A New Method for Non-invasive Estimation of Human Muscle Fibre Type Composition in Athletics

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29:2; 7-16, 2014

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ABSTRACT

The composition of an individual's muscles is a talent predictor in athletics. However, the invasive technique of muscle biopsy, the accepted means for determining muscle fibre population, is unsuitable for talent identification. Non-invasive possibilities tested to date are also unsatisfactory. Recently, proton magnetic resonance spectroscopy (^{1}H-MRS) has been used to measure muscle metabolites including carnosine, which is present in different concentrations in type-I (slow twitch) and type-II (fast twitch) fibres. The authors aimed to determine if this means is suitable for estimating muscle fibre composition. Carnosine levels measured by ^{1}H-MRS in the gastrocnemius muscles of athletes and control subjects and then compared to untrained subjects who underwent both ^{1}H-MRS and muscle biopsies significantly correlated to the area occupied by type II fibres in explosive event athletes, with endurance athletes registering lower levels compared to the reference population. Similar trends were found in both young and ex-athletes. The authors conclude that the method is valuable for estimating muscle fibre composition but point out its limitations, including cost. This article has been adapted from a manuscript previously published by the Public Library of Science.

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Introduction

An important element of talent for specific athletics disciplines is undoubtedly the fibres of which a muscle is composed. In humans, skeletal muscle fibres exist in two main categories: the fatigue-resistant slow-twitch (ST) or type-I fibres, and the fatigue-sensitive fast-twitch (FT) or type-II fibres[1]. Classical papers from the 1970s[2,3] established that excellence in sports with short and long exercise durations requires a high proportion of FT and ST muscle fibres, respectively. With humans there is an ongoing nature versus nurture debate about whether a fibre can be modified into another type. However,

the effect of specific types of exercise training on the transition between ST and FT fibre populations is probably limited[4]. Therefore, measurement of the muscle fibre type composition can be a tool for talent identification and for defining an athlete's optimal exercise duration in athletics and many other sports. Because of the invasive nature and high sampling variance of the muscle biopsy method, a non-invasive alternative to measure muscle fibre type composition would be useful.

Several attempts have been made to determine muscle fibre type composition in a non-invasive way in resting muscles: magnetic resonance imaging (MRI)[5-7], phosphorus magnetic resonance spectroscopy (^{31}P-MRS)[8] or tensiomyography (TMG)[9] (contracting muscles). These results were either equivocal or in the case of contracting muscles, too dependent on training status and fatigue. Consequently, this suggests that MRI, ^{31}P-MRS and TMG are less suitable to reliably estimate muscle fibre type composition.

Recently, ^{1}H-MRS has been used to measure muscle metabolites, such as intra- (IMCL)[10] and extra-myocellular lipids (EMCL)[10], trimethylamonium (TMA)[11] and carnosine[12]. Two important requirements to use a metabolite for the estimation of muscle fibre composition, are that the concentrations are markedly different between type-I and II fibres, and are largely independent of extrinsic factors such as diet and training. With this in mind, carnosine seemed to be a good candidate.

The dipeptide carnosine is present in high concentrations and is a relatively stable characteristic of human skeletal muscle (approximately 10% variation over a three-month period[13] and a high resemblance between dizygotic and especially monozygotic twins[14]). Only high-dose beta-alanine supplementation for several weeks can change the muscle carnosine content[12]. Short-term exercise training has little or no impact on muscle carnosine levels[15-18]. However, muscle fibre type is a major determinant of carnosine levels with FT fibres containing twice as much carnosine as ST fibres[19,20], explaining why marathon runners have low muscle carnosine content[21]. In untrained subjects, positive correlations have been found between FT fibre proportion and carnosine content, using muscle biopsies[22,23].

The aim of the current study was to develop a new and non-invasive estimation method of fibre type composition in human muscles, based on proton magnetic resonance spectroscopy (^{1}H-MRS) measurement of muscle carnosine content.

Methods

Subjects

A total of 262 subjects volunteered to participate in this cross-sectional study. The study population consisted of 170 controls (80 males and 90 females) and 92 athletes (76 males and 16 females). None of the subjects were vegetarian or had taken beta-alanine in the three months prior to the start of the study. All subjects gave their written informed consent with the study being approved by the local ethics committee (Ghent University Hospital, Belgium).

The reference population was physically active, but not involved in competitive sport or organised training.

The athletes were divided in three subgroups: 1) 14 talented young male track-and-field athletes, 2) 64 active elite athletes (48 males, 16 females) and 3) 14 male ex-athletes. All active and ex-athletes (groups 2 and 3) were or had been competing at an international level, with 19 winning a medal at the Olympics, World or European Championships. The active elite athletes were recruited from triathlon (n=6) and track-and-field (n=71). The track-and-field athletes were assigned to one of the following disciplines; 100-200m, 400m, 800m, 1500m, 3000m-marathon, jumps, throws, decathlon, using the IAAF scoring tables of athletics[24]. The young talented (n=14) and former athletes (n=14) were divided into an explosive and endurance group.

Muscle fibre typing

Muscle biopsies were taken at rest from the gastrocnemius of 12 males of the reference group, with a 14 Gauge true-cut biopsy needle (Bard Magnum Biopsy gun; Bard, Inc., New Jersey, USA). With use of an ultrasonograph for guidance (Ultrasonography Pro Sound SSD-5000, ALOKA Co., Ltd., Tokyo, Japan. with probe UST-5545, frequency 5-13MHZ), three muscle samples were taken following local anaesthesia (lidocaine 1%, Linisol®). The samples were frozen in nitrogen-cooled isopentane and embedded in Tissue-Tek for immunohistochemical analysis. The samples were stained for myosin heavy chain isoforms and analysed according to DeBOCK et al[25].

Muscle carnosine content

The carnosine content of the gastrocnemius muscle of all 262 subjects was measured with a proton magnetic resonance spectroscopy (^{1}H-MRS), as previously described[26]. With the subjects lying in the supine position the lower leg was fixed in a holder with the angle of the ankle at 20° plantar flexion. All the MRS measurements were performed with a 3 Tesla whole body MRI scanner (Siemens Trio, Erlangen) equipped with a spherical knee-coil. Single voxel point-resolved spectroscopy[1] sequence with the following parameters was used: repetition time (TR)= 2,000ms, echo time (TE) = 30ms, number of excitations = 128, 1,024 data points, spectral bandwidth of 1.200Hz, and a total acquisition time of 4.24 min. The absolute carnosine content (in millimolar; mM) was calculated as described by BAGUET et al[26].

Previous studies showed a variation coefficient of gastrocnemius carnosine content over a six-week period of 11.9% in untrained[13] and 13.2% in trained subjects[12,26]. Given the higher carnosine concentrations in men compared with women[27], Z-scores were used, instead of absolute values. The Z-scores for both genders were calculated using the mean and standard deviation of the reference population.

Statistical Analysis

The correlation in Figure 2 was evaluated by a Pearson correlation. Independent sample T-tests were used to compare the muscle carnosine content between explosive and endurance

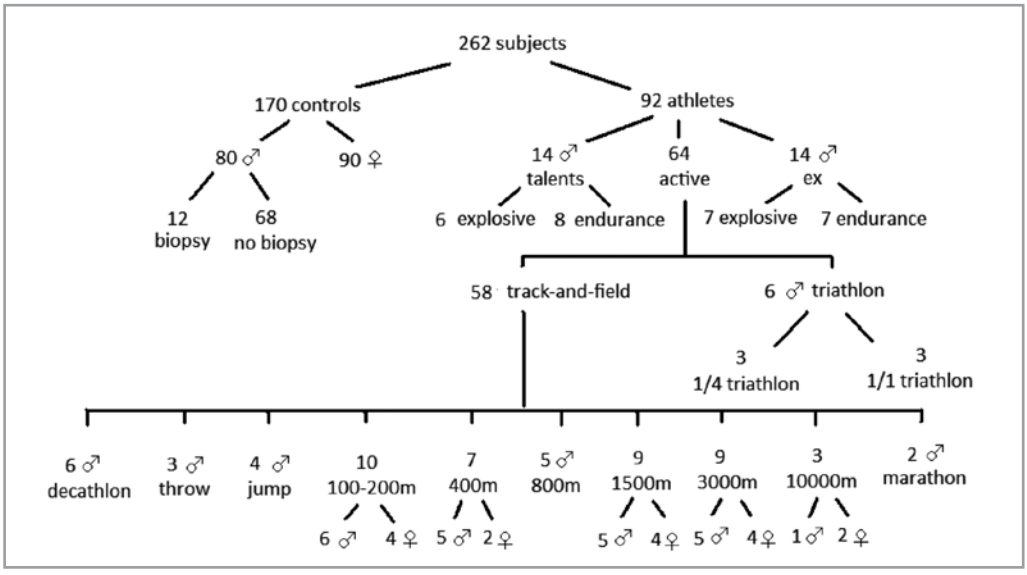

Figure 1: Overview of subject population (Numbers of subjects per group and by gender are shown. Muscle carnosine content was measured in all 262 subjects and muscle biopsies were taken from 12 untrained males.)

athletes in the talents, elites and ex-athletes (SPSS statistical software, SPSS 17.0, Chicago, IL). Values are presented as means ± SD with significance assumed at $p \leq 0.05$. The sigmoidal curve was designed with SigmaPlot 11 (Systat Software Inc.).

Results

Relationship between muscle carnosine content and fast-twitch fibre area

In the 12 untrained subjects, the biopsy-determined percentage of fast twitch fibres ranged between 29 and 62%. Figure 2 shows a strong positive correlation ($p=0.009$ and $r=0.714$) between ^{1}H-MRS-based carnosine concentration, expressed in Z-scores, and the percentage of fast twitch fibres in gastrocnemius muscle.

Muscle carnosine content in active elite athletes

Within the elite athletes, muscle carnosine measured with ^{1}H-MRS was ~25% higher ($p<0.001$) in the explosive athletes (i.e. sprinters) than the reference untrained population. In turn, carnosine was ~35% lower ($p<0.001$) than normal in typical endurance athletes (i.e., 3000m to marathon runners and triathletes) (Figure 3). Sprinters (100-400m) had, on average, a 1.9-fold higher carnosine content than marathon runners and triathletes ($p< 0.001$). All 100m-400m runners had a higher muscle carnosine content than the population mean and all of the triathletes and marathon runners had a lower carnosine concentration than the average of the reference population. Athletes competing in disciplines requiring both sprint and endurance capacities, such as decathletes, showed intermediate carnosine levels. We observed the same pattern in female athletes (data not shown), although absolute carnosine concentrations were consistently lower in women than in men, in agreement with previous reports[27]. Figure 4 displays all male and female active elite runners (n=45) ranked according to their best running distance. Interestingly, a negative sigmoidal ($R^2=0.9810$), rather than linear relation was found between the logarithm of the best running distance and muscle carnosine content, expressed in Z-scores.

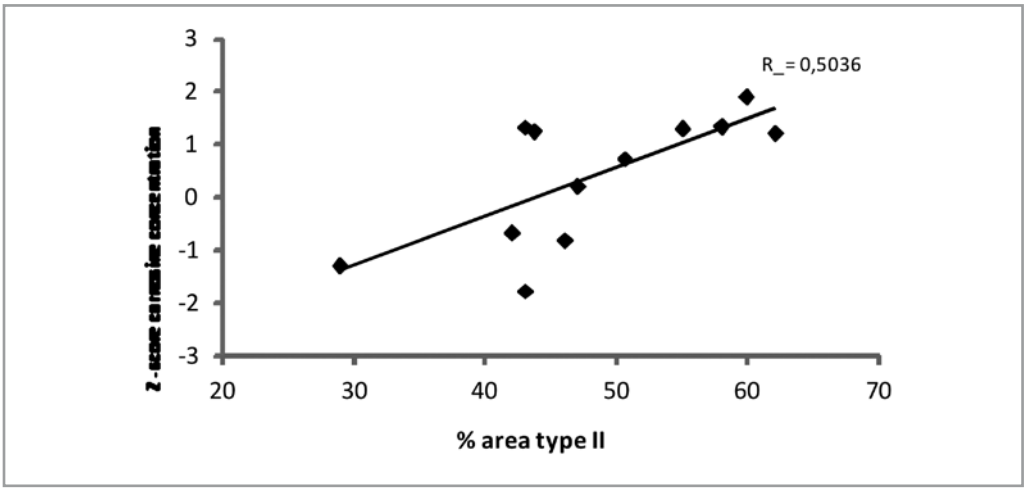

Figure 2: Correlation between muscle carnosine content and percentage area occupied by type-II fibres in 12 untrained subjects (The X-axis displays the percentage of the total area occupied by type-II fibres. The muscle carnosine content (expressed in Z-scores) is shown on the Y-axis. A significant positive correlation between muscle carnosine content and percentage area occupied by type-II fibres is demonstrated.)

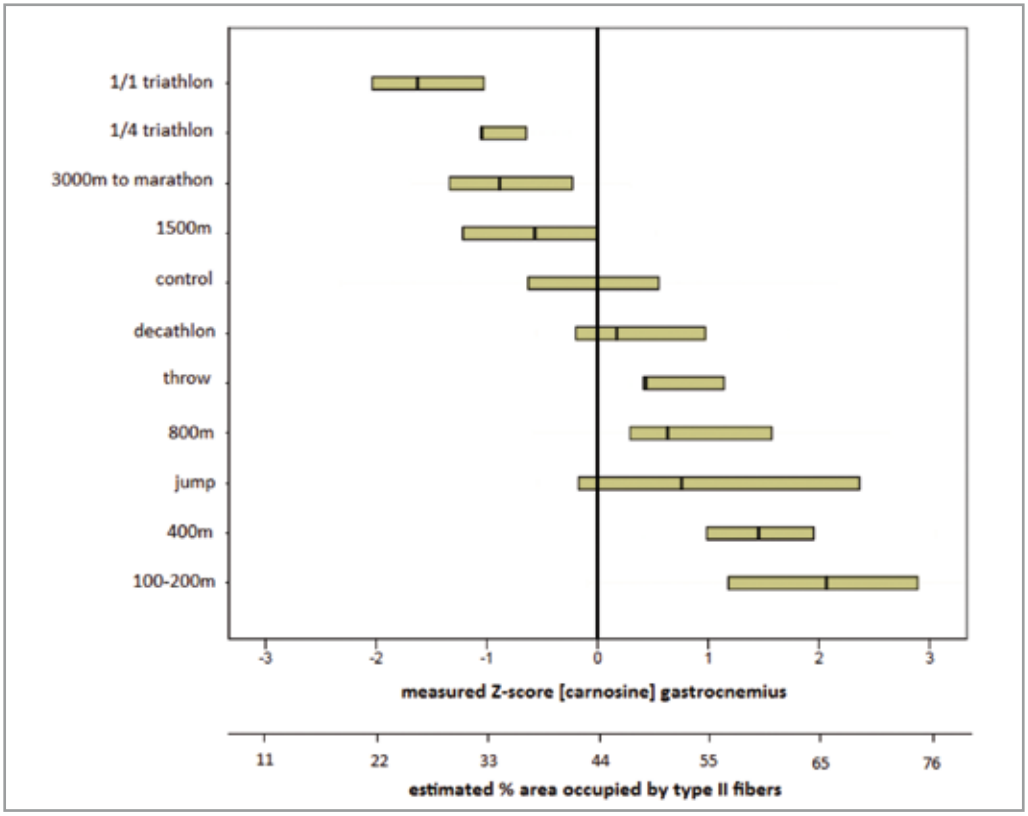

Figure 3: Carnosine content of gastrocnemius muscle in track-and field athletes and triathletes compared to an untrained control population (Muscle carnosine content in various small groups of male elite athletes (n= 64) and in a the male control population (n=170) ranked from low to high. Numbers per group are given in Figure 1. The primary X-axis shows the measured carnosine content (expressed in Z-scores), while the secondary X-axis displays the estimated percentage area occupied by type II fibres (derived from Figure 2). The vertical line represents the median of the control population. The medians (small vertical lines) and first and third quartile are shown by group.)

Muscle carnosine content in young and former athletes

The muscle carnosine concentration remained significantly different between ex-sprinters (n=7) and ex-endurance athletes (n=7) (p=0.01), who had discontinued training for many years. Moreover, a similar difference (p=0.03) was observed in young talents between explosive (n=6) and endurance (n=8) athletes (figure 5).

Discussion

In order to develop a new non-invasive method to estimate muscle fibre composition in humans, muscle carnosine content of 92 Belgian track-and-field athletes and 170 controls was measured using ^{1}H-MRS. Sprint athletes (100m-400m) all exhibited higher carnosine levels since these distances require a higher percentage of fast-type muscle fibres[3,28], as opposed to endurance athletes (1500m-marathon), who expressed a lower carnosine concentration. This is consistent

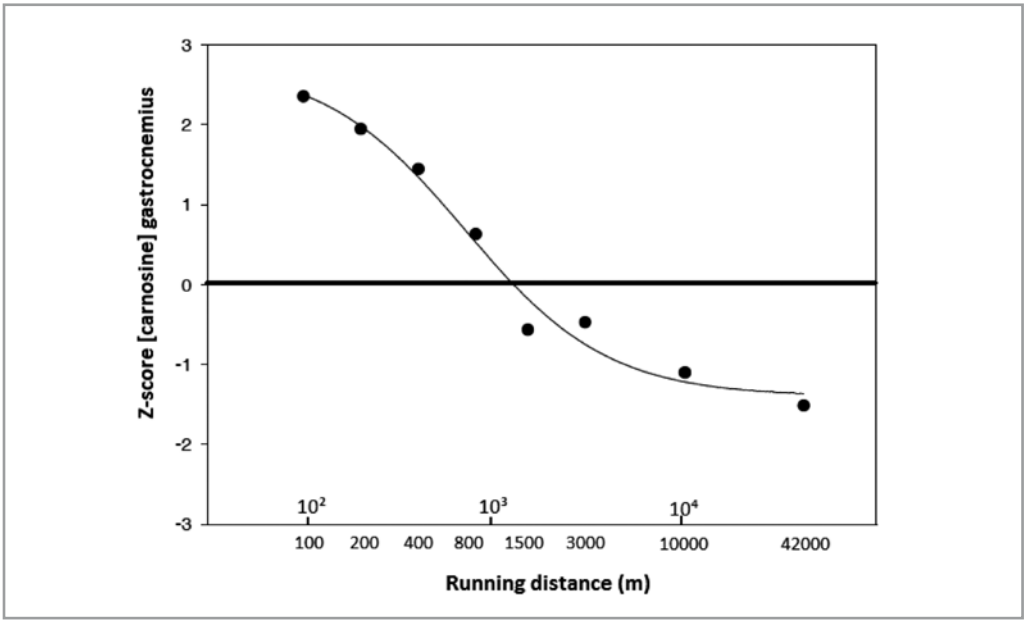

Figure 4: Carnosine content of the gastrocnemius muscle in male and female active elite runners, according to their best running distance (All male (n=29) and female (n=16) elite runners were ranked according to their best running distance (using the IAAF scoring tables). The X-axis displays running distance (m) in a logarithmic fashion and the Y-axis shows the carnosine content, expressed in Z-scores. A Y-value of zero corresponds to the average of the male/female reference population. The median Z-score per group is shown and the best sigmoidal fit is presented (R^2=0.9810).)

since these events require a higher percentage of slow twitch fibres[3,28]. It is interesting to note that the 800m runners' carnosine levels where roughly between the sprint and endurance athletes, as indicated by the steepest part of the sigmoidal curve (midpoint ~1000m).

Of the explosive athletes, the 100m sprinters had a mean Z-score of +2.28. This means that the odds of finding a person in the general Belgian population with this fibre typology is approximately 1 in 100. When taking into account the many factors that define sprint talent (anthropometry, trainability, reaction time, etc.), this illustrates why talent detection and identification is very important and at the same time very difficult.

Higher carnosine content in sprinters is not an acute response to intensive training, but rather a reflection of the predominant percentage of fast twitch muscle fibre type. This observation is supported since a significant difference of muscle carnosine concentration was seen when comparing ex-sprinters and ex-endurance athletes. Moreover, a similar significant difference also existed when comparing young (14-18 years old) sprinters to endurance athletes. These young athletes are still at the start of their career and their accumulated training history is several thousands of hours less than their elite adult colleagues, suggesting that the muscle carnosine content is probably largely genetically determined (Figure 5), as is the muscle fibre type composition. Indeed, in 2012 BAGUET et al found higher correlations in muscle carnosine in monozygotic (r=0.86) compared to dizygotic (r=0.51) twins[14] . Therefore, we believe that this new method may prove useful in the identification of talents in sports where muscle fibre type composition is a determining factor.

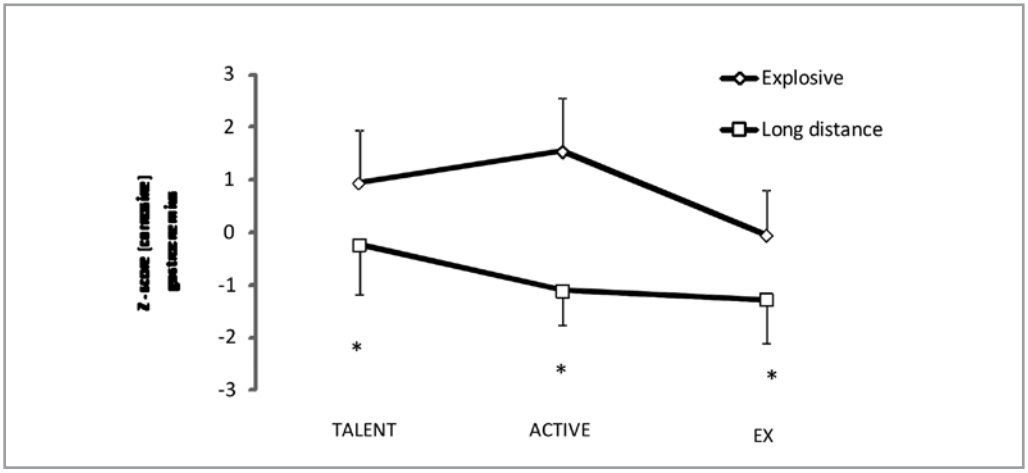

*Figure 5: Comparison of the gastrocnemius carnosine content in young talented male athletes (n=14), active elite athletes (n=19) and ex-athletes (n=14) (The Y-axis shows the carnosine content (expressed in Z-scores). The diamonds represent explosive athletes (talents n= 6; active n= 12 ; ex n= 7) and the squares represent endurance runners (talents n= 8; active n=14; ex n=7). Data are shown as means ± standard deviation. *Different from explosive athletes (p≤ 0.05).)*

VAN DAMME et al[29] explored the performance constraints in elite decathletes and concluded that performances on different sub-disciplines, like the 100m and 1500m, correlate negatively, partly because of the conflicting muscle fibre type requirements. Moreover, excellence in a particular discipline (specialist) is detrimental for overall decathlon performance (generalist). Our findings seem to agree with both of these points. With respect to muscle carnosine, disciplines like the 100m and 1500m indeed have antagonistic requirements (Figure 4). Additionally, the six elite decathletes we measured (Figure 3) had intermediate carnosine levels within a relatively narrow range, suggesting that they were all generalists, rather than specialists.

This novel method provides a non-invasive estimation of human muscle fibre type composition. Important advantages of this method are;

- it does not induce damage to the muscle like the biopsy method,
- it can be used outside of a laboratory,
- it is infinitely repeatable and applicable to special populations (i.e. elite athletes).

In turn, besides the muscle damage caused, a disadvantage to biopsies is the fact that their results are not very representative. Typically, when a biopsy is taken the fibre typing is done on a tissue sample representing less than 0.01% of the total muscle mass[30] that contains only a couple of hundred fibres and even fewer motor units. Therefore, a single biopsy, is not an ideal estimator of the whole muscles fibre type distribution[31,32] and multiple biopsies are required to adequately estimate the muscle fibre type distribution[33,34].

The current NMR-based ^{1}H-MRS method typically samples 10-15ml or grams of muscle, including both superficial and deep parts of the muscle representing approximately 5% of the entire muscle. MRS-based carnosine quantification has a relatively good repeatability in both untrained[13] and trained[12,26] humans. Another advantage compared to the biopsy method, is that the MRS technique is not labor intensive; the scanning and analysis are performed in 30 min or less (effective scan time ~20 min). The MRS-based technique therefore tackles most of the disadvantages of the biop-

sy method that have kept the technique from evolving from a research tool towards a routine screening method for predicting, and steering athletic success[35].

A potential weakness of this current method is that it is based on indirect estimation through quantification of a single metabolite, carnosine, which is a typical metabolite of FT fibres. Certain nutritional interventions such as beta-alanine supplementation[12,36] can influence the muscle carnosine content without altering the fibre type composition, which will disrupt the relationship between muscle fibre type composition and carnosine content. Beta-alanine supplementation in the three months preceding the test was therefore treated as an exclusion criterion in the present study.

Some important considerations on the practical use of this method are:

- it is not applicable prior to or during puberty, due to the influence of pubertal hormones on muscle carnosine[14],
- its dependence on the availability of a 3 Tesla NMR scanner,
- the rental of a NMR scanner is often expensive.

Conclusion

The use of ^{1}H-MRS based carnosine quantification is a valuable non-invasive approach to estimate muscle fibre type composition. This conclusion is based on the close level of agreement with the performance characteristics of various small groups of elite athletes. This fast and easy method may have useful applications in talent identification and sport discipline (re)orientation. More than 40 years after the initial discovery by GOLLNICK & SALTIN[3], documenting on the extremely large proportion of ST fibres in the muscles of 1972 Olympic marathon champion Frank Shorter (USA) and other truly elite distance runners, it seems that the important role of muscle fibre type composition in defining athletic success is ready to be translated into practical application in athletics.

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