MUSCLE GLYCOGEN DEPLETION PATTERN AND METABOLIC RESPONSE IN BULLS AFTER BULLFIGHTING

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ABSTRACT: *Muscle metabolism and glycogen depletion pattern were investigated in 12 4-year old bulls after bullfighting in order to establish their muscular adaptation to exercise and the order of recruitment of muscle fibers. Biopsies were taken immediately after bullfighting from the gluteus medium muscle at an absolute depth of 50 mm to perform histochemistry and biochemistry. Glycogen depletion pattern was evaluated by means of periodic acid Schiff's stain and muscle glycogen and lactate concentrations were determined fluorometrically. Muscle pH was also determined. During bullfighting, a marked intervention of the glycolytic pathways occurred, with glycogen breakdown, lactate formation and muscle acidosis. Glycogen depletion pattern showed a recruitment order of I→IIA→IIB. Total muscle glycogen content after bullfighting was mainly dependent on the percentage of type IIB fibers with high content in glycogen. In summary, the results showed the high anaerobic metabolism during bullfighting, in spite of the main intervention of type I and IIA fibers, which could have been linked to psychological stress and catecholamines release. Furthermore, the low pH could be indicative of a reduced muscle buffer capacity, possible due to the lack of energy precursors and incapacity for ADP rephosphorilation.*

Key words: Bovine, exercise, glycogen, metabolism, muscle

PATRÓN DE DEPLECIÓN GLUCOGÉNICA Y RESPUESTA METABÓLICA MUSCULAR A LA LIDIA EN TOROS BRAVOS

RESUMEN: *Se ha investigado el metabolismo muscular y el patrón de depleción glucogénica en 12 toros bravos tras la lidia para establecer su adaptación al ejercicio y el orden de intervención de las fibras musculares. Se obtuvieron biopsias musculares tras la lidia, en el músculo glúteo medio, a una profundidad absoluta de 50 mm, para realizar análisis histoquímicos y bioquímicos. El patrón de depleción glucogénica se evaluó mediante la tinción del ácido peryódico de Schiff, las concentraciones musculares de glucógeno y lactato se cuantificaron mediante fluorimetría y se midió el pH muscular. Durante la lidia, se produjo una intervención importante de las vías glucolíticas, con formación de lactato y acidosis muscular. El patrón de depleción glucogénica mostró un orden de contracción fibrilar I→IIA→IIB. El contenido muscular total de glucógeno tras la lidia dependió fundamentalmente del porcentaje de fibras IIB con contenido elevado en glucógeno. En resumen, durante la lidia existe un metabolismo muscular anaerobio intenso, a pesar de la intervención preferencial de las fibras I y IIA, resultados que podrían haberse debido al efecto del estrés psicológico y liberación de catecolaminas. El bajo pH tras la lidia indicaría una capacidad tamponadora reducida, posiblemente debido a la escasez de precursores energéticos e incapacidad de refosforilación del ADP.*

Palabras claves: Bovinos, ejercicio, glucógeno, metabolismo, músculo

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INTRODUCTION

Although bullfighting is a kind of exertion, there is a paucity of scientific information concerning physiological adaptation to this intense exercise. In the last years, we have been analyzing the hematological (1,2), and plasma biochemical (3) response to bullfighting, as well as muscle composition and metabolism (4,5,6,7) in these animals. Bullfighting lasts for more than 15 minutes (8), and therefore, a predominance of oxidative metabolism vs. anaerobic glycolysis in muscle is expected (9). However, in a previous research, we found marked glycogen depletion, with post-bullfighting concentrations lower than 60 mmol/kg d.w. and with intense lactate production, higher than 200 mmol/kg d.w. (4,6). These findings emphasized the great dependence of bovine locomotor muscle of anaerobic pathways for energy resynthesis. This fact could be the result of limitations in the availability of oxygen and/or in the capacity to use it. The main factors involved might be the cardiorespiratory functionality, blood hemoglobin concentration, transit time of the blood in the muscle, capillarization, muscle myoglobin concentration and the number of mitochondria in the muscle fibers (10).

In knowledge of the authors, the heart and respiratory rates during bullfighting have not been recorded, because of the inherent difficulty of management of this bovine breed. We have observed that most of the bulls undergo asphyxia episodes during exercise, which were expressed as incapacity for movement and continuous panting (3). In addition, Villafuerte et al. (1) found a non-significant trend to decreased red blood cells and hemoglobin concentration after bullfighting. That was associated with the hemorrhage and it is tenting to speculate about the deterious effect of the reduced blood volume in oxygen transport and aerobic pathways in the locomotor muscles. On the other hand, locomotor muscles of the bulls are demonstrated to have high activities of glycolytic enzymes (LDH, lactate dehydrogenase; PHOS, glycogen phosphorylase) and low activities of oxidative enzymes (CS, citrate synthase; HAD, 3-OH-acetyl coenzyme A dehydrogenase) (5). Accordingly, it appears than a combination of cardiorespiratory, hematologic and muscular factors makes bovine muscle anaerobic during exercise.

Other factor to take into account is the influence of the emotional stress. In fact, catecholamines stimulate glycolytic pathways, with glycogen breakdown and lactate production. Glycogen depletion patterns are used to indicate how muscle fibers are recruited during work (11). From histochemical ATP-ase stains, muscle fibers are classified as type I, IIA and IIB. Type I fibers have low and type IIA and IIB high contractile velocity. Additionally, type I fibers have greater oxidative potential than type II fibers and type IIB fibers have lower oxidative potential than type IIA (5,6,7,12,13). Catecholamines strongly influence glycogen breakdown on oxidative fibers, while maximum intensity exercise causes glycogen breakdown in glycolytic fibers (14,15,16).

This research aims to describe glycogen depletion pattern in bulls after bullfighting in locomotor muscles, in order to explain which type fibers are involved in this kind of exercise, and to analyze the effect of the emotional stress. Because of the high muscle lactate concentrations found previously after bullfighting (4), it was hypothesized that this exercise produces a more intense recruitment of fast-twitch fibers in the locomotor muscles.

MATERIAL AND METHODS

Bulls

A total of 12 four-year old healthy bulls were included in the study. They belonged to two different farms (6 from each one).

Muscle sampling

Only muscle biopsies could have extracted immediately after bullfighting, with a biopsy needle using the percutaneous technique of Bergström. The most active muscles during bullfighting are unknown. It has been shown that the equine gluteus medium muscle is active in all types of exercise. Because of the easy accessibility, this muscle was also chosen in the bulls. Biopsies were taken 15 cm caudodorsally to the tuber coxae, in the middle of the line between tuber coxae and ischii tuber. The samples were withdrawn from either the right or the left side. All the biopsies were obtained at an absolute depth of 50 mm, since the animals had similar weights (mean weight 566.3 ± 20 Kg). Immediately after extraction, samples were divided in two fractions, for histochemistry and biochemistry.

Histochemistry

Samples for histochemistry were rolled in talcum power before being frozen and stored at –80°C until analyzed. After, they were cut into 10 μm thick cross-sections in a cryostat at –20°C (*2800 Frigout E, Reicher-Junt)* and histochemically stained for myosin ATP-ase activity after both alkaline (pH 10.3) and acid (pH 4.3 and 4.6) preincubation. Three main muscle fiber populations were then identified: I, IIA and IIB (7,17). About 200 fibers were counted on photomicrographs from each biopsy to establish muscle fiber composition.

A semi-quantitative assessment of glycogen content in the fibers was obtained by the technique of Periodic- Acid Schiff (PAS). According to their staining intensity, the cross-sections

were considered as high (H), medium (M) and low or empty (L) in glycogen content, then, they were compared to the photomicrographs showing the fiber composition.

Biochemistry

Muscle samples were immediately frozen and stored at –80°C until analyzed. They were freeze-dried (*Hetosic type CSD2, Heto Lab Equipment, Denmark)*, dissected free of fat, blood and connective tissue with a dissection microscope and weighted. The weight ranged between 1.4 and 1.8 mg in all the cases (1.65 ± 0.23 mg). Total glycogen concentrations (GLY) were analyzed by boiling part of the muscle biopsy for 2 hours in 1 ml of 1 M ClH in order to hydrolyze GLY into glucose residues. Muscle lactate concentrations (LA) were determined after perchloride acid extraction. Both muscle GLY and LA were measured according to the methods described by Lowry and Passoneau (18) with fluorometrical techniques. Muscle pH was determined after sample homogenization in KCl buffer with iodoacetate, to prevent additional glycolysis (19).

Statistical analysis

All the results are presented as mean±SD. The correlations between GLY depletion pattern and muscle GLY, LA and pH were investigated by a linear correlation analysis (*Pearson product-moment correlation)*. When significant correlations were observed, a simple regression study was performed. Level of significance was set at $P<0.05$.

RESULTS

No significant differences were found between the two farms, so all the data were processed together. Muscle GLY and LA concentrations after bullfighting were 54.57±27 mmol/Kg d.w. and 255.9±56 mmol/Kg d.w. respectively. Muscle pH after bullfighting was 5.895±0.11, ranging between 6.030 and 5.720.

GLY depletion pattern after bullfighting is presented in Figure 1. Most of the type I fibers were almost E in GLY (63.01%), while most of the type IIA fibers had a M content in GLY (72.22%). Most of the type IIB fibers showed a H content in GLY (85.9%).

The results of the correlation analysis between the percentages (%) of the three main fiber populations with (H), (M) and (L) GLY content are showed in Table 1. The % of type I fibers stained (H) was positively correlated with the % of type I fibers stained (H) and negatively with the % of type IIB fibers stained (H). Likewise, a positive correlation between the % of type I fibers stained (M) and the % of type IIA fibers stained (L) was found. Finally, a negative relation between the % of type I fibers stained (L) and the % of type IIA fibers stained (M) was observed. The results of the correlation analysis of the GLY depletion pattern and the metabolic response to bullfighting are presented in Table 2. Muscle GLY concentrations were mainly dependent on GLY stored in type IIB fibers stained (H) for GLY. The relationship between both variables followed this expression: % type IIB fibers stained (H): -16.4 + 0.91 GLY (Figure 2a). The main determining factor of muscle pH was the % of type I fibers stained (M) in GLY, according to this expression: % type I fibers stained (M): 230.1 – 37.19 pH (Figure 2b). No significant correlations were found between the muscle GLY, LA and pH after bullfighting (Table 3).

DISCUSSION

In agreement with previous studies, bullfighting induced a marked recruitment of glycolytic pathways with LA formation (4,5,6,7,13). McVeigh et al. (20) found that resting GLY concentrations in bovine muscle were about 250 mmol/Kg d.w., although means higher than 300 mmol/Kg d.w. could be measured if they were fed a high-carbohydrate diet. If these concentrations are considered as baseline for muscle GLY content, our bulls could have undergone a GLY reduction near of 75%. Some evidences have been found that the availability of muscle GLY in horses could be a limiting factor for performance for both endurance (21) and anaerobic exercises (22), and the same might be true for the exercising bulls.

Despite the high LA formation, no significant relationship was found between muscle GLY and LA. These results could have derived from LA diffusion rate from muscle fibers to bloodstream. It has been demonstrated in horses that a limitation of the LA diffusion rate exists, depending on muscle LA concentrations, muscle capillarization and muscle oxidative profile, volemia and monocarboxylate transporters (23). A similar fact could be expected in bovine muscles, although in knowledge of the authors, the influence of these factors and the existence of lactate transporters in muscle fibers have not been determined yet.

An unexpected finding was the low muscle pH, in spite of the quick extraction of muscle biopsies to avoid additional glycolysis. Snow et al. (24) reported muscle LA concentrations of 149 and 204 mmol/Kg d.w. in Thoroughbred racehorses after several bouts at galloping velocities, with blood pHs of 6.5 and 6.3, respectively. If we consider these data, a muscle LA concentration of 250 mmol/Kg d.w. would produce a muscle pH of 6.0, slightly higher than the mean pH found in the bulls after bullfighting (5.895). Therefore, it seems that bovine muscle presents a high glycolytic capacity together with a limited

buffer capacity. Muscle buffer capacity is mainly determined by the amount of phosphocreatine, bicarbonate and carnosine and it increases in response to physical training (25). A previous research established that bullfighting induces significant adenosine triphosphate (ATP) depletion (4). The lack of energy precursors in bovine muscle, as ATP and phosphocreatine, could have acted as limiting factor of muscle buffer capacity.

Earlier studies in horses have shown that fiber recruitment within the gluteus muscle is related to speed, and the fibers are recruited from type I to IIA to IIB as the intensity or duration of exercise increases (11). In supramaximal and maximal exercises, carried out above or at maximum oxygen uptake, GLY depletion is more intense in type IIA and IIB fibers (15,16). In the present research, GLY depletion pattern of bulls after bullfighting followed this order: I→IIA→IIB. This pattern has been found in submaximal exercises and after psychological stress (11,14). However, it was unexpected that a main intervention of types I fibers would generate high muscle LA concentrations. Energy resynthesis in these fibers are dependent on oxidative breakdown of blood glucose and/or the muscle GLY, and β-oxidation of free fatty acids (26). Up to now, the main limiting factors of performance in bullfighting bulls remain speculative, although muscle glycolytic and buffering capacities should be considered.

The correlations between muscle GLY depletion pattern and muscle metabolic response to bullfighting emphasized the influence of both exercise and psychological stress. The correlations between the % of types I, IIA and IIB fibers stained (H) highlighted the preferential intervention of fast-twitch fibers, although it can not be

Table 1. Correlations between the % of the three main muscle fiber populations stained high (H), medium (M) and low (L) for glycogen in the gluteus medium muscle of 12 bullfighting bulls after bullfighting (significant correlations are in bold; level of significance P<0.05; ns no significant).
Tabla 1. Correlaciones entre los % de las diferentes poblaciones fibrilares musculares con tinción alta (H), media (M) y baja (L) para glucógeno en el músculo glúteo medio de 12 toros bravos tras la lidia (correlaciones significativas en negrita; nivel de significación P<0.05; ns no significativo).

	I (H)	*I (M)*	*I (L)*	*IIA (H)*	*IIA (M)*	*IIA (L)*	*IIB (H)*
I (H)	1.000						
I (M)	0.670 ns	1.000					
I (L)	0.110 ns	-0.640 ns	1.000				
IIA (H)	**1.000**	0.670 ns	0.110 ns	1.000			
IIA (M)	-0.140 ns	0.490 ns	**-0.930**	-0.140 ns	1.000		
IIA (L)	-0.450 ns	**0.950**	0.840 ns	-0.450 ns	-0.750 ns	1.000	
IIB (H)	**-0.850**	-0.190 ns	-0.610 ns	-0.850 ns	0.580 ns	-0.080 ns	1.000

Table 2. Correlations between the total muscle concentrations of glycogen (GLY), lactate (LA) and pH in the gluteus medium muscle and the % of the three main fiber populations stained high (H), medium (M) and low (L) in 12 bulls after bullfighting (significant correlations are in bold; level of significance P<0.05; ns no significant).
Tabla 2. Correlaciones entre la concentración muscular de glucógeno (GLY), lactato (LA) y pH en el músculo glúteo medio y el % de las tres poblaciones fibrilares musculares con tinción alta (H), media (M) y baja (L) en glucógeno en 12 toros bravos tras la lidia (correlaciones significativas en negrita; nivel de significación P<0.05; ns no significativo).

	GLY	*LA*	*pH*
I (H)	-0.720 ns	-0.080 ns	-0.340 ns
I (M)	-0.200 ns	-0.060 ns	**-0.870**
I (L)	-0.600 ns	-0.290 ns	0.010 ns
IIA (H)	-0.720 ns	-0.080 ns	-0.340 ns
IIA (M)	0.750 ns	0.610 ns	-0.330 ns
IIA (L)	-0.130 ns	-0.160 ns	0.140 ns
IIB (H)	**0.860**	0.160 ns	0.320 ns

assumed that only theses fibers were recruited during bullfighting. Since the excitability threshold for fiber recruitment increases progressively from type I to type IIA to type IIB, it is physiologically improbable that type IIB fibers recruitment occurred without type I recruitment. However, the type I fibers could have been more dependent on blood glucose and free fatty acids during exercise.

In the present study, the influence of psychological stress has been shown by significant correlations between % of type I stained (L) and % of type IIA fibers stained (M). Furthermore, muscle GLY concentrations were mainly dependent on the content of GLY in type IIB fibers. In order to quantify the influence of psychological factors on muscle GLY breakdown, a comparative study between GLY depletion pattern in postural and locomotor muscles is required.

In summary, muscle GLY depletion in bullfighting bulls showed the influence of both exercise and psychological stress. GLY breakdown occurred mainly by glycolytic pathways, with LA formation and muscle acidosis. During maximal exercises, the loss of muscle functionality and the disruption of muscle fibers (rhabdomyolysis) can be linked to the lack of energy precursors for ADP rephosphorilation and muscle acidosis. Therefore, the control of stressful procedures during transport and handling of bullfighting bulls are strongly encouraged. Thus, bovine muscle could dispose of more GLY during bullfighting, although enhanced respiratory, cardiovascular and muscular capacities could be reached by physical training in order to improve physical performance.

Figure 1. Glycogen depletion pattern in the gluteus medium muscle in 12 bulls after bullfighting (■high glycogen; ▒ medium glycogen; □ low or empty glycogen).

Figura 1. Patrón de depleción glucogénica en el músculo glúteo medio de 12 toros bravos tras la lidia (■concentración alta de glucógeno; ▒ concentración media de glucógeno; □ concentración baja de glucógeno).

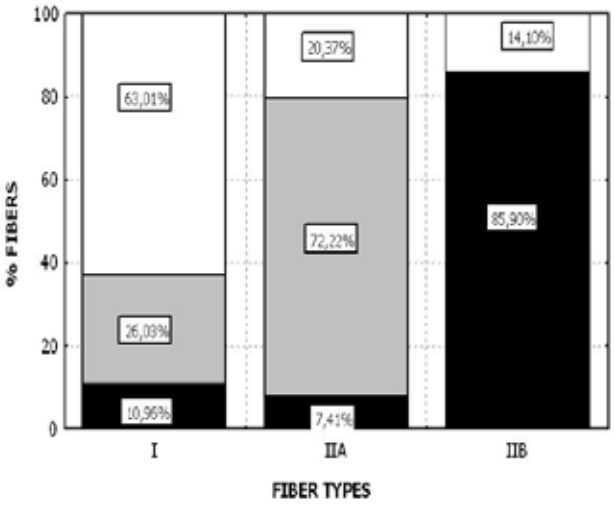

Table 3. Correlations between the total muscle concentrations of glycogen (GLY), lactate (LA) and pH in the gluteus medium muscle in 12 bulls after bullfighting (ns: no significant).

Tabla 3. Correlaciones entre las concentraciones musculares de glucógeno (GLY), lactato (LA) y pH en el músculo glúteo medio en 12 toros bravos tras la lidia (ns: no significativo).

	GLY	*LA*	*pH*
GLY	1.000	0.590 ^{ns}	-0.290 ^{ns}
LA		1.000	-0.490 ^{ns}
pH			1.000

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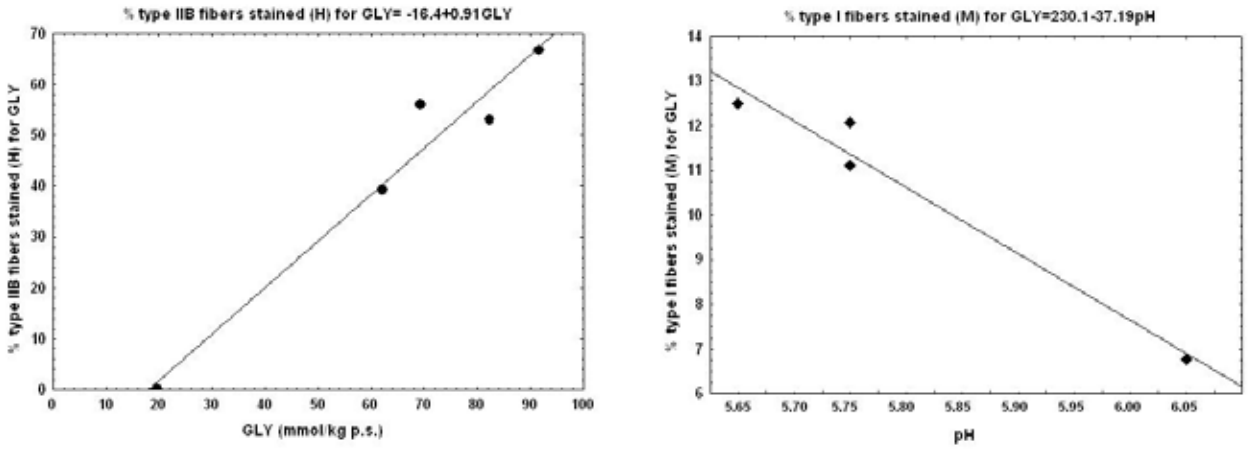
Figure 2. Regression analysis of the % of type IIB fibers stained high (H) in glycogen and muscle glycogen concentration and the % of type I fibers stained medium (M) in glycogen and muscle pH in the gluteus medium muscle of 12 bulls after bullfighting.

Figura 2. Análisis de regresión del % de fibras tipo IIB con tinción alta (H) para glucógeno y concentración muscular de glucógeno y % de fibras tipo I con tinción media (M) para glucógeno y pH muscular en 12 toros bravos tras la lidia.



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