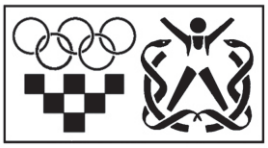

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GENDER DIFFERENCES IN GLUCOSE BLOOD LEVELS IN RATS AFTER FORCED SWIMMING EXERCISE

SPOLNE RAZLIKE U RAZINI GLUKOZE U KRVI KOD ŠTAKORA NAKON VJEŽBE FORSIRANOG PLIVANJA

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SUMMARY

The aim of this study was to investigate possible differences in blood glucose levels between male and female rats immediately after acute bout of forced swimming exercise. Adult male Wistar rats (weight 300-350 g) were divided into two groups by gender: males (n =8) and females (n =8). All the rats were given standard rat chow and tap water ad libitum and were housed at 25±3oC on a 12-hour dark/light cycle. Both groups of rats were exposed to forced swimming stress daily, for 6 days. Duration of each swimming session progressively increased from 5 minutes on the first day to 30 minutes on sixth day, allowing adaptation to swimming conditions. The rats were forced to swim in plastic tanks (90 cm wide, 120 cm deep) containing tap water (temperature ca. 25 degrees C). The depth of water was 40 cm. Seventh day we performed acute bout of 40 minutes swimming exercise. Animals were fasted 12 hours before start of last swimming sessions to obtain fasting blood glucose levels. Preexercise blood samples were taken immediately before last swimming session (7th day) and postexercise samples immediately after the last swimming session from rat's tail vein. Glucose levels in blood were determined using Optium Xceed™ Diabetes Monitoring System (Abbot). Before last swimming session male rats had slightly lower glucose levels in comparation with female rats, but this difference was not statistically significant (3.77vs4.64 mmol/l). Acute bout of forced swimming exercise raised blood glucose level and established values in postexercise period were significantly higher in both study group in comparation to values before exercise. Male rats had greater postexercise glucose blood levels (11.85 mmol/l) in comparation with female rats (6.26 mmol/l). Our findings document the existence of gender impact on the glucose postexercise concentrations confirming the differences in the energy substrates utilization and glucose metabolism regulation during and after exercise.

Key words: glucose, gender, exercise, rats

SAŽETAK

Cilj ove studije bio je istražiti moguće razlike razine glukoze u krvi između muških i ženskih štakora odmah nakon akutnog opterećenja vježbom forsiranog plivanja.: Odraslih muški štakori soja Wistar (prosječne mase 300-350 g) su bili podijeljeni u dvije skupine po spolu: muški (n= 8) i ženski (n = 8). Svi štakori su dobili standardnu, komercijalnu hranu za štakore i vodu iz slavine ad libitum, a bili su smješteni na 25 ± 3 ° C na 12-satnom tamno / svijetlo ciklusu. Obje skupine štakora bila su izložene vježbama forsiranog plivanja,dnevno u trajanju od 6 dana. Trajanje svake sesije plivanja postupno se povećavalo sa 5 minuta prvog dana do 30 minuta šestog dana , čime se postigla prilagodba životinja na uslove plivanja. Sedmi dan smo izveli akutno opterećenje 40 minutnog forsiranog plivanja. Štakori su plivali u plastičnim spremnicima (90 cm širine, 120 cm dubine) koji su sadržavali vodu iz slavine (temperature približno 25 stupnjeva C). Dubina vode iznosila je 40 cm. Životinjama nismo davali hranu 12 sati prije početka posljednje sesije plivanja kako bi dobili razine glukoze u krvi natašte. Uzorci krvi za određivaje glukoze uzeti su neposredno prije posljednje sesije plivanja (7.dan) i neposredno nakon posljednje sesije plivanja iz repne vene štakora. Razine glukoze u krvi utvrđene su pomoću Optium ™ Xceed Dijabetes sustava praćenja (Abbot) . U uzetim uzorcima krvi neposredno prije posljedenje sesije plivanja utvrđene su nešto niže razine glukoze kod muških u usporedbi s ženskim štakorima, ali ta razlika nije bila statistički značajna (3.77vs4.64 mmol /l). Akutno opterećenje vježbom forsiranog plivanja podiglo je razinu glukoze u krvi i srednje vrijednosti u uzorku uzetom neposredno nakon posljednje sesije plivanja bile su značajno više u obje skupine u usporedbi s vrijednostima prije vježbanja. Muški štakori imali su značajno veću razinu glukoze u krvi (11,85mmol / L) u usporedbi sa ženskim štakorima (6,26 mmol / L). Naši rezultati ukazuju na uticaj spola na razinu glukoze nakon fizičkog opterećenja potvrđujući spolne razlike u iskorištavanju energetskog supstrata i i regulaciji metabolizma glukoze u toku i nakon vježbanja.

Ključne riječi: glukoza, spol, vježbanje, štakori

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INTRODUCTION

Exercise, as bodily movement produced by skeletal muscle, results in corresponding caloric expenditure. This energy utilization lead to weight loss or weight maintenance, which is an important factor in prevention and management of many diseases, among the most important are obesity, heart diseases and diabetes mellitus. It improves glycemic control, decreases the atherogenic lipidic profile, and reduces weight and cardiovascular risk (18). In designing any type of exercise prescription professionals need to take in consideration general status of subject as well as many physiological, social and behavioural factors. This factors are mostly conditioned by gender differences. It is well known fact that there are gender differences in the prevalence and prognosis of the many disease where exercise could be used to modify risk or as therapeutic procedure (15). Some studies also showed gender differences in body physiological systems response after exercise prescription (10,12). The basis of this difference may underlie in existing differences between males an females in type of muscle fuel stores and fuel utilization during exercise (16). Previous data showed that during exercise of submaximal intensity, women oxidize a greater proportion of lipid relative to carbohydrate than men (4). There is no clear data about differences in postexercise utilization, but established differences in intramuscular fuel utilization during exercise and quantity of glycogen intramuscular stores, could affect postexercise glucose utilization differently in men and women.

The other factor that could influence gender differences in postexercise glucose blood level is insulin action. Improved insulin action is an important metabolic consequence of both acute and chronic exercise. Greater insulin sensitivity has been observed in women compared with men at rest (13), during exercise (2), and after a meal (14). But it is still unknown if this differences contribute in insulin action and blood glucose level immediately post-exercise

The aim of this study was to investigate possible differences in blood glucose levels between male and female rats immediately after acute bout of forced swimming exercise.

METHODS

Experimental animals

Adult male Wistar rats (weight 300-350 g) were divided into two groups by gender: males (n=8) and females (n=8). All the rats were given standard rat chow and tap water ad *libitum* and were housed at 25±3oC on a 12-hour dark/light cycle.

All the experimental procedures were performed at Medical faculty Sarajevo, Department of Physiology and previously approved by the Ethic Committee of Medical Faculty Sarajevo.

Exercise protocol

Before we performed acute bout of forced swimming exercise we exposed rats to daily swimming session aimed to the acclimatization to water conditions and swimming, as type of exercise. On that way we avoid impact of additional, stress factors on glucose response. Both groups of rats were exposed to forced swimming stress daily, between 10.00 AM to 11.00 AM, for 6 days. Duration of each swimming session progressively increased from 5 minutes on the first day to 30 minutes on sixth day. The rats were forced to swim in plastic tanks (width 90 cm, depth 120 cm), containing tap water (temperature of ≈ 25°C). The depth of water was 40 cm. A maximum of two rats, same sex, were allowed to swim together.

Seventh day we performed acute bout of swimming exercise. Rats swam 40 minutes till exhaustion at the same conditions as in period of adaptation. Animals were fasted 12 hours before start of last swimming sessions to obtain fasting blood glucose levels.

The body weight of each rat was measured before starting of accommodation period and after the last swimming session.

Blood collection

Pre-exercise blood samples were taken immediately before last swimming session (7th day) and post-exercise samples immediately after the last swimming session from rat's tail vein. The tail was thoroughly washed with warm water and dried with a clean towel before the sample was taken.

Glucose blood level measurements

Glucose levels in blood were determined using Optium Xceed™ Diabetes Monitoring System (Abbot). After cutting the top of the tail vein with scissors, freely flowing blood drop was putted on Optimum Blood Glucose electrode. Blood glucosa level was displayed on monitor.

Statistical analyses

Data are presented as mean ± standard deviation (SD). Analysis was performed using SPSS package 16.0 (SPSS Inc., Chicago, Illinois, USA). Values of measured parameters before an after exercise were compared using non-parametric equivalent to paired t-tests, Wilcoxon test. Differences in mean values between groups were assessed using the Mann-Whitney test. A two-tailed p value < 0,05 was considered significant.

RESULTS

There were statistically significant increase in body weight measured after swimming period, compared with values at the beginning of the experiment, in both study groups.

Table 1. Body weight in swimming and control group, before exercise and after last swimming session

Tablica1. Tjelesna masa plivajuće (eksperimentalne) i kontrolne grupe štakora prije i nakon treninga plivanja

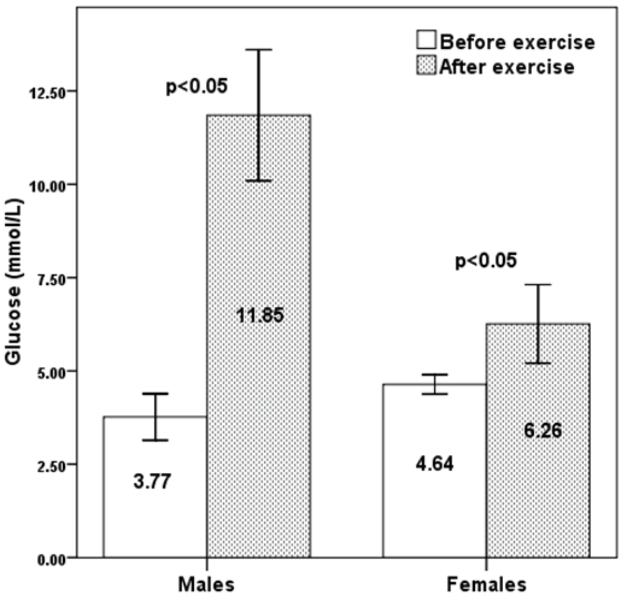
	Weight before	Weight after	p-value
Males (N=8)	339.7±47.7	353.8±51.9	<0.05
Females (N=8)	239.2±19.7	249.4±19.6	<0.05
p-value	<0.05	<0.05	

Values are means ±SD; N- number of rats

Table 2. Blood glucose levels in swimming and control group, before and after last swimming session
Tablica 2. Koncentracija glukoze u krvi kod plivajuće (eksperimentalne) grupe i kontrolne grupe prije i nakon treninga plivanja

	Glucose before	Glucose after	p-value
Males (N=8)	3.8±0.6	11.9±1.8	<0.05
Females (N=8)	4.6±0.3	6.3±0.1	<0.05
p-value	NS	<0.05	

Values are means ±SD; N- number of rats



Values are means ±SD

Figure 1. Blood glucose levels in male and female rats, before and after last swimming session
Slika 1. Koncentracija glukoze u krvi kod muških i ženskih štakora prije i nakon treninga plivanja

Before last swimming session (preexercise) male rats had slightly lower glucose levels in comparison with female rats, but this difference was not statistically significant (3.77vs4.64 mmol/l) (Table 2). Acute bout of forced swimming exercise raised blood glucose level and established values in postexercise period were significantly higher in both study group in comparison to values before exercise.

Glucose levels measured in blood samples taken immediately after last swimming session showed pronounced gender differences. Male rats had greater postexercise glucose serum levels (11.85 mmol/l) in comparation with female rats (6.26 mmol/l).

DISCUSSION

Gender differences in energy utilization in rest and during exercise are well known but data of the studies performed on humans and animals are inconsistent (1,2,14). Very few investigations have established the glucose response during recovery from endurance exercise. The aim of present study was to investigate possible differences in blood glucose levels between male and female rats after forced swimming exercise (postexercise).

Our results showed that acute bout of forced swimming exercise raised blood glucose concentrations and established values in postexercise period were significantly higher in both study group in comparison to values before exercise. Glucose concentrations measured in blood samples taken immediately after last swimming session showed greater absolute increase in male rats in comparison with female rats. We also found significant increase in body weight measured after swimming period, compared with values at the beginning of the experiment, in both study groups.

Our results regarding postexercise glucose response are similar to results of earlier investigation from our laboratory (9) that outlined the serum concentrations in trained male athletes during recovery from a 30 minutes exercise on cycle ergometer at 50% VO2max following an overnight fast. When compared to resting levels, and with values measured at the end of exercise (30.minute), post-exercise concentrations of glucose increased but the level of glucose in all measurements was in reference range (3.3-6.3 mmol/l). There are limited number of investigations of post-exercise glucose response in experimental animals. Ivy et al. (8) were among the first to describe gender differences in fuel utilisation during exercise in rats. They showed that males had significantly higher resting muscle glycogen levels. Exercise resulted in significant glycogen depletion in both sexes but males utilized approximately 50% more glycogen during the exercise bout (running for 5 min at 1.7 mph at a 17% grade) than females. Authors consider that the greater glycogen utilization in red and white fast-twitch muscle during exercise by males could represent a true sex difference but could also be attributable in part to the males having performed more work as a result of 20% greater body mass. No sex difference was observed in the rates of muscle glycogen repletion after exercise.

We also concern that gender differences in postexercise blood glucose levels underlie in different fuel utilisation during exercise that have reflection on glucose levels at the start of recovery period. In accordance with this are the results of most recently study on rats which confirmed previous findings that during exercise females (rats and humans) preferentially utilize fat and males carbohydrates (6). Horton et al. (7) conducted a study to assess gender differences in fuel metabolism during long-duration exercise. Fuel oxidation was measured using indirect calorimetry and blood samples were drawn for circulating substrate and hormone levels. Results indicated that females expended more total energy from fat oxidation (50.9%) than that of men (43.7%), but less total energy from carbohydrates (45.7% for women and 53.1% for men). In addition to differences in fuel metabolism, males also had higher circulating levels of catecholamines.

Additionally, gender differences could be partly provocate also by different peripheral insulin sensitivity. Aerobic training lead to increase peripheral insulin sensitivity and ability of insulin to suppress endogenous glucose production (3). Anatomic and biochemical changes in trained skeletal muscle lead to increase of glucose uptake like enhanced blood flow and translocation of GLUT4 vesicles to cell surface membranes (16). Results of some studies showed that women with greater insulin sensitivity in rest have less capacity to improve insulin action during exercise (11).

Our results are in accordance with results of Horton et al.(14). Authors conducted one of the few studies reporting hormone and substrate responses during two hours of recovery from a prolonged exercise bout (cycling, 2 h, 40% VO_{2max}) in comparably trained males and females with similar fitness capacities (64.4 and 55.5 $mL \cdot kg^{-1} \cdot min^{-1}$, respectively). No gender differences were observed in the substrate responses (i.e. FFAs, lactate), but greater glucose concentrations at all time points in males. Gender differences were also noted for post-exercise insulin levels which increased in males but decreased in

females. No differences were observed in the post-exercise cortisol responses for males and females.

Insulin levels increased to counteract elevated plasma glucose levels, but it is unclear from which occurred first. In contrast to this results are the results of Tarnopolsky et al (17) who identified gender differences in the prolonged post-exercise response for plasma glucose, FFAs, insulin, and GH in trained male and female runners completing a 15.5 km run at 65% VO_{2max}. These researchers observed increases in glucose and insulin levels 15 min post-exercise in females, with no changes observed in males.

Considering fact that cited studies performed on humans showed that there are numerous potential confounding factors as menstrual cycle phase, training status, energy and macronutrient intake prior to measurements we assume that that the use of experimental exercise models can be way to avoid them.

CONCLUSIONS

Our findings document the existence of gender impact on the glucose postexercise concentrations confirming the differences in the energy substrates utilization and glucose metabolism regulation during exercise. Experimental exercise protocols could be used to avoid numerous confounding factors that could influence humoral response during exercise.

ACKNOWLEDGMENT

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