FAST AND SLOW MYOSINS AS MARKERS OF MUSCLE INJURY

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Key words: muscle injury, serum muscle markers, fast myosin, slow myosin.

Word count: 3000
ABSTRACT

**Objective:** The diagnostic of muscular lesions suffered by athletes is done through a clinical diagnosis accompanied by confirmation tests using imaging techniques on the lesion (ultrasonography and/or magnetic resonance) as well as laboratory techniques for blood analysis that detect usually the presence of non-specific muscle markers. The aim of the study is the evaluation of fast and slow-twitch fibres injury using specific muscle markers for those fibres.

**Methods:** Blood samples were obtained from 51 non-sport people and 38 sportsmen with skeletal muscle injury. Western blood was performed to determine fast and slow myosin and creatin kinase (CK). Skeletal muscle damage was diagnosed according to physical examination, ultrasonography and magnetic resonance and biochemical markers criterion.

**Results:** The imaging tests have been shown to be excellent for the detection and confirmation of Grade II and III lesions. However, Grade I lesions often remain unconfirmed by these techniques. Grade I lesions show higher fast myosin than slow myosin with very low CK increase. Grade II and III show high values of both myosin.

**Conclusions:** The evaluation of fast and slow myosin in the blood 48 hours after the lesion occurs has been seen to be a good parameter for the detection of Type I lesions specially, based on the fact that fast myosin is an exclusive skeletal muscle marker. The correct diagnosis of Grade I lesions can promote prevention of the injury’s progression in athletes undergoing continual training sessions and competitions, thus aiding sports physicians in their decisions.
Key terms for indexing purpose: muscle injury, serum muscle markers, fast myosin, slow myosin
INTRODUCTION

Muscle is sensitive to the protocols of contraction and work to which it is submitted, since its structure is prepared to support these protocols and to adapt to new situations of force. However, if integrity is affected, to a greater or lesser extent, by overload, with tears occurring which we call muscle lesions. These lesions can, again to a greater or lesser degree, produce incapacity to continue exertion of the force. Extenuating unaccustomed exercise and high-force eccentric action leads to skeletal muscle damage, with changes in muscle structure and function. It induces damage to muscle fibre membranes [1, 2] myofibrillar disruption [3] and sarcoplasmic reticulum vacuolization [4].

Such exercise-induced muscle damage activates a cascade of reactions that result in an activated skeletal muscle protein metabolism. The protease calpain is activated immediately after exercise. Calpain initiates the metabolic turnover of myofibrillar proteins by releasing them from their filamentous structure [5]. Although calpain does not degrade actine and myosin it contributes to their release [6]. This allows the detection of such proteins in peripheral blood after cleavage, using specific assays such as troponin I (TnI) and myosin heavy chains (MHC) [7,8]. Sorichter et al. [9] exposed the features of an ideal marker of skeletal muscle fibre injury. One of these was that the marker should be absolutely muscle-fibre specific to allow reliable diagnosis of skeletal fibre type injury. None of the markers analyzed by these authors is muscle-type specific. The markers habitually used, such as creatin kinase, heart-Fatty Acid Binding Protein (h-FABP), myoglobin (Mb), TnI or α-actin [10], in addition to not being totally specific for skeletal muscle, reach a maximum value before 10 hours have elapsed after the origin of the lesion and decrease considerably before 24 hours have elapsed after the stress situation. The greater part of lesions is produced on holidays, so that it is very easy for these 10-12 critical hours for carrying out analysis of the patient to have elapsed. Often, the recently produced lesions are not accompanied by pain, and a day later can be enough for the markers of low molecular weight to have degraded and left no trace in the serum. The troponins are proteins that are very specific in terms of fibre type, being of low molecular weight but susceptible of being rapidly proteolyzed, which may be the reason why they have a very short half-life in blood [11].

Skeletal muscle is a tissue with a heterogeneity of fibers types, type I and type II, the proportion of which varies with the type of muscle and even within the different regions of the type of muscle [12]. Some of the contractile proteins present different isoforms according with the type of fiber. One of these is myosin, which can present different heavy and light isoforms, according to whether the fiber type is fast or slow [13].

Myosin presents an ideal profile as a parameter to study and is directly assignable to the grade of the lesion since, due to its high molecular weight, its appearance in blood can only be explained by a fibre lesion. Fast myosin is only characteristic of fast skeletal muscle, while slow myosin is common to skeletal and cardiac muscle. The maximum value of slow myosin in blood has been measured by Schiaffino and Reggiani [14] and presents its maximum 48 and 72 hours after the lesion.
The aim of the present paper is to evaluate muscle lesions, using as markers fast and slow myosins present in the serum of athletes 48 hours after having suffered a lesion. The efficiency of this marker will be compared with the detection of the lesion by ultrasonography (US), magnetic resonance (MR) and other traditional serum markers.
RESULTS

1. MR Images:

Figure 1 shows three MR images of different grades of muscle lesion. 1A): Grade I lesion in the upper part of the posterior side of the right thigh. We observe, in the axial image, a zone of muscle oedema (signal increase) transduced by the recent biceps femoris tear (arrows). 1B): Grade II lesion in the medial-distal part of the posterior side of the left thigh. We observe, in the axial image, an area of oedema (signal increase) and fibrillar defect transduced by the recent biceps femoris lesion (long head) (arrows). 1C): Grade III lesion in the distal part of the anterior side of the thigh. We observe, in the coronal image, a large area of oedema (signal increase) and extensive fibrillar defect of the rectus femoris, in the distal part (arrows).

2. US images

Figure 2 shows the ultrasonographic correspondence of the lesions shown in Figure 1. 2A): Grade I lesion. The US shows, in transverse section, the area of fibrillar defect located between the biceps femoris and the semitendinous. 2B): Grade II lesion. The US shows, in transverse section, the most extensive area of the fibrillar defect and haematic suffusion in the long head of the biceps femoris. 2C): Grade III lesion. The US shows, in longitudinal section, complete muscle defect of the rectus femoris (arrows).
3. Clinical diagnosis, marker enzymes, and myosin in normal and injured muscles

<table>
<thead>
<tr>
<th>Diagnostic</th>
<th>Number of samples</th>
<th>US</th>
<th>MR</th>
<th>CK (U/l)</th>
<th>MYOSIN (µg/l)</th>
<th>Fast/Slow ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>51</td>
<td>----</td>
<td>----</td>
<td>102±8</td>
<td>625±62</td>
<td>1535±166</td>
</tr>
<tr>
<td>Grade I</td>
<td>12</td>
<td>(-) o (+)</td>
<td>----</td>
<td>202±22</td>
<td>2880±159</td>
<td>1281±197</td>
</tr>
<tr>
<td>Grade II</td>
<td>16</td>
<td>++</td>
<td>++</td>
<td>482±47</td>
<td>3432±402</td>
<td>3722±700</td>
</tr>
<tr>
<td>Grade III</td>
<td>10</td>
<td>+++</td>
<td>+++</td>
<td>739±245</td>
<td>8055±2200</td>
<td>6518±124</td>
</tr>
</tbody>
</table>

TABLE I. Comparison of the results obtained by different technologies of muscle injuries diagnostic.
Data are expressed as means ± SEM. Statistical Anova showed to be extremely significant for every parameter (columns). P< 0.001

Table I shows the normal values of the sera of the control group of athletes and of the athletes with different grades of muscle lesions. Our results indicate that in the normal state the concentration of fast and slow myosin in blood did not surpass 1000 µg/ml of fast myosins and 2000 of slow myosins, showing a fast/slow ratio of 0.3. The patients diagnosed with Grade I lesions, which are not imaged by US or MR, showed high levels of fast myosin (greater than the slow myosins), showing a fast/slow ratio greater than 2. The CKs were found practically within the limits of normality. In the Grade II and III lesions, which were diagnosed by US and MR, an increase in both fast and slow myosins was observed, although a fast/slow ratio near 1 was exhibited. The concentration of slow myosins in comparison with fast myosins increased in direct proportion with the seriousness of the lesion. The CKs also showed an increase in the same direction as the muscle lesion, increasing in the same way as the slow myosins, showing itself to be a very good marker for type II and type III lesions, especially.
DISCUSSION

The different human muscles are made up of a mixture of slow and fast fibres, approaching 50%, unlike what occurs in some animals who have muscles with 90% of only fast fibres and others with 90% of only slow fibres. Concretely, the vastus lateralis of young athletes between the ages of 15 and 18 years and of caucasian race have 36.5% of slow type fibres and 63.5% of fast type fibres, and of these, 52.3% are type IIa, 8.1% type IIb and 3.1% of type IIc. [18] The existence of mixed muscles in humans makes lesions the cause of the entrance of slow and fast myosins into blood. However, due to the fact that resistance to lesions and to fatigue in the two types of fibres is not the same, slow or fast myosin heavy chain (MHC) could be found in blood according to the type of lesioned fibres. In general, the rapid fibres are more easily fatigued and more sensitive to the lesion. Therefore, we can expect that the fast fibres that tire more rapidly release fast MHC before the slow fibres in the face of less intense exercises. The slow MHCs will flow under more fatiguing conditions and probably their presence in blood will be the expression of a more important lesion.

On the other hand, the presence of fast MHC in blood signals the fact that only skeletal muscle is affected and thus constitutes an absolutely specific marker. The presence of slow MHC could indicate the presence of lesion in skeletal and/or heart muscle. However, given the fact that the patient types undergoing the test are athletes in which a cardiac lesion is ruled out, the detection of slow MHC in blood would respond as a slow fibre lesion marker, with the consequences that this information would be able to contribute.

We have developed a method to detect myosins in blood based on specific recognition by fast and slow myosin antibodies. We studied a group of athletes who practice different sports and who presented for consultation with muscle pains. A medical examination was given to these patients, as well as an US, an MR, and certain blood analyses which included evaluation of the presence of CK used as a usual muscle marker, as well as slow and fast myosins. We also studied 51 people who did not practice any sport either sporadically or for pleasure and who were catalogued as normal.

Thus, as shown, 48 hours after the onset of the muscle problem, the CK activity show a small increase in the Grade I lesions. Only fast myosin is a marker with very high values suggesting that Grade I lesions are mainly produced in Tipe II fibres. The results of the US and MR in the Grade I lesions are confused/ambiguous in some cases, yielding negative or positive results that fail to assure total recognition of the lesion. The US and MR techniques in Grade II and Grade III lesions are highly effective, since they detect the lesions via images.

In the Grade II lesions, the serum markers show an increases in CK activity, with the greatest increases being those of both types of myosin, which can reach as much as 10 times the normal value (in some case). The Grade III lesions are well detected by the CK activity, and also by both types of myosin.
Our conclusions are that the use of fast myosin provides a highly sensitive marker for Grade I lesions, at least equal to the MR sounding and greater than US and clinical diagnosis. On the other hand, fast myosin is a totally specific marker for skeletal muscle, a property not shared by any of the markers in use today, such as CK and myoglobins. Myosin also presents the advantage of being more sensitive and more stable in blood, since it presents a maximum 48 hours after the lesion and lasts longer, so that it is easier to use it in diagnoses not carried out at that moment, and it can be used as a parameter to follow the evolution of the muscle lesion. Therefore, we conclude that the determination of fast and slow myosins is a good system to aid the diagnostic of muscle lesions, especially for those that are difficult to detect by other procedures.

ACKNOWLEDGEMENTS

The authors wish to express their thanks to the volunteers who participated. We also thank the CEARE and the IDIBAPS of the Faculty of Medicine for their cooperation. The study was financed mainly by two grants from the Consell Català de l’Esport (Catalan Sports Council of the Generalitat of Catalunya) and a small collaboration from the Consejo Superior de Deportes (Higher Council for Sports of the MEC) and from the FISS of the Instituto Carlos III (Red de Centros RCMN-C03708).
REFERENCES

FIGURES

Figure 1
Magnetic resonances of different grades of muscle lesion:
A) Grade I lesion. B) Grade II lesion. C) Grade III lesion.

Figure 2
Ultrasonography of different grades of muscle lesion:
A) Grade I lesion. B) Grade II lesion. C) Grade III lesion.
"What is already known on this topic?"

Skeletal muscle has different myosin isoforms depending on the type of fibres that compose every muscle. The contractile properties of these fibers depend on the existence and combination of such different isoforms of myosin, tropomyosin, troponins and other proteins.

Up to now, only myosin and troponin I from slow-twitch muscle have been used as a marker of muscle injury. However these measures only give information about slow-twitch muscle injuries.

The use of ß-actine as an injury marker gives only information about any skeletal and heart muscle fibre injuries, as creatine kinase do.

"What this study adds"

The analysis of myosin from fast and slow-twitch fibers in serum, gives information about the injuries in every type of fibre. For the first time it is described a method to perform the evaluation. The study adds information about the importance of that measure compared to other methods (ecography and RMN) and the grade of muscle injury.

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Br J Sports Med published online December 10, 2007

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doi: 10.1136/bjsm.2007.037945corr1

There was an error in the article by Guerrero et al published in the July issue of the journal (Guerrero M, Guiu-Comadevall M, Cadefau JA, et al. Fast and slow myosins as markers of muscle injury. Br J Sports Med 2008;42:581–4). Table 1 was omitted from the article. The table is reproduced online at http://bjsm.bmj.com/supplemental/.