and muscular glycogen concentration (Fig. 8.3.2.2; Table 8.3.2.2) in the group which ingested ethanol was expected. Molima et al. (1991) demonstrated that glucose takeover by gastrocnemian muscle in rats significantly decreased after chronic alcohol consumption.

If our results were to be compared to the other researches' regarding the administration of alcohol (Budohoski și colab., 1984; Molina and colab., 1991; Spolarics and colab., 1994; Thong and Graham., 2002, 2002; Keijzers și colab., 2002), we are the first who have noticed

that Red Bull administration determined related effects with alcohol, these being a decrease of glucose concentration (Figure 8.3.2.2, Table 8.3.2.2) and glycogen stores (Fig 8.3.2.2, Table 8.3.2.2).

Moreover, we have noticed that when the two drinks are administered together, they have synergistic effects, determining a highly significant decrease.

Ultrastructurally, the main modification that appears in all treated groups consists in the presence of some enlarged spaces between muscle fascicles, in which free nuclei and an amorphous material can be found. Also, there appears some slight changes in myocytes, as evidenced through the presence of small vacuolisations at the level of the ,,I" bands, which contain actin myofilaments (Fig. 8.3.2.5 b; Fig. 8.3.2.6 b; Fig. 8.3.2.7 b).

Partial conclusions

The main conclusion of this part of the study is that Red Bull decreases the physical endurance to effort, due to biochemical changes (glycogen storage and muscular glucose concentration decrease) and ultrastructural modifications (vacuolisation and expansions of the structural elements from the reticular triads, alterations of actin myofilaments). As a result, the producer's promises concerning the increase of the physical performances after energy drink consumption are not confirmed (at least in the endurance case).

Red Bull with alcohol consumption should be avoided, especially by athletes, because our results show that this kind of combination leads to an intense physical exhaustion.

6.3.3. Changes induced by Red Bull consumption in the cardiac muscle

Our results show that glucose concentration has increased in all groups after treatment (Fig. 8.3.3.1 a, Table 8.3.3.1), although it is significantly increased only in the RBE group. Moreover, the experiments indicate that, following the treatment, a clear increase of glycogen concentration also occurred in all groups, as shown in Fig. 8.3.3.1 b and Tab. 8.3.3.1. It can be observed that the glycogen concentration is significantly increased in the RB group.

It is believed that, in the RB group, the increase of glucose and glycogen concentration is caused by two ingredients of the energy drink, caffeine and taurine. Normally, caffeine causes calcium release from intracytoplasmic stores (Kuo *et al*., 2007) and activation of AMPK (AMP-activated protein kinase) *via* CaMKK (Calmodulin-dependent protein kinase kinase-β) (Fogarty

et al., 2010). AMPK promotes the uptake and use of glucose in cardiomyocytes (Daskalopoulos *et al.*, 2016). Besides, AMPK either inhibits glycogen synthesis by phosphorylation of glycogen synthase, or activates glycogen degradation by phosphorylation of glycogen phosphorylase (Jeon, 2016). However, chronic activation of AMPK, as has happened in our study, may increase glycogen synthesis by increasing glucose uptake and glucose-6-phosphate formation. This induces allosteric activation of glycogen synthase that can overcome inhibitory phosphorylation by AMPK (Hunter *et al.*, 2011). Moreover, taurine increases glucose uptake, glycolysis and glycogen synthesis in the heart of adult rats (Lampson *et al.*, 1983).

Tabel 8.3.3.1. The combined effects of the physical effort (swim test) with Red Bull and/or ethanol consumption on some biochemical parameters in the myocardium

Parameters	Myocardium			
	C	**RB**	**E**	**RBE**
Glucose (mg/g tissue/)	0.65±0.13	0.75±0.1	0.72±0.08	1.34±0.16*
Glycogen (mg/g tissue/)	1.29±0.15	2.9±0.47*	1.65±0.19	1.64± 0.216
Cholesterol (mg/g tissue/)	10.61±1.31	2.14±0.16***	2.20±0.33***	2.07±0.24***
Protein (mg/g tissue/)	98.52±1.35	99.49±2.14	105.33±1.59*	99.55±2.39
LDH activity (umoles pyruvate/g tissue/ min)	0.015±0.0004	0.014±0.0009	0.015±0.0002	0.011±0.0001
ALT activity (μg pyruvate/g tissue/ hour)	7289.08±385.5	9151.49±572.19*	8470.95±385.13	7453.11±183.08
AST activity (μg pyruvate/g tissue/ hour)	1970.25±143.4	2616.58±169.48*	2578.59±191.5*	2731.27±118.64**

C-Control; RB-Red Bull; E-Ethanol; RBE-Red Bull and ethanol. n=7 in all groups. The results are expressed as mean value ± standard error of the mean (Xm±ES).

Glucose **RBE***p < 0.05**;** Glycogen **RB***p < 0.05; Cholesterol **RB****p < 0.01**; E****p < 0.01**; RBE*****p < 0.001**;** Proteins **E***p < 0.05**;** ALT activity **RB***p < 0.05**;** AST activity **RB***p < 0.05**; E***p < 0.05**; RBE****p < 0.01**.**

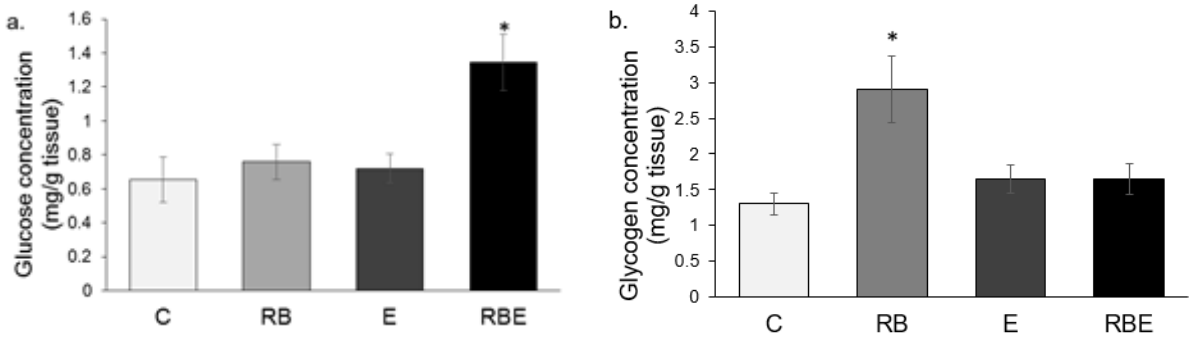

Figura 8.3.3.1. Changes induced by the combined physical effort (swim test) and Red Bull and/or ethanol consumption on the concentration of glucose and glycogen in the myocardium. (a) Glucose and (b) glycogen concentration in the myocardium (*p < 0.05 *vs* C). C-control, RB-Red Bull, E-Ethanol, RBE-Red Bull and Ethanol. n=7 in all groups. The results are expressed as mean value ± standard error of the mean (Xm±ES).

In our experiment, ethanol has led to a slight increase in glucose and glycogen concentration (Fig. 8.3.3.1 a, Tab. 8.3.3.1). It is known that ethanol decreases the sensitivity to insulin, and this is mediated in the heart muscle by TNFα (Tumour necrosis factor-α) and/or IL-6 (interleukin 6), that induce JNK (Jun N-terminal kinases) activation, which inhibits the path Akt-AS160-GLUT4 (Lang *et al.*, 2014).

The combined administration of Red Bull and ethanol produced a significant increase of glucose concentration (Fig. 8.3.3.1 a, Table 8.3.3.1). This outcome was expected since the independent administration of each of these components had led to an increase of the glucose concentration in the myocardium.

The concentration of cholesterol was significantly diminished in all groups, as shown in the results presented in Fig. 8.3.3.2 and Table 8.3.3.1. This effect can in turn be a cause of the myocardial dysfunctions reported in the literature in chronic consumption of EDs and alcohol.

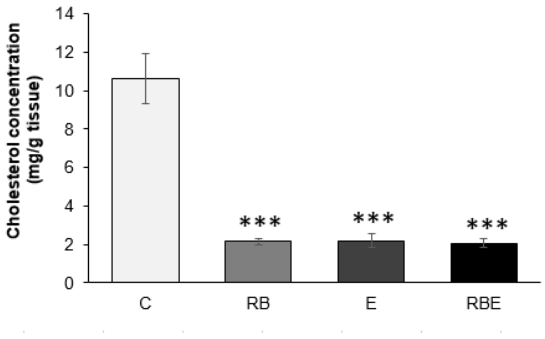

Figure 8.3.3.2 Changes of total cholesterol concentration in the myocardium (*p<0.05 *vs* C; **p<0.01 *vs* C; ***p < 0.001 *vs* C) after physical effort (swim test), Red Bull and Ethanol administration. C, RB, E and RBE are the control, Red-Bull, Ethanol and Red-Bull Ethanol groups respectively. n=7 in all groups. The results are expressed as mean value ± standard error of the mean (Xm±ES).

The reduction of the cholesterol concentration induced by Red Bull (Fig. 8.3.3.2, Table 8.3.3.1) may be caused by the elevated taurine and/or niacin contained by this drink.

Such a change is somewhat normal because both taurine and niacin are two compounds

used in the prevention and progression of atherosclerosis (Xu *et al.*, 2008; Ruparelia *et al.*, 2011). More precisely, it is known that taurine reduces serum cholesterol (Chen *et al.*, 2012) and niacin reduces serum cholesterol and triglycerides, and increases HDL concentration (Barter, 2011).

Decreased cholesterol in myocardium following chronic ethanol treatment (Fig. 8.3.3.2, Table 8.3.3.1) was also reported by Godfrey *et al*. (2015) and Hu *et al*. (2013), but the results have never been explained. The physiological significance of the phenomenon is unknown.

The combined administration of Red Bull and ethanol has induced an even higher reduction of cholesterol in the myocardium compared to the independent administration of the two components (Fig. 8.3.3.2, Tabel 8.3.3.1).

The most serious ultrastructural modifications observed in the heart tissue of the rats treated with EDs are those that indicate the occurrence of myocyte necrosis. Most nuclei have a normal shape, but for some myocytes, the myofibril arrangement has a loose structure and the space between them is occupied by many large (swollen) mitochondria displaying a rarefied matrix and dilated cristae, which makes us assume that oxidative metabolism is affected. All of these morphological alterations are correlated with the measured biochemical alterations in glucose, glycogen, cholesterol and AST and ALT activities reported in our study (Fig. 8.3.3.5, Fig. 8.3.3.6, Fig. 8.3.3.7).

Partial conclusions

Our results explain, to a certain extent, the symptoms described in the literature by those who consume energy drinks in large quantities or for a long period of time. We particularly refer to the accumulation of glycogen in the myocardium, which can disrupt the cardiac activity and may favour the occurrence of tachycardia, palpitations, cardiac arrhythmias, hypertension and even death (Yew and Laczek, 2007, Lattavo *et al.*, 2007; Babu *et al.*, 2008; Clauson *et al.*, 2008). Also, lowering the cholesterol concentration may in turn be a cause of myocardial dysfunctions reported in the literature following the chronic consumption of energy drinks and alcohol.

Athletes, as well as active people should avoid both the consumption of energy drinks and their consumption in combination with alcohol. Our results show that the two beverages may, in time, irreversibly affect the myocardium, causing necrosis.

9. General discussion and final conclusions

The present study is the first complex study which focuses especially on the metabolic and ultrastructural effects upon body, induced on the physical effort and particularly on sport background, by the chronic and acute Red Bull consumption and Red Bull/ethanol combination.

In the first part of the study we presented the newest literature data concerning the effects determined by the chronic and acute consumption of the energy drinks and their main ingredients (caffeine and taurine).

The researches performed in the experimental part had two major objectives: (1) evaluation of the acute administration of Red Bull and the manner in which certain blood biochemical parameters are affected and also the blood pressure and the pulse in young athletes or in young people without regular physical activities, during the physical exercises performing; (2) evaluation of the effects of Red Bull, ethanol and Red Bull/ethanol upon body and the manner in which the chronic consumption influences some biochemical and ultrastructural parameters in rats (the tissues used were blood, liver, skeletal and cardiac muscle).

For humans, the consumption of Red Bull is affecting especially the hemodynamic parameters, for both athletes and untrained people who do physical effort, because of this energy drinks can represent a risk factor in development cardiovascular diseases.

Chronic consumption of Red Bull, ethanol and Red Bull/ethanol combination affected all the tissues studied.

Our biochemical and ultrastructural results obtained after the Red Bull administration largely explain the symptoms described in the literature as appearing after chronic or excessive consumption of energy drinks. The glucose and glycogen concentration in all the tissues studied are among the affected biochemical parameters. It is to be remarked, especially, the decrease in glucose concentration and the glycogen storage from the skeletal muscle. That explains the physical performance decrease in the endurance effort case. In myocardium, the situation was reversed: the glycogen and glucose concentration significantly increased after the treatment. The glycogen storage in myocardium can disturb the cardiovascular system activity and favour the appearance of tachycardia, palpitations, cardiac arrhythmias and high blood pressure and can even lead to death (Yew and Laczek, 2007; Lattavo *et al*., 2007; Babu *et al*., 2008; Clauson *et al.*, 2008).

The increase of the cholesterol concentration in liver and blood, as well as its decrease

in the myocardium can be causes for hepatic and cardiac disfunctions noticed in the literature after the chronic consumption of energy drinks.

Red Bull induced ultrastructural modifications in all the tissues studied. In liver, it produced the distension of SER (smooth endoplasmic reticulum), in the skeletal muscle it determined myofibrilar alterations, disarray and diminution of the muscle fibers, while in the myocardium lysis areas could be observed. The most common ultrastructural modification present in all tissues is represented by the rarefaction and dilatation of the mitochondrial matrix and cristae. The mitochondrial alterations, associated with the impairment of the oxidative phosphorylation can explain the low physical resistance at swimming and makes us believe that in humans it can produce the same effects.

One of the experimental groups was treated with alcohol because in the last years the combination of alcohol and energy drinks has become a trend for young people. We wanted to establish whether this kind of combination is more dangerous than individual administration of those two drinks. Our results obtained for the alcohol chronic treatment confirmed what is already known from literature. The alcohol affected all the organs studied, at both biochemical and ultrastructural level (Fernandez-Sola *et al.*, 1994; Cheng și *et al.*, 2001; Worrall *et al.*,., 2001; Lang *et al.*, 2005; Siegmund *et al.*, 2005; Kutuk and Basaga, 2007; Preedy *et al.*, 2007; Law *et al*., 2012; Ayala *et al*., 2014; El Hajj *et al.*,2014).

The combination of alcohol and energy drinks affected the body more than the individual drinks do. The cumulative effect of the two drinks was observed for glucose, glycogen, cholesterol, protein concentration and some enzyme activities in all tissues studied. Furthermore, the Red Bull and ethanol combination determined the myocyte necrosis of the myocardium. Therefore, in time, the heart damage can be irreversible. Biochemical and ultrastrutural modifications explain the low physical resistance of animals.

In humans, Red Bull affected especially the hemodynamic parameters in both athletes and untrained people with physical effort. Therefore, energy drinks can represent risk agents for the development of cardiovascular diseases.

Consequently, on the basis of the experimental results, we can draw some general conclusions concerning the metabolic effects of energy drinks and their combination with alcohol.

1. Chronic consumption of energy drinks deeply affect especially the carbohydrate

metabolism. The glucose and glycogen decrease in skeletal muscles explains the physical performance decrease in the endurance effort case and contradict the producers, which promise the opposite. The glycogen storage in the myocardium can affect the cardiovascular system activity and explains the negative effects associated with energy drink consumption reported in literature (tachycardia, palpitations, cardiac arrhythmias, high blood pressure);

2. We can affirm that both Red Bull and combinations of Red Bull with ethanol affect movement qualities in a significant way because the consumption is lowering the resistance to endurance physical effort.

3. Red Bull produces ultrastructural modifications in all tissues (smooth endoplasmatic reticulum dilatation in liver, myofibrile disarrangement and diminution of the skeletal muscular fibres, appearance of myocardium lysis areas). The common ultrastructural modification is represented by the rarefaction and dilatation of the matrix and mitochondrial cristae. These phenomena most likely stand at the basis of some metabolic disorders and decrease of the physical resistance during the sport performing;

4. The energy drinks deeply affected the liver. The increase of the hepatic cholesterol in addition to the increase in number and dimensions of the lipid droplets after Red Bull consumption does not exclude the possibility that, in time, the energy drink consumption favours the development of the non-alcoholic hepatic steathosis.

5. The negative effects of the energy drinks exceed the benefits induced by sport upon body. Even though athletes hope for a physical performance improvement by energy drinks, various ultrastructural and biochemical alterations produced by Red Bull in our experiment show that, in reality, the whole body activity is disturbed.

6. The energy drinks and alcohol operate largely synergistic and sometimes even complementary. In this combination, the effects of the two drinks are almost always exacerbated. Therefore, their consumption must be discouraged at any age.

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