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Cyclic movement frequency is associated with muscle typology in athletes

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There is a continuing research interest in the muscle fiber type composition (MFTC) of athletes. Recently, muscle carnosine quantification by proton magnetic resonance spectroscopy (1H-MRS) was developed as a new non-invasive method to estimate MFTC. This cross-sectional study aims to better understand estimated MFTC in relation to (a) different disciplines within one sport; (b) cyclic sport exercise characteristics; (c) within-athlete variability; and (d) athlete level. A total of 111 elite athletes (74 runners, 7 triathletes, 11 swimmers, 14 cyclists and 5 kayakers) and 188 controls were recruited to measure muscle carnosine in gastrocnemius and deltoid muscle by 1H-MRS. Within sport disciplines, athletes were divided into subgroups (sprint-, intermediate-, and endurance-type). The controls were used as reference population to allow expression of the athletes’ data as Z-scores. Within different sports, endurance-type athletes systematically showed the lowest Z-score compared to sprint-type athletes, with intermediate-type athletes always situated in between. Across the different sports disciplines, carnosine content showed the strongest significant correlation with cyclic movement frequency ($R = 0.86$, $P = 0.001$). Both within and between different cyclic sports, estimated MFTC was divergent between sprint- and endurance-type athletes. Cyclic movement frequency, rather than exercise duration came out as the most determining factor for the optimal estimated MFTC in elite athletes.

It is a classic and inherent aspect of exercise physiology: human skeletal muscle is not a homogenous tissue, but a combination (observed in variable proportions) of different cell types, namely fast-twitch (FT: type IIa and IIx) and slow-twitch (ST: type I) fibers. These skeletal muscle fiber types show a large diversity in physiological characteristics that define their contractile performance. Although cross-sectional area and maximal force ($P_0$) are not consistently found to differ between fiber types, FT have a much higher (5- to 10-fold) maximal shortening velocity, and consequently also peak power ($= force \times velocity$) as compared to ST fibers (Schiappino & Reggiani, 2011). On the other hand, ST fibers have the advantage that they are very resistant to fatigue. During repeated contractions, FT fibers fatigue within seconds to minutes at most, while ST fibers have been shown to keep contracting at initial force level almost indefinitely (hours to days), if fuel and oxygen remain provided (Stephenson et al., 1998).

Interestingly, there are large inter-individual differences in muscle fiber type composition (MFTC), as some individuals have skeletal muscles composed of merely 20–30% ST fibers and others of up to 95% ST fibers (Simoneau & Bouchard, 1989). Within an individual, one specific muscle (e.g., soleus) can contain more ST fibers than another muscle (e.g., triceps brachii). However, when an individual, based on MFTC of one muscle, is identified as slow (= i.e., having more ST fibers than population average), being “slow” will then hold true for all other muscles of that individual (Vikne et al., 2012). This finding is one of the elements contributing to the idea that MFTC is genetically determined. There is an ongoing debate on whether a fiber can modify into another type in humans due to training or detraining (Ingalls, 2004). Transition between IIa and IIx can certainly occur in humans, but transition between type I and II is much less documented in humans (Staron et al., 1990; Ingalls, 2004). Twin studies have suggested that at least half of the inter-individual variation in MFTC is genetically determined (Komi et al., 1977; Simoneau & Bouchard, 1995) and the first genetic polymorphisms that define MFTC have been identified (Vincent et al., 2007).

With MFTC having a large implication on human muscle contractility and performance, and as it is at
least by a considerable part genetically determined, an athlete’s MFTC has a major impact on almost every aspect of his/her sport scientific guidance, such as, training advice and talent identification. Indeed, classical studies (Gollnick et al., 1972; Tesch & Karlsson, 1985; Aagaard et al., 2011) investigated MFTC in athletes of different sport disciplines to determine the relationship with performance. These studies concluded that within a certain sport (e.g., track-and-field) in the disciplines with short exercise duration and high exercise intensity, athletes consistently display a high proportion of FT fibers, and athletes participating in disciplines with long exercise duration at low-intensity were characterized by a high percentage of ST fibers (Gollnick et al., 1972; Costill et al., 1976; Saltin et al., 1977; Tesch & Karlsson, 1985; Aagaard et al., 2011). Although there is a reasonable amount of information on track-and-field, many sports remain sparsely studied and the majority of studies report information on the vastus lateralis muscle only. Additionally, it has to be stated that these studies were usually performed on a limited number of athletes, on mediocre athletes, on athletes that were no longer competing, or on single subjects (case studies). The former two limitations also apply to the recent paper on a former World Champion sprinter (Trappe et al., 2015). These limitations have led to a fragmentary literature, which does not allow us to make accurate predictions nor applications in the field. We currently miss, for instance, information to define the optimal MFTC of a 50 m swimmer. Would it be similar to a 100–200 m runner (i.e., high FT proportion) because both exercise modes last less than half a minute? Or would it be more similar to a distance runner, because short-distance swimming resembles more distance running when considering movement frequency (steps or strokes per second) and muscle power?

A major reason for the fragmentary information in the literature is that for MFTC determination, the “gold standard” is the (immuno) histochemical evaluation of an invasive muscle biopsy, which produces a limitation to sample high numbers of high-level athletes during their active career. Recently, Baguet et al. (2011) developed a new non-invasive method to estimate MFTC, based on proton magnetic resonance spectroscopy (1H-MRS) measurement of muscle carnosine. Carnosine is a dipeptide present in high concentrations in human skeletal muscle and is typically present in FT fibers and only to a lesser extent in ST fibers (Harris et al., 2006; Blancquaert et al., 2015). Therefore, muscle carnosine content is positively related to the percentage area of FT fibers in that muscle (Baguet et al., 2011). A first evaluation showed a good validity of this technique in track-and-field athletes (Baguet et al., 2011).

We here present a large database of over 650 MRS-based muscles scans of arm and leg muscles of elite athletes in different cyclic sports (running, swimming, cycling, kayaking) and non-athletic controls. We first hypothesized that sprint- and endurance-type athletes have a clear distinction in estimated MFTC in the different cyclic sports. A second aim was to investigate the most determining factor (exercise duration or cyclic movement frequency) for the optimal estimated MFTC in elite athletes. Furthermore, the existence of an across-muscle phenotype was explored in control subjects and swimmers. In a last aim, we compared estimated MFTC of sprint and endurance runners to see whether a higher level of running is characterized by a more extreme estimated MFTC.

Materials and methods

Subjects

A total of 299 subjects volunteered to participate in this cross-sectional study. The study population consisted of 188 controls [98 males (average age: 22 ± 2 years) and 90 females (average age: 21 ± 2 years)]. Furthermore, 111 elite Belgian athletes (89 males and 22 females) were recruited. The controls were not specifically trained, but some of them took part in some form of recreational exercise. The athletes consisted of five subgroups: (a) 74 runners, (b) 7 triathletes, (c) 11 swimmers, (d) 14 cyclists, and (e) 5 kayakers. All athletes were or had been competing at national and/or international level. Table 1 shows some details about the level of the athletes. The 74 runners were assigned to one of the following disciplines: sprint-type (SPR-T) (100–400 m), intermediate-type (INT-T) (800 m), or endurance-type (END-T) (>1500 m), based on their highest score using the International Amateur Athletic Federation (IAAF) scoring tables of athletics. The 11 swimmers were divided into three subgroups: sprint-type (SPR-T) (50–100 m), intermediate-type (INT-T) (200 m), and endurance-type (END-T) (400–1500 m), based on their Fédération Internationale de Natation (FINA) points on their best swimming performances. The 14 cyclists were specialized in three different disciplines: track cycling (TRACK or SPR-T), road cycling

### Table 1. Number and level of athletes in the different sport disciplines. Women are represented between parenthesis

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>European level</th>
<th>World level</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Runners</strong></td>
<td>74 (21)</td>
<td>20</td>
<td>17</td>
</tr>
<tr>
<td>SPR-T</td>
<td>25 (7)</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>INT-T</td>
<td>11</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>END-T</td>
<td>38 (14)</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td><strong>Triathletes</strong></td>
<td>7</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td><strong>Swimmers</strong></td>
<td>11</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>SPR-T</td>
<td>4</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>INT-T</td>
<td>5</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>END-T</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Cyclists</strong></td>
<td>14 (1)</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td><strong>Kayakers</strong></td>
<td>5</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td><strong>Cyclists</strong></td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><strong>Road</strong></td>
<td>8</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td><strong>Climb</strong></td>
<td>5</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td><strong>Kayakers</strong></td>
<td>5</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>SPR-T</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>INT-T</td>
<td>4</td>
<td>3</td>
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</tbody>
</table>
Muscle carnosine quantification by ¹H-MRS

Muscle carnosine content was measured by proton magnetic resonance spectroscopy (¹H-MRS) in soleus and gastrocnemius medialis muscles in all 299 subjects. In 47 control subjects, 11 swimmers and 5 kayakers, carnosine content was additionally measured in deltoid muscle. All the MRS measurements were performed on a 3-T whole body MRI scanner (Siemens Trio, Erlangen), as described by Baguet et al. (2010). The subjects were lying in supine position. To measure the calf muscles, the lower leg was fixed in a spherical knee-coil, while a shoulder coil was used to measure carnosine in the deltoid muscle. Single voxel point-resolved spectroscopy (PRESS) sequence with the following parameters was used; repetition time (TR) of 2000 ms, echo time (TE) of 30 ms, number of excitations is 128, 1024 data points, spectral bandwidth of 1200 Hz, and a total acquisition time of 4.24 min. The average voxel size for the soleus, gastrocnemius, and deltoid muscles, the lower leg was fixed in a spherical knee-coil, while a shoulder coil was used to measure carnosine in the deltoid muscle. Single voxel point-resolved spectroscopy (PRESS) sequence with the following parameters was used; repetition time (TR) of 2000 ms, echo time (TE) of 30 ms, number of excitations is 128, 1024 data points, spectral bandwidth of 1200 Hz, and a total acquisition time of 4.24 min. The average voxel size for the soleus, gastrocnemius, and deltoid muscles was 40 mm x 12 mm x 30 mm, 40 mm x 12 mm x 30 mm and 40 mm x 13 mm x 30 mm, respectively. For the calf muscles, the absolute carnosine content (mM) was calculated as described before by Baguet et al. (2010). For deltoid muscle, the integral of the C2-H peak (at ~8 ppm) was quantified relative to the water peak integral (x1000) and calculated as arbitrary units (Gualano et al., 2012). A variation coefficient for repeated measurements within the same day (Ozdemir et al., 2007) were 4.3% (soleus), 7.6% (gastrocnemius), and 6.6% (deltoid), while the biological variability (variation coefficient within a 4- to 6-week period) (Baguet et al., 2009; Bex et al., 2014) was 9.8% (soleus), 14.2% (gastrocnemius), and 13.3% (deltoid). All the carnosine concentrations were converted to muscle-specific Z-scores, based on the normal distribution of our data. The absolute value of Z-score represents the distance between the individual score of an athlete and the population mean (muscle and gender specific) in units of the standard deviation. Z-score is negative when the individual score is below the mean, positive when above. The mean and SD for the control population was calculated for each muscle type and sex, in order to allow athletes’ Z-score calculation.

Exercise characteristics

The typical cyclic movement frequency and exercise duration for the respective sport disciplines are represented in Fig. 1.

Movement frequency is the number of repetitive movement cycles per second, expressed in Hz. One movement cycle refers to an entire ipsilateral movement pattern of a limb before it becomes repeated. For running this means one stride (two steps), for cycling one complete pedal revolution, for front crawl and kayaking this means two arm strokes (one with the left, one with the right). The data on movement frequency are well documented for running

Fig. 1. The typical cyclic movement frequency (a) and exercise duration (b) range for the respective sport disciplines.

Statistics

To investigate the relation between estimated MFTC and cyclic sport exercise characteristics, Pearson correlation was calculated between the mean Z-scores of the athlete populations, movement frequency of the different disciplines, and the mean exercise duration of the different disciplines. A paired sample T-test was done to investigate the across-muscle phenotype by comparing the Z-score of carnosine content in the arm and the leg within the group of swimmers. Pearson correlation was also used between leg and arm muscles in the controls and swimmers. To examine estimated MFTC in relation to athlete level, an independent sample T-test was performed to compare the Z-score of carnosine content between two levels of athletes (<1050 IAAF points and >1050 IAAF points) in either sprint and endurance running. The athletes with an IAAF score above the 1050 points were almost all participants at international or even world championships. All analyses were done with SPSS statistical software (SPSS 21, Chicago, Illinois, USA). All values are reported as mean ± SD and statistical significance was set at P < 0.05.

Results

Athletes with different specialization (SPR-T, INT-T, and END-T) in various cyclic sports

For gastrocnemius muscle, the Z-scores of the different athletes are represented in Fig. 2. Within each sport discipline, the SPR-T has the highest Z-score
compared to INT-T and END-T. In the runners, the SPR-T had a Z-score of 1.68, the INT-T had 0.52, and the END-T had -0.91. The Z-score of the triathletes was -1.32. In the SPR-T, INT-T, and END-T swimmers a z-score of 0.59, 0.27, and -1.73 was found, respectively. For cycling, the road cyclists had a Z-score of -0.88, while the climbers had 2.03. Only one track cyclist participated and had a Z-score of 3.92. The INT-T kayakers showed a Z-score of 0.00, while the SPR-T kayaker had a Z-score of 1.12.

For deltoid muscle within the swimmers and kayakers, the highest Z-score was found in the SPR-T athletes and the lowest Z-score in the END-T athletes. The SPR-T, INT-T, and END-T swimmers showed a Z-score of -1.28, -1.37, and -1.73, respectively. In the SPR-T and INT-T kayakers a Z-score of 0.01 and -0.33 was found, respectively.

Carnosine content as indirect estimation of MFTC is linked to cyclic movement frequency

Fig. 1a and b show respectively the typical cyclic movement frequency and duration that are observed in the different sport disciplines. We then related movement frequency and duration to the Z-score for carnosine of the most relevant measured muscle group in the different athlete populations (i.e., gastrocnemius of runners and cyclists, deltoid of swimmers and kayakers). A strong and significant positive correlation was found between Z-score of carnosine content and cyclic movement frequency across the different sports and their disciplines (R = 0.86, P < 0.001) (Fig. 3a). No significant correlation was found between Z-score of carnosine content and exercise duration (R = -0.58, P = 0.06) (Fig. 3b). Without the SPR-T cyclist (which was only one subject), similar results were found with R = 0.78 (P = 0.008) for movement frequency and R = -0.51 (P = 0.13) for exercise duration. No correlation was found between movement frequency and exercise duration (R = -0.23, P = 0.50).

Across-muscle phenotype

We explored whether carnosine Z-scores of leg muscles and arm muscle within the same subject were correlated. Within the control group (N = 32), a significant positive correlation was found between Z-scores of leg muscles (mean of soleus and gastrocnemius muscles) and arm muscle (deltoid) (R = 0.37, P < 0.05). A similar correlation was found within the group of the swimmers (R = 0.81, P < 0.01) (Fig. 4). However, the linear trend line was shifted downwards for the swimmers: the control group had a mean Z-score of 0.14 ± 0.94 in leg muscles and 0.10 ± 0.99 in arm muscle, while the swimmers showed a lower Z-score in arm compared to leg muscles (−1.56 ± 0.93 vs 0.29 ± 1.09, respectively, P < 0.01).

Level of athletes

Within running, the SPR-T and END-T runners were divided into two groups based on their level (threshold of more and less than 1050 IAAF points on their best running performance). For SPR-T runners, a higher level of athletes was characterized with a higher Z-score than the low level athletes [2.02 ± 0.98 (15 athletes) vs 1.16 ± 1.12 (10 athletes), respectively, P = 0.05]. However, there was no difference in Z-score between the higher and lower level of END-T runners [−0.89 ± 0.82 (26 athletes) vs −0.93 ± 0.59 (12 athletes), respectively, P = 0.89].

Discussion

To date, integrated information within a single study design on MFTC in a large pool of active athletes is
not available. This is likely due to the invasive nature of a muscle biopsy, which is still considered as gold standard for measuring MFTC. With a new non-invasive estimation technique (Baguet et al., 2011), based on MRS-determined muscle carnosine content (expressed as Z-scores relative to population average), we were able to build a coherent and extensive database of estimated MFTC in active elite athletes from different cyclic sports.

A first aim of this study was to compare estimated MFTC in athletes with different specialization (SPR-T, INT-T, and END-T) within various cyclic sports. Our results confirmed that all explosive athletes (SPR-T) had the highest carnosine levels and thus the greatest estimated area of FT fibers, compared to END-T athletes, with INT-T athletes always situated intermediate to SPR-T and END-T. Sprint and endurance events within a sport require different muscle characteristics (e.g., fatigue profile, power output, etc.), which is confirmed by these results. Within cycling, END-T cyclists correspond to multistage road cycling including frequent climbing (e.g., Tour de France, Giro, etc.), whereas INT-T cyclists correspond to single day flat road races, and SPR-T cyclist to track cycling. The findings of our study will help to differentiate and (re)orientate athletes to the best discipline within sports based on their estimated MFTC.

In a second aim, we made a comparison of estimated MFTC between different cyclic sports. We explored four popular leg and/or arm muscle-driven cyclic locomotion types in sports: running, swimming, cycling, and kayaking. First, a detailed analysis of usual movement frequency and duration of the different sports and their disciplines was conducted. Remarkably, when all sports are combined in one analysis, there was no significant correlation between exercise duration and muscle carnosine content. However, a very strong correlation was found between movement frequency and carnosine content. This suggests that the dominant possession of FT muscle fibers, and their fivefold higher contractile velocity and maximal power properties, is mainly deterministic for the performance level in those cyclic sports with high movement frequency (running, cycling) and much less so in sprint disciplines of sports with slower frequency (swimming, kayaking). This occurred despite the existence of distinctions between SPR-T and END-T athletes within these sports. Until now, exercise duration was assumed to be the most determining factor for defining the optimal MFTC of athletes (Gollnick et al., 1972; Costill et al., 1976), but this study showed that movement frequency may be a more relevant element dictating desired muscle properties in different sport disciplines.

Whether possessing the right MFTC is due to the genetic aspect, rather than the effect of training has been subject of extensive investigation. Vikne et al. (2012) suggested the existence of an across-
muscle phenotype, supporting the importance of the heritability component. In our study, we could confirm a significant positive correlation between the carnosine Z-scores in the leg and the carnosine Z-scores of the arm muscles. Yet, the swimmers displayed a downward shift toward less carnosine (thus higher estimated proportion of ST fibers) in their deltoid muscle compared to the legs. Multiple years of daily training in a relatively slow swimming movement may have caused a shift to more ST fibers or to a greater area of ST fibers in their arm muscle compared to the leg muscles in the same individuals, and compared to the arm muscles of a control population. This finding is in agreement with biopsy-based findings by Tesch and Karlsson (1985), showing that a higher proportion of ST fibers is found in muscles that are frequently used during long-term endurance training. At the moment, most studies about MFTC are cross-sectional, which makes it hard to determine causality. However, there are already some longitudinal interventions which showed a transition from FTx to FTa and to a lesser extent from FT to ST fibers (Staron et al., 1990; Ingalls, 2004). These collective findings suggest that a combination of environment (training) and genetics determine the muscle-specific MFTC of athletes.

In the last research question of this study, we examined whether higher level runners have a more extreme estimated MFTC, i.e., whether more successful SPR-T runners deviate more from population average (more positive Z-score) than less successful SPR-T runners, and more successful END-T runners have more negative Z-scores than less successful counterparts. Interestingly, our data could confirm the former, but not the latter. Thus, the better SPR-T runners (IAAF scores above 1050) were characterized by even higher carnosine levels, but no differences were found between the two levels of END-T runners. To reach world level, SPR-T runners need the highest abundance in FT fibers to produce the highest possible muscular power output, which is a well-established limiting factor in sprint running performance. Within endurance running, it can be hypothesized that a high proportion of ST fibers is critical to reach a certain level, but that it is not the limiting factor to become a world-class athlete. This is in line with the relatively high movement frequency of endurance running (cfr supra), which can be especially high during the final lap(s) in track running (the last 400 m can be run in under 53 s in a 10K race). Consequently, even END-T runners likely need a reasonable proportion of FT fibers to become successful.

One limitation of the study was the small sample size of some subgroups of athletes. This was on the one hand due to the limited availability of elite athletes in Belgium (i.e., kayakers). On the other hand, BA supplementation in elite athletes, especially in swimmers and (track) cyclists, was one of the main reasons to exclude participants from the study. Another limitation was that the $^1$H-MRS methodology was validated against the biopsy technique by Baguet et al. (2011) only in the calf muscles. This technique was then further optimized to measure carnosine in the deltoid muscle (Bex et al., 2014). However, muscle carnosine concentrations in the arm muscles have not yet been compared to MFTC measured by the histochemical analysis of muscle biopsies.

**Perspectives**

The current study was conducted with a non-invasive methodology, namely $^1$H-MRS quantification of muscle carnosine, developed by Baguet et al. (2011). Within different sports, a systematic distinction in estimated MFTC was found between the sprint- and endurance-type athletes. Furthermore, our findings across different sports and disciplines suggest that movement frequency, rather than exercise duration, is a more important deterministic factor for optimal MFTC. We hope to have raised a renewed interest in the importance of muscle fiber typology in sports, and we consider the currently explored non-invasive estimation methodology a useful and applicable method in sport science practice.

**Key words:** Cyclic sports, contractile properties, carnosine.

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**Conflict of interest**

There are no conflicts of interest.

**Supporting Information**

Additional Supporting Information may be found in the online version of this article.

**Table S1.** Duration range for the different sport disciplines. BR = Belgian Record. WR = World Record.
Non-invasive muscle typing in athletes

References


