210 - VERIFICATION OF A PROTOCOL EFFECT OF CARBOHYDRATES SUPERCOMPENSATION IN MUSCULARAND HEPATIC GLYCOGEN IN RATTUS NORVEGICUS

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INTRODUCTION:

The incapacity of the skeletal muscle produce high levels of muscular power or mantain these levels in the time is called muscular tiredness (ASCENSÃO et al., 2003). This is followed by a series of physiological and metabolic changes. Among the metabolic factors we can indicate the glucose, lipoprotein levels, enzymes, urea, uric acid, among others. On the other hand, the nutrition is an indispensable tool in the sportive practice, exerting a long influence in the called endurance exercise training. There is no doubt that the carbohydrate consume (CHO) before and during endurance exercise will delay the tiredness appearance, saving hepatic and muscular glycogen, and suppling glucose directely for the muscles in activity (BARCELOS, et al., 2003; MINÉ, 2005).

It is known that as most intensive the activity is, lesser will be the time of its lenght, whereas the body will go on tiredness. The glucose energy synthesizes the ATP, permiting the nutrition during the physical exercise, but it is enough to maintain the activity just for some minutes. There after it is used the stock of CHO(glycogen). Finishing that energetic stock and the body is still in activity, and having a balance between the consume and the oxygen spent, it begins the use of fat, more exactly tryglicerides, to prolong the physical activity; if there is no balance between the spent and consume of the oxygen, the body goes on tiredness and happens the interruption of the exercise (MINÉ, 2005).

Nowadays, we know that the consume of CHO is essencial in endurance exercise for the high performance maintenance (LAMB; KNUTTGEN; MURRAY, 1994) and that the glycogen depleting with the exercise followed by a rich diet in carbohydrate results in a increase of muscular glycogen for concentrations well above those normally maintained in a fed condition (NAKATANI et al., 1997). Normally, each 100 muscle grams contains about 1,7g of glycogen; the overload with glycogen permits its accumulation from 4 to 5g (COYLE, 2007).

Therefore, several studies have suggested that the supercompensation of carbohydrates is an efficient method to increase the energetic tissues' stocks. The ingestion of CHO has to be emphasized before, during and after the physic exercises for then, help on the person's performance, delay the muscular tiredness and replace the glycogen in the muscle (LIBERALI; MATOS, 2008).

MATERIALS AND METHODS:

Animals: For the experience, were used 59 Wistar Rattus norvegicus male rats, deriving from the Itajaí Valley University facility. All the procedures during the study were approved by the Ethics Committee in Animal Research of that University, observing the ethics principles of animal experimentation and the Biosafety. Every day, the amount of fodder consumed by the animals of each cage was checked, beeing replaced according to the necessity. The water was provided at libitum.

During the pre-experimental period (7 days), the animals were separated in coletive cages with 5 animals in each cage, having commercial fodder and water ad libitum. This period was an adaptation of the animals to the operation and the environment. After that stage, the animals were weighed, marked with numbers and different colors for each type of treatment and, subdivided in the experimental groups.

Experimental groups: The animals were separated in four groups: DN: With a diet of swimming and supercompensation (n= 15), SDN: Without supercompensation diet and with swimming (n= 15), DSN: With a supercompensation diet and without swimming (n= 15) and SDSN: Without supercompensation and without swimming diet (n= 14).

Program: 1st Stage (animals adaptation) – during 7 days, the animals were housed in cages for adaptation. All animals were sbmitted to swimming for 2 minutes, so that all pass the same “stress”.

2nd Stage (adaptation to the liquid environment) – this had the aim of minimize the intensive stress due to the water and the tank depth. The animals of the first and second group were submitted to progressive swimming sessions, so as to the activities started with 20 minutes , by adding 10 minutes to each day, until reach 60 minutes, which lenght remained until the end of the first week.

3rd Stage (supercompensation of carbohydrates program) – in this stage, the animals of the second and third group were submitted to the CHO supercompensation period. First, the groups were submitted on a diet for a glycogen depletion, containing low tenors of CHO and rich in fat. For that, the commercial fodder was replaced by Brazil nuts, ad libitum, because they contain, for each 10g of the product, 1g of carbohydrates, 1g of protein, 6g of total fat and 2g of unsaturated fat.

During the subsequent three days, the animals of the groups above, were submitted on a rich diet of CHO. Besides the commercial fodder, it was added a mix of glucose to the animals water (each 20ml of the product produced 23g of carbohydrates). During that period, the animals of the second group were submitted only on that diet, without swimming practice (DSN group), on the contrary of the third group of animals, which besides the supercompensation diet were submitted to swimming practice (DN group). The animals of the first group continued on the normal diet, however submitted to swimming and, the SDSN group remained on a normal diet and without physic activity. At the end of those six days, the animals were submitted on a day called “event” (swimming for two hours and thirty minutes without stopping). At the end of the “event”, the animals of each group were sacrificed.

Sacrifice and sample extraction: after the experimental period, the animals were sacrificed in CO2 and through the median laparotomy, the liver and the soleus and gastrocnemius muscles for determination of the tissue glycogen were removed. The organs were weighed, stored in closed containers and kept in freezer under 4° C for later biochemical analysis.

Biochemical Analysis: For the glycogen analysis, firstly the samples were weighed many times, using an analytical scale after using the greenhouse, until obtaining the dryed weigh and, after that, they were macerated.

The glycogen was determined by a calorimetric method by using a commercial enzimatic kit – Glucose PAP Liquiform of Labtest Diagnóstica S.A. It was used aproximately 0,2 g of the macerated sample, incubated in amyloglucosidase 1%, in phosphate plug 0,1M, pH 7,0, in a recipient in water at 55° C during 2 hours. Before being centrifugated (2000 rpm forr 5 minutes) it was added 0,4 ml of nitric acid 3 M and 2 ml of phosphate plug 0,6 M. After centrifugated, 100 ml of the floating were incubated with 2 ml of the oxidases glucose reagent and the tube remained in a recipient immersed in water at 37°C for 15 minutes. The measures by absorbency were proceeded in a Spectrophotometer UV Visible, using wave length of 500 nm. For that it was made the calibration curve with Glucose PAP Liquiform of Labtest Diagnostica S.A. standard, to proceed the Spectrophotometer measurement.

RESULTS AND DISCUSSIONS:

The glycogen concentrations in a muscle did not present meaningful differences among the variety of experimental groups (Fig. 1). Althoug the comparison of the muscular glycogen concentration accumulated among the different groups (DN, SDN, DSN, and SDSN) does not show meaningful difference, the physical activity and the glycogen overload show a clear tendency of raise of that nutrient stocks.

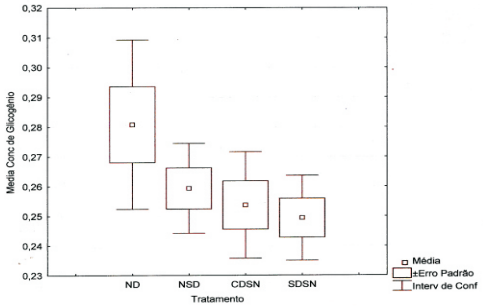

Fig. 1: Relation between the treatments and average amount of muscular glycogen (mg/dl)

Lima – Silva et al. (2007) checked that, after the reduction of muscular glycogen with manipulation exercise-diet, followed by three days of a rich diet in carbohydrate, the stocks of muscular glycogen increased aproximately, 60 % in relation to a mixed diet. Rankin (2001) says that it is an agreement in the scientific environment that the ingestion of food rich in CHO for many days improves atheletes' performance in training programs which demand endurance exercise repetition and short duration. That effect is attributed to an initial increase of the muscular glycogen stocks, which does not happen when the food is poor in CHO. Similar fact is observed when the food ingestion is before or during the endurance exercise. Neverthless, it does not exist any scientific evidence that a rich diet in CHO is better that a moderate diet in this nutrient, in cases of endurance exercise.

High levels of muscular glycogen in trained animals are showed by Barcelos et al. (2003). The muscular glycogen levels found in this research were similar for those authors. However, for the hepatic issue, the amount of the present study was twice higher than those found by them, probably due to the fact that our data did not happen at the end of each stage, what can possible made that difference.

The mecanism of the glycemic maintenance ensured the analysed groups to support similar glucose levels, however through different metabolic strategies. The trained group, probably because accustomed to exercise, showed a tendency in use other energetic substrate, like fat, promoting a lesser silken glucose depletion and consequently a higher preservation of that substrate in the muscle. The trained organism suffers positive adjustment in relation to a higher use of fat as an energetic substrate. On the other side, the control group, which does not dispose those adjustments, remains mobilizing glycogen as a source of glucose to maintain its ideal base levels (SILVEIRA et al., 2007).

The Fig. 2 represents the average of glycogen concentration (mg/dl) in the liver, in the various groups. The comparison of the glycogen level concentration accumulated among the different groups (DN, SDN, DSN AND SDSN) was carried out through the analysis of the (ANOVA), followed by the Turkey Test, showing expressive difference between animals with swimming in relation to the group without swimming, according demonstration in picture 3.

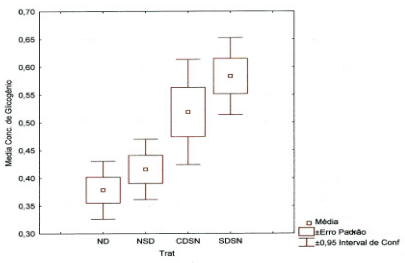

Fig. 2: Relation between the treatments and the average amounts of hepatic glycogen (mg/dl)

	Trat.	{1}	{2}	{3}	{4}
1	DN		0,871	**0,030**	**0,001**
2	SDN	0,871		0,115	**0,004**
3	DSN	**0,030**	0,115		0,518
4	SDSN	**0,001**	**0,004**	0,518	

Picture 3: Turkey test for the hepatic glycogen amounts in the various experiments

According to Afonso et al. (2003), the liver is a fundamental organ in the organism homeostasis, with a high metabolic rate and sensible to external stimulus, and this may answer to the agents that cause the stress with depleting of glycogen levels. On the study presented by the authors, the acute exercise caused more hepatic glycogen depleting, besides more cardiac and muscular glycogen mobilization in the postexercise condition, which corroborates with the obtained results.

The most significative physiological responses happened in the hepatic tissue related to the supercompensation. During the experiment, the muscles showed increasing values in glycogen tenor and the hepatic tissues showed higher glycogen tenors than the muscular tissue, according to Lima et al. (2003), Guyton and Hall (2006) and Afonso et al. (2003) informations. According to those authors, the hepatic tissue retain much glycogen and these are higher than the muscular tissue.

Previous studies showed that the physical training can promote the hepatic glycogen accumulation (GOBATO, 1993; GOMES, 2002) due to a change on the pattern of the energetic substrates mobilization by the organism (SILVEIRA et al., 2007). According to Rogatto et al. (2004), the intense and intermittent physical exercise seems to have more influence on the CHO and fat metabolisms, owing to the mobilization of muscular and hepatic glycogen stocks and the increase of glucose and AGL

concentrations.

CONCLUSION:

In the present study, the training that was used was not able to promote meaningful changes in the muscular glycogen, because the animals of the control group did not show glycogen concentration different of the trained rats group and of those that had the supercompensation diet. The similar glucose levels were maintained through glycemic homeostasis maintenance mechanisms, however through different metabolic strategies.

Our results showed a tendency that the maintenance of high muscular glycogen concentration is extremely important, mainly with sports athletes with high performance, on which the maximum performance is constantly required. So, the regular physical training, as well as adequated and balanced food, can influence positively on the muscular glycogen stocks increase.

In general, the training protocols (intensity, duration and frequency) are much different among the studies, and it can exemplify, in part, the different results. Much part of the experimental models of physical activities for animals have been based on aerobic exercises, prolonged and low-moderated intensity, which generates doubts about the possible effects of more intensive and intermitent efforts on the organic responses of exercised animals (ROGATTO, 2004; FIGUEIRA et al., 2007).

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VERIFICATION OF A PROTOCOL EFFECT OF CARBOHYDRATES SUPERCOMPENSATION IN MUSCULARAND HEPATIC GLYCOGEN IN RATTUS NORVEGICUS

ABSTRACT:

The nutrition is a indispensable tool in the sportive practice, exerting a long influence in the called endurance exercise training. The consume of carbohydrates (CHO) before and during prolongened exercise will delay the tiredness, saving hepatic and muscular glycogen, and providing glycogen directly for the muscles in activity. However, it is necessary to check the effect of a protocol for the supercompensation of CHO, which can be used by many athletes to delay the muscular tiredness. Therefore, it was proposed an experience in which were used 59 Wistar male rats (Rattus norvegicus). The experience was consisted by three stages: 1st Stage: 7 days for adaptation. 2nd Stage: Progressive swimming sessions were carried out with the animals of the first

and third group, so as to the activities started with 20 minutes, and the 3rd Satge: supercompensation program of CHO. After the experimental period, it was checked the amount of the hepatic and muscular glycogen concentration. The muscular glycogen accumulated between the different groups did not present statistic differences, however, the physical activity and the glycogen overload show a tendency of increase of its stock. The most meaningful physiological results happened in the hepatic tissue relating to the supercompensation. During the experience, the soleus and gastrocnemius muscles showed increasing values in glycogen tenors and the hepatic tissue showed glycogen tenors higher than the muscular tissues. Our results showed a clear tendency of the high concentrations of muscular and hepatic glycogen maintenance through a rich diet in CHO.

KEYWORDS: supercompensation, carbohydrates, muscle

VERIFICATION DE L'EFFET D'UN PROTOCOLE DE SUPERCOMPENSATION DE CARBOHYDRATES SUR LE GLYCOGENE MUSCULAIRE ET HEPATIQUE APPLIQUE AU RATTUS NORVEGICUS

RESUME:

L'alimentation est un outil indispensable à la pratique sportive et excerce une grande influence sur les sports dits d'endurance. La consommation de carbohydrates (CHO) avant et durant les exercices prolongés retardera la présence de fatigue, économisant le glycogène hépatique et musculaire, et fournissant la glycose directement aux muscles en activité. Néanmoins, il semble nécessaire de vérifier l'effet d'un protocole pour la supercompensation de CHO, lequel peut être utilisé par un grande nombre d'athlètes pour retarder la fatigue musculaire. Pour cela, nous proposons une expérience lors de laquelle 59 rats mâles (Rattus norvegicus), d'espèce Wistar, ont été utilisés. L'expérience a été composée de trois étapes. 1ère étape : 7 jours d'adaptation. 2ème étape : des séances progressives de natation ont été réalisées avec les animaux du premier et du troisième groupe, sachant que les activités ont commencé avec une durée de 20mn, et la 3ème étape: programme de supercompensation de CHO. Après la période expérimentale, la concentration de glycogène hépatique et musculaire a été quantifiée. Le glycogène musculaire accumulé entre les divers groupes n'a pas présenté de différence statistique, cependant, l'activité physique et la surcharge de glycogène démontrent une tendance à l'augmentation des réserves de ce dernier. Les réponses physiologiques plus significatives sont apparues dans le tissus hépatique correspondants à la supercompensation. Pendant l'expérience, les muscles soléaire et gastrocnémien ont présenté des valeurs croissantes en taux de glycogène et le tissus hépatique a presenté des taux de glycogène supérieurs à ceux du tissus musculaire. Nos résultats ont montré une nette tendance de maintien de concentrations élevées de glycogène musculaire et hépatique dû à une alimentation riche en CHO.

MOTS-CLÉS: supercompensation, carbohydrates, muscle.

LA VERIFICACIÓN DEL EFECTO DE UNO PROTOCOLO DE SUPERCOMPENSACIÓN DE CARBOIDRATOS EN GLICÓGENO MUSCULAR Y HEPÁTICO EN RATTUS NORVEGICUS.

RESUMEN:

La nutrición es una herramienta imprescindible adentro de la práctica desportiva, ejerciendo uma larga influencia en los deportes llamados de larga duración. La consumición de los carboidratos (CHO) antes y durante ejercicios alargados irá a retrasa lo aspecto de la fatiga, ahorrando el glicógeno hepático y muscular, y abasteciendo la glucosa directamente a los músculos en actividad. Sin embargo, ve si la necesidad para verificar el efecto de un protocolo para la supercompensación de CHO, a las cuales puede ser utilizado por muchos atletas por la retrasa la fatiga muscular. Por lo tanto, un experimento fue propuesto donde 59 ratóns masculinos fueram utilizadas (Rattus norvegicus), ascendencia de Wistar. El experimento fue compuesto por tres fases: 1a fase: 7 días para la adaptación. 2a fase: Sesiones graduales de la natación habían sido efectuadas con los animales del primero y el tercero grupo, siendo que las actividades habían sido iniciadas con 20 minutos y la 3a fase: programa del supercompensación de CHO. Después del período experimental, fue cuantificado la concentración del glicógeno hepático y muscular. El glicógeno muscular acumulado entre los diversos grupos no presentó diferencia estadística, sin embargo, la actividad física y la sobrecarga del glicógeno demonstro una tendencia del aumento de las reservas de esto. Las respuestas más significativas fisiológicas habían ocurrido en el tejido hepático refería a la supercompensación. Durante el experimento, los musculos sóleo y gastrocnêmio habían presentado valores de aumento en textos del glicógeno y el tejido hepático presentó los textos del glicógeno superiores a de lo tejido muscular. Nuestros resultados habían demostrado la tendencia clara del mantenimiento de altas concentraciones del glicógeno muscular y hepático con una dieta rica en CHO.

PALABRAS-LLAVE: supercompensación, carboidratos, músculo

VERIFICAÇÃO DO EFEITO DE UM PROTOCOLO DE SUPERCOMPENSAÇÃO DE CARBOIDRATOS NO GLICOGÊNIO MUSCULAR E HEPÁTICO EM RATTUS NORVEGICUS

RESUMO:

A nutrição é uma ferramenta indispensável dentro da prática desportiva, exercendo uma longa influência nos chamados esportes de longa duração. O consumo de carboidratos (CHO) antes e durante exercícios prolongados irá retardar o aparecimento da fadiga, economizando glicogênio hepático e muscular, e fornecendo glicose diretamente para os músculos em atividade. Contudo, vê se a necessidade de verificar o efeito de um protocolo para a supercompensação de CHO, que pode ser utilizado por muitos atletas para retardar a fadiga muscular. Para tanto, foi proposto um experimento em que foram utilizados 59 ratos machos (Rattus norvegicus), linhagem Wistar. O experimento foi composto por três fases: 1a Fase: 7 dias para a adaptação. 2ª Fase: Sessões progressivas de natação foram realizadas com os animais do primeiro e do terceiro grupo, sendo que as atividades foram iniciadas com 20 minutos e a 3a Fase: programa de supercompensação de CHO. Após o período experimental, foi quantificada a concentração do glicogênio hepático e muscular. O glicogênio muscular acumulado entre os diferentes grupos não apresentou diferença estatística, porém, a atividade física e a sobrecarga de glicogênio demonstram uma tendência de aumento das reservas deste. As respostas fisiológicas mais significativas ocorreram no tecido hepático referentes à supercompensação. Durante o experimento, os músculos sóleo e gastrocnêmio apresentaram valores crescentes nos teores de glicogênio e o tecido hepático apresentou teores de glicogênio superiores ao do tecido muscular. Nossos resultados mostraram a tendência clara da manutenção de concentrações elevadas de glicogênio muscular e hepático através de uma dieta rica em CHO.

PALAVRAS-CHAVES: supercompensação, carboidratos, músculo

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