UNIVERSITEIT GENT

FACULTY OF MEDICINE AND HEALTH SCIENCES

Department of Movement and Sports Sciences

NMR-BASED APPLICATIONS IN ELITE SPORTS PERFORMANCE

Tine BEX

Thesis submitted in fulfillment of the requirements for the degree of Doctor in Physical Education

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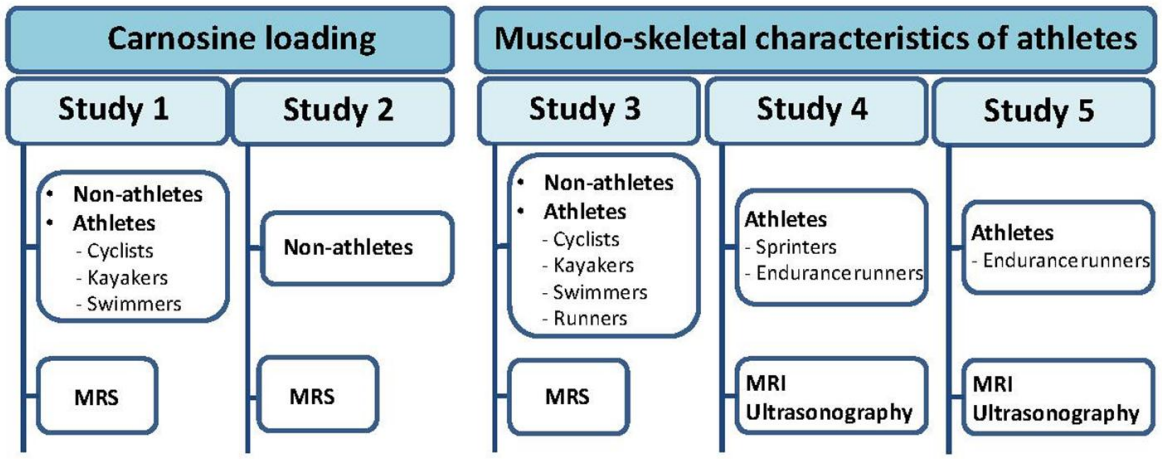
English summary

Nuclear magnetic resonance (NMR) scanners can be used in athlete populations because of its non-invasive nature. Both spectroscopy (MRS) and imaging (MRI) are harmless techniques which can be used in several applications towards sport science.

The studies in this PhD thesis included two major topics. In the first section (study 1 and 2), the focus was put on a better understanding of the muscle carnosine loading protocol. Secondly, study 3, 4 and 5 investigated musculo-skeletal characteristics of the athlete itself. The figure below schematically presents the 5 studies in this thesis.



Part 1 – Carnosine loading

Carnosine, a dipeptide consisting of beta-alanine (BA) and L-histidine, is a specific metabolite present in skeletal muscles. Interesting characteristics (e.g. proton buffer, calcium regulator) can make higher carnosine concentrations beneficial for athletes. Due to supplementation with the rate-limiting precursor BA, muscle carnosine content can increase up to 80 %. However, only 2-3 % of the total amount of ingested BA is incorporated into muscle carnosine. Therefore, the question was raised which determinants can influence the BA uptake and as such the carnosine loading efficiency.

Study 1 started with a methodological research to measure carnosine in specific muscles of the upper body by ^{1}H-MRS. The reproducibility in the deltoid muscle was good, which enables the comparison of muscle carnosine content within one subject in different parts

of the body. In a second part of this study, BA supplementation-induced changes in carnosine concentrations were analyzed in different athlete populations. It turned out that all trained muscles showed higher increases in carnosine concentrations compared to untrained muscles. As a result, the suggestion was made that exercise training could enhance the carnosine loading efficiency.

Study 2 was perfomed to examine whether the increased effectiveness of BA supplementation in trained muscles is due to an acute or chronic response of exercise training. It was found that both acute high-volume and high-intensity training protocols in non-specifically trained subjects were able to augment the carnosine loading efficiency, concluding that acute exercise training is a determinant of the carnosine loading protocol with a possible additive effect of chronic trained muscles.

Part 2 – Musculo-skeletal characteristics of athletes

It has been shown that fast-twitch (FT) fibers contain more carnosine compared to slow-twitch (ST) fibers. Using this relationship, carnosine concentration measurements by ^{1}H-MRS can be used to indirectly estimate muscle fiber type composition (MFTC). There are large inter-individual differences in MFTC between athlete populations, which is a valuable topic for sport scientific guidance.

In **study 3**, MFTC-data of different athlete populations were collected and analyzed. A first finding observed within sports is that explosive athletes contain more muscle carnosine compared to endurance-type athletes. Furthermore, across different sports, muscle carnosine concentration, as estimation of MFTC, is linked to cyclic movement frequency, rather than exercise duration. This suggests that cyclic sports with high movement frequency require athletes with a dominant possession of FT muscle fibers. Another finding in this study is that a higher level of athletes is not essentially translated to a more extreme MFTC. In endurance running, it was shown that the higher-level athletes had an equal amount of ST fibers compared to the lower-level athletes. In contrast, more successful sprinters were characterized by a higher percentage of FT fibers compared to less successful sprinters.

In running, there is a clear distinction in MFTC between sprint and endurance runners. However, the question was raised whether other musculo-skeletal characteristics differ

between sprint and endurance runners of the same ethnicity. As success in sprint and endurance performance is determined by different factors (e.g. power production vs. running economy), it could be hypothesized that other discriminants (besides MFTC) are present between sprinters and endurance runners. **Study 4** investigated different musculo-skeletal leg characteristics in sprint and endurance runners of Caucasian ethnicity. This study discovered that sprinters have mainly greater muscle volumes in the lateral and proximal leg muscles. No differences were found in skeletal properties, nor in architectural characteristics between the two groups. Furthermore, it was demonstrated that higher hamstring muscles volumes were directly related to better sprint performance.

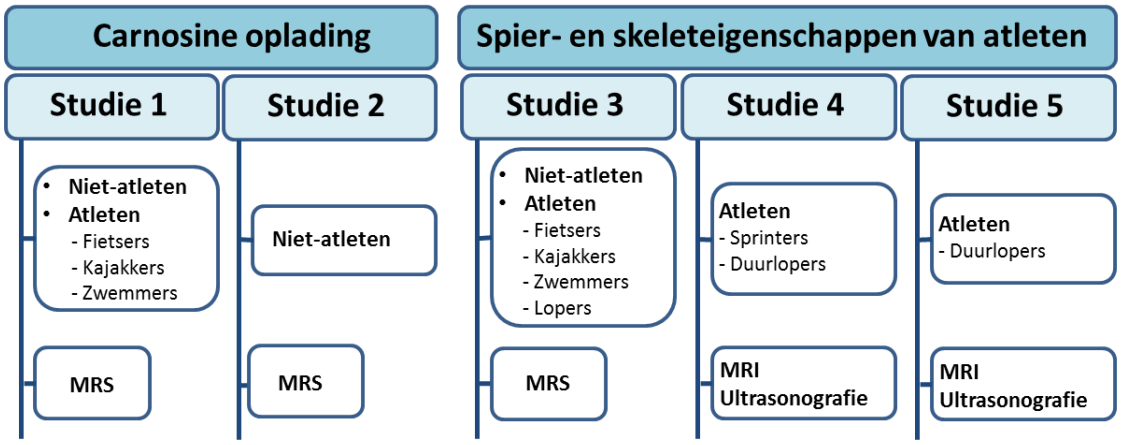
Taking a closer look to endurance running, the dominance of East-African runners is outstanding. Therefore, the body morphology of endurance runners of different ethnicity was examined in **study 5**. It was found that East-African endurance runners had longer legs and longer shanks compared to Caucasian endurance runners. Furthermore, muscle mass and volumes of the lower leg were found to be lower in East-Africans compared to Caucasians.

Altogether, this thesis contributed to new insights in the applications of NMR in sport science. A first application was situated in the field of sport nutrition by investigating carnosine loading strategies. Additionally, MRS and MRI were used to reveal specific muscle characteristics in different athlete populations.

Nederlandse samenvatting

Nuclear magnetic resonance (NMR) is handig voor gebruik bij atleten omwille van het non-invasief karakter. Zowel spectroscopy (MRS) als imaging (MRI) zijn onschadelijke technieken die gebruikt kunnen worden voor toepassingen in de sportwetenschappen.

De studies in dit doctoraat zijn vervat in twee belangrijke onderwerpen. Ten eerste dragen studies 1 en 2 bij tot een beter inzicht in het protocol naar spiercarnosine oplading. Ten tweede zullen studies 3, 4 en 5 de skeletale en spierkarakteristieken van atleten onderzoeken. Op de figuur hieronder kan je de 5 studies schematisch terugvinden.



Deel 1 – Carnosine oplading

Carnosine, een dipeptide bestaande uit beta-alanine (BA) en L-histidine, is een specifiek metaboliet aanwezig in skeletspieren. Omwille van enkele interessante functies (zoals protonen buffer en calcium regulator) kunnen hogere carnosine concentraties voordelig zijn voor atleten. Bij supplementatie van de snelheidsbeperkende precursor BA zullen de carnosine concentraties stijgen tot 80 %, maar slechts 2-3 % van de totale hoeveelheid ingenomen BA zal uiteindelijk omgezet worden tot spiercarnosine. De vraag is dan ook welke determinanten de opname van BA en dus de efficiëntie van de carnosine oplading beïnvloeden.

Studie 1 startte met een methodologisch onderzoek om carnosine te kunnen bepalen met ^{1}H-MRS in spieren van het bovenlichaam. De reproduceerbaarheid in de deltoideus

spier was goed, wat de mogelijkheid gaf om spiercarnosine concentraties te vergelijken binnen één subject in verschillende delen van zijn lichaam. In een tweede deel van de studie werden veranderingen in spiercarnosine concentraties na BA supplementatie onderzocht in verschillende atletenpopulaties. Daaruit bleek dat alle getrainde spieren grotere stijgingen hadden in carnosine concentraties vergeleken met ongetrainde spieren. Vandaar werd gesuggereerd dat training mogelijks de efficiëntie van carnosine oplading kan verhogen.

Om te onderzoeken of de verhoogde efficiëntie van BA supplementatie in getrainde spieren te wijten was aan een acute of chronische respons op inspanning, werd **studie 2** uitgevoerd. Daar werd gevonden dat zowel acute training protocols met een groot volume als een hoge intensiteit bij niet-specifiek getrainde atleten de efficiëntie van de carnosine oplading verhoogden. Op basis daarvan kan geconcludeerd worden dat acute training een determinant is van het carnosine oplading protocol, met een mogelijk extra positief effect bij chronisch getrainde spieren.

Deel 2 – Spier- en skeleteigenschappen van atleten

Het is aangetoond dat snelle spiervezels meer carnosine bevatten dan trage spiervezels. Derhalve zal het meten van carnosine met ^{1}H-MRS gebruikt kunnen worden om spiervezeltypesamenstelling (SVTS) op een indirecte manier te bepalen. Er bestaan grote inter-individuele verschillen in SVTS bij atletenpopulaties wat ervoor zorgt dat dit een interessant onderwerp is voor de sportwetenschappelijke begeleiding van atleten.

In **studie 3** werd de SVTS van verschillende atletenpopulaties verzameld en geanalyseerd. Een eerste bevinding, wanneer we kijken binnen een bepaalde sport, is dat explosieve atleten meer spiercarnosine bevatten dan uithoudingsgetrainde atleten. Verder werd ook aangetoond dat, overheen verschillende sporten, spiercarnosine concentraties als inschatting van SVTS eerder gelinkt zijn aan de frequentie van sporten dan aan de duur. Dit suggereert dat cyclische sporten met een hoge bewegingsfrequentie nood hebben aan atleten met een dominante hoeveelheid snelle vezels. Een andere bevinding was dat atleten van een hoger niveau niet per se beschikken over een meer extreem SVTS. In duurlopen vonden we een gelijke hoeveelheid trage vezels terug tussen een lager en hoger niveau van atleten. Bij sprinters daarentegen bleken de meer succesvolle sprinters

gekarakteriseerd te zijn met een hoger percentage aan snelle vezels vergeleken met minder succesvolle sprinters.

In atletiek is het duidelijk dat sprinters en duurlopers over een uiteenlopende SVTS beschikken. De vraag blijft echter of er andere spier- of skeleteigenschappen zijn die verschillen tussen sprinters en duurlopers van dezelfde etniciteit. Succes in sprint- en duurprestatie wordt bepaald door verschillende factoren (met name het leveren van vermogen vs. een goede loopeconomie). Vandaar kan verondersteld worden dat er andere discriminante eigenschappen aanwezig zijn (naast SVTS) bij sprinters en duurlopers. **Studie 4** onderzocht de verschillende skeletale en spierkarakteristieken bij sprinters en duurlopers van Kaukasische etniciteit. Deze studie toonde aan dat sprinters grotere spiervolumes hebben in de proximale en laterale spieren van het been. Er werden geen verschillen gevonden tussen de twee groepen wat betreft skeletale of architecturale eigenschappen. Verder werd er aangetoond dat grotere volumes van de hamstrings gerelateerd waren aan betere sprint prestaties.

Verdergaand op het duurlopen kan er besloten worden dat Oost-Afrikaanse lopers momenteel buitengewoon goed zijn. Vandaar werd de lichaamsmorfologie van duurlopers van verschillende etniciteit onderzocht in **studie 5**. Er werd gevonden dat Oost-Afrikaanse duurlopers langere benen en langere onderbenen hebben vergeleken met Kaukasische duurlopers. Verder bleken de spiermassa en spiervolumes van het onderbeen kleiner te zijn bij Oost-Afrikaanse in vergelijking met Kaukasische duurlopers.

Samenvattend kunnen we stellen dat dit doctoraat bijgedragen heeft tot het creëren van nieuwe inzichten in toepassingen van NMR in de sport. Een eerste toepassing situeert zich binnen het domein sportvoeding door onderzoek naar carnosine oplading strategieën. Daarnaast werden MRS en MRI gebruikt om bepaalde spiereigenschappen in verschillende atletenpopulaties te onderzoeken.

I

Introduction

1. Background

Nuclear magnetic resonance (**NMR**) scanners are widely used and present in almost every hospital. Furthermore, during international events (like Olympic Games), medical services like NMR scanners are available to provide easily accessible medical treatment and to minimise time out for the athletes (Bethapudi et al 2013). And thus, the use of diagnostic imaging in sports is very popular. So far, there are only a few non-clinical applications of NMR regarding sports. The benefit of NMR lies in its non-invasive nature which is especially usefull for **athlete** populations. This thesis contains different non-clinical applications of NMR in the framework of athletes and therefore could be translated to the sport practice.

2. NMR as tool in sport practice

In this first section, the most useful applications of NMR will be clarified as well as the basic principles. The NMR scanner is a useful and harmless method which can be used in athlete populations. Therefore, this methodology is used across all the studies of this thesis.

2.1. Imaging

Today, nuclear magnetic resonance (NMR) has become a popular and powerful analytical technology that has found a variety of applications in many disciplines of scientific research. The principles of NMR were used in the chemical and structural analysis of molecules and tissues. Magnetic resonance imaging (MRI) is a harmless technique using no ionising radiation, which is in contrast with other medical imaging tests like computed tomography (CT scans) which delivers high doses of radiation. MRI is used primarily in medical settings to produce high quality images of soft tissues such as the brain, heart, and muscles and to discover tumors in many organs. Beside the medical applications, MRI can be used in different branches of sport practice. The imaging application is commonly used in **injured athletes**, but also for setting up interactive atlases of human **anatomy** with references of bones and muscles this new method is gaining popularity.

2.2. Spectroscopy

Magnetic resonance spectroscopy (MRS) is a useful non-invasive method that provides in vivo biochemical information and can be used for metabolite analysis. The most detected nuclei, based on their high abundance in humans, are phosphorus (^{31}P) en protons (^{1}H). However, MRS studies should keep in mind the quite low signal-to-noise ratio. The ^{1}H-MRS detection limit is around 1 mM and by using water and fat suppression techniques, a number of metabolites present in quite low concentrations (< 10 mM) can be detected (Tomanek 2013).

MRS can be used in the field of **nutrition** or to evaluate **exercise** and **health condition**s by analyzing concentrations of different molecules, like glycogen, creatine, carnosine, adenosine triphosphate, lactate, choline, N-acetyl aspartate etc (Cox 1996; Boesch and

Kreis 2001; Madan et al 2015). Nutritional intervention studies (e.g. supplementation of creatine) can be evaluated by MRS to investigate increases in specific metabolites. Furthermore, the use of MRS for drug analysis has the advantage to quantitate drug conjugates and metabolites simultaneously (Komoroski et al 2000). This method also can be utilized for identifying and monitoring recovery by altered neurophysiology in case of sport-related **injury**, like concussion, or for detecting muscle injuries and exercise stress after high-intensity exercise performance. Specific metabolites, like carnosine, can be linked to specific **muscle properties** and in this manner, MRS can provide useful information to the sport practice.

2.3. NMR basics

2.3.1. NMR hardware

NMR experiments require in the first place a superconducting magnet, which is able to generate a strong homogeneous field (between 0.5 and 7 Tesla (T)) over a large volume (B_0). In this thesis, all experiments were done with a whole body NMR scanner with a field strength of 3 T. A higher magnetic field goes together with an increasing signal-to-noise ratio, allowing higher quality spectra (Cox 1996; Hornak 1996).

A second important component of a NMR scanner is a radiofrequency (RF) coil. This transmitter/receiver system has as primary function to transmit pulses (= external magnetic field (B_1)) and receive MR signals. Different kind of coils (e.g. surface coils, volume coils) can be used depending on the region of interest and the application (Cox 1996; Hornak 1996). In our studies, knee and shoulder coil were chosen to measure in the calf and shoulder muscles (Fig 1).

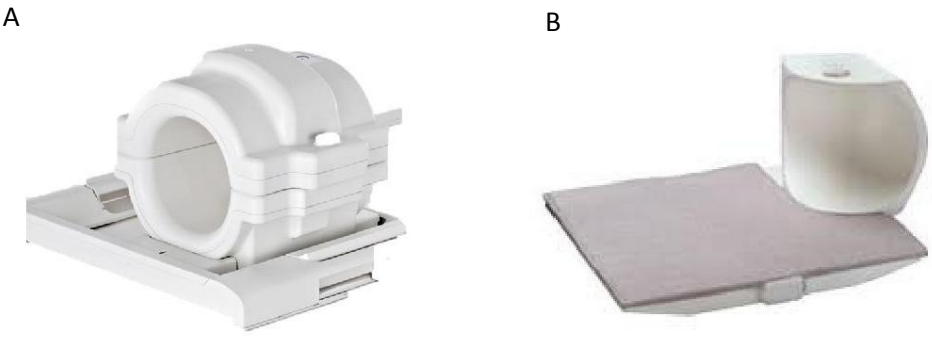


Figure 1. A (RF) knee coil (A) and shoulder coil (B).

2.3.2. NMR physics

Certain atomic nuclei, such as hydrogen-1 (proton, ^1H), has a specific property, called spin. Placing these protons in an external magnetic field, the spin vector of the nuclei will line up with (= parallel or low energy configuration) or against (= antiparallel or high energy configuration) the magnetic field (B_0) (Fig 2A). Furthermore, a proton will also freely rotate around B_0 with a particular frequency (= Larmor frequency) (Fig 2B). Protons are the most abundant nuclei in the human body and thus a muscle can be seen as a group of spins. As most protons will be oriented in the direction of B_0 (because of the low energy status) (Fig 2C), a net magnetization vector (M_0) will align according to B_0 (Z-axis) (Fig 2D).

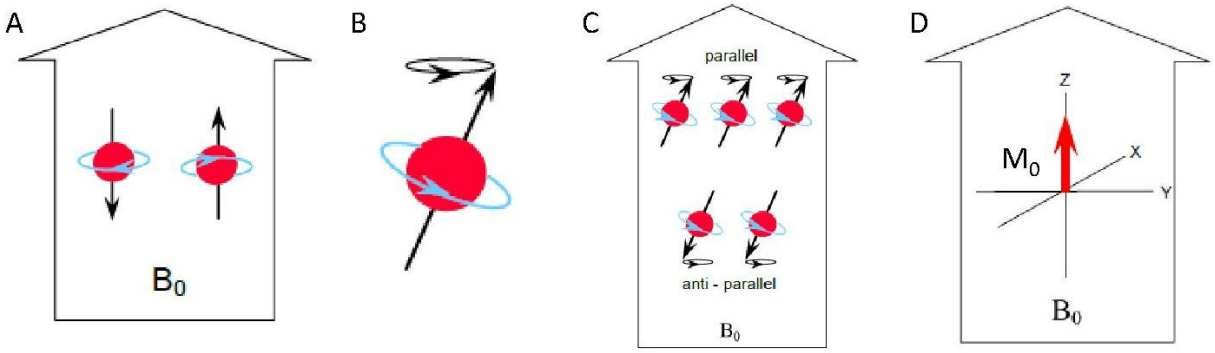


Figure 2. Schematic illustration of spin system: (A) Each proton can align parallel or antiparallel to the magnetic field B_o, (B) the Larmor frequency of each proton, (C) Group of spins, parallel or antiparallel and (D) net magnetization vector (M_0) (Blink et al (2004)).

By sending radiofrequency energy, induced by the RF coil, these protons can be excited in a controlled way. In this manner, M_0 will be out of balance and will flip away from the Z-axis toward the XY-plane (Fig 3A). During excitation, the nuclear spins will absorb energy of the RF pulse, which transform them in a high energy condition.

After excitation, relaxation will start to ensure M_0 returning to its equilibrium, which can be described by two processes, known as T_1 (longitudinal relaxation) and T_2 (transverse relaxation). The longitudinal relaxation (T_1) is defined as the time required to M_Z to achieve 63 % of its equilibrium value (Fig 3B). The same mechanism happens for T_2 relaxation, with T_2 the time which describes the return to 37% of its equilibrium of the transverse magnetization, M_{XY} (Fig 3C). Both values of T_1 and T_2 depends on the type of tissue (e.g. fat, water).

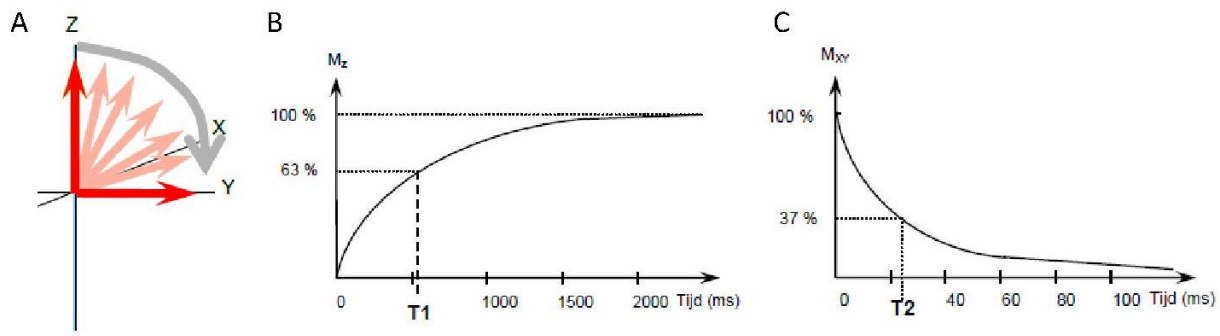


Figure 3. Excitation and relaxation process: (A) M_0 flipping from the Z-axis towards the XY-plane, (B) T_1 longitudinal relaxation and (C) T_2 transverse relaxation (Blink et al (2004)).

During the relaxation process, M_0 causes an alternating voltage, which will be received by the RF coil. This resulting signal, called the Free Induction Decay (FID), results into a frequency spectrum following Fourier transformation. The relative frequency position of nuclei, known as the chemical shift, depends on magnetic field strength and is measured using the dimensionless unit, parts per million (ppm). ^{1}H-MRS is based on the chemical shift, which is the difference between the resonance frequency of the nucleus and the reference nucleus (usually tetramethylsilane (TMS)). MRI is based on the variations in a number of parameters (as T1 en T2) between different tissues in normal and pathological conditions which can be used for image contrast (Cox 1996; Hornak 1996; Blink 2004).

2.3.3. Quantification of carnosine

In the studies of this thesis, ^{1}H-MRS was used to measure the metabolite carnosine. Therefore, a voxel with a volume around 14 ml was placed in the muscle of interest (e.g. soleus, gastrocnemius and deltoid muscle)(Fig 4).

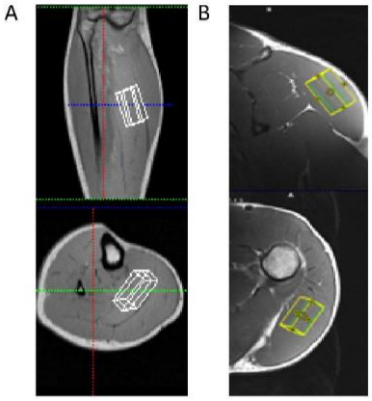


Figure 4. Voxel placement in soleus (A) and deltoid (B) muscles on coronal and transverse images.

The protons on the imidazole ring of carnosine produces 2 peaks in the proton spectrum, located at 7.0 (C5) and 8.0 (C2) ppm (Fig 5). It has been shown that C2 peak has a better test-retest reproducibility and is less susceptible to orientation effects than C5 (Derave et al 2007; Ozdemir et al 2007), which is the reason to use C2 peak for carnosine quantification.

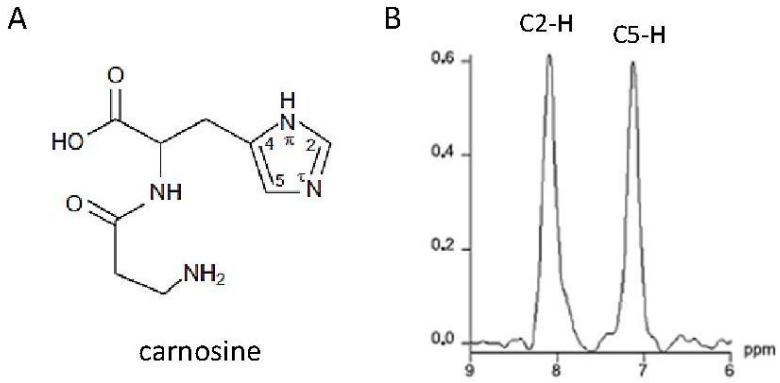


Figure 5. Chemical structure of carnosine (A) and a muscle proton spectrum (B) with carnosine located at 7 ppm (C5-H) and 8 ppm (C2-H).

Carnosine concentration can be expressed in absolute or relative values. Absolute quantification of muscle carnosine is based on a phantom with a known carnosine concentration. This phantom is used as external standard. Carnosine concentrations can also be expressed in relative terms, by relating the area under the curve (= integral) of the C2 peak of carnosine to the water peak.

3. Carnosine and carnosine loading

The following section will take a closer look to a specific metabolite, namely carnosine. Recently, this molecule has become popular in scientific research (with over 100 papers published in the last year). Carnosine plays an important role in the first three papers of this thesis, thereby this part will elucidate the basics of this fascinating metabolite in a nutshell.

3.1. Carnosine metabolism

In 1900, carnosine (beta-alanyl-L-histidine) was discovered by a Russian chemist, called Vladimir Gulewitch. The name is coming from 'Carnis', which is Latin for meat and fish. As carnosine is a cytoplasmic dipeptide abundantly present in human skeletal muscle (5-8 mmol/l wet weight), this name is accurately chosen. Methylated variants of carnosine, namely anserine or balenine are also found in skeletal muscles of animals, but not in human skeletal muscles. These three compounds (carnosine, anserine and balenine) together are named the histidine-containing dipeptides (Boldyrev et al 2013). Carnosine is synthesized from the precursors L-histidine and beta-alanine (BA) by the enzyme carnosine synthase. Harris et al (2006) were the first to demonstrate that BA is the rate-limiting factor for the dipeptide synthesis. The breakdown of carnosine is catalyzed by carnosinase both in blood circulation (CN1) and in tissues (CN2), while carnosine synthesis takes mainly place in skeletal muscles. BA is available from the hydrolysis of carnosine, ingested by daily food (like meat and fish). Furthermore, BA can be augmented by oral supplementation and a small part of BA is endogenously obtained from degradation of uracil in the liver. Since BA is not synthesized in the muscle, it is provided via an efficient carrier system with TauT and PAT1 as BA transporters (Everaert et al 2013). Figure 6 shows an overview of the carnosine metabolism.

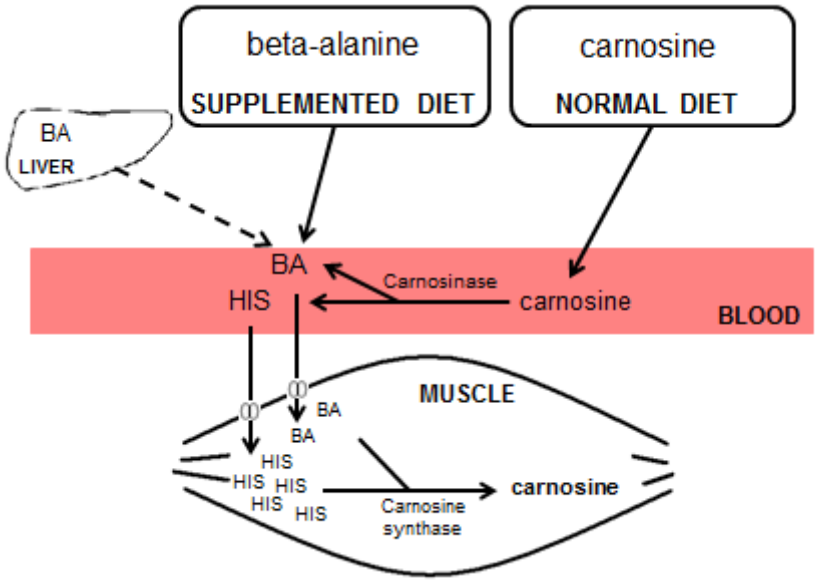


Figure 6. Schematic illustration of the carnosine metabolism.

The large amount of carnosine in skeletal muscles raise the question towards the functions of this specific metabolite. First of all, the role as **proton buffer** is probably the most studied one. During high-intensity exercise, intramyocellular pH can drop from values around 7.0 at rest to values as low as 6.3. Metabolites that are able to accept H^+, can function as proton buffers (e.g. inorganic phosphate, bicarbonate, proteins). Histidine has a pKa of 6.1 which is in the range of the physiological pH of the muscle. Binding histidine with BA, the pKa (Ka = acid dissociation constant) of the imidazole ring raises to 6.83. This optimal pKa, together with high concentrations makes carnosine a useful buffer in skeletal muscle. The intracellular buffering capacity of carnosine is thus regulated by the nitrogens atoms of the imidazole ring and contributes approximately 4.5 and 9.4 % in slow-twitch (ST) and fast-twitch (FT) fibers in human vastus lateralis, respectively (Mannion et al 1992).

Carnosine as **calcium regulator** is another often mentioned function. During muscle contractions cross-bridges between actin and myosin are formed. To allow this formation, Ca^{2+}, released from the sarcoplasmic reticulum, is required to bind to troponin. Dutka et al (2012) demonstrated that elevated muscle carnosine levels can increase Ca^{2+} sensivity and release in human muscle fibers in vitro. Recently, Swietach et al (2013; 2014) suggested an interesting link between the pH buffering capacity and the calcium handling of carnosine. This mechanicsm, called the carnosine shuttle, is based on the capacity of

carnosine to act as a mobile buffer by exchanging Ca^{2+} and H^+. Both protons and calcium ions can competitively bind to this mobile buffer. The hypothesis is that increased H^+ production at the sarcomere site can induce unloading of Ca^{2+}, which can consecutively increase cross-bridge formation. Unloading of H^+ from carnosine at the sarcoplasmic reticulum is in his turn enhanced by Ca^{2+} release, which improves the subsarcolemmal H^+ concentrations (reviewed by Blancquaert et al (2015))(Fig 7).

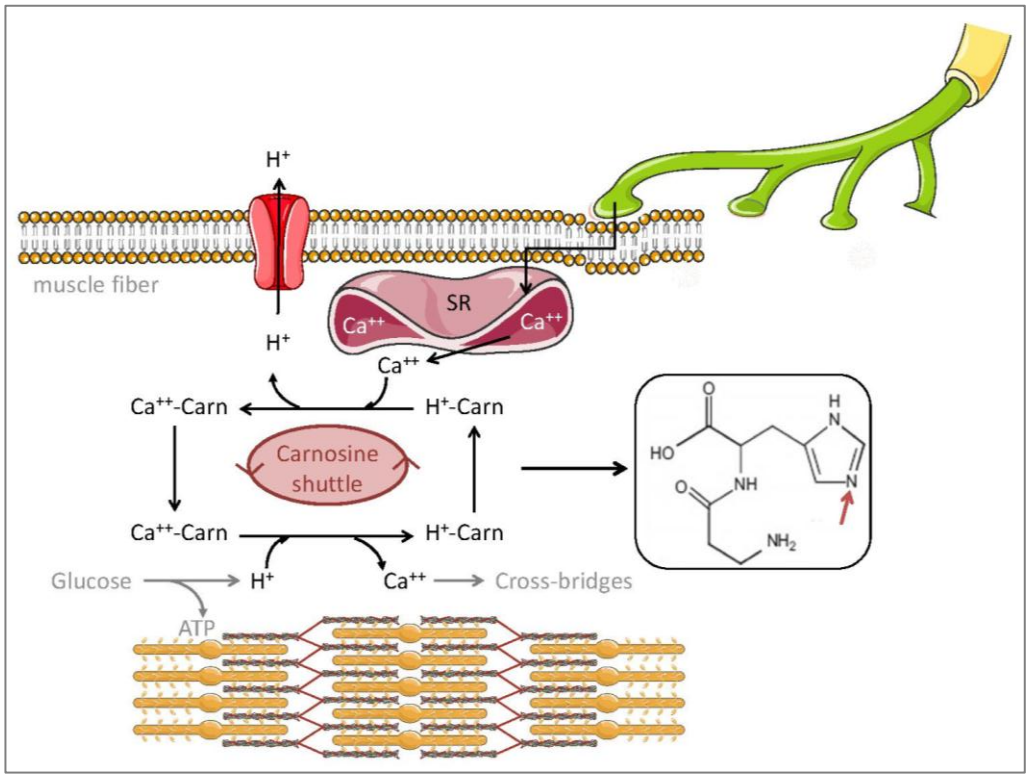


Figure 7. Hypothesis on the ergogenic mechanism of carnosine in skeletal muscle, based on the findings of Swietach et al (2013). It represents carnosine as a mobile buffer by exchanging Ca^{2+} and H^+ between sarcomere site and the subsarcolemmal region. Molecular structure of carnosine is shown with the arrow to the competitive binding site of H^+ and Ca^{2+} (reviewed by Blancquaert et al (2015)). (SR = sarcoplasmatic reticulum)

Other possible functions are based on the **antiglycation** and **antioxidant** properties of carnosine, which suggests therapeutic effects of carnosine towards certain disorders.

3.2. Determinants of muscle carnosine content

There are large inter-individual differences in carnosine content in human skeletal muscle, while the intra-individual variation is limited. The amount of baseline muscle carnosine is influenced by several determinants, which will be discussed below (Fig 8).

Firstly, the effect of **age and gender** is clearly demonstrated in the study of Baguet et al (2012). For both males and females, muscle carnosine reaches a peak value around 18 years old and starts to decrease during aging, with the highest decline shortly after young adolescence. Furthermore, after puberty, males show higher carnosine content compared to females in two different muscles (gastrocnemius and soleus muscle), which is in line with previously reported findings (Everaert et al 2011).

The influence of **diet** on muscle carnosine content is rather limited. However, people who were long-term vegetarian showed lower concentrations compared to omnivores (26 % lower in gastrocnemius muscle) (Everaert et al 2011). Furthermore, BA tablets as supplement induces high increases in carnosine content (40 – 80 %), which will be discussed later (see 3.3 Beta-alanine supplementation).

Muscle fiber type composition plays a key factor in baseline carnosine content (Abe 2000). The metabolite carnosine shows a strong fiber type disparity. On single fiber level, Harris et al (1998) showed that FT fibers contain twice as much carnosine compared to ST fibers (Hill et al 2007). A study of Baguet et al (2011b) with different athlete populations found that sprinters had more carnosine than endurance athletes, which was also the case in young talents and former athletes. In this cross-sectional study, it has to be mentioned that there can be a small additive effect of training towards more or less FT fibers, which can have an influence on the carnosine concentrations. Although, based on the review of Harris et al (2012), training by itself seems not to directly affect muscle carnosine contents.

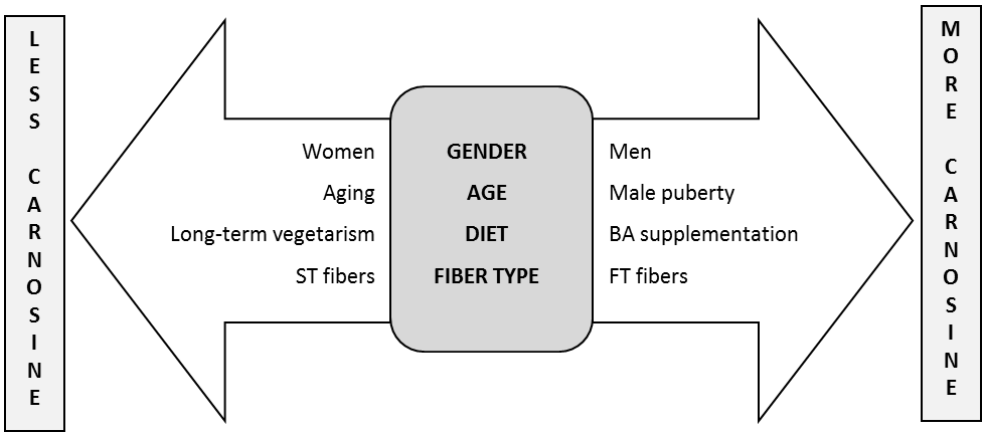


Figure 8. Determinants of baseline muscle carnosine content, with specification towards more or less baseline carnosine concentrations.

3.3. Beta-alanine supplementation

Elevated muscle carnosine concentrations by BA supplementation can have a beneficial effect on high-intensity exercise performance, which is the reason why this supplement has become extremely popular among competitive athletes. This part will summarize the currently available information regarding the optimization of the BA supplementation protocol and the link between BA and performance enhancement. Even though this supplement is widely used, less is known about the mechanisms that regulates the uptake and storage in skeletal muscle.

3.3.1. Carnosine loading protocol

BA supplementation (4 – 6.4 g/day) during 4 – 10 weeks augments muscle carnosine concentrations with 40 – 80 %. Recently, a dose-response relationship was identified between BA intake and carnosine increase (Stellingwerff et al 2012). These results suggested that carnosine loading is mainly determined by the total amount of ingested BA, with no differences between high doses during short-term supplementation vs. low doses spread over a longer period. In sport settings, the former would be most useful, because athletes can be loaded in a shorter period. Supplementation can be done with pure BA (P-BA) or slow-release BA (SR-BA), who were both equally effective (Stegen et al 2013). Current supplementation strategy is to take several little dosages (around 10 mg/kg body weight) throughout the day to reduce the risk on paresthesia symptoms (Harris et al 2006). Taking SR-BA tablets minimizes possible side-effects by slower absorption kinetics, giving the possibility to take a larger dose of BA without adverse effects (up to 1.6 g/dose, 4 times/day) (Décombaz et al 2012).

During prolonged competition, it would be useful for athletes to maintain their elevated carnosine levels. Stegen et al (2014) established an intermediate dose of 1.2 g/day as optimal maintanance dose for keeping muscle carnosine stores elevated after a loading phase. The wash-out of BA is rather a slow process that takes 6 – 20 weeks before muscle carnosine levels return to baseline values (Baguet et al 2009).

3.3.2. Determinants of carnosine loading

The loading efficiency of carnosine, calculated by dividing the molar carnosine increase by the total molar intake of BA, is very low. Only 2.8 % of total ingested BA is incorporated into muscle carnosine, assuming that 40 % of body mass is muscle mass. A small part (1 – 2 %) of BA is excreted in the urine, but approximately 95 % of the ingested BA has an unknown metabolic fate (Stegen et al 2013). Strategies to optimize the loading protocol should be investigated to generate a better understanding into the metabolism. Yet, there are already a few known factors, influencing the carnosine loading process.

A first determinant is the effect of **meal coingestion**. Stegen et al (2013) revealed that the BA supplementation efficiency was enhanced by meal coingestion (up to 3.0 %). The authors suggest that insulin could stimulate the muscle carnosine synthesis process and therefore higher increases were found in carnosine concentrations when combining meal and BA tablets, compared to taking BA tablets in between the meals.

Exercise training could be another potential determinant of carnosine loading, although there are no data supporting this. This is by analogy with creatine, another widely used supplement, which showed a higher creatine increase in the trained leg, compared to the untrained leg after creatine supplementation (Harris et al 1992). The same was found by Robinson et al (1999) who demonstrated elevated creatine accumulation after one bout of exhaustive exercise. To date, literature on the effect of exercise training during BA supplementation is limited. Kendrick et al (2009) did not manage to find an influence of isokinetic strength training on carnosine loading, comparing a trained leg and an untrained leg (Fig 9). Training consisted of 10 x 10 maximal knee-extensions, 3 – 4 times per week, over 4 weeks. Based on these findings, it was concluded that training had no effect on the efficiency of BA supplementation.

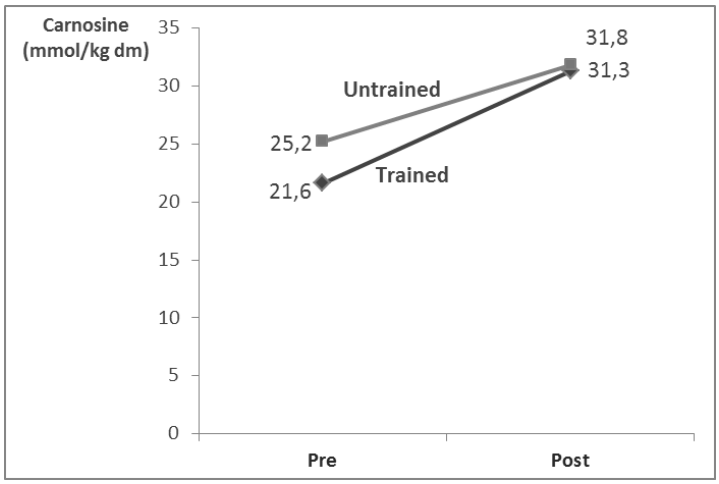


Figure 9. The increase in carnosine concentration in the trained leg and untrained leg after 4 weeks of BA supplementation. Training consisted of 10 x 10 maximal contractions, 3 – 4 training sessions per week (based on Kendrick et al (2009)).

Aims: Chronic BA suppementation is effective in increasing muscle carnosine content. However, the loading scheme is long and poorly efficient. Strategies to improve efficiency are currently explored and may include concurrent exercise training. Exploring the latter is a first aim of this PhD thesis and will be discovered in study 1 and 2.

3.3.3. Ergogenic effects of elevated carnosine content

Based on the functions of carnosine as proton buffer and calcium regulator, elevated carnosine contents can have a beneficial effect on sport performance. However, not all performance studies demonstrated an enhancement after BA supplementation. The meta-analysis of Hobson et al (2012) bundled the equivocal findings combining BA supplementation and exercise performance (Fig 10). Based on this review, it can be concluded that high-intensity exercises between 1 – 4 minutes can benefit from increased muscle carnosine levels. Exercises lasting less than 1 minute show no exercise improvement after BA supplementation. For long duration exercise (> 4 minutes), too little studies were performed at the moment, but a likely positive effect was shown. Recently, Chung et al (2014) examined the effect of BA supplementation on a 1-h time-trial in cyclists, but there was no beneficial effect on performance, although muscle carnosine concentrations were doubled due to supplementation. The review of Blancquaert et al (2015) summarized the most recently published articles, including some

results in aquatic sports. The conclusion was in accordance with the findings formulated by Hobson et al (2012) as the most significant improvements were found in 200m swimming performance, which lasts around 2 minutes. Furthermore, BA supplementation does not seem to affect performance in team sports, because no beneficial effects were found in repeated sprint performances and intermittent activities (Derave et al 2007; Smith-Ryan et al 2012; Ducker et al 2013).

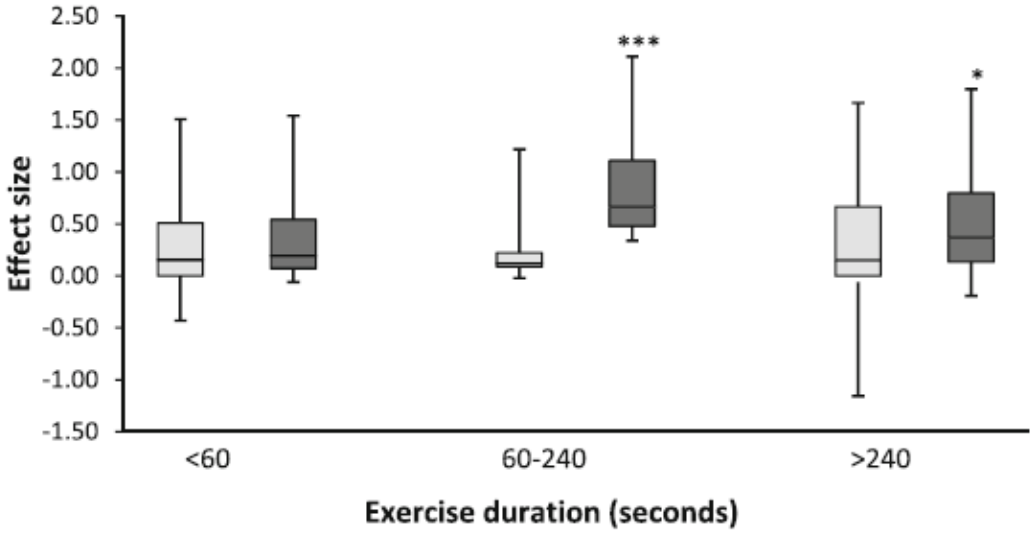


Figure 10. The effect size of placebo and BA groups when subdivided by exercise duration. Light gray represents placebo groups and dark grey represents BA groups. * denotes significantly greater than placebo with $P < 0.05$, *** denotes signficantly greater than placebo with $P = 0.001$ (Hobson et al 2012).

Another important question is whether BA supplementation is equally effective in elite athletes as in sub-elite athletes, as most of the papers are conducted at recreationally athletes. The potentially confounding factor of training status on the ergogenic effect of carnosine loading could be in accordance to nitrate, another popular supplement (Jones 2014). Based on a recent study (de Salles Painelli et al 2014), it is established that BA supplementation has a beneficial potential in highly trained subjects, which supports the value of this popular supplement in athletic populations.

4. Muscle fiber type composition

This part of the introduction will go back to the basics of muscle physiology and will introduce muscle fiber type composition. This will subsequently be linked to the topic of carnosine of part 3 and to the topic of MRS of part 2.

4.1. Muscle fiber types

Human skeletal muscle consists of a mixture of two different cell types: type I or slow-twitch (ST) fibers and type II or fast-twitch (FT) fibers. FT fibers are further categorized in fast oxidative glycolytic (IIa) and fast glycolytic (IIx) fibers. Muscle fibers are innervated by motor neurons, who are responsible to stimulate these fibers to contract. Three classes of motor neurons are identified: (1) motor neurons containing type I fibers, characterized by high amount of impulses, low frequency and long duration sets; (2) motor neurons stimulating type IIx fibers, described by moderate activity of impulses, high frequency and short duration sets; (3) motor neurons consisting of type IIa fibers have properties in between the others (Schiaffino and Reggiani 2011).

The contractile performance of the different muscle fiber types are defined by a broad variability in mechanical parameters (e.g. shortening velocity, fatigue profile and power delivery). Following the force-velocity relationship, it is known that on one hand a high shorten velocity goes together with low force production and on the other hand, generating high force results in a low shorten velocity. Therefore, different muscle fibers should optimize both parameters to ensure maximum power. As seen in the force-velocity relationship, FT fibers can deliver a greater force at a certain velocity, generating greater power values. Furthermore the maximal velocity and force were greater in FT fibers, resulting in twice as high peak powers compared to ST fibers (Fig 11) (Bottinelli et al 1996; Schiaffino and Reggiani 2011). Besides mechanical power, the energy metabolism of the fiber types shows a different profile. ST fibers are characterized by a high fatigue resistance and generate ATP by oxidative mitochondrial processes. FT fibers rely on the glycolytic system to achieve ATP very fast (Schiaffino and Reggiani 2011). To handle these specific metabolic activities, muscle fibers optimize their characteristics, with a higher capillary density and greater number of mitochondria in ST fibers.

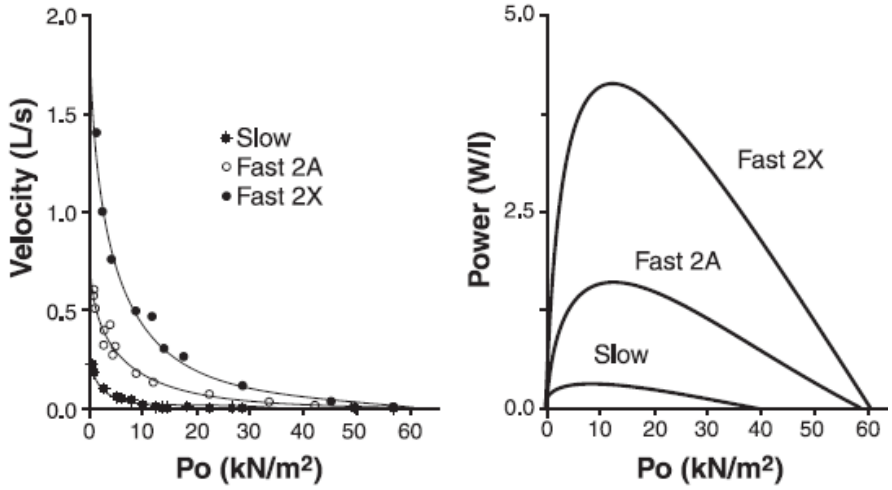


Figure 11. Force-velocity and force-power curves of human skeletal muscle fibers (slow (ST), fast 2A (FTa) and fast 2X (FTx)) (Bottinelli et al 1996). Peak power values increase orderly from slow to fast 2A and fast 2X (reviewed by Schiaffino and Reggiani (2011)).

Muscle fiber type composition is the distribution of both muscle fiber types within muscles (Fig 12). Most people have an equal distribution of ST and FT fibers, but there are large inter-individual differences in MFTC. Additionally, a wide intra-individual diversity in MFTC is found, depending on the primary function of a certain muscle. Muscles responsible for postural stability (e.g. soleus) will consist of a greater proportion of ST fibers, while some other muscles (e.g. triceps) are characterized by a high percentage of FT fibers for ensuring dynamic movements in sport practice. However, most muscles in the human body fulfill both tonic and phasic functions, and showed no clear preponderance of one fiber type (Johnson et al 1973).

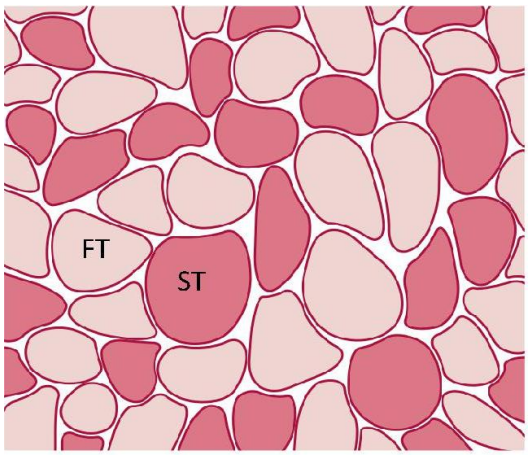


Figure 12. Human skeletal muscle represented as a mixture of ST fibers and FT fibers.

4.2. Nature-nurture debate

Whether having a specific MFTC is determined by genetic factors or whether the plasticity of fibers is influenced by training/detraining is commonly discussed. Simoneau and Bouchard (1995) summarized to what extent MFTC is controlled by inherited components and the environment (Fig 13). It was suggested that about 45 % of the variance in MFTC distribution between people is due to the genetic aspect and 40 % is declared to environmental factors. A small part (15 %) is explained by the error component of the methodology (= technical variance).

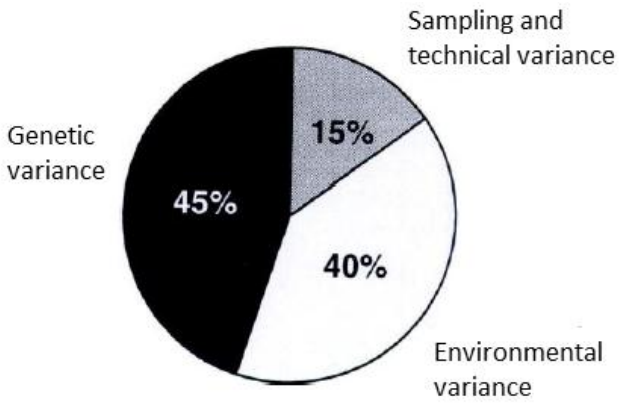


Figure 13. Estimates of the different variances (technical, genetic and environmental) influencing muscle fiber type compositions between individuals (Simoneau and Bouchard 1995).

The presence of a genetic effect on MFTC was shown by Komi et al (1977). This study demonstrated identical muscle fiber compositions in monozygotic twins, but not in dizygotic twins. Vincent et al (2007) identified the first genetic polymorphism (ACTN3) associated with fiber type distribution. Furthermore, Vikne et al (2012) showed an interrelationship in various muscles from one individual. Although the initial composition in muscle fiber types is genetically determined, the final outcome in MFTC will be a combination of nature and nurture. As Simoneau and Bouchard (1995) suggested, a part of MFTC will be influenced by the environment (e.g. exercise training). Some longitudinal interventions were able to demonstrate a transition from FTx to FTa and to a lesser degree from FT to ST fibers, indicating muscle fibers can modify into another type (Andersen and Henriksson 1977; Simoneau et al 1985; Staron et al 1990; Ingalls 2004; Aagaard et al 2011). Whether one individual is more sensitive for adaptation of MFTC by

training than an other can also be partly due to genetics, suggesting the existence of a gene-environment interaction.

4.3. Distribution of MFTC in different sport populations

There is an ongoing research interest in MFTC in different sports. However, most important studies on elite athletes were done in the seventies and eighties by the muscle biopsy method. This commonly used method is not ideal for athletes, which is the reason for the limited studies and the limited subjects in the studies. Table 1 gives an overview of the literature of MFTC in different sports and figure 14 is a visual summary of the distribution in MFTC in different sports and their disciplines. This figure illustrates the fragmentary literature and for some sports (e.g. cycling) the literature do not specify the level and subdisciplines of the athletes (e.g. track cycling, road cycling).

4.4. Measurement of muscle fiber type composition

Until now, literature on a great pool of elite athletes is lacking. The reason for this is the invasive method to determine MFTC, namely the muscle biopsy. Other methods to estimate MFTC, like the jump test by Bosco et al (1983), were based on performance tests. Although the high test-retest reliability on the jump test, the results could be affected by motivational factors as well as training and fatigue. Therefore, the need to an useful, non-invasive alternative to measure MFTC in resting conditions remained high. Recently, Baguet et al (2011b) investigated a new method, based on ^{1}H magnetic resonance spectroscopy (^{1}H-MRS). In what follows, we take a closer look at the pros and contras of the muscle biopsy and the ^{1}H-MRS method.

4.4.1. Muscle biopsy

Muscle biopsy, also known as the ‘gold standard’, is an invasive technique to measure MFTC. This technique is commonly used for diagnosis of myopathies and the understanding of the structure and function of skeletal muscle. The muscle biopsy allows to quickly collect muscle tissue samples immediately before, during and after a bout of exercise. Furthermore, this method is able to investigate the effect of a nutritional intervention combined with different exercise protocols (Shanely et al 2014). This method could also analyze differential responses of muscle fiber types in muscle wasting, leading

to a better understanding of some specific disorders (Ciciliot et al 2013). The biggest advantage is that muscle biopsies enables to have an overall view of the muscle function (e.g. capillarisation, percentage and area of different fiber types) and of a much larger repertoire of biochemical methods (e.g. specific protein and gene expression) which is very useful in different intervention studies in the field of sports. However, this method has some disadvantages to determine MFTC. First of all, in a single biopsy a small part of the whole muscle (0.01 %), containing only a few of hundreds of fibers, is analyzed for fiber typing (Albracht et al 2008). This method is an insufficient estimator of the total muscle mass, which is the reason for the rather low representativity of this technique. To estimate MFTC appropriately, numerous biopsies are required, but analyzing these biopsies is also very labor-intensive. Furthermore, the muscle biopsy causes damage to muscles, which make it not suitable for elite athletes.

4.4.2. ^{1}H-MRS

^{1}H-MRS is a new technology to estimate MFTC. Some other attempts with the NMR scanner have previously been made: determining T1 and T2 relaxation times using MRI or measuring inorganic phosphate by phosphorus magnetic resonance spectroscopy (^{31}P-MRS). However, none of these methods succeeded to estimate MFTC. ^{1}H-MRS is a technique based on measuring carnosine, a specific stable metabolite which is more present in FT fibers compared to ST fibers. It was shown from biopsy samples that the carnosine concentration was twice as high in FT compared to ST fibers (Harris et al 1998; Tallon et al 2007; Hill et al 2007; Kendrick et al 2009). Furthermore, some studies (Mannion et al 1995; Suzuki et al 2002) showed a (tendency to a) significant positive correlation between biochemically-determined carnosine levels and fraction FT fibers in human muscle biopsies. Baguet et al (2011b) were the first who compared ^{1}H-MRS measurement of carnosine to the 'gold standard', namely the muscle biopsy. In this study, subjects were first measured with ^{1}H-MRS to quantify their carnosine concentrations and afterwards a biopsy was taken from the same subjects. A positive correlation between muscle carnosine content and percentage area occupied by FT fibers by ^{1}H-MRS was found, which is the basis to use this new methodology to indirectly estimate MFTC. Important to note is that athletes could not take BA in the last three months before the measurement and also special attention is needed for vegetarians, as the influence of diet

on carnosine levels is not yet clear. However, this promising technique has some advantages compared to the muscle biopsy. First of all, it is a non-invasive, painless method which can be repeatedly done without damaging the muscle. This makes it very useful in athlete populations and children. Secondly, a higher amount of muscle mass, which contains both superficial as deeper parts of the muscle, is analyzed compared to the muscle biopsy, ensuring a better representativity. The test-retest variability for measuring carnosine is quite low, with a variation coefficient within the same day of 4.3 % (soleus) and 7.6 % (gastrocnemius) (Ozdemir et al 2007). The biological variability within a 6 week period was 9.8 % (soleus) and 14.2 % (gastrocnemius) (Baguet et al 2009). A third benefit: this technique is not labor-intensive and provided with some experience, the measurement and analysis lasts only 30 minutes. As this new method counters most of the disadvantages of the muscle biopsy, it can be a promising technique for the future. Nonetheless, this new method remains an estimation of MFTC and requires the availability of a NMR scanner, which makes this method not easily transported.

Aims: Muscle fiber type composition (MFTC) shows enormous innate inter-individual differences. The understanding of MFTC in athletes could have large implications for sport sciences, especially for training information, injury risk and talent identification. Until now, muscle fiber type remains an intriguing topic in relation to sport and together with the development of a new non-invasive method, the question raises whether it would be possible to have an overview of MFTC in athletes both within and between different sports. This could lead to new insights why there are big differences in MFTC between athletes. This aim will be explored in the third study of this thesis.

Table 1. Overview of % ST fibers and % area ST fibers in deltoideus (DELT), vastus lateralis (VL) and gastrocnemius (GASTRO) muscles in controls and athletes of different cyclic sports.

Sport discipline	Muscle	Subjects	% ST fibers	% area ST fibers	Literature
Untrained controls	DELT	12	46.0 ± 6.8		(Gollnick et al 1972)
		12	50 ± 9	44 ± 10	(Tesch and Karlsson 1985)
	VL	12	36.1 ± 5		(Gollnick et al 1972)
		12	43 ± 9	40 ± 9	(Tesch and Karlsson 1985)
		4	53 ± 6.4		(Baumann et al 1987)
	GASTRO	19	57.7 ± 2.5	60.0 ± 2.7	(Costill et al 1976a)
		11	52.6	56	(Costill et al 1976b)
Athletics (SPRINT)	VL	8	49 ± 7.1		(Baumann et al 1987)
		1	29		(Trappe et al 2015)
		1	26.0	21.9	(Gollnick et al 1972)
	GASTRO	2	24	23.5	(Costill et al 1976a)
Athletics (MIDDLE DISTANCE)	DELT	9	49 ± 8	44 ± 8	(Tesch and Karlsson 1985)
	VL	8	58.9 ± 3.7		(Gollnick et al 1972)
	GASTRO	18	61.8 ± 2.9	62.1 ± 2.6	(Costill et al 1976b)
		7	51.9	46.5	(Costill et al 1976a)
Athletics (LONG DISTANCE)	VL	9	67 ± 10	67 ± 10	(Tesch and Karlsson 1985)
	GASTRO	14	79.0 ± 3.5	82.9 ± 3.1	(Costill et al 1976b)
		5	69.4	62.3	(Costill et al 1976a)
Swimming	DELT	5	74.3 ± 5.7		(Gollnick et al 1972)
	VL	5	57.7 ± 9.3		(Gollnick et al 1972)
Cycling	DELT	4	50.7 ± 4.4		(Gollnick et al 1972)
	VL	4	61.4 ± 5.9		(Gollnick et al 1972)
		14	~70	~69	(Aagaard et al 2011)
		19	56 ± 3		(Coyle et al 1992)
		13	80 ± 8.3		(Baumann et al 1987)
		20	55		(Hopker et al 2013)
Kayaking	DELT	9	71 ± 11	63 ± 15	(Tesch and Karlsson 1985)
	VL	9	41 ± 10	33 ± 10	(Tesch and Karlsson 1985)

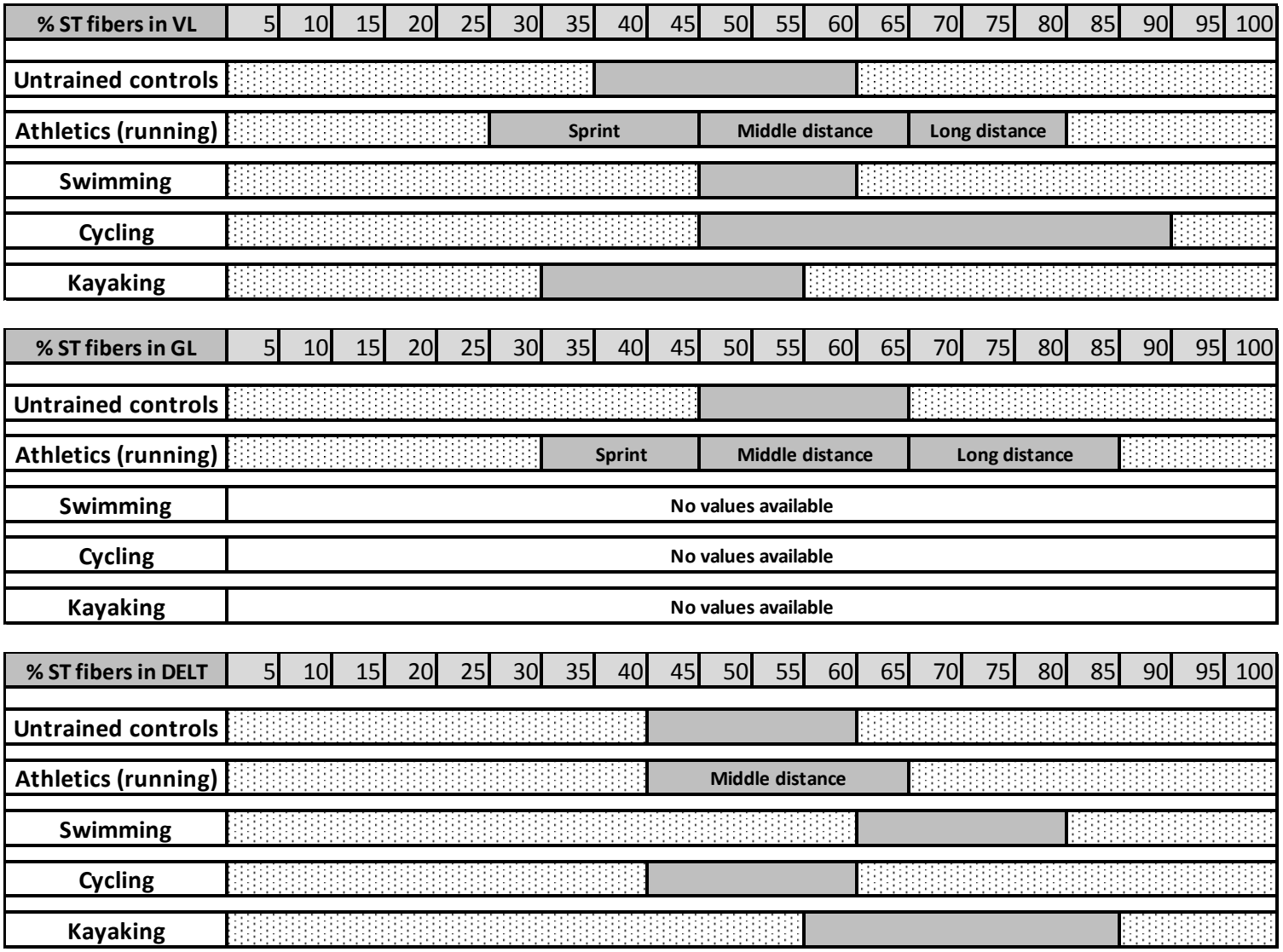


Figure 14. Schematic overview of percentage ST fibers in vastus lateralis (VL), gastrocnemius (GASTRO) and deltoideus (DELT) muscles in different cyclic sports (based on table 1).

5. Sprint performance

In this part, the focus shifts to sport performance and in this chapter, especially sprint running performance. In the fourth paper of this thesis, MRI is used to have a closer look to the musculo-skeletal characteristics of sprinters. There are many factors leading to success in sprinting, but the focus in this chapter will be more on the biomechanical parameters as their influence on the velocity-curve of sprint running is quite important. However, besides this, other factors (e.g. energy systems, reaction time etc.) are affecting sprint performance, but these factors will not be discussed here.

The chance to become an exceptional sprint athlete is influenced by numerous factors. One of the crucial factors for success in sprint running performance is the ability of skeletal muscles to produce force at a high velocity, which is strongly influenced by genetics (e.g. ACTN3) (Eynon et al 2013). Understanding success in sprint performance demands on one hand a detailed insight in the velocity-curve of sprint running and on the other hand knowledge of the specific musculo-skeletal properties of the leg of sprint athletes.

5.1. Phases of the velocity curve

Sprint performance is a multifactorial and complex process, which can be divided in three main phases: acceleration, constant velocity and deceleration (Fig 15) (Volkov and Lapin 1979; Mero et al 1992).

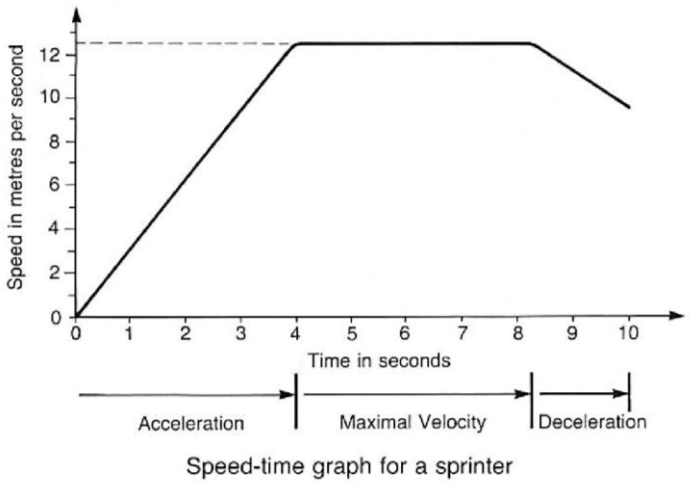


Figure 15. The running velocity curve (from IAAF "Introduction to Coaching Theory") with three main phases in a 100m sprint: (1) acceleration phase, (2) maximal velocity phase and (3) deceleration phase.

The **acceleration phase** is characterized by an increase in stride length, stride rate and flight time, while contact time will decrease. Contact time is divided into braking and propulsion phases, based on the negative and positive horizontal reaction forces. During the acceleration phase of sprinting, the propulsive force is large and generated for a long period, which emphasizes the importance of strength during this phase (Mero et al 1992). To produce such a large power output in the sprint start, the total amount of muscle mass is a key factor (van Ingen Schenau et al 1994).

Running velocity is the result of stride length and stride rate, with the most critical role for stride rate at maximal velocities. During **constant velocity** sprinting, the leg extensor muscles showed a peak activity in the braking phase, which is mainly due to the recruitment of the rectus femoris muscle as hip flexor. During the propulsion phase, the biceps femoris and gastrocnemius muscles are moderately active and seem to play a primary role in this phase. Furthermore, muscle activitation during the propulsion phase is markedly lower than during the braking phase by an increased recoil of elastic energy. This suggests the importance of elastic properties of the muscles in increasing explosive force production (Cavagna et al 1971). The requirement to accelerate and decelerate the rotational velocity of the entire leg, relative to the trunk is the limited factor for the maximal velocity (van Ingen Schenau et al 1994). This is influenced by muscle mass (absorbing and generating energy) on one hand and moment of inertia on the other hand. As these elements are conflicting requirements, leg characteristics of sprinters should be optimized to reach the maximal velocity.

In the **deceleration phase** during a 100m race, a decrease of stride rate take place in combination with a small increase in stride length. Contact and flight time increases also at the end of the race. The shape of the velocity-time curve is influenced by many variables (e.g. wind, utilization of energy sources) which makes the interpretation of the deceleration rather difficult (Mero et al 1992). The deceleration phase should be minimized as this phase is detrimental for performance. The existence of the deceleration phase is more a physiological than a biomechanical conundrum. As mentioned before, human skeletal muscles consist of a mixture of fiber types with different properties. FT fibers can combine high velocity contractions together with great power production, but only for short duration exercises (like 100m sprint). Sprinters, performing long sprint runs

(up to 400m) should also possess the fatigue resistant ST fibers to restrain the deceleration.

Overall, the recent study of Morin et al (2012) concluded that 100m performance is highly related to velocity variables, rather than force capabilities. However, it is challenging for the human body to optimize both parameters to ensure elite performance.

5.2. Body morphology

The question is now how body morphology of sprinters influences the different phases and thus how the musculo-skeletal characteristics of the leg are optimized to deal with conflicting requirements, such as muscle mass versus moment of inertia.

5.2.1. Skeletal properties

Some studies already examined different skeletal geometrical parameters of sprinters (Abe et al 2000; Lee and Piazza 2009; Karamanidis et al 2011). No differences were found between a group of high level and low level sprinters in different skeletal characteristics of the lower leg, ankle and foot (Karamanidis et al 2011). Only a moderate relationship was found between shank length and 100m personal best, with lower length for the higher level sprinters. Thigh and lower leg length also showed no differences between sprinters, endurance runners and untrained subjects (Abe et al 2000). Lee and Piazza (2009) were the first to demonstrate differences in skeletal measures between sprinters and non-sprinters, namely higher mean toe lengths and shorter shank lengths. They suggest that longer toes can enhance forward acceleration, by increasing the ‘gear ratio’ (lever arm of the ground reaction fource (GRF) to the lever arm of the Achilles’ tendon) and contact time.

5.2.2. Muscle properties

One of the most determining factors for sprinting is power generation, which is directly related to muscle fiber type, muscle volume/mass and muscle fiber length. The impact of these different parameters on sprint performance will be clarified below.

5.2.2.1. Muscle fiber type

As described earlier (section 4.4), sprinters are characterized by a higher percentage FT fibers compared to endurance runners. Besides the number of FT fibers, the cross-sectional area of FT fibers is also higher in leg extensor muscles compared to endurance runners (Gollnick et al 1972; Costill et al 1976a). Thorstensson et al (1976) investigated a correlation between percentage of FT fibers and contraction velocity of the knee extensor muscles. As FT fibers are able to deliver high power outputs in combination with these high contractile velocities, an abundance of these type of muscle fibers will determine sprint performance.

5.2.2.2. Muscle volume/mass

Muscle volume/mass is known as one of the most important determinants for power generation. Some studies already tried to investigate muscle volumes in sprinters, although this was mostly done by interpreting muscle thickness, analyzed with ultrasonography. Abe et al (2000) suggested a difference in muscle shape in sprinters, compared to endurance runners. They found greater muscle thickness in upper portion of the anterior thigh and not in the lower portion (70% thigh length). These results were confirmed in the study of Kumagai et al (2000), who found greater muscle thicknesses in the upper portion of the leg, both in the anterior (quadriceps) and posterior (hamstrings) part in high-level sprinters, compared to low-level sprinters. Additionally, greater muscle thickness was found in the high-level sprinters in gastrocnemius lateralis, but not in gastrocnemius medialis. However, Kubo et al (2011) could not support this, as they did not find differences in muscle thickness in the knee extensors between sprinters and untrained controls, except for the medial side. For plantar flexors, muscle thickness was greater in all muscles (soleus, gastrocnemius lateralis and gastrocnemius medialis) in sprinters compared to controls.

Hoshikawa et al (2006) proposed an important role, beside the knee extensors, for the knee and/or hip flexors in relation to sprint running performance. They found that the hip flexors, with the psoas major as key muscle, seem to be essential as power generators for accelerating the swing leg during sprint running. However, no relation was found between quadriceps and hamstrings and 100m race performance, which is in contrast

with the results of Kumagai et al (2000) and the suggestions that the hamstrings are a critical muscle group during sprinting (Mero et al 1992; Schache et al 2012).

5.2.2.3. Muscle architectural parameters

Shortening velocity of muscle fibers as a parameter of sprint running performance, is partly determined by architectural properties, like muscle fascicle length and pennation angle (Wickiewicz et al 1984). Abe et al (2001) developed a schematic illustration to explain in which way longer fascicle lengths can have an impact on sprint performance. In a longer fascicle (A) each sarcomere can shorten less by sharing the shortening distance compared to a shorter fascicle (B), implicating that the longer fascicle (A) can shorten at a slower velocity and thus can generate a greater force based on the sarcomeres force-velocity curve (Fig 16). It has already been shown that the plasticity of muscle geometry exists and thus differences in architectural characteristics between athletic populations might be at least partly due to different training regimes (Blazevich 2006).

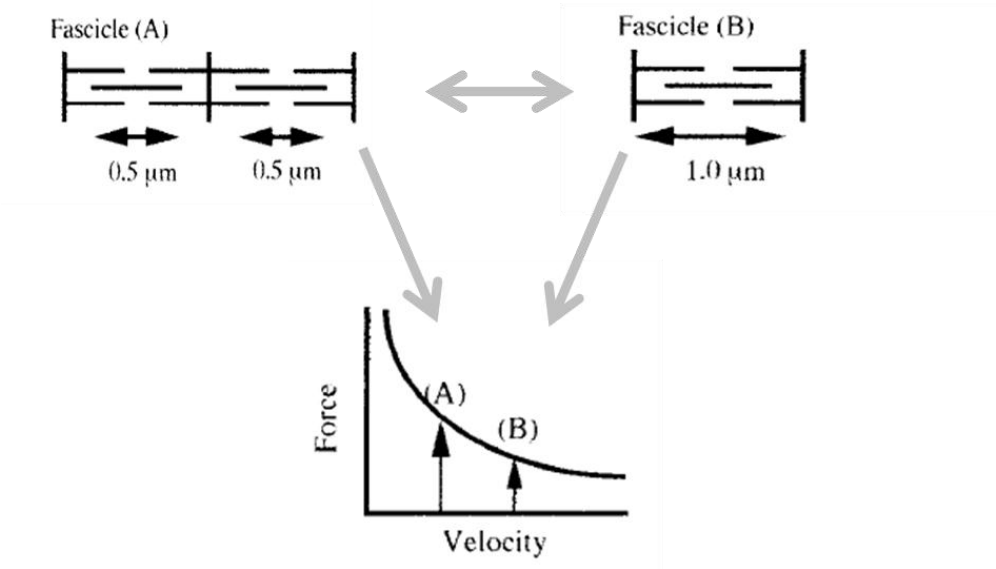


Figure 16. The effect of longer fascicle length on the force-velocity relationship. Shortening distance is 1.0 µm and thus fascicle (B) need to shorten 1.0 µm, while fascicle (A) can share the shortening distance between its sarcomeres. In this way, fascicle (A) can shorten at a lower velocity than fascicle (B) and can deliver more force, following the force-velocity curve (figure adapted from Abe et al (2001)).

In sprinters, longer fascicle lengths in vastus lateralis, gastrocnemius medialis and gastrocnemius lateralis muscles were found compared to endurance runners, confirming the relationship between sprint performance and differences in muscle fascicle length

(Abe et al 2000). However, no differences in fascicle length in gastrocnemius muscles were found between high-level and low-level sprinters, indicating that differences in the highest sprint ability were not affected by architectural properties (Karamanidis et al 2011).

Aims: Sprint running performance is characterized by an interaction of parameters, which should be all optimized to reach elite performance. However, a question remains to what extent the body morphology can differ within one ethnicity. Therefore, the third aim of this thesis is to describe the musculo-skeletal leg characteristics of sprinters compared to endurance runners, to clarify the most distinguished differences.

6. Endurance running performance

In the last part of this introduction, superiority in endurance running will be explained. How it is established and how the human body is adapted to deliver extreme endurance performances. Endurance running is strongly determined by physiological factors and therefore this perspective is predominantly described. Biomechanical issues (e.g. vertical and horizontal forces etc.) will not be discussed in this thesis. This part also focus on the dominance of East-African runners which has been a fascinating phenomenon for years. In paper 4 and 5 of this thesis, the characteristics of the leg of endurance athletes of both Caucasian and East-African runners were investigated by MRI. This is in order to map differences in body morphology of endurance athletes of various ethnicity.

Excellence in endurance running is regulated by a complex interaction of factors. It is yet clear that both training and innate aspects are necessary to become an exceptional endurance runner. Some research associated already some candidate genes, such as ACE to endurance performance in Caucasian populations, but this is still not replicated in African populations (Wilber and Pitsiladis 2012; Tucker et al 2013).

6.1. Critical factors influencing endurance running performance

Superior endurance running depends on three critical factors: (1) maximal oxygen uptake (VO_{2max}), (2) fractional utilization of VO_{2max} and (3) running economy (Larsen 2003).

(1) **Maximal oxygen uptake (VO_{2max})** is seen as one of the most important factors in competitive distance running and a high VO_{2max} is created by the combination of natural endowment and training effort. In the study of Pollock (1977) VO_{2max} values were measured in the range of 71.3 to 84.4 ml/min/kg. The highest value (84.4 ml/min/kg) was found in an outstanding endurance runner and this is one of the highest VO_{2max} reported for a runner. However, no differences were found in VO_{2max} between elite African runners and Caucasian athletes with homogeneous race performances (Saltin et al 1995b; Weston et al 2000), suggesting that a high VO_{2max} is required for a certain level, but it is not the major factor that distinguishes elite athletes (Myburgh 2003).

(2) The ability to sustain a high percentage of VO_{2max} over a given distance (**fractional VO_{2max}**) is another frequently pointed predictor of endurance performance. Weston et al

(2000) suggested a higher fractional utilization of VO_{2max} in African endurance runners compared to Caucasians. However, a recent study of Tam et al (2012) could not confirm the same results in athletes of a higher level, suggesting that the dominance of East-African runners cannot be explained by differences in fractional VO_{2max}. It can be concluded that fractional utilization of VO_{2max} is related to endurance performance, but it is not the main discriminant that can explain differences on elite performance.

(3) Endurance running performance depends on the metabolic demand, which can be expressed either as energy cost of running (C_r) or **running economy (RE)**. C_r is the energy spent per unit distance, while RE is the energy demand at a given running speed (Lacour and Bourdin 2015). Optimum performance in any endurance running event requires efficient utilization of available energy. Some studies showed already a better RE in African endurance runners compared to Caucasian runners, suggesting that the lower energy cost may account for the differences between ethnic groups (Saltin et al 1995b; Weston et al 2000; Lucia et al 2006; Lucia et al 2008). In contrast, similar low-average oxygen cost of running was found in elite Kenyan and European marathon runners (Tam et al 2012), even as in black and white South African populations (Coetzer et al 1993). Although there is still some inconsistency in the literature, RE is seen as one of the key factors in endurance running success and is a better predictor of performance than VO_{2max} in elite runners (Saunders et al 2004).

6.2. Body morphology

To optimize the earlier mentioned parameters, endurance runners possess a specific body morphology. It is not yet clear whether skeletal or either muscle properties underlie elite endurance performance.

6.2.1. Skeletal properties

Limb dimensions have been addressed as possible effects on RE. There might be a relationship between leg length and stride length, suggesting that leg length influences RE (Anderson 1996). This is based on the assumption that the metabolic cost of running is partly determined by the angular inertia of the legs, which is on his turn influenced by the length of the legs. However, the effect of leg length on RE has only been established

indirectly. Furthermore, a large variation in RE was found in a heterogenous group of distance runners with similar leg length (Williams and Cavanagh 1987). When comparing different ethnicities, longer shank lengths were found in East-African runners compared to their white counterparts (Lucia et al 2006). Kunimasa et al (2014) demonstrated longer body segments at both thigh (7.3 %) and shank (8.4 %) in Kenyan compared to Japanese runners with similar body height. A recent study of Sano et al (2015) confirmed these results for shank lengths, and even in relative scales, greater shank length to height were found in Kenyan compared to Japanese runners. This can be attributed to the existence of racial differences in body proportions, with longer extremities in African vs. Caucasians (Wagner and Heyward 2000).

6.2.2. Muscle properties

6.2.2.1. Muscle fiber type

It has been suggested that a higher proportion of ST muscle fibers is associated with better RE and is thus beneficial for endurance performance (Williams and Cavanagh 1987). However, on elite level no differences were found between elite Kenyan and Scandinavian runners (Saltin et al 1995a). Furthermore, superior endurance running performance of black athletes was not due to a greater proportion of ST fibers (Coetzer et al 1993; Weston et al 2000). Regarding the high running speeds in world-class endurance performances (even for marathon running), the percentage and properties of FTa fibers should be mentioned. To summarize, possessing a high percentage of ST fibers is needed to reach elite endurance level, but it is not the explanatory factor for success of African runners.

6.2.2.2. Muscle volume/mass

Body mass, especially leg mass, can have an influence on RE. Myers and Steudel (1985) showed that adding a mass to the limbs has a negative effect on cost of locomotion. In this respect, slender limbs with low masses are identified as potentially responsible for superior economy in African runners. Some studies showed already lesser leg thickness in East-African runners. In the study of Lucia et al (2006) a 9 % difference in calf circumference was found between elite African and Spanish runners. Kong and de Heer

(2008) confirmed these results in Kenyan runners, suggesting that their slim limbs may attribute to a lower moment of inertia and thus requiring less muscular effort for swinging the limbs. Saltin et al (2003) showed a relationship between mean lower leg thickness and RE in Kenyan and Danish runners (Fig 17).

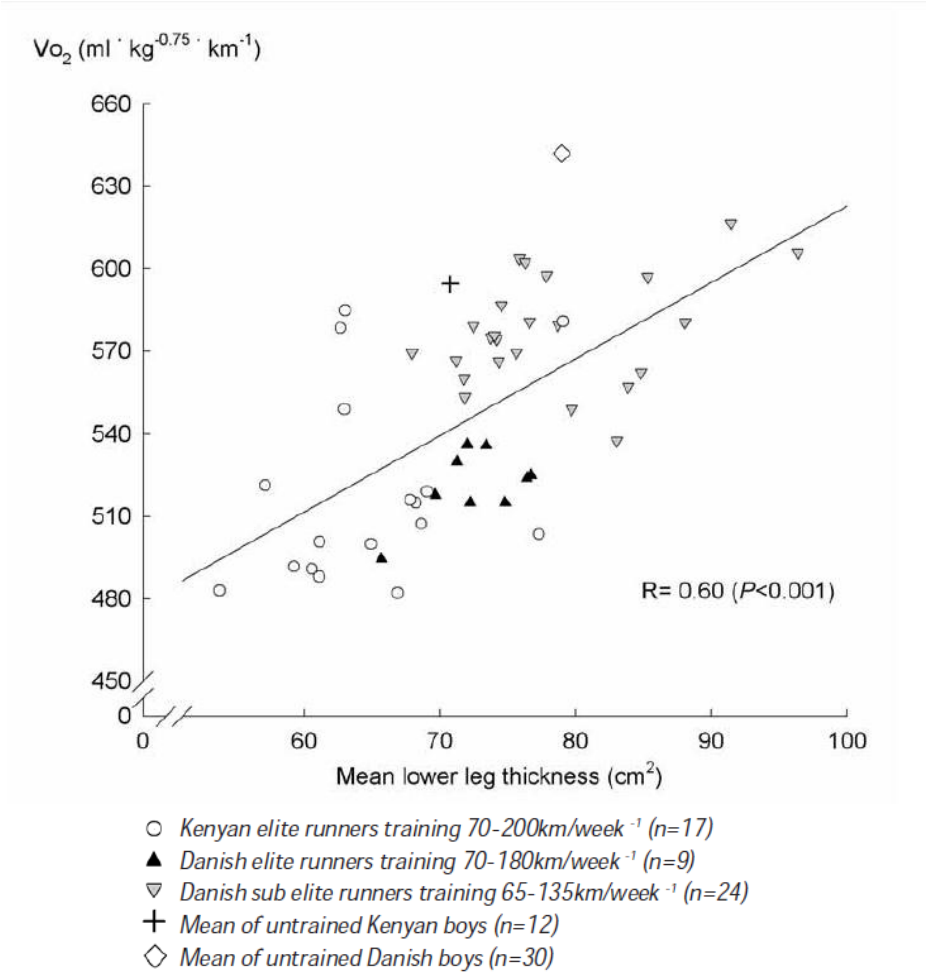


Figure 17. Relationship between mean lower leg thickness and running economy in various groups of runners. Untrained Kenyan and Danish boys are added for comparison but they are not included in the calculated R-value (Saltin 2003).

6.2.2.3. Muscle architectural parameters

Shorter muscle fascicles, together with greater pennation angles were suggested to reduce the metabolic energy cost in running. In the study of Abe et al (2000), endurance runners had shorter fascicles and greater pennation angles in vastus lateralis and gastrocnemius muscles compared to sprinters. Even within a group of endurance runners differences were found, with shorter fascicle lengths and greater pennation angles in

gastrocnemius medialis muscle in Kenyan compared to Japanese runners (Sano et al 2015). A shorter fascicle will posess fewer sarcomeres, requiring less metabolic energy for contraction (Blazevich 2006). Greater pennation angles ensure fascicles to work closer to optimal force-production lengths. These muscle properties support fatigue resistance and metabolic efficiency, which would be beneficial in endurance performance. Recently, Murach et al (2015) illustrated that architectural characteristics of endurance athletes are not entirely determined by genetic predisposition and thus can be affected by endurance training, suggesting the existence of architectural plasticity.

Aims: Superior endurance performance of East-African runners is a hot topic. To date, some differences have already been revealed, but it is still an open question which is the main reason for their superiority. Therefore, the fifth study will start with an overall analysis of body morphology to discover the main discriminants between East-African and Caucasian endurance runners.

7. Experimental aims and outline of the thesis

The original research of this thesis consists of 5 studies, which all make use of NMR equipment for MRI or MRS applications in healthy sport populations. Study 1 and 2 are linked to the carnosine loading protocol and had the aim to optimize this, while study 3, 4 and 5 focus on the characteristics of the body morphology of the athlete itself. Study 5 will be presented in the general discussion due to the preliminary nature of the data. Figure 18 is a reflection of the different studies showing the studied population and the overarching methodology.

As seen in the introduction, the carnosine loading protocol is not yet optimized, because of the low efficiency of BA uptake during supplementation. A possible determinant, namely exercise training, is until now poorly understood. This is in contrast with other supplements and therefore, **study 1 and 2** were performed. The aims of both studies are to investigate the effect of exercise on BA supplementation efficiency.

- The purpose of study **study 1** is to investigate whether BA supplementation can cause higher carnosine concentrations in trained compared to untrained muscles in different athlete populations, starting with a methodological analysis to measure carnosine in the upper body.
- **Study 2** examines the impact of acute exercise on untrained subjects to see if it is sufficient to enhance the loading efficiency. In addition this study aims to investigate which exercise modalities are most beneficial.

In the second part of the original research, **study 3, 4** and **5** focus on the musculo-skeletal characteristics of the athlete.

- In **study 3,** the aim is to investigate MFTC in different athlete populations and relate MFTC to specific muscle characteristics as well as sport characteristics.

- The aim of **study 4** is to examine the differences in the musculo-skeletal leg characteristics between sprinters and endurance runners of Caucasian ethnicity to discover the main discriminants for either sprint or endurance running performance.

- **Study 5** has the purpose to investigate the ethnic differences between Caucasian and East-African endurance runners by analysing the leg morphology of these athlete populations.

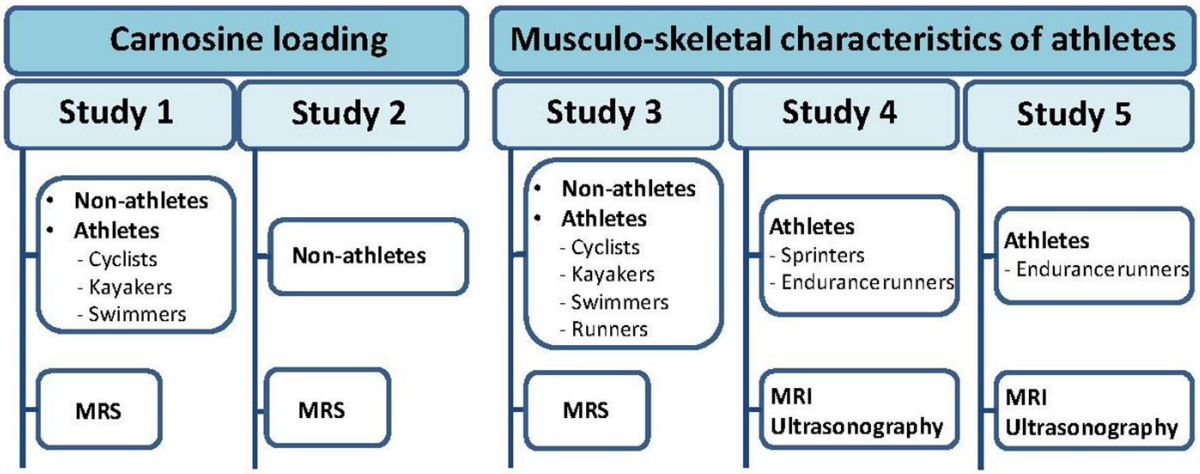


Figure 18. Overview of the original research of the thesis

II

Original Research

Study 1

Muscle carnosine loading by beta-alanine supplementation is more pronounced in trained vs. untrained muscles

Bex T, Chung W, Baguet A, Stegen S, Stautemas J, Achten E, Derave W

J Appl Physiol (2014) 16:204-209

ABSTRACT

Purpose. Carnosine occurs in high concentrations in human skeletal muscle and assists working capacity during high-intensity exercise. Chronic beta-alanine (BA) supplementation has consistently been shown to augment muscle carnosine concentration, but the effect of training on the carnosine loading efficiency is poorly understood. The aim of the present study was to compare muscle carnosine loading between trained and untrained arm and leg muscles.

Methods. In a first study (n=17), reliability of carnosine quantification by proton magnetic resonance spectroscopy (^{1}H-MRS) was evaluated in deltoid and triceps brachii muscles. In a second study, participants (n=35; 10 nonathletes, 10 cyclists, 10 swimmers and 5 kayakers) were supplemented with 6.4g/day of slow-release BA for 23 days. Carnosine content was evaluated in soleus, gastrocnemius medialis and deltoid muscles by ^{1}H-MRS. All the results are reported as arbitrary units.

Results. In the nonathletes, BA supplementation increased carnosine content by 47% in the arm and 33% in the leg muscles (NS). In kayakers, the increase was more pronounced in arm (deltoid) vs. leg (soleus + gastrocnemius) muscles (0.089 vs. 0.049), whereas the reverse pattern was observed in cyclists (0.065 vs. 0.084). Swimmers had significantly higher increase in carnosine in both deltoid (0.107 vs. 0.065) and gastrocnemius muscle (0.082 vs. 0.051) compared to nonathletes.

Conclusions. We showed that 1) carnosine content can be reliably measured by ^{1}H-MRS in deltoid muscle, 2) carnosine loading is equally effective in arm vs. leg muscles of nonathletes, and 3) carnosine loading is more pronounced in trained versus untrained muscles.

KEYWORDS: histidine-containing dipeptides, muscle contractions, sport supplements

INTRODUCTION

There is a growing interest in the molecule carnosine and its role in skeletal muscle in the field of exercise physiology (Derave et al 2010; Artioli et al 2010; Sale et al 2010). Carnosine (β-alanyl-L-histidine) is a dipeptide synthesized from the precursors L-histidine and beta-alanine (BA) by carnosine synthase (Boldyrev et al 2013). It is stored in high concentrations in human skeletal muscle (~5 mmol/kg wet muscle) (Derave et al 2010). The chronic oral ingestion of BA, the rate-limiting precursor in carnosine synthesis, has been shown to elevate the muscle carnosine content by 40 – 80 % (Harris et al 2006; Hill et al 2007; Derave et al 2007; Baguet et al 2009; Baguet et al 2010a; Stellingwerff et al 2012). Interestingly, the muscle carnosine loading strategy is ergogenic in high-intensity exercise (for meta-analysis see Hobson et al. (Hobson et al 2012)), which is likely related to its function in skeletal muscle, as a proton buffer (Baguet et al 2010b) and calcium regulator (Dutka et al 2012).

Since augmented muscle carnosine has many applications in sports and possibly also in health (del Favero et al 2012; Sale et al 2013), it is necessary to generate a better understanding of the determinants of muscle carnosine loading. First, the absolute increase in muscle carnosine is strongly dependent upon the total BA amount supplemented over a certain period of time (Stellingwerff et al 2012). Meanwhile, Stegen et al. (2013) demonstrated that coingestion of BA with meals can beneficially influence muscle carnosine loading, suggesting that insulin could play a role in muscle carnosine loading.

It could be hypothesized that exercise training can also facilitate muscle carnosine loading, by analogy with creatine, another popular nutritional supplement, for which a higher increase in creatine concentration was demonstrated in the trained compared to the untrained leg after creatine supplementation (Harris et al 1992; Robinson et al 1999). In the latter study, one-legged training consisted of 1 hour hard exercise per day for 7 days. To date, the effect of concomitant training during BA supplementation on the carnosine loading efficiency is poorly understood. Only one training study is available (Kendrick et al 2009), which found that carnosine loading after BA supplementation was not influenced by a limited volume of one-legged isokinetic training, consisting of 10 x 10 maximal

isokinetic contractions, 3 to 4 training sessions per week, over 4 weeks (Kendrick et al 2009).

The present study aims to explore the effects of training on BA-induced muscle carnosine loading in an observational rather than interventional design. For this purpose we used a novel training study approach, in which we investigated the effects of BA on carnosine content in arms and legs in nonathletes versus athletes who train either upper body or lower body, or both.

We recently developed a non-invasive method for quantification of carnosine in human lower leg muscles using proton magnetic resonance spectroscopy (^{1}H-MRS) (Ozdemir et al 2007). To our knowledge, ^{1}H-MRS -based metabolite quantification in a single muscle of the upper body has never been investigated, neither for creatine nor for carnosine loading. In the present study, we first investigated in which large muscle of the upper body carnosine is reliably measurable by ^{1}H-MRS. Then, we hypothesized that muscle carnosine loading via BA supplementation is of the same magnitude in arm and leg muscles in a non-active control group. Finally and most importantly, we aimed to investigate whether carnosine loading would be more pronounced in trained versus untrained muscles, by comparing loading in arm and leg muscles that are specifically trained in different athlete groups.

MATERIALS AND METHODS

This study consists of 2 parts. For study A, carnosine was measured in 2 muscles of the arm to investigate in which muscle of the upper body carnosine is reliably measurable by ^{1}H-MRS. Study B was a nutritional intervention study focusing on the difference in loading between arm and leg muscles in nonathletes and different athlete populations.

Study A

Subjects: Seventeen students (15 males and 2 females) volunteered to participate in this study. All subjects were physically active and were involved in different sports (athletics, football, swimming, cycling…). The subjects' age, weight, and height were 22.0 ± 1.0 yr, 72.4 ± 6.4 kg, and 178.0 ± 8.9 cm, respectively.

Study protocol: Eleven subjects (9 men and 2 women) were measured twice on the same day to test the methodological variation. The subjects were measured, taken out of the scanner and immediately back in the scanner and were measured again. Six subjects underwent 2 tests, separated by 3 – 4 weeks, to test the additional biological variation of muscle carnosine content over that period. In the seventeen subjects, muscle carnosine content was measured separately in deltoid and triceps brachii muscles by ^{1}H-MRS, as specified below.

Study B

Subjects: Thirty-five men (10 healthy nonathletes, 10 road cyclists, 10 swimmers and 5 flat-water kayakers) participated in this study. Table 1 shows some details about the training history and current training load of the athletes. All the athletes were well-trained at baseline (recreational and regional class athletes) and trained at least 8 hours a week in their specific sports (cycling, swimming or kayaking) during the supplementation period, whereas the nonathletes were inactive throughout. All subjects had a normal diet and none of them was a vegetarian. None of the subjects took supplements in the 3 months prior to the study and during the study, except from the supplement as part of the experimental intervention.

Study protocol: All the participants were supplemented for 23 days with 6.4 g slow release beta-alanine (SR-BA) (4x/day 2 tablets of 800 mg, Carnosyn, Natural Alternatives

International). None of the subjects reported side effects due to the supplementation. Before and after the supplementation period muscle carnosine content was measured by ^{1}H-MRS in soleus, gastrocnemius medialis and deltoid muscles. Due to the better reproducibility of the deltoid in study A, we decided to exclude triceps brachii in study B.

Both studies were approved by the local ethical committee (Ghent University Hospital, Ghent, Belgium). All subjects gave their written informed consent to take part in the studies and were aware that they were free to withdraw from the experiment at any point.

Table 1: Baseline values of the four groups of study B and details of the training of the athletes.

	Nonathletes	Cyclists	Kayakers	Swimmers
Number of subjects	10	10	5	10
Age (yr)	22.0 (± 2.3)	29.0 (± 8.9)	21.7 (± 3.4)	19.4 (± 1.0) *
Height (cm)	178.0 (± 7.8)	177.0 (± 5.9)	185.6 (± 5.4)	178.0 (± 5.6)
Weight (kg)	72.4 (± 10.3)	71.8 (± 9.1)	81.2 (± 10.9)	70.3 (± 5.2)
Years of training (yr)		3 - 10	4 - 15	6 - 14
Training sessions/week		4.7 (± 1.0)	6.0 (± 2.0)	6.9 (± 2.2)
Training hours/ week (h)		11.5 (± 3.4)	9.6 (± 2.6)	11.8 (± 3.5)

There are no significant differences between the groups, except for age in the swimmers. Swimmers' age was significant lower than the age of the cyclists and kayakers (* $p < 0.05$). Data are means ± SD.

Muscle carnosine quantification by ^{1}H-MRS

All the MRS measurements were performed on a 3-T whole body MRI scanner (Siemens Trio, Erlangen). Carnosine content was measured by ^{1}H-MRS, as described by Baguet et al. (2010a). The subjects were lying in supine position. To measure the calf muscles, the lower leg was fixed in a spherical knee-coil with the angle of the ankle at 20° plantar flexion. A shoulder coil was used to measure carnosine in the deltoid and triceps muscle (Fig 1A). Single voxel point-resolved spectroscopy (PRESS) sequence with the following parameters was used; repetition time (TR) of 2.000 ms, echo time (TE) of 30 ms, number of excitations is 128, 1.024 data points, spectral bandwidth of 1.200 Hz, and a total acquisition time of 4.24 min. The average voxel size for the soleus, gastrocnemius, deltoid and triceps brachii muscle was 40 mm x 12 mm x 30 mm, 40 mm x 12 mm x 30 mm, 40 mm x 13 mm x 30 m and 30 mm x 9 mm x 27 mm, respectively. The line width of the

water signal for the soleus, gastrocnemius, deltoid and triceps brachii muscle was on average respectively 25.5 Hz, 26.8 Hz, 27.4 Hz and 25.2 Hz, following shimming procedures. The integral of the C2-H peak (at ~8 ppm) was quantified relative to the water peak integral (x1000) and reported as arbitrary units (Gualano et al 2012) (Fig 1B).

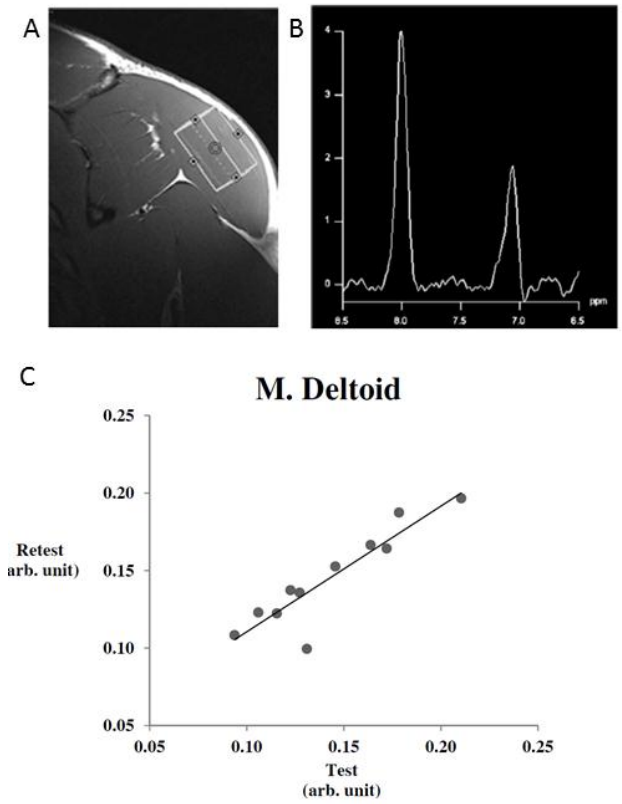


Figure 1. A: Representative image (coronal plane) of voxel placement in the deltoid muscle. **B**: C2 and C5 proton resonances of the imidazole ring of carnosine can be identified at ~8ppm and ~7ppm, respectively, in the proton MRS spectrum of the human deltoid muscle (representative spectrum). **C:** Study A: Reliability of the carnosine concentration in the deltoid muscle, measured twice on the same day. * $p < 0.05$. ICC = 0.903.

The reason why we could not calculate into millimolar concentrations is because the shoulder coil was a receive-only coil, whereas the knee coil was a transmit/receive coil. With a receive-only coil it is not possible to use the phantom method to calculate the millimolar concentrations, as we usually do. In order to allow comparison between the leg muscles and the deltoid, we expressed the concentration relative to the water peak (arbitrary units) for all muscles. We found significant correlation coefficients for the water signal pre and post supplementation in the three muscles groups: 0.684 ($p < 0.001$) for soleus, 0.632 ($p< 0.001$) for gastrocnemius and 0.661 ($p < 0.001$) for deltoid. The variation coefficients were respectively 4,23% for soleus, 4,59% for gastrocnemius and 7,1% for

deltoid. There was no significant difference in water signals between pre and post supplementation for the different groups in the different muscles. These results showed that muscle water content was not influenced during supplementation which gives us the possibility to use these arbitrary units.

Statistics

The reliability of measuring carnosine in a single muscle of the upper body in study A was evaluated by Intraclass Correlation Coefficient. The variation coefficient (CV) was calculated by following equation: CV = (SD/Mean)*100.

For study B, a one-way ANOVA was performed to compare the difference in baseline muscle carnosine concentration and the absolute carnosine increase after BA supplementation between the 4 groups. A post hoc analysis (Tukey) was done for multiple group comparison and a paired sample t-test was done to investigate the difference in the absolute carnosine increase within the groups. All analyses were done with SPSS statistical software (SPSS 21, Chicago, IL). All values are reported as mean ± SD and statistical significance was set at $p < 0.05$.

RESULTS

Reliability of arm muscle carnosine quantification (Study A).

The CV of the carnosine concentration was calculated for each muscle. Deltoid muscle had a smaller variation than the triceps brachii (6.6% vs. 9.2%: Table 2). Intraclass correlation coefficients (ICC) were significant for both deltoid (ICC: 0.903, $p < 0.01$) (Fig 1C) and triceps muscle (ICC: 0.656, $p = 0.01$), respectively. Over a time period of 4 weeks, we found a CV of 13.3% in the deltoid (Table 3) and a correlation coefficient of 0.734 ($p = 0.03$).

Table 2: The coefficient of variation of the carnosine content in deltoid and triceps muscle measured twice on one day

Deltoid	Measurement 1	Measurement 2	Mean	Stdev	CV (%)
1	0.146	0.153	0.149	0.005	3.3
2	0.211	0.197	0.204	0.010	4.8
3	0.131	0.099	0.115	0.022	19.4
4	0.164	0.166	0.165	0.002	1.1
5	0.123	0.137	0.130	0.010	8.0
6	0.178	0.187	0.183	0.006	3.5
7	0.172	0.164	0.168	0.006	3.3
8	0.106	0.123	0.115	0.012	10.5
9	0.127	0.136	0.132	0.006	4.5
10	0.116	0.122	0.119	0.005	4.0
11	0.094	0.108	0.101	0.010	10.2
Mean	0.142	0.145			**6.6**
Stdev	0.035	0.031			

Triceps	Measurement 1	Measurement 2	Mean	Stdev	CV (%)
1	0.204	0.167	0.185	0.027	14.3
2	0.201	0.201	0.201	0.000	0.1
3	0.155	0.126	0.141	0.021	14.9
4	0.116	0.110	0.113	0.004	3.9
5	0.169	0.170	0.170	0.001	0.6
6	0.132	0.167	0.150	0.025	16.7
7	0.196	0.176	0.186	0.015	7.9
8	0.174	0.182	0.178	0.006	3.3
9	0.149	0.104	0.126	0.031	24.9
10	0.137	0.136	0.136	0.001	0.4
11	0.137	0.167	0.152	0.021	14.0
Mean	0.161	0.155			**9.2**
Stdev	0.030	0.031			

Table 3. The coefficient of variation of the carnosine content in deltoid muscle at week 0 and 4

Deltoid	0w	4w	Mean	Stdev	CV (%)
1	0.069	0.102	0.086	0.023	27.29
2	0.149	0.133	0.141	0.011	8.02
3	0.131	0.129	0.130	0.001	1.09
4	0.089	0.111	0.100	0.016	15.56
5	0.133	0.168	0.151	0.025	16.44
6	0.089	0.076	0.083	0.009	11.14
Mean	0.110	0.120			**13.3**
Stdev	0.032	0.031			

BA-induced carnosine loading in arm vs leg muscles (Study B).

For the nonathletes, there was a significant increase in carnosine concentration in both arm (deltoid) and leg muscles (soleus + gastrocnemius) after supplementation, but the absolute and relative increase in carnosine in the arm vs. leg muscles showed no significant difference ($p = 0.286$ and $p = 0.230$, respectively) (Fig 2). The relative increase was 47.44 ± 32.29% in arm muscle and 33.01 ± 23.71% in the leg muscles.

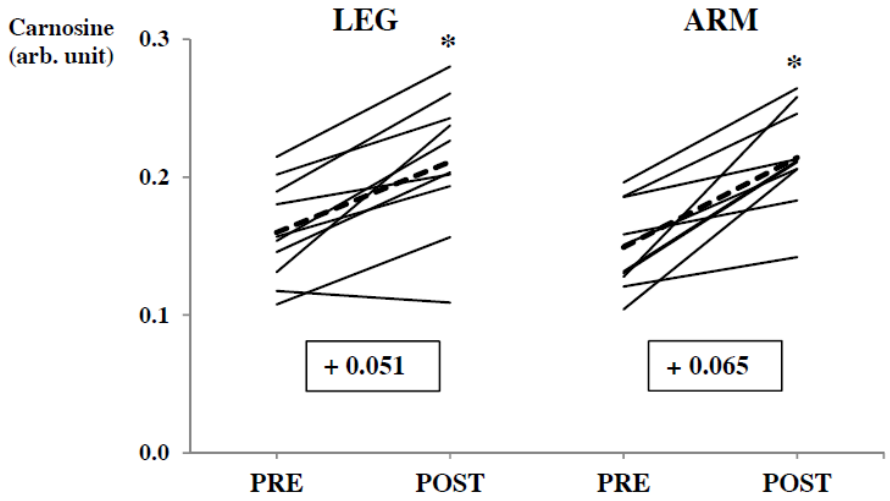


Figure 2: Study B: Absolute increase in muscle carnosine concentration in arms (deltoid) and legs (average of soleus and gastrocnemius) in the nonathletes.* $p < 0.05$ vs pre.

There were no differences between the athletic groups concerning height and weight (Table 1). Yet, swimmers were significantly younger than the cyclists ($p = 0.019$). There were no differences in baseline deltoid carnosine concentration between the groups. Baseline carnosine concentration in the leg muscles (soleus + gastrocnemius) was significantly lower in the cyclists than in nonathletes ($p = 0.028$) and kayakers ($p = 0.047$).

In gastrocnemius muscle, the absolute increase in carnosine (expressed as arbitrary units) was significantly higher in the cyclists (0.082 ± 0.035) and the swimmers (0.076 ± 0.015), compared to the nonathletes (0.043 ± 0.026) and the kayakers (0.035 ± 0.014) (Fig 3 and 4). The swimmers (0.107 ± 0.029) had a higher absolute increase in the deltoid muscle in comparison to cyclists (0.065 ± 0.040) and the nonathletes (0.065 ± 0.035) (Table 4). There were no differences between the groups in the soleus. For mean muscle carnosine in the legs (soleus and gastrocnemius), the cyclists (0.084 ± 0.027) and swimmers (0.082 ± 0.017) had a higher absolute increase than the nonathletes (0.051 ± 0.031). We found no significant correlations between training volume or years of training and increases in carnosine concentration with BA supplementation within nor across athlete groups.

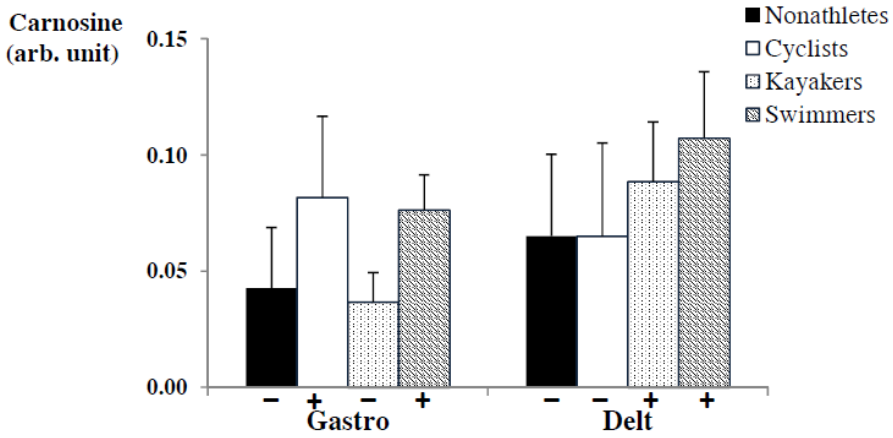


Figure 3: Study B: Absolute increase in muscle carnosine concentration in deltoid and gastrocnemius muscle in the nonathletes, cyclists, kayakers and swimmers. Plus and minus signs indicate whether this limb is actively trained (+) or not (-) in this group.

In the kayakers, their deltoid muscle (0.089 ± 0.026) had a higher absolute increase than their leg muscles (soleus + gastrocnemius) (0.049 ± 0.019, p = 0.001). An opposite pattern was observed with the cyclists where the leg muscles (0.084 ± 0.027) showed a tendency to a higher absolute increase than the deltoid muscle (0.065 ± 0.040, p = 0.078).

Taking all the subjects together (n = 35), there was a negative correlation between baseline carnosine content and the absolute increase in muscle carnosine in the leg muscles (soleus + gastrocnemius) (p = 0.020; r = -0.404) and deltoid muscle (p = 0.005; r = -0.476) following BA supplementation. The lower the baseline muscle carnosine content was, the higher was the increase in muscle carnosine, following supplementation. For

each group individually, there was only a significant negative correlation between baseline carnosine content and the absolute increase in the deltoid muscle in the cyclists group ($p = 0.010$; $r = -0.765$).

Table 4: Muscle carnosine concentration pre and post supplementation and absolute increase in muscle carnosine concentrations after BA supplementation in the nonathletes, cyclists, kayakers and swimmers.

M. Soleus	Pre	Post	Δ (post – pre)
Nonathletes	0.149 (± 0.029)	0.209 (± 0.055)	0.060 (± 0.041)
Cyclists	0.098 (± 0.032)	0.183 (± 0.035)	0.085 (± 0.023)
Kayakers	0.155 (± 0.066)	0.216 (± 0.041)	0.061 (± 0.027)
Swimmers	0.140 (± 0.033)	0.227 (± 0.032)	0.087 (± 0.023)

M. Gastrocnemius	Pre	Post	Δ (post – pre)
Nonathletes	0.171 (± 0.050)	0.214 (± 0.052)	0.043 (± 0.026)
Cyclists	0.121 (± 0.043)*†	0.202 (± 0.039)	0.082 (± 0.035)*†
Kayakers	0.178 (± 0.038)	0.214 (± 0.030)	0.037 (± 0.013)
Swimmers	0.157 (± 0.042)	0.233 (± 0.040)	0.076 (± 0.015)*†

M. Deltoideus	Pre	Post	Δ (post – pre)
Nonathletes	0.149 (± 0.032)	0.214 (± 0.036)	0.065 (± 0.035)
Cyclists	0.142 (± 0.036)	0.207 (± 0.027)	0.065 (± 0.040)
Kayakers	0.135 (± 0.033)	0.223 (± 0.028)	0.089 (± 0.026)
Swimmers	0.131 (± 0.039)	0.238 (± 0.045)	0.107 (± 0.029)*$

Data are means ± SD. * $p < 0.05$ versus control group. † $p < 0.05$ versus kayakers. $ $p < 0.05$ versus cyclists

DISCUSSION

The first goal of our study was to determine in which muscle of the upper body carnosine can be reliably measured via ^{1}H-MRS. For both the deltoid and triceps brachii muscles, the C2-peaks (at ~8 ppm) in the proton MRS spectra were sufficiently large to allow quantification. We found relatively low CVs (6.6 % for deltoid and 9.2 % for triceps brachii) in the test-retest condition within the same day, indicating that the carnosine concentration in the arm can be reliably measured. When adding the biological variability (3-4 weeks apart), the CV in the deltoid increased from 6.6 % to 13.3 %, which is in agreement with Baguet et al. (2009), who found a variation of 9.8 % in the soleus muscle and 14.2 % in the gastrocnemius muscle, when measured several weeks apart, compared to 4.3 % and 7.6 %, respectively, when measured twice on the same day (Ozdemir et al 2007). This first part of the study gave us the opportunity to compare supplementation-induced changes in muscle carnosine concentrations within one subject in different parts of their body in the second part of the study. Due to the better reproducibility of the deltoid, we decided to exclude triceps brachii in study B.

A subsequent goal was to compare BA-induced muscle carnosine loading in arm vs. leg muscles in a non-active control group. To date, the increase of carnosine concentration after BA supplementation has not yet been investigated in the upper body musculature, despite several reports of ergogenic effects in exercise types that entirely (Tobias et al 2013) or predominantly (de Salles Painelli et al 2013) depend on this part of the body. Our results indicate that BA supplementation is equally effective in raising carnosine concentrations in upper vs. lower body muscles in nonathletes. Based on the above results, we started measuring athletes from different sports disciplines to compare carnosine loading in specifically trained and untrained muscles. We selected three well-trained athletic populations: swimmers, kayakers and cyclists. Kayakers almost exclusively train their upper body, cyclists the lower body whereas swimmers train the entire body. It was hypothesized, based on the positive effects of exercise training on muscle creatine loading (Harris et al 1992; Robinson et al 1999), that trained muscles would have a higher absolute increase in carnosine concentration when compared to untrained muscles. In line with this hypothesis, the results of current observational study clearly indicate that there is an effect of training on the carnosine loading as we observed a nearly doubling of

the increase in muscle carnosine content after supplementation in specifically trained muscles compared to the untrained muscles (77.95% vs. 42.88%, respectively). In soleus, we did not find a greater accumulation in cyclists compared to nonathletes and kayakers. Soleus is a tonic muscle that is recruited very frequently also in daily activities like walking and standing in nonathletes. Therefore the difference in activity degree between the groups is smaller for soleus than for gastrocnemius. In the gastrocenemius, we found a higher absolute increase in carnosine concentrations in the swimmers and cyclists compared to the nonathletes. Vice versa, the deltoid of the swimmers had a higher absolute increase in carnosine concentration in comparison to the nonathletes. This was further supported when comparing loading after supplementation in different muscles within each athlete group: cyclists were able to accumulate more carnosine in their leg muscles (soleus + gastrocnemius) than arm (deltoid) muscles, whereas a reverse pattern was observed in kayakers. Even though we did not conduct a training intervention (only nutritional intervention) in the current study, these results confirm that trained muscles are more efficiently loaded with carnosine than untrained muscles (Fig 4).

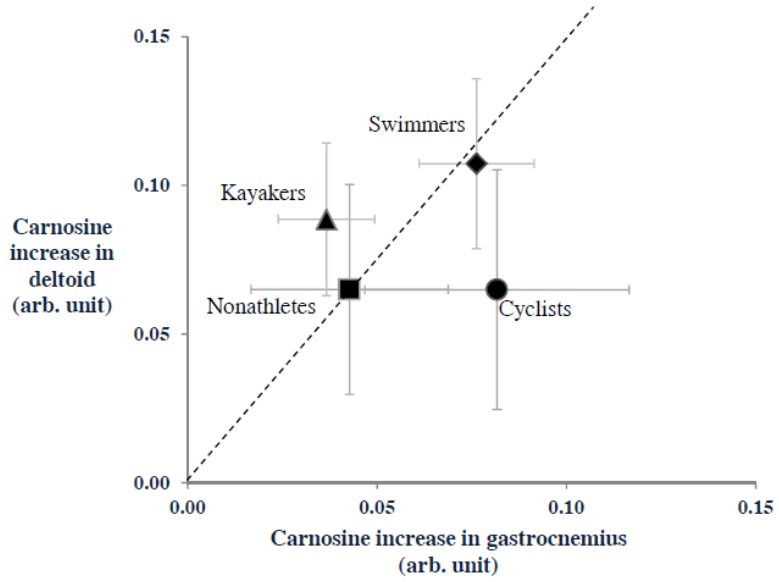


Figure 4: Study B: Absolute increase in muscle carnosine concentration in deltoid and gastrocnemius muscle in the cyclists, nonathletes, kayakers and swimmers.

Our findings seem to be in contrast with Kendrick et al. (2009) who found no difference in carnosine loading between a trained and untrained leg in a single-leg training study. However, it is worthy to note that training volume (10 x 10 maximal knee extensions, 3 –

4 times per week) was limited in that study. The athletes in our study trained at least 8 hours per week in their specific sport. If we estimate the amount of contractions, the current study had approximately 130x the number of contraction cycles compared to Kendrick's study. Kendrick et al. (2009) probably did not undertake enough contractions to really assess whether training had an effect on muscle carnosine synthesis. We suspect that the apparent contradiction between both studies is attributable to differences in training modalities, which should be subject to further investigation. The observed differences in carnosine loading in trained vs. untrained muscles could either be attributed to the acute effects of muscle contractile activity, i.e. the effect of the actual exercise during the supplementation period, or to the beneficial structural and metabolic properties of muscle induced by prior training. The current study design is not able to distinguish between training status vs. exercise effects. However, an advantage of the current study design is that –similar to the one-leg training studies- the comparison occurs within one subject, ensuring identical nutritional and environmental factors, and identical circulating hormone and BA concentrations. Yet in contrast with the one-leg training studies, effects of larger training load and volumes can be investigated in the currently adopted design.

Although this is still the first study to document a training effect on muscle carnosine loading, it is tempting to speculate on its underlying physiological mechanisms. A possible explanation for the acute effect of exercise training is the increased blood flow in contracting muscles, which results in better BA delivery to the muscle cells. Another possibility is that there is a contraction-induced stimulation of transporters such as TauT and PAT1, which are expressed in human skeletal muscle (Everaert et al 2013), to take up BA in the myocyte. This would be in accordance with other transsarcolemmal metabolite transporters, such as the glucose and fatty acid transporter (GLUT4 and FAT/CD36), which are recruited by a contraction stimulus (Krook et al 2004; Jeppesen et al 2011). On the other hand, if it is the result of the training status, rather than the contractile activity, it could be related to the endurance training-induced increases in the capillary density (Prior et al 2003), which ensures also better BA delivery in the trained muscles. Consequently, this same training-induced alteration can also increase the expression of

transporters involved in BA uptake and enzymes involved in carnosine synthesis (Everaert et al 2013). However, all the proposed explanations are speculative at present.

From a practical point-of-view, this scientific finding can be translated into attractive implications to athletes. It seems wise to supplement BA during a period of substantial training volume, rather than in a rest or recovery period, in order to optimize supplementation effectiveness throughout a training season. In addition, the physiological carnosine loading mechanism is probably more effective in trained vs. untrained individuals. This is opposite to some other ergogenic supplements, where biological activity and effectiveness is often less pronounced in a trained population.

In summary, we managed to measure carnosine reliably and non-invasively in a single muscle of the upper body (deltoid) by ^{1}H-MRS. We also showed that muscle carnosine loading via BA supplementation is of the same magnitude in arm and leg muscles in a non-active control group. Finally, we demonstrated that carnosine loading is more pronounced in the trained versus untrained muscles of athletes. These findings suggest that training is a possible determinant of carnosine loading, but it remains to be determined whether these effects are due to the acute exercise effects and/or to chronic adaptations of training.

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Study 2

Exercise training and beta-alanine-induced muscle carnosine loading

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ABSTRACT

Purpose. Beta-alanine (BA) supplementation has been shown to augment muscle carnosine concentration, thereby promoting high-intensity exercise performance. Trained muscles of athletes have a higher increase in carnosine concentration after BA supplementation compared to untrained muscles, but it remains to be determined whether this is due to an accumulation of acute exercise effects or to chronic adaptations from prior training. The aim of the present study was to investigate whether high-volume (HV) and/or high-intensity (HI) exercise can improve BA-induced carnosine loading in untrained subjects.

Methods. All participants (n=28) were supplemented with 6.4 g/day of BA for 23 days. The subjects were allocated to a control group, HV or HI training group. During the BA supplementation period, the training groups performed 9 exercise sessions consisting of either 75–90 min continuous cycling at 35–45% W_{max} (HV) or 3 to 5 repeats of 30s cycling at 165% W_{max} with 4 min recovery (HI). Carnosine content was measured in soleus and gastrocnemius medialis by proton magnetic resonance spectroscopy.

Results. There was no difference in absolute increase in carnosine content between the groups in soleus and gastrocnemius muscle. For the average muscle carnosine content, a higher absolute increase was found in HV (+ 2.95 mM; P = 0.046) and HI (+ 3.26 mM; P = 0.028) group compared to the control group (+ 1.91 mM). However, there was no additional difference between the HV and HI training group.

Conclusions. HV and HI exercise training showed no significant difference on BA-induced muscle carnosine loading in soleus and gastrocnemius muscle. It can be suggested that there can be a small cumulative effect of exercise on BA supplementation efficiency, although differences did not reach significance on individual muscle level.

KEYWORDS: beta-alanine, muscle contractions, sport supplements, carnosine loading

INTRODUCTION

When a nutritional supplement is ingested with the aim to raise its concentration and accumulation in skeletal muscle cells, it is necessary to understand what factors are controlling the myocellular uptake and storage of that molecule. Literature on creatine supplementation shows that muscle creatine loading is enhanced when muscle activity is increased during creatine ingestion (Harris et al 1992; Robinson et al 1999). Harris et al. (1992) showed a higher increase in creatine content in a trained leg compared to an untrained leg after supplementation combined with acute exercise, while Robinson et al. (1999) showed that a single bout of exhaustive exercise before creatine supplementation can already markedly augment muscle creatine accumulation. However, it is not clear whether the potentiating effect of contractile activity on muscle loading of nutritional supplements is specific to creatine only, or a more universal mechanism. In the latter case, it could also affect carnosine loading via beta-alanine (BA) supplementation.

In recent years, there has been an increasing interest in BA as nutritional supplement in athlete populations (Harris and Stellingwerff 2013; Sale et al 2013; Blancquaert et al 2015). Chronic BA supplementation (4 – 10 weeks) has consistently been shown to elevate muscle carnosine concentrations by 40 – 80 % (Harris et al 2006; Baguet et al 2009; Stellingwerff et al 2012), which can be beneficial for high-intensity exercise performance (Hill et al 2007; Hobson et al 2012). Carnosine, a dipeptide of L-histidine and BA, occurs in high concentrations in human skeletal muscles (Boldyrev et al 2013). The ergogenic effect of elevated carnosine concentrations is possibly based on its functions as proton buffer (Baguet et al 2010b) and calcium regulator (Dutka et al 2012) or a combination of these functions (Swietach et al 2013; Swietach et al 2014) in skeletal muscle. BA is the rate-limiting precursor to increase muscle carnosine levels and therefore supplementation with BA is the most effective way to increase muscle carnosine levels. Until now, the parameters to optimize the supplementation strategy (e.g., dose or timing of intake) are not fully understood and should be further investigated to result in clearer guidelines for athletes. Furthermore, there are large inter-individual differences in carnosine loading effectiveness that are hardly understood at present (Chung et al 2014).

In recent years, only a few studies investigated the effect of exercise and training on muscle carnosine loading. Kendrick et al. (2009) did not find a difference in a trained vs. untrained leg after 4 weeks of training combined with BA supplementation. However, in this study training consisted of isokinetic training with limited training volume (10 x 10 maximal contractions, 3 to 4 training sessions per week, over 4 weeks). In contrast, Bex et al. (2014) demonstrated that trained muscles (e.g. arm muscles of kayakers and leg muscles of cyclists) showed a higher accumulation of carnosine compared to the untrained muscles (e.g. leg muscles of kayakers and arm muscles of cyclists). Hence, both within and between athletes, trained muscles had approximately two-fold higher carnosine loading compared to untrained muscles for the same BA intake. This study used experienced athletes who trained at least 8 h per week in their specific sport, which suggests that exercise and/or training status appears to be a feasible determinant of muscle carnosine loading. As the study of Bex et al. (2014) was observational rather than interventional, it was unclear whether these effects are due to either the acute response of exercise or the chronic adaptations of muscle induced by prior training, or a combination of both. The precise role of training intensity, duration and volume on exercise-induced carnosine loading should be further revealed. Implementation of different acute exercise protocols can give a better insight on which of these various factors affect the effectiveness of the carnosine loading protocol.

It is already known that training by itself does not influence muscle carnosine content (Mannion et al 1994; Kendrick et al 2008; Kendrick et al 2009; Baguet et al 2011a), but the effect of exercise training on the increase in muscle carnosine content by BA supplementation is not yet clear. Therefore, the main purpose of this study is to investigate whether intramuscular carnosine loading following BA supplementation is influenced by training volume versus training intensity.

MATERIALS AND METHODS

Participants

Twenty-eight men volunteered to participate in this study. All gave their written informed consent and the study was approved by the local ethical committee (Ghent University Hospital, Ghent, Belgium). None of the subjects were vegetarian. All subjects were non-specifically trained, but some of them took part in some form of recreational exercise 1 to 3 times per week (jogging, cycling, etc.). None of the subjects were engaged in regular organized training. Subjects were divided in either a control group or training group. The control (non-training) group consisted of 10 subjects, who were asked to be inactive throughout the intervention period. The control group's age, weight, and height were 22.0 ± 2.3 yr, 72.4 ± 10.3 kg, and 178.0 ± 7.8 cm, respectively. The subjects in the training group were allocated to a high-volume (HV) (n = 9) or high-intensity (HI) (n = 9) group, matched for age, weight, height, muscle carnosine concentration, VO_{2max} and maximal power output at the graded exercise test (W_{max}). The age, weight, and height were 21.6 ± 1.5 yr, 77.0 ± 7.6 kg, and 180.0 ± 4.0 cm for the HV group and 21.7 ± 2.1 yr, 80.4 ± 14.9 kg, and 180.0 ± 6.0 cm for the HI group respectively.

The supplementation protocol lasted 23 days and involved ingesting 6.4 g/day (2x 800 mg tablets, 4 times daily with at least 2 h apart) of slow-release BA (Carnosyn, Natural Alternatives International). The supplement batch tested negative for contamination from prohibited substances by an independent drug surveillance laboratory (HFL Sport Science, Cambridgeshire, UK). All subjects were advised to take the tablets together with meals and the subjects in the training groups were also asked to take one of the doses just prior to their training sessions. None of the subjects reported side effects due to the supplementation. Muscle carnosine concentration was measured before and after supplementation by proton magnetic resonance spectroscopy (^{1}H-MRS) in soleus and gastrocnemius medialis muscles in all subjects and an incremental cycling test was performed in the training group before and after supplementation.

Experimental protocol

Preliminary incremental cycling test

Each subject in the training group performed a maximal ramp exercise test on an electrically-braked cycling ergometer (Lode, Groningen, Netherlands). Oxygen consumption was measured continuously via a computerized breath-by-breath system (JaegerOxycon Pro, Hoechberg, Germany). Pedalling frequency was kept between 75 and 80 rpm. After a warm-up of 3 min at 50 W, the work rate was increased by 35 W/min to the point the subjects failed to continue to pedal at 75 rpm. Maximal power output at the graded exercise test (W_{max}) corresponded to the mean value achieved over the last 30 s of the incremental cycling test.

Training protocol

The training protocol consisted of 9 sessions spread over 21 days, with 1 - 2 days of recovery between the training sessions (Table 1). Both groups performed training on Mondays, Wednesdays and Fridays for 3 weeks. For the HI group, training consisted of repeated 30 s maximal cycling bouts at 165 % of W_{max}, interspersed with 4 min of recovery (cycling at 40 – 70 W at their preference). Training progression was implemented by increasing the number of repeats from 3 to 5 repetitions. For the HV group, training consisted of 75 - 90 min continuous cycling at an intensity corresponding to 35 - 45 % of W_{max}. Training progression in the HV group was implemented by increasing duration of exercise throughout the weeks.

Table 1: Parameters of the high-volume (HV) and high-intensity (HI) training protocols.

Parameter	HV	HI
Work intensity	35 - 45 % of W_{max}	165 % of W_{max} (‘all out’)
Exercise protocol (per session)	75 - 90 min of continuous exercise	3 - 5 repeats x 30 s ‘all out’ 4 min active recovery
Total training time per session	75 – 90 min	1.5 - 2.5 min (intervals only) 18.5 - 27.5 min (incl. recovery)
Total training time over 3 weeks	743 min	18 min (intervals only) 225 min (incl. recovery)

Determination of muscle carnosine content

Before and after supplementation, the carnosine content was measured in soleus and gastrocnemius medialis muscles by proton magnetic resonance spectroscopy (^{1}H-MRS), as previously described by Baguet et al. (2010a). As seen in the study of Bex et al (2014), the plantar flexors showed a higher increase in carnosine content in the cyclists. The subjects were lying in supine position on their back and the lower leg was fixed in a spherical knee coil. All the measurements were performed on a 3-T whole body MRI scanner (Siemens Trio, Erlangen, Germany). Single voxel point-resolved spectroscopy (PRESS) sequence with the following parameters was used: repetition time (TR) = 2,000 ms, echo time (TE) = 30 ms, number of excitations = 128, 1,024 data points, spectral bandwidth of 1,200 Hz, and a total acquisition time of 4.24 min. The average voxel size for the soleus and gastrocnemius medialis muscles was 40 x 12 x 30 mm and 40 x 12 x 30 mm, respectively. Following shimming procedures, the linewidth of the water signal was on average 25.1 and 26.7 Hz for soleus and gastrocnemius medialis muscles, respectively. The absolute carnosine content was calculated as described before by Baguet et al. (2010a). A variation coefficient for repeated measurements within the same day (Ozdemir et al 2007) were 4.3 % (soleus), 7.6 % (gastrocnemius) and 4.7 % (average of soleus and gastrocnemius), while the biological variability within a 6 week period (Baguet et al 2009) were 9.8 % (soleus), 14.2 % (gastrocnemius) and 9.5 % (average of soleus and gastrocnemius).

Statistics

A 3 x 2 general linear model repeated measures ANOVA was performed to evaluate the muscle carnosine content after BA supplementation, with “intervention group” (control, HV and HI) as between-subjects factor and “time” (pre vs. post) as a within-subjects factor. In case of a significant interaction effect, a 2 x 2 general linear model repeated measures ANOVA was performed between the different intervention groups. A 2 x 2 general linear model repeated measures ANOVA was performed to evaluate the changes in performance data, with “training group” (HV and HI) as between-subjects factor and “time” (pre vs. post) as a within-subjects factor. Pearson correlation was calculated between baseline carnosine content and absolute increase in muscle carnosine. Pearson correlation was also used to see if changes in muscle carnosine content correlated with

changes in W_{max} or VO_{2max}. All analyses were done with SPSS statistical software (SPSS 21, Chicago, IL). All values are reported as mean ± SD and statistical significance was set at $P < 0.05$. Trends were being identified with statistical significance set at P between 0.05 en 0.10.

RESULTS

Muscle carnosine loading

There was a significant increase in carnosine concentration in soleus, gastrocnemius and mean of both muscles in response to BA supplementation in the control, HV and HI training group (Table 2).

Table 2: Muscle carnosine content (mM) of the soleus, gastrocnemius and mean of both muscles, pre and post supplementation. Values are means (± SD) of 10 subjects in the control group, 9 subjects in the high-volume (HV) and 9 subjects in the high-intensity (HI) training group.

M. Soleus	Pre	Post	Interaction	Pre vs. Post
Control group	4.54 (± 0.62)	6.67 (± 1.60)		
HV group	5.06 (± 1.05)	7.95 (± 1.21)	0.201	< 0.001
HI group	5.13 (± 1.09)	8.13 (± 1.80)		

M. Gastrocnemius	Pre	Post	Interaction	Pre vs. Post
Control group	6.63 (± 2.10)	8.33 (± 1.92)		
HV group	8.71 (± 1.35)	11.72 (± 1.63)	0.080	< 0.001
HI group	8.64 (± 1.38)	12.17 (± 2.55)		

Mean both muscles	Pre	Post	Interaction	Pre vs. Post
Control group	5.59 (± 1.25)	7.50 (± 1.57)		
HV group	6.88 (± 1.15)	9.84 (± 1.32)	0.039	< 0.001
HI group	6.89 (± 0.93)	10.15 (± 1.88)		

Data are means ± SD.

Table 2 showed no significant time x group interaction effect in soleus (Fig 1A). In the gastrocnemius, a tendency to significant interaction effect (P = 0.080) was visible and in the mean of the 2 muscles a significant interaction effect (P = 0.039) was found. For gastrocnemius, there was a higher increase in HV group (+ 3.01 mM; P = 0.044) and HI group (+ 3.53 mM; P = 0.052) compared to the control group (+ 1.69 mM) (Fig 1B).

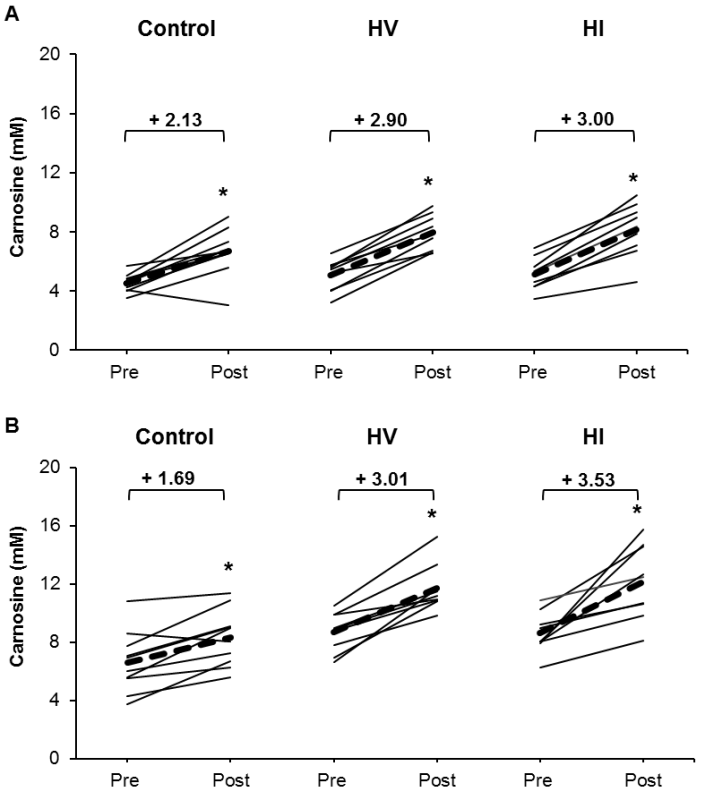

Figure 1. Absolute increase in muscle carnosine concentration (mM) in soleus muscle **(Fig 1A)** and gastrocnemius **(Fig 1B)** in the control group, HV and HI training groups. Thin solid lines represent individual subjects and bold dashed lines represent group average. *P < 0.05 vs. pre.

Similar results were found in the mean of the 2 muscles, namely a higher absolute increase in the HV group (+ 2.95 mM; P = 0.046) and HI group (+ 3.26 mM; P = 0.028), compared to the control group (+ 1.91 mM) (Fig 2). The absolute increase in carnosine concentration in the HV and HI training group was not significantly different from each other in either gastrocnemius (P = 0.583) nor mean of the 2 muscles (P = 0.572).

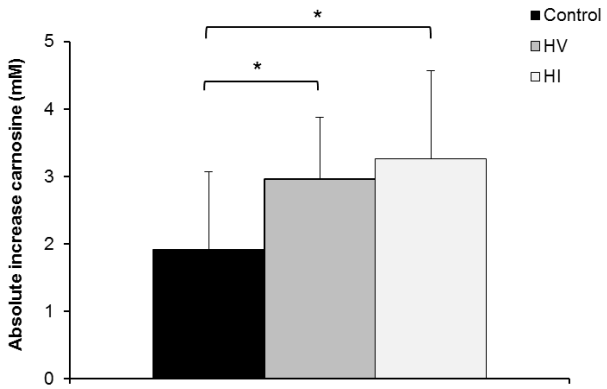

Figure 2. Absolute increase in muscle carnosine concentration (mM) in the mean of the 2 muscles in the control group, HV and HI training group. *P < 0.05 vs. control group.

There was no correlation between baseline carnosine content and the absolute increase in muscle carnosine in soleus muscle (P = 0.493; r = 0.17) and gastrocnemius muscle (P = 0.386; r = -0.22), following BA supplementation. Furthermore, changes in muscle carnosine content were not correlated with changes in W_{max} (soleus: P = 0.831; r = 0.06, gastrocnemius: P = 0.207; r = 0.32) or VO_{2max} (soleus: P = 0.771; r = -0.07, gastrocnemius: P = 0.326; r = 0.25).

Incremental VO_{2max} test

Following training, W_{max} was improved during the incremental VO_{2max} test by 2.2 % in the HV group and 4.5 % in the HI group (main effect of time, P < 0.01) (Pre: 46.6 ± 7.6 vs. Post: 49.5 ± 9.8 ml/min/kg), but there was no difference between the groups. VO_{2max} increased after training by 7.0 % in the HV group and 5.0 % in the HI group (main effect of time, P < 0.05) (Pre: 369.8 ± 46.1 vs. Post: 381.1 ± 38.8 W), with no difference between the groups. No effect of training on the differences in maximal heart rate were found in both groups (Pre: 193.5 ± 8.2 vs. Post: 191.2 ± 9.0 bpm).

DISCUSSION

The main purpose of this study was to investigate whether training volume or training intensity can enhance the efficacy of BA supplementation towards muscle carnosine loading. The potential effects of exercise on muscle metabolite loading could be in accordance with creatine, another nutritional supplement (Harris et al 1992; Robinson et al 1999). Both one bout of exercise (Robinson et al 1999) and one hour training per day for seven days (Harris et al 1992) resulted in higher creatine loading in trained legs compared to untrained legs following creatine supplementation. For BA, the role of exercise on muscle carnosine loading needs further clarification as there is inconsistency between the results of Kendrick et al. (2009) and Bex et al. (2014). The current interventional study aimed to resolve the equivocal results and tried to get a better understanding of muscle carnosine loading effectiveness. Concerning the increase in carnosine concentration in this study, a higher absolute increase was found in both HV and HI group compared to the control group for the average muscle carnosine. These results confirm the data of Bex et al. (2014), although the increases were smaller in current study. The effects in the study of Bex et al. (2014) could also be partly the result of prior training status, namely training-induced increases in capillary density and higher expression of transporters and enzymes. As the current study conducted a training intervention in untrained subjects, the effect of prior training status was eliminated. Possibly an additive effect of exercise on trained muscles causes the highest BA supplementation efficiency. This would implicate that trained athletes who train during BA supplementation have more pronounced muscle carnosine loading than untrained subjects who train.

The current study implemented two different training protocols to take a closer look at the exercise modalities and the inherent possible underlying mechanisms involved in exercise potentiation of muscle carnosine loading. The HV and HI training protocols used in the current design were inspired by the study of Gibala et al. (2006). This study demonstrated that these training protocols cause similar effects on training-induced increases in muscle oxidative capacity, buffering capacity and glycogen content. Given the large difference in training volume, their data demonstrated that HV and HI are comparable to induce rapid adaptations in skeletal muscle and exercise performance. The

results of the VO_{2max} test in current study suggest that indeed both groups had a small but similar response on the different training sessions.

One group (HV group) performed continuous endurance training during the training period, while training in the HI group consisted of repeated 30 s maximal exercise bouts. The rationale for making these groups is based on some possible hypotheses. One likely explanation for exercise-induced carnosine loading would be the activity-related increase in blood flow and capillary recruitment, leading to higher interstitial BA concentrations, which are then available for transsarcolemmal transport and intracellular storage. This mechanism is likely more sensitive to exercise duration and could have been an explanation if effects had been more pronounced in HV than HI exercise. Yet, other mechanisms may be involved, such as the translocation and recruitment of transporters (TauT and PAT1) to take up BA in the myocytes in response to reactive oxygen species and/or contractile signaling (Everaert et al 2013). This would be in accordance with the glucose (GLUT4), creatine (CRT) and fatty acid transporter (FAT/CD36), which are recruited to the sarcolemma by a contraction stimulus (Krook et al 2004; Derave et al 2006; Jeppesen et al 2011). It is expected that this recruitment is more sensitive to exercise intensity, and therefore more intensely activated by HI exercise. The results of this study showed an equal increase in muscle carnosine concentrations in both training groups, suggesting that none of the above possibilities can be excluded at present. At physiological level, the precise mechanism to optimize BA-induced carnosine loading requires further investigation. It is possible that a combination of the above described or yet other mechanisms are responsible for enhancing the BA supplementation efficiency. Further research should include muscle biopsies to get a better insight in the mechanisms of exercise on carnosine loading efficiency. The advantage of muscle biopsies relates to the analysis on pooled single fiber level, which could determine the effect of the training stimulus in a muscle. In this study, ^{1}H-MRS- based carnosine quantification is used instead of muscle biopsies, because it is a non-invasive technique that has a good repeatability in untrained (Baguet et al 2009) and trained (Derave et al 2007; Baguet et al 2010a) humans. Furthermore, a greater part of the muscle is analyzed compared to muscle biopsies (Albracht et al 2008). However, from a practical viewpoint, these results can be translated

into better guidelines for athletes, because both the HV and HI exercise stimuli seem to enhance the efficiency of the BA supplementation protocol.

Despite revealing new strategies to increase the efficiency of the BA supplementation protocol, the carnosine loading effectiveness remains low. Stegen et al. (2013) were the first to calculate BA supplementation efficiency by dividing the molar increase in muscle carnosine by the total ingested molar amount of BA. Only 2.80 % of ingested BA is actually incorporated into muscle carnosine (assuming that 40 % of body mass is muscle mass). Recently, the study of Stegen et al. (2013) showed that meal co-ingestion was able to increase the efficiency of BA supplementation, while Bex et al. (2014) revealed that exercise and/or training status had an additional effect on the loading efficiency (up to 5.82 % in trained muscles) (Stegen et al 2013; Bex et al 2014). In the current study, the loading efficiency of the HV and HI group was 5.17 % and 5.67 %, while it was 3.49 % in the control group. Further research on BA metabolism is needed to get an understanding of the metabolic fate of the majority of the ingested BA.

Furthermore, this study confirms the high interindividual variability in response to BA supplementation, with some subject displaying a supplementation-induced doubling of muscle carnosine content, yet with also two rare cases of non-responders (one in soleus and one in gastrocnemius of the control group). In our experience of the 109 subjects that have been supplemented chronically with BA in the various studies of our lab (Derave et al 2007; Baguet et al 2009; Stegen et al 2013; Bex et al 2014; Chung et al 2014), only one did not show an increase in soleus carnosine content and only three did not show gastrocnemius carnosine loading. Even though this number of ‘non-responders’ is rather small compared to other supplements (e.g. creatine), one could wonder why some subjects do not respond. The current study aimed to identify a key factor that influences interindividual variability in muscle carnosine loading efficiency. Given the high remaining interindividual variability, we conclude that there must be other elements, in addition to training (which was controlled in this study) that define responsiveness to BA supplementation. Future studies on the pharmacokinetics and metabolism of BA could shed light on this issue.

In summary, this study showed no significant difference on muscle carnosine loading after HV and HI exercise training. However, a beneficial effect on the efficiency of the muscular BA uptake and carnosine accumulation may occur, although this was not statistically confirmed on soleus and gastrocnemius muscles separately.

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Study 3

Cyclic movement frequency determines muscle typology in athletes

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Scan J Med Sci Sport (submitted)

ABSTRACT

*Purpose***.** There is a continuing research interest in the muscle fiber type composition (MFTC) of athletes. Recently, muscle carnosine quantification by proton magnetic resonance spectroscopy (^{1}H-MRS) was developed as a new non-invasive method to estimate MFTC. This cross-sectional study aims to establish the relationship between exercise characteristics and the corresponding estimated MFTC for athletes, both within and between different cyclic sports.

Methods. 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 ^{1}H-MRS. Within sport disciplines, athletes were divided in subgroups (sprint-, intermediate- and endurance-type). The controls were used as sex-specific reference population to allow expression of the athletes' data as Z-scores.

Results. 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).

Conclusions. Both within and between different cyclic sports, estimated MFTC was divergent between sprint- and endurance-type athletes. Cyclic movement frequency, rather than movement duration came out as the most determining factor for the optimal estimated MFTC in elite athletes.

KEYWORDS: cyclic sports, contractile properties, carnosine

INTRODUCTION

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 (CSA) 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 x velocity) as compared to ST fibers (Schiaffino and 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 and 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 and 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 athletes' MFTC has a

major impact on almost every aspect of his/her sport scientific guidance, such as, training advice, injury risk prevention and especially for talent identification. Indeed, classical studies (Gollnick et al 1972; Tesch and 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 vice versa for ST fibers (Gollnick et al 1972; Costill et al 1976a; Saltin et al 1977; Tesch and 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 100-200m 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 (2011b) developed a new non-invasive method to estimate MFTC, based on proton magnetic resonance spectroscopy (^{1}H-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 (Blancquaert et al 2015). Therefore, muscle carnosine content is positively related to the percentage area of FT fibers in that muscle (Baguet et al 2011b).

A first evaluation showed a good validity of this technique in track-and-field athletes (Baguet et al 2011b).

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 (movement 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 and 90 females) and 111 elite Belgian athletes (89 males and 22 females). The controls were not specifically trained, but some of them took part in some form of recreational exercise. The athletes consisted of 5 subgroups: 1) 74 runners , 2) 7 triathletes, 3) 11 swimmers, 4) 14 cyclists and 5) 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 1 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 in 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 specialised in three different disciplines: track cycling (TRACK), road cycling (ROAD)(flat races) or climbing (CLIMB)(road cycling with mountain stages). One kayaker of the 5 was a 200 m specialist (SPR-T), while the other 4 were 1000 m specialists (INT-T).

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

	Total	European Level	World Level
Runners	74 (21)	20	17
SPR-T	25 (7)	5	3
INT-T	11	3	2
END-T	38 (14)	12	12
Triathletes	7	3	3
Swimmers	11	2	1
SPR-T	4	1	
INT-T	5	1	1
END-T	2		
Cyclists	14 (1)		14
TRACK	1 (1)		1
ROAD	8		8
CLIMB	5		5
Kayakers	5		4
SPR-T	1		1
INT-T	4		3

None of the subjects was vegetarian nor took beta-alanine supplements in the 3 months prior to the study. Data for some of the runners, triathletes and control subjects have already been included in a previous report (Baguet et al 2011b), but these groups were expanded. The study was approved by the local ethical committee (Ghent University Hospital, Ghent, Belgium). All subjects gave their written informed consent to take part in the study.

Muscle carnosine quantification by ^{1}H-MRS

Muscle carnosine content was measured by proton magnetic resonance spectroscopy (^{1}H-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. (2010a). 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 2.000 ms, echo time (TE) of 30 ms, number of excitations is 128, 1.024 data points, spectral bandwidth of 1.200 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 m, respectively. For the calf muscles, the absolute carnosine content (mM) was calculated as described before by Baguet et al. (2010a). 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 – 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.

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

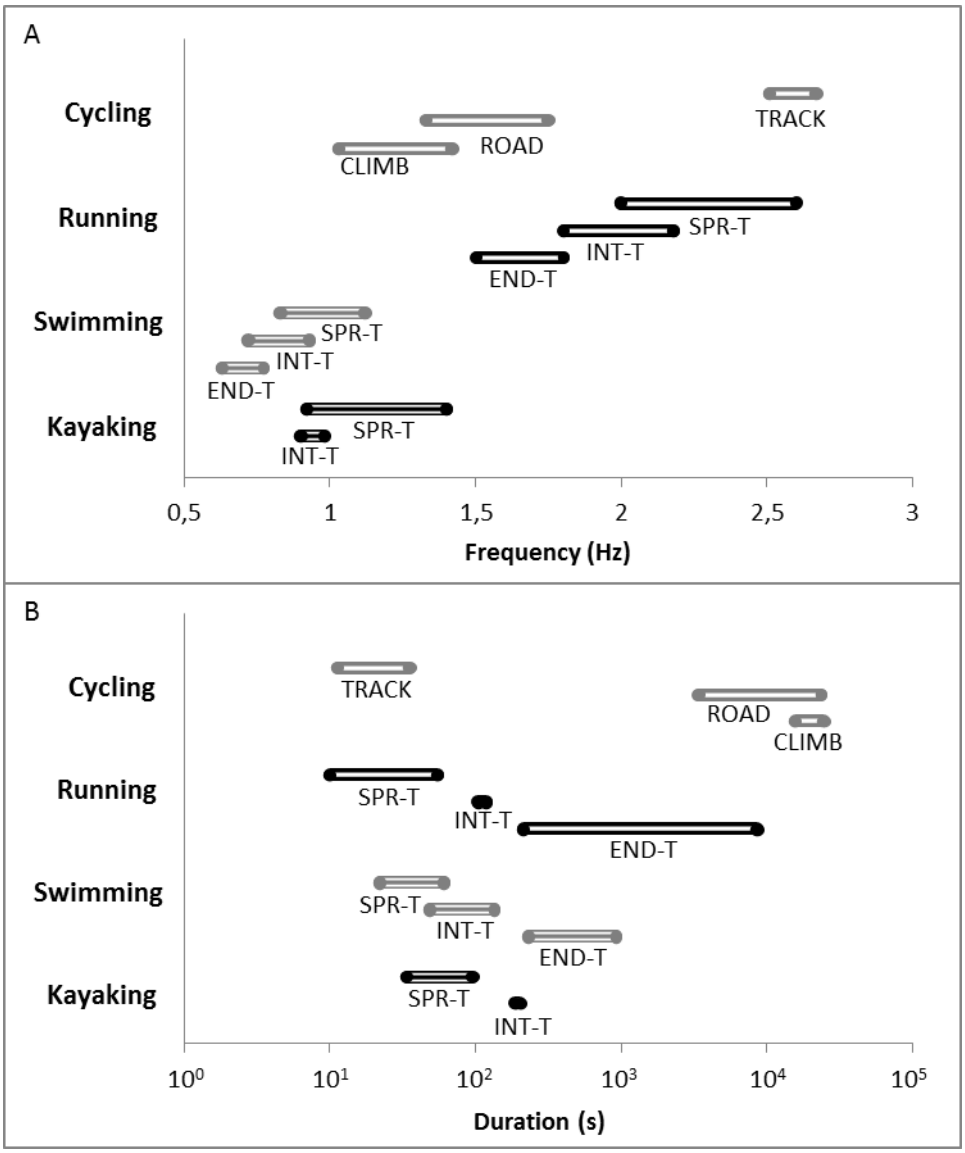

Figure 1: The typical cyclic movement frequency (1A) and duration (1B) range for the respective sport disciplines.

The step frequency in the different running disciplines is well documented (Gajer et al 2007; Salo et al 2011; Wilber and Pitsiladis 2012). The term 'step frequency' is used for one foot contact to the next contralateral foot contact. We calculated the 'stride frequency' which means two consecutive steps. For swimming, the stroke rates were based on male competitors at the 1996 Olympic Games and the 1998 World

Championships (Maglischo 2003). A stroke rate includes two arm strokes, one with the right and one with the left in front crawl. Some studies analyzed the pedaling cadence in professional road and track cycling (Lucia et al 2001; Lucía et al 2001; Dorel et al 2005). For kayaking, a recent review of McDonnell et al (2013) was used. The movement durations for the different sport disciplines were defined and represented in Table 2.

Table 2: Duration range for the different sport disciplines. BR = Belgian Record. WR = World Record.

Sport disciplines	Duration ranges
Running • SPR-T • INT-T • END-T	• BR men and women 100m – 400mH • BR men and women 800m • BR men and women 1500m – marathon
Swimming • SPR-T • INT-T • END-T	• BR men 50m freestyle – 100m breaststroke • BR men 100m freestyle – 200m breaststroke • BR men 400m – 1500m freestyle
Cycling • TRACK • ROAD • CLIMB	• BR track record women 200m – 500m • 2014 UCI Road WC Men Time Trial – Road Race • Tour de France Mountain stage (Single bout)
Kayaking • SPR-T • INT-T	• WR men 200m K2 – 500m K1 • WR men 1000m K2 – 1000m K1

Statistics

Pearson correlation was calculated between the mean Z-scores of the athlete populations, movement frequency of the different disciplines and the mean movement duration of the different. Pearson correlation was also used between leg and arm muscles in the controls and swimmers. 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. A paired sample T-test was done to investigate the difference in the Z-score of carnosine content within the group of swimmers. All analyses were done with SPSS statistical software (SPSS 21, Chicago, IL). 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 figure 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.

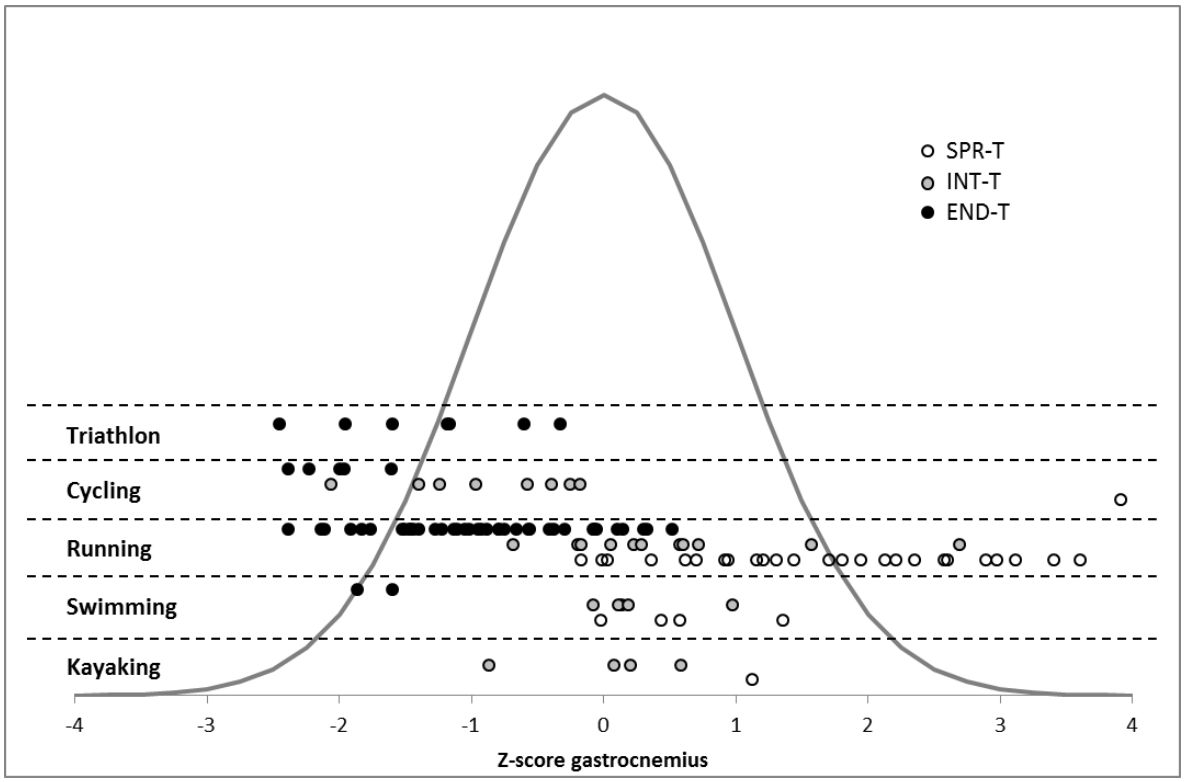

Figure 2: Individual Z-scores for gastrocnemius muscle of all the athletes, divided in SPR-T, INT-T and END-T groups.

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 en 1B 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 movement duration (R = -0.58, P = 0.06) (Fig 3B).

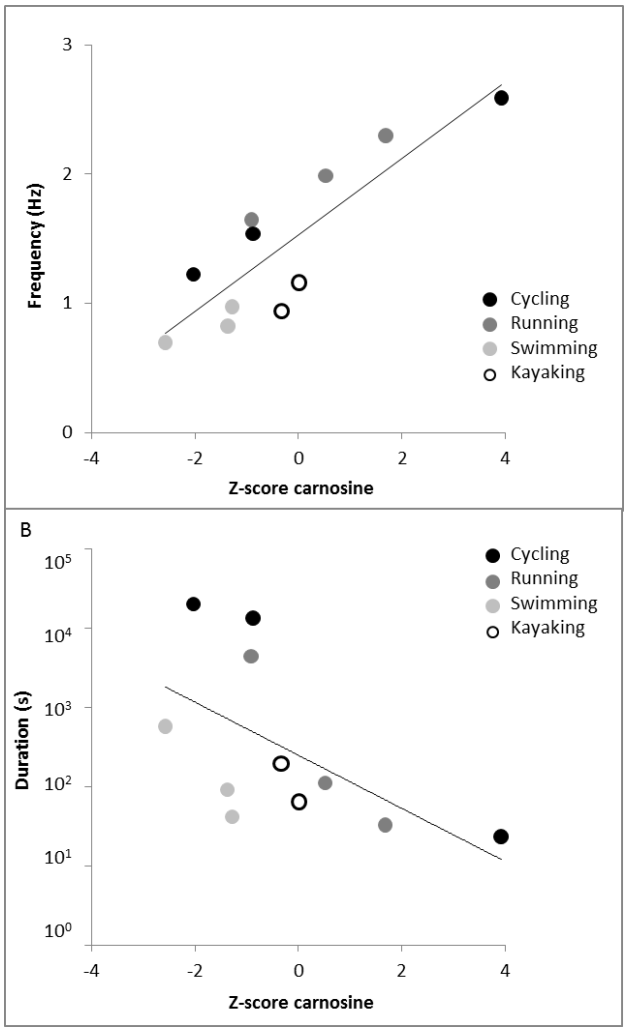

Figure 3: Correlation between Z-score of carnosine content and cyclic movement frequency across the different sport disciplines (R = 0.86, P = 0.001) (3A) and movement duration (R = -0.58, P = 0.06) (3B).

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 movement duration. No correlation was found between movement frequency and movement 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).

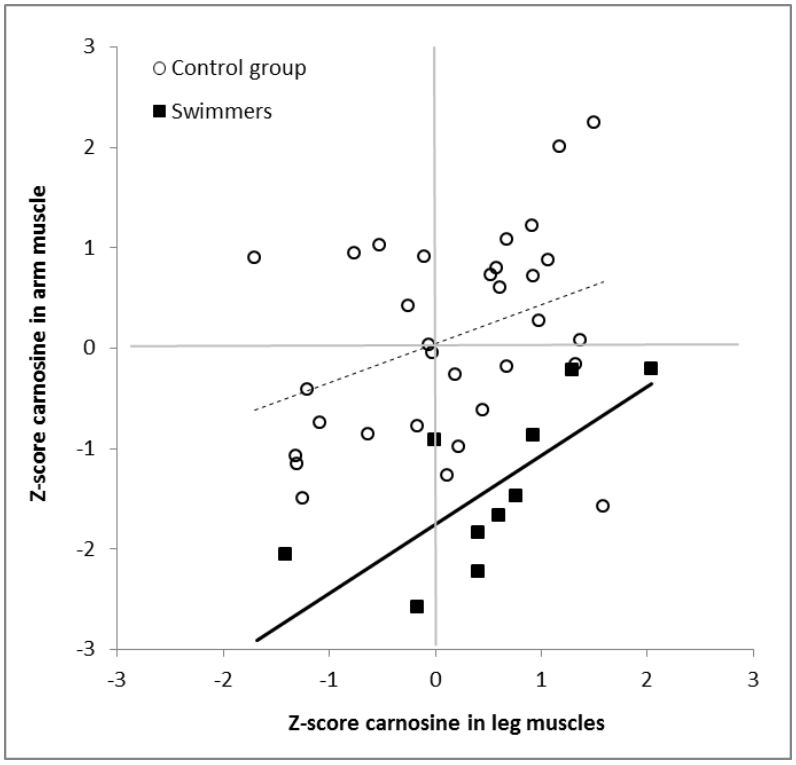

Figure 4: Correlation between Z-score of carnosine content in leg muscles and arm muscle in the control group (R = 0.37, P < 0.05) and the swimmers (R = 0.81 , P < 0.01).

Level of athletes.

Within running, the SPR-T and END-T runners were divided in 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) (Fig 5).

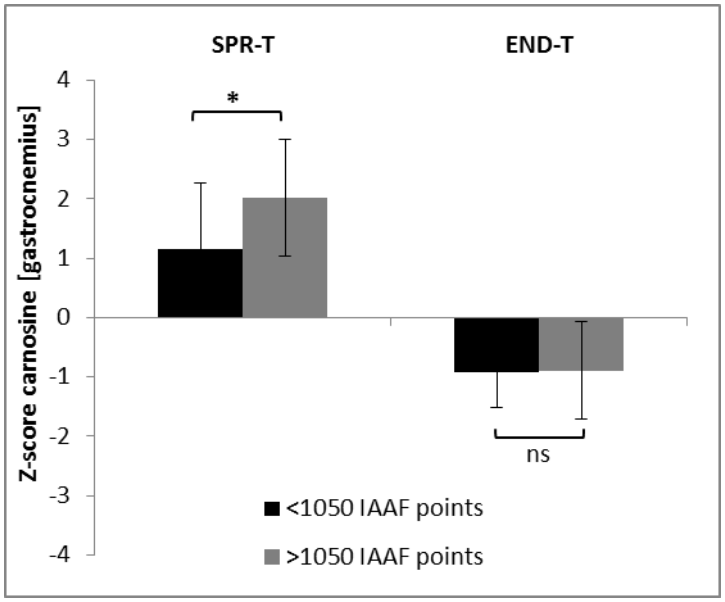

Figure 5: Z-score in the SPR-T and END-T runners, who were divided in two groups based on their level (more and less than 1050 IAAF points). * $p \leq 0.05$.

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 2011b), 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 corresponds to multistage road cycling including frequent climbing (e.g. Tour de France, Giro, etc.), whereas INT-T cyclists corresponds 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 movement 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 5-fold 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, movement duration was assumed to be the most determining factor for defining the optimal MFTC of athletes (Gollnick et al 1972; Costill et al 1976a), 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 towards 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 400m can be run in under 53sec in a 10K race). Consequently, even END-T runners likely need a reasonable proportion of FT fibers to become successful.

PERSPECTIVES

Current study was conducted with a non-invasive methodology, namely ^{1}H-MRS quantification of muscle carnosine, developed by Baguet et al (2011b). 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 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.

ACKNOWLEDGEMENTS

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Study 4

Discriminant musculo-skeletal leg characteristics between sprint and endurance elite Caucasian runners

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Aerts P, De Clercq D, Derave W

Scan J Med Sci Sport (submitted)

ABSTRACT

Purpose. Excellence in either sprinting or endurance running requires specific musculo-skeletal characteristics of the legs. This study aims to investigate the morphology of the leg of sprinters and endurance runners of Caucasian ethnicity.

Methods. Eight male sprinters and eleven male endurance runners volunteered to participate in this cross-sectional study. They underwent magnetic resonance imaging and after data collection, digital reconstruction was done to calculate muscle volumes and bone lengths. Ultrasonography was used to determine muscle architecture.

Results. Sprinters have a higher total upper leg volume compared to endurance runners (7340 vs. 6265 cm^3). Specifically, the rectus femoris, vastus lateralis and hamstrings showed significantly higher muscle volumes in the sprint group. For the lower leg, only a higher muscle volume was found in the gastrocnemius lateralis for the sprinters. No differences were found in pennation angle or fascicle length, nor relative bone lengths. There was a significant positive correlation between ratio hamstrings/quadriceps volume and best running performance in the sprint group.

Conclusion. Sprinters and endurance runners of Caucasian ethnicity showed the greatest distinctions in muscle volumes, rather than in muscle orientation or skeletal measures. Sprinters show higher volumes in mainly the proximal and lateral leg muscles than endurance runners.

KEYWORDS: running, morphological properties, performance

INTRODUCTION

The question whether a runner is more suited for sprint vs. endurance disciplines and whether he has the talent to obtain national or even international top level, depends in part on the musculo-skeletal characteristics of the legs. Some of these characteristics are almost entirely dependent on genetic predisposition and some are additionally trainable in a certain direction. A main limiting factor for sprinting is power generation, which is directly related to muscle fiber type, length and muscle volume (Mero et al 1992; Young et al 1995). The latter may likely influence the inertial properties of the limb segments. On the other hand, endurance running is characterized by a high muscle oxidative capacity and an excellent running economy, influenced by several factors, such as reduced inertia of the legs (Weyand and Davis 2005). According to these limiting factors, runners that excel in either sprinting or endurance running should have divergent musculo-skeletal leg characteristics.

This study will provide a direct and extensive morphological comparison between sprint and endurance runners within a single ethnic group (Caucasian), conducted with combined ultrasonography and MRS and MRI-based analyses. A first, already well-known, discriminant relevant for sprinting vs. endurance running is muscle fiber type composition. Classical studies in the 70s (Gollnick et al 1972; Costill et al 1976a) indicated that elite level in endurance and sprint running is characterized by a high and low percentage of slow-twitch (type I) fibers respectively in the gastrocnemius muscle.

A second potential discriminant is muscle volume, which is a dominant determinant of power generation (Bamman et al 2000; Trappe et al 2001). For this reason, it can be hypothesized that sprinters should have higher muscle volumes, at least in some critical muscles, particularly recruited during the acceleration phase of sprint running. Abe et al (2000) showed greater muscle thickness in upper portion of the anterior thigh, but not in the lower portion (70% thigh length) for sprinters compared to endurance runners, which could be a reflection of differences in muscle shape of the upper leg. These data were confirmed by Kumagai et al (2000) within a population of sprinters, with a greater muscle thickness in the upper thigh (both quadriceps and hamstrings) in the best sprinters. It has been suggested that muscle shape (thicker upper portion of quadriceps and hamstrings)

is associated with better sprint performance (Abe et al 1999; Abe et al 2000; Kumagai et al 2000). Also high power of ankle plantar flexors (calf muscles) is required for generating high ground-reaction forces during sprinting (Mero et al 1992). Endurance running performance, by contrast, is probably hampered by high volumes of the lower leg, because a good mechanical efficiency (economy) is aided by a leg morphology which distributes mass closer to the hip joint, inducing smaller inertia during the swing phase (Saunders et al 2004). In addition, the relative contribution of the sizes of the different muscles within the total leg muscle volume in sprinters vs. endurance runners is poorly understood. More detailed analyses are essential to complete our knowledge of the different muscle and muscle groups to explore the difference between sprinters and endurance runners (e.g. medial vs. lateral, lower leg vs. upper leg, anterior vs. posterior).

In addition to muscle volume, the architecture of the lower leg muscles is thought to play a determining role. Muscle shortening velocity is not only determined by its fiber type, but also by its fiber length, determined by the number of sarcomeres in series. Moreover, the larger the pennation angle, the lower the shortening velocity. Indeed, Abe et al. (2000) showed that sprinters have a higher fascicle length and a smaller pennation angle of the gastrocnemius muscles than endurance runners. Whether this is reflected in differences in physiological cross-sectional areas (PCSA) is not yet investigated.

This study aims to directly compare the physiological and functional anatomical characteristics of the leg of male sprinters and endurance runners of Caucasian ethnicity in order to discover the largest discriminants.

MATERIALS AND METHODS

Subjects

Eight male sprinters (60m indoor - 200m) and 11 male endurance runners (3000m – 5000m) volunteered to participate in this cross-sectional study. All athletes were competing in major Belgian national and/or international running competitions. The runners were classified based on their best running performance according to the International Amateur Athletic Federation (IAAF) scoring system. This IAAF classification allows intra- and inter-individual comparisons of performance obtained in different events. The best IAAF score of each runner was used as the individual running performance. Personal best performances ranged between 6.95 and 7.07 sec for 60m or 10.68 and 10.99 sec for 100m in sprinters and 7.54 and 8.53 min for 3000m or 13.32 and 14.55 min for 5000m in endurance runners. The study was approved by the local ethical committee (Ghent University Hospital, Ghent, Belgium) and all subjects gave their written informed consent.

MRI

Axial spin-echo T1-weighted MR images were acquired of both legs simultaneously while subjects lay supine in a 3-T whole body MRI scanner (Siemens Trio, Erlangen, Germany). Images were collected using a repetition time of 9.64 ms, echo time of 2.45 ms and slice thickness of 1.20 mm. A matrix size of 440 x 269 mm was used for all scans. Three overlapping series (a total of 224 slices) covered a field of view starting proximally at the iliac crest and running down to the distal part of the calcaneus. After data collection, the MR images were transferred to 3D slicer for digital reconstruction.

Two-Dimensional Measurements

The 3D Slicer software version 3.6 (www.slicer.org; an open source software; Brigham and Womens Hospital, Boston, MA) was used to measure length of thigh, shank and leg length. Thigh length was defined as the distance between the tips of greater trochanter and lateral condyle of the femur (Fig 1). Shank length was defined as the distance between the medial condyle of the tibia to the tip of the medial malleolus (Fig 1). Leg length was defined as the distance between the tip of greater trochanter of femur to the tip of the medial malleolus of the tibia.

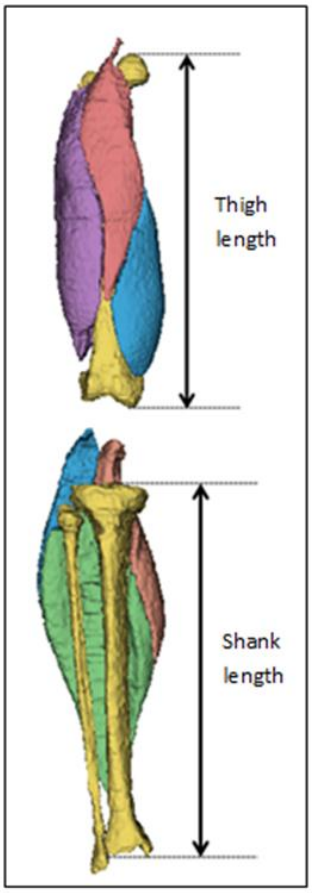

Figure 1. 3D reconstructions of upper and lower leg with the different muscles (right leg, ventral view). Thigh length and shank length are defined.

Three-Dimensional Measurements

The MRI images were displayed and manually segmented into anatomically significant structures. Muscle volumes were measured using the LabelStatistics module of Slicer 3.6, after manually selecting the muscles on transverse slices (the contrast of muscle tissue is too low for fully automatic segmentation). Figure 2 demonstrates how the different muscles were determined on a axial slice in upper leg (Fig 2a) and lower leg (Fig 2b) and on a coronal slice (Fig 2c). The volume measurements for the muscles were expressed in centimeters cubed (cm3). We focused on the major flexor and extensor muscle groups. The 7 muscles or muscle groups traced were the vastus lateralis and intermedius (VL + VI), vastus medialis (VM), rectus femoris (RF), hamstrings (HAMSTR) (semimembranosus, semitendinosus, biceps femoris long head, biceps femoris short head), gastrocnemius medialis (GM), gastrocnemius lateralis (GL), and soleus (SOL). Total upper leg volume (combined bone and muscle volume) was defined from the tip of the pelvis to the tip of

the lateral condyle of the femur. Total lower leg volume (combined bone and muscle volume) was defined from the tip of the tibia to the tip of the medial malleolus of the tibia.

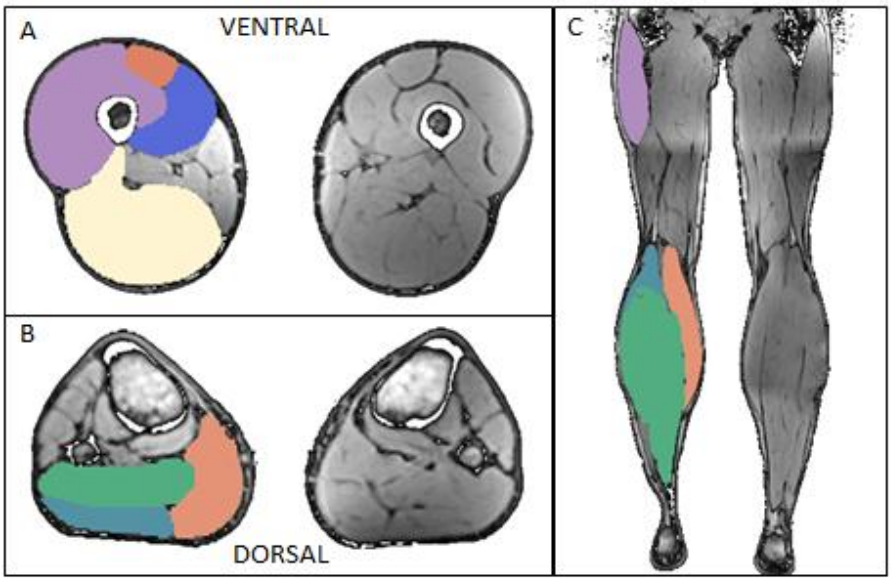

Figure 2. Axial slice in upper leg (A) and lower leg (B) and coronal slice of total leg (C, dorsal view).

The open source software pyFormex was used to determine moments of inertia. Due to the lack of direct measurements of the tissue densities, we first computed the homogenized density y^i_{home} with i being the index identifying upper and lower leg. Calculation were based on one subject from which both bone and entire limb surfaces were segmented. All muscles were assumed to have the same density ($\gamma_{musc} = 1059.7\ kg/m^3$) (Mendez and Keys 1960). The apparent density of the bones was also taken from literature ($\gamma_{bone} = 1700\ kg/m^3$) (Nigg and Herzog 1994). All centers of mass were computed on the volume embedded by each surface. Each volume was filled with tetrahedrons, so the center of mass can be calculated by the centroids of each element weighted on the volumes. The inertia I_{limb} was computed with respect to a transverse axis through the center of mass of each limb assumed fully filled with muscles from which we then subtracted the inertia I_{bone} of the bone geometry having a fictitious density equal to the difference between the muscles density and the apparent density of the bone $\gamma *_{bone} = \gamma_{bone} - \gamma_{musc}$.

Measurement of muscle architecture

Ultrasonography (Telemed UAB, Vilnius, Lithuania) was used to determine VL, GL and GM muscle architecture (muscle thickness, pennation angle and fascicle length). VL, GL and GM are superficial muscles and therefore mostly used for analyses by ultrasonography. A 5.0- to 10-MHz linear transducer (HL9.0/40/128Z) was used to visualize the proximal and distal aponeurosis of VL, GL and GM. The ultrasound probe was placed perpendicular above the muscle belly at 9 anatomical sites (anterior thigh at 30%, 50% and 70% thigh length, starting at the greater trochanter of the femur for VL and posterior lower leg at 20%, 25% and 30% proximal level of shank length, starting at the medial condyle of the tibia for GL and GM).

Images were saved on the ultrasound (US) unit for later measurements (Fig 3). Echowave II Software (TELEMED Ltd, Vilnius, Lithuania) was used for measuring distances and angles after all images were obtained. On each image the two best discernible parts of fascicles were digitized. Muscle thickness was defined as the distance between the deeper and upper aponeurosis. The pennation angle was defined as the mean of the enclosed angles between the muscle fascicles and the superficial and deep aponeurosis. Fascicle length was obtained by multiplying muscle thickness with the sine of the pennation angle. Physiological cross-sectional area (PCSA) is calculated by the following formula: PCSA = muscle volume/fiber length (by Alexander and Vernon, 1975).

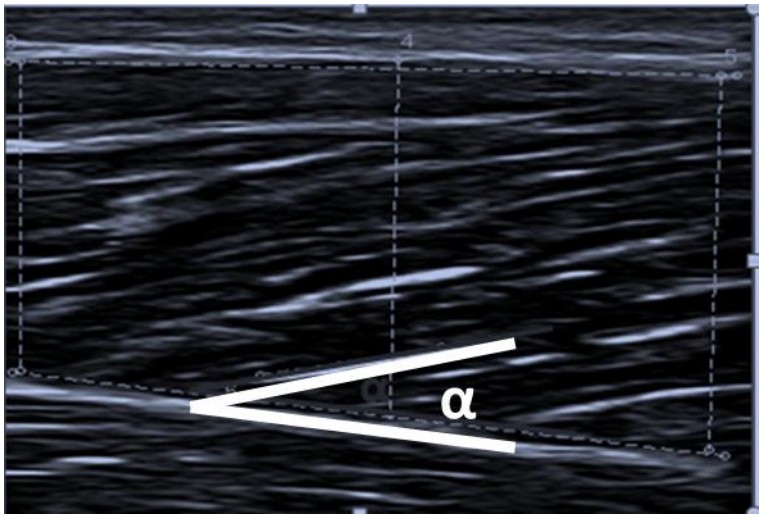

Figure 3. Image of muscle architecture by ultrasonography, with α as pennation angle.

Determination of muscle carnosine content

Carnosine content was measured in SOL and GM by 1H-MRS, as previously described by Baguet et al. (2010a). These muscles were chosen because carnosine in the calf muscles could be determined by 1H-MRS, which is not the case for upper leg muscles. The subjects were lying in supine position and the lower leg was fixed in a spherical knee coil. All the measurements were performed on a 3-T whole body MRI scanner (Siemens Trio, Erlangen, Germany). Single voxel point-resolved spectroscopy (PRESS) sequence with the following parameters was used: repetition time (TR) = 2,000 ms, echo time (TE) = 30 ms, number of excitations = 128, 1,024 data points, spectral bandwidth of 1,200 Hz, and a total acquisition time of 4.24 min. The average voxel size for SOL and GM was 40 x 12 x 30 mm and 40 x 12 x 30 mm, respectively. Following shimming procedures, the linewidth of the water signal was on average 24.0 and 25.5 Hz for SOL and GM, respectively. The absolute carnosine content (mM) was calculated as described before by Baguet et al. (2010a).

Statistics

An independent sample T-test was performed to compare the different parameters between the sprinters and endurance runners. Pearson correlation was calculated between the IAAF scores and the different parameters. All analyses were done with SPSS statistical software (SPSS 21, Chicago, IL). All values are reported as mean ± SD and statistical significance was set at $P < 0.05$.

RESULTS

Anthropometric characteristics

Sprinters and endurance runners were similar in performance level (IAAF score) and body height, but the sprinters' body mass, BMI and fat percentage were higher (Table 1). As indicated in Table 2, sprinters and endurance runners showed no differences in length of either the body segments thigh and shank, nor the total leg. The ratio of these body segments to body height and the ratio of thigh and shank length to leg length were similar in both groups. Due to a comparable body height and comparable body segments lengths, there was no need to normalize other parameters to body height and further analyses could be done with absolute values.

Table 1: Physical characteristics and running performance.

	Sprinters (n = 8)	Endurance runners (n = 11)
Age (year)	23.6 ± 4.2	23.5 ± 3.7
Height (cm)	178.0 ± 5.6	180.4 ±3.5
Body mass (kg)	74.8 ± 6.4	64.0 ± 4.7 *
Body mass index	23.6 ±1.8	19.7 ±1.2 *
Fat percentage (%)	9.0 ±1.5	7.2 ±1.9 $
Running performance (IAAF score)	984.6 ±77.4	1013.2 ± 107.2

Data are means ± SD. * p < 0.05 versus sprinters. $ p = between 0.05 - 0.10 versus sprinters.

Table 2: Body segments and ratio of different body segments.

	Sprinters (n = 8)	Endurance runners (n = 11)
Leg length (cm)	86.4 ± 4.3	87.6 ± 2.3
Length thigh (cm)	47.2 ± 2.7	48.1 ±1.2
Length shank (cm)	39.6 ± 2.0	40.0 ± 1.3
Ratio leg length/height	0.485 ±0.012	0.486 ±0.011
Ratio length thigh/height	0.265 ±0.008	0.267 ±0.006
Ratio length shank/height	0.222 ±0.007	0.222 ± 0.006

Data are means ± SD. * p < 0.05 versus sprinters. $ p = between 0.05 - 0.10 versus sprinters.

Muscle volumes parameters

The total upper leg volume was significantly higher for the sprinters compared to the endurance runners (17 %, P = 0.002) (Fig 4). Specifically, the RF, VL + VI and HAMSTR showed significantly higher muscle volumes (Table 3A). The RF contributed to on average 11 %, the VL + VI 44 %, the VM 16 % and the HAMSTR 29 % of the total analyzed thigh muscle volume, with no differences between the two groups. Center of mass (COM) was defined in the direction of gravity and expressed in percentage of the total leg length. COM of the different muscles of the upper leg showed no differences between the groups (Table 3A).

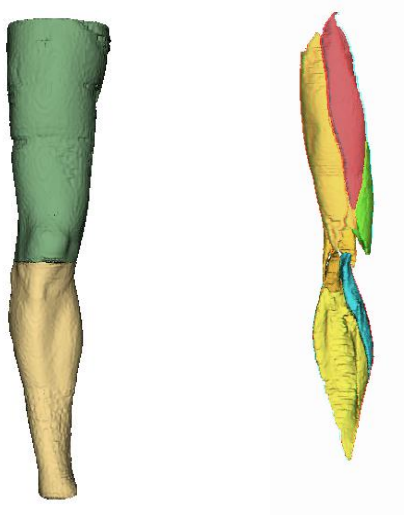

Figure 4. 3D reconstruction of total leg and different muscles (right leg, ventral view).

Table 3B shows the data of the lower leg. Sprinters had a significantly higher muscle volume in the GL (21 %, P = 0.017) and a tendency to a higher muscle volume in SOL (17 %, P = 0.091) compared to endurance runners. Total lower leg volume and GM volume were similar in both groups. No differences were found in muscle distribution or COM in the different muscles of the lower leg. The percentage differences between sprinters and endurance runners in the different muscles volumes were 26 % for RF, 23% for VL, 21 % for GL and HAMSTR, 17% for soleus, 14 % for VM and 9 % for GM. The transverse moment of inertia of the lower leg at knee joint and the upper leg at hip joint were not significantly different between sprinters and endurance runners (0.13 ± 0.02 vs. 0.12 ± 0.02 kg.m², P = 0.102 (lower leg) and 0.59 ± 0.10 vs. 0.52 ± 0.07 kg.m², P = 0.105 (upper leg)).

Table 3: Muscle volumes, ratios and center of mass (COM) of upper leg (A) en lower leg (B). Ratios were expressed in percentage of the total analyzed thigh volume (RF, VL + VI, VM and HAMSTR). COM was defined in the direction of gravity and expressed in percentage of the total leg length.

A	Upper leg	Sprinters (n = 8)	Endurance runners (n = 11)
Volume (cm³)	RF	372.92 ± 27.58	295.10 ± 44.97 *
	VL + VI	1478.25 ± 172.05	1204.48 ± 159.92 *
	VM	530.80 ± 88.02	464.64 ± 77.43
	HAMSTR	966.83 ± 132.08	799.31 ± 117.92 *
	Total volume	7339.67 ± 652.04	6265.06 ± 584.53 *
Ratio (%)	RF	11.23 ± 1.24	10.74 ± 1.67
	VL + VI	44.14 ± 1.77	43.59 ± 2.06
	VM	15.79 ± 1.33	16.77 ± 1.43
	HAMSTR	28.83 ± 1.95	28.91 ± 2.04
COM (%)	RF	23.73 ± 3.74	23.06 ± 2.33
	VL + VI	30.68 ± 4.06	30.32 ± 2.00
	VM	35.51 ± 4.20	35.38 ± 2.35
	HAMSTR	36.48 ± 3.96	36.26 ± 1.78
	Total volume	37.29 ± 3.76	36.87 ± 2.03

Data are means ± SD. * p < 0.05 versus sprinters. $ p = between 0.05 - 0.10 versus sprinters.

B	Lower leg	Sprinters (n = 8)	Endurance runners (n = 11)
Volume (cm³)	SOL	496.46 ± 76.29	424.91 ± 92.05 $
	GM	281.88 ± 60.18	259.14 ± 52.45
	GL	206.20 ± 32.15	170.08 ± 27.49 *
	Total volume	2815.89 ± 195.14	2662.31 ± 302.09
Ratio (%)	SOL	50.45 ± 4.28	49.56 ± 3.54
	GM	28.55 ± 3.66	30.42 ± 3.59
	GL	21.00 ± 2.48	20.01 ± 1.23
COM (%)	SOL	82.01 ± 2.82	82.26 ± 1.76
	GM	69.73 ± 3.57	69.65 ± 1.89
	GL	67.86 ± 3.67	67.83 ± 1.67
	Total volume	83.28 ± 2.99	83.83 ± 1.57

Data are means ± SD. * p < 0.05 versus sprinters. $ p = between 0.05 - 0.10 versus sprinters.

There were significant positive correlations between ratio HAMSTR/QUADS muscle volume and IAAF scores in the sprint group (R = 0.811, P = 0.015) and in the group with all athletes together (R = 0.532, P = 0.019), but no correlation was found in the endurance group alone.

Skeletal muscle architecture

There were no group differences in pennation angle or fascicle length of the VL, GM en GL. PCSA in the VL showed a tendency to a higher value in sprinters compared to endurance runners, but no differences were found in PCSA of GM and GL (Table 4).

Table 4: Pennation angle, fascicle length, PCSA. Physiological cross-sectional area (PCSA) is calculated by the following formula: PCSA = muscle volume/fiber length (by Alexander and Vernon, 1975).

		Sprinters (n = 8)	Endurance runners (n = 11)
Pennation angle (°)	VL	13.39 ± 2.4	11.87 ± 2.6
	GM	13.47 ± 1.8	15.50 ± 3.1
	GL	11.94 ± 2.2	11.32 ± 2.2
Fascicle length (cm)	VL	111.98 ± 23.4	110.6 ± 26.7
	GM	85.45 ± 9.0	77.68 ± 19.8
	GL	88.91 ± 18.54	75.92 ± 18.0
PCSA	VL	13.75 ± 3.5	11.3 ± 2.2 $^{\$}$
	GM	3.30 ± 0.6	3.55 ± 1.0
	GL	2.40 ± 0.5	2.30 ± 0.4

Data are means ± SD. * $p < 0.05$ versus sprinters. $^{\$}$ p = between 0.05 - 0.10 versus sprinters.

Muscle carnosine concentration

Muscle carnosine concentration was approximately 60% higher in sprinters compared to endurance runners for SOL (5.74 ± 2.14 vs. 3.62 ± 0.94 mM, P = 0.035) and for GM (10.82 ± 2.57 vs. 6.61 ± 1.15 mM, P = 0.002).

Fig 5 shows all individual Z-scores of sprinters and endurance runners on a representative selection of different musculo-skeletal parameters, with muscle carnosine in GM as the most discriminant feature.

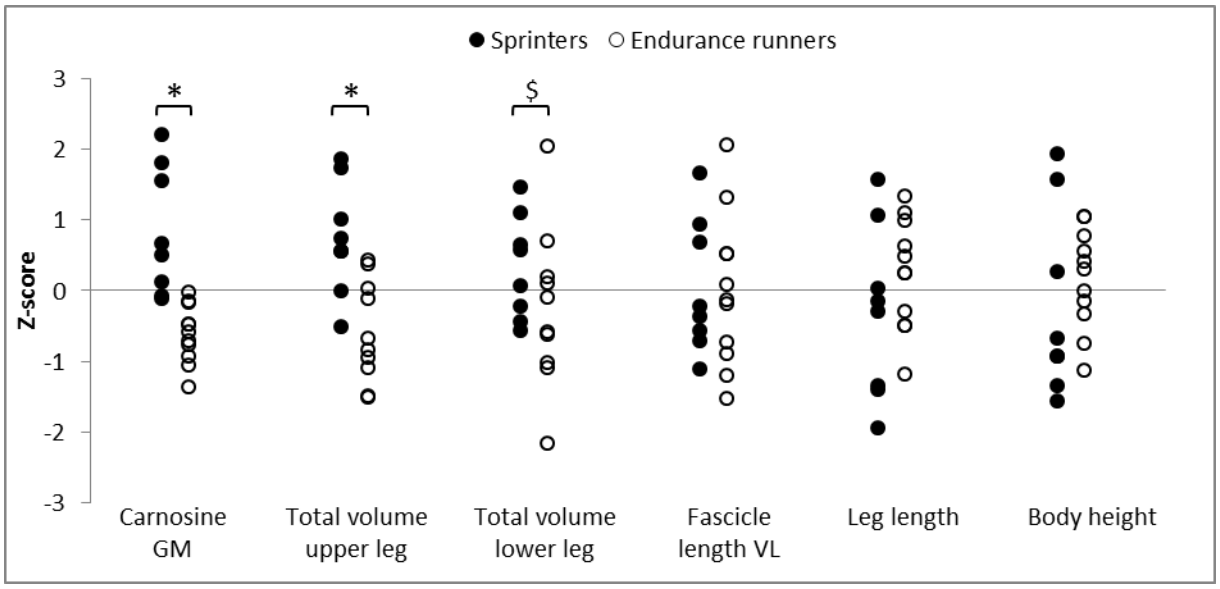

Figure 5. All individual Z-scores of sprinters (black circles) and endurance runners (open circles) on a representative selection of different musculo-skeletal parameters. * $p \leq 0.05$ and $^{\$}$ p = between 0.05 – 0.10.

DISCUSSION

Excellence in sprint or endurance running requires specific musculo-skeletal properties of the leg. At cellular level, it is well known that skeletal muscles of sprinters are characterized by a high percentage fast-twitch fibers and that endurance runners possess a high proportion of slow-twitch fibers. In this study, muscle carnosine concentration, as indirect estimation of percentage area occupied by type II fibers (Baguet et al 2011b), was 60% higher in sprinters. This confirms the extreme distinction in muscle fiber type composition between these two groups of athletes. However, the question remains at macroscopic morphological level which of the characteristics are specifically divergent between sprint and endurance runners.

In sprinting, the determining factor for performance is power production, which is regulated by the combination of force and velocity (Mero et al 1992). To optimize power generation, the muscles specifically recruited during sprint running should be primarily developed, with greater volumes as result. This is in contrast to endurance running where lower volumes in upper and lower leg were expected to ensure low energy cost for the swinging phase. The results of this study showed indeed higher muscles volumes in the sprint specific muscles (e.g. VL+VI, RF, HAMSTR), which resulted in higher total upper leg volume. However, at individual muscle level no differences were found in VM. This is in contrast to the results in the study of Kubo et al (2011) who found greater thickness of the medial side of the knee extensors, but this was compared to untrained subjects. It has been suggested that the VM muscle among the knee extensor muscles is important for stabilizing functions both during sprinting and endurance running (Toumi et al 2007). The VL is a more power delivering muscle, suggesting an important role for this muscle during sprinting and therefore the VL is more distinguished between sprinters and endurance runners than the VM.

Furthermore, a significant higher GL and a clear trend for larger volume in SOL was found in the lower leg, but with surprisingly no significant greater total lower leg volume between sprinters and endurance runners. A possible explanation for finding no differences in total lower leg volume between these athlete populations is that both sprint and endurance running require optimal muscle volumes in the lower leg with a

positive effect towards power delivering (Mero et al 1992; Trappe et al 2001), but not too heavy for swinging the legs (Saunders et al 2004).

As stated before, power generation is the most important factor for sprint running performance and is determined by force and velocity. Longer fascicle lengths will cause higher shortening velocities and can therefore contribute to a higher power output during sprinting. The study of Abe et al (2000) already showed that sprinters had higher fascicle lengths and lower pennation angles compared to endurance runners. However, we were not able to confirm the data of this study. PCSA, which is indirect determined by fascicle lengths and muscle volumes, tended to be higher in the VL. Therefore, it can be concluded that sprinters are able to deliver high power outputs due to a higher force production (higher PCSA by greater muscle volumes) and not by higher contraction velocities (equal fascicle length). Furthermore, muscle fiber type seems to play a major role, because FT fibers are able to deliver more force at a higher velocity, which directly influences power production.

Additionally, some studies suggested a different shape of the upper leg between sprinters and endurance runners (Abe et al 1999; Abe et al 2000; Kumagai et al 2000), but this cannot be confirmed by our results, as there were no differences in distribution of the different muscles or in the position of the center of mass of the different muscles. It has to be mentioned that earlier studies were done with ultrasonography, which gives an estimation of muscle volume, while in this study muscle volumes were calculated on MRI-based 3D reconstructions of serial 2D slices, which is a more powerful methodology.

It has already been suggested that the hamstring and psoas major muscles play an important role during sprinting (Mero et al 1992; Hoshikawa et al 2006; Schache et al 2012). Hamstrings volume is indeed larger in sprinters than in endurance runners. Hamstrings eccentrically deliver large power peaks when decelerating the swing leg just before ground contact (Schache et al 2012). A positive correlation between HAMSTR/QUADS ratio and IAAF scores in sprinters was found. Although causality of this relationship is uncertain, it could mean that a high hamstring muscle mass, relative to quadriceps muscle mass, is advantageous for sprint running performance, more than for endurance running performance.

Nevertheless, the ratio of total hamstrings volume to total quadriceps volume averaged nearly 1:3 in earlier studies (Tate et al 2006). This study found 2.5 fold lower volumes in hamstrings compared to quadriceps in both sprinters and endurance runners. This means that, although very strong hamstrings are required for sprinting, H/Q ratio is about equal in sprinters and endurance runners indicating the importance of knee stability and prevention of knee injuries during running (Hirokawa et al 1991).

It can be concluded that the results in this study, generated by MRI-based anatomical 3D reconstruction, indicate that the differences between sprint and endurance Caucasian runners are situated in muscle volumes, rather than muscle orientation, shape or skeletal measures. Higher muscle volumes were mainly found in proximal and lateral leg muscles for the sprinters. However, the major discriminant between these athlete populations of the same ethnicity remains estimated muscle fiber type composition. Our data also support the notion that the hamstrings muscles are an important factor in sprint running performance.

PERSPECTIVES

The current study is the first to describe the musculo-skeletal leg characteristics between sprinters and endurance runners of the same ethnicity (Caucasian) with MRI, allowing single bone and single muscle level analysis. This information source should allow more accurate biomechanical simulations of running, based on true athletic legs of sprinters and endurance runners. Until now, most biomechanics analysis software (e.g. Visual 3D) is based on Dempster's anthropometic data (Dempster 1955). Further research on a higher level of athletes is required on whether body morphology shows more distinctions between sprinters and endurance runners. This can create some new insights to the main musculo-skeletal characteristics contributing in sprint or endurance performance.

ACKNOWLEDGEMENTS

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III

General Discussion

1. Carnosine loading protocol

As known from the introduction, intramuscular carnosine loading by BA supplementation is not yet a very efficient process. Until now, around 95 % of the BA intake has a unknown metabolic fate. A first determinant of carnosine loading is already established by Stegen et al (2013). Meal and BA coingestion augments the loading efficiency from 2.4 % to around 3 %, which still remains a low effectiveness. **Study 1 and 2** of this thesis investigated another potential determinant, namely exercise training. This suggestion was made in analogy with another popular supplement, namely creatine (Harris et al 1992; Robinson et al 1999). However, BA supplementation in combination with exercise training was only investigated in one study with no effect of isokinetic training on carnosine loading between a trained and untrained leg (Kendrick et al 2009). As the literature is quite limited, study 1 and 2 were performed to have a closer look at the possible influence of exercise training on the carnosine loading.

1.1. Exercise as potential determinant

BA was given to a group of nonathletes and three different athlete populations (swimmers, cyclists and kayakers) in **study 1**. This gives the possibility, within a cross-sectional study design, to compare carnosine loading both between athlete populations and within athletes. Only a dietary intervention took place in this study, without influencing the training programs of the athletes. However, a minimal training load of around 8 hours per week was required from the athletes to participate in the study. This study found two-fold higher carnosine loading after BA supplementation in trained muscles, compared to untrained muscles. Even within one athlete population (kayakers, cyclists) big differences were found between arm and leg muscles in the increase in carnosine concentrations. The loading efficiency in trained muscles was up to 5.80 % (Table 1). The question raised whether this was due to the acute response of exercise training during the study or whether training over many years was responsible for chronic adaptations of muscle, leading to higher carnosine loading efficiency. Therefore, **study 2** deals with this by taking non-specifically trained subjects who were subjected to exercise training. In this manner, the effect of training status on BA supplementation efficiency was eliminated. Furthermore, different acute training protocols (high-volume (HV) vs.

high-intensity (HI)) were selected to investigate which protocol would be most successful in enhancing the effectiveness of the carnosine loading process. The results of study 2 suggested higher carnosine concentrations after BA supplementation in the training groups compared to the non-training group. The efficiency of the BA-induced carnosine loading was 5.17 and 5.67 % in the HV and HI training group, respectively (Table 1). These data confirm the results of study 1 that exercise training is a determinant of the carnosine loading protocol and that exercise training during supplementation could enhance this process. As the two training protocols were equally efficient, the conclusion could be made that both training interventions could promote the increases in carnosine concentrations.

Table 1. BA-induced carnosine loading and the effect of acute or chronic exercise on the loading efficiency.

	Acute effect of exercise	Chronic effect of exercise	Efficiency (%)	Data
Nonathlete – No exercise	-	-	3.49	(Study 1–2)
Nonathlete – Exercise	+	-	5.17 – 5.67	(Study 2)
Athlete – No exercise	-	+	/	
Athlete – Exercise	+	+	5.80	(Study 1)

To summarize, the highest carnosine loading efficiency was found in the trained muscles of the athletes of study 1, but with only a small difference in the nonathletes of study 2 who trained 3 times per week in either HV or HI training protocols. Therefore, it could be concluded that the most important mechanism will be the acute effect of exercise on carnosine loading, but a possible additive effect of exercise on trained muscles could cause the highest BA supplementation efficacy. As there were no differences between HV and HI exercise training, no extra information could be gathered on the mechanisms behind the better loading. So until now, one study could not demonstrate an effect of exercise training on the BA uptake (Kendrick et al 2009), although the two studies in this thesis could.

1.2. Possible mechanisms of exercise on carnosine loading efficiency

Based on the three studies that already were performed on the topic BA supplementation and exercise training, some hypotheses could be made on the mechanisms of exercise training on the carnosine loading efficiency.

During exercise, a first **acute response** is the increase in perfused capillaries, providing an increase in nutrient delivery (Rattigan et al 2006). During BA supplementation, this activity-related increase in perfusion could cause higher BA concentrations, leading to a higher availability of BA for transport to and storage in muscle cells (Mechanism 1). Furthermore, during exercise there is translocation of the GLUT4 glucose transporters to take up glucose in a faster way. The GLUT4 proteins are recruited from intracellular sites by muscle contractions and than they move to the cell surface, where they are responsible for transport of glucose into the muscle cells (Krook et al 2004; Holloszy 2008). In accordance with the GLUT4 transporters, carnosine loading efficacy could be enhanced by activity-related translocation of transporters (PAT1 and TauT) for BA uptake in the myocytes (Mechanism 2).

Long-term exercise training elicits adaptations in many physiological systems. A first metabolic adaptation in skeletal muscle is the increase in blood flow capacity. Exercise training increases the number of capillaries per square millimeter of muscle with the largest increase in capillary density in high oxidative muscles by endurance training and in more glycolytic muscles by sprint interval training. Changes in capillarity could explain only partly the increases in blood flow capacity, because blood flow capacity is induced by a mixture of vascular adaptations (e.g. capillarity, growth and remodeling of arterioles, altered control of vascular resistance…). Depending on the type of training and the muscle fiber recruitment patterns associated with it, the relative contribution of these adaptations could cause the higher blood flow capacity during exercise (Laughlin and Roseguini 2008). In this way, a first possible chronic response of exercise training that could be beneficial for the carnosine loading process is the higher capillary density in trained muscles. This adaptation could result in a higher blood flow to active muscles and provide a greater surface area for the exchange of BA (Mechanism 3). Furthermore, it could be hypothesized that longterm training could cause a higher expression of

transporters/enzymes for BA uptake and a more efficient carnosine synthase enzyme (Mechanism 4). Taking these explanations together, 4 different mechanisms may regulate the higher carnosine loading due to chronic or acute exercise (Fig 1).

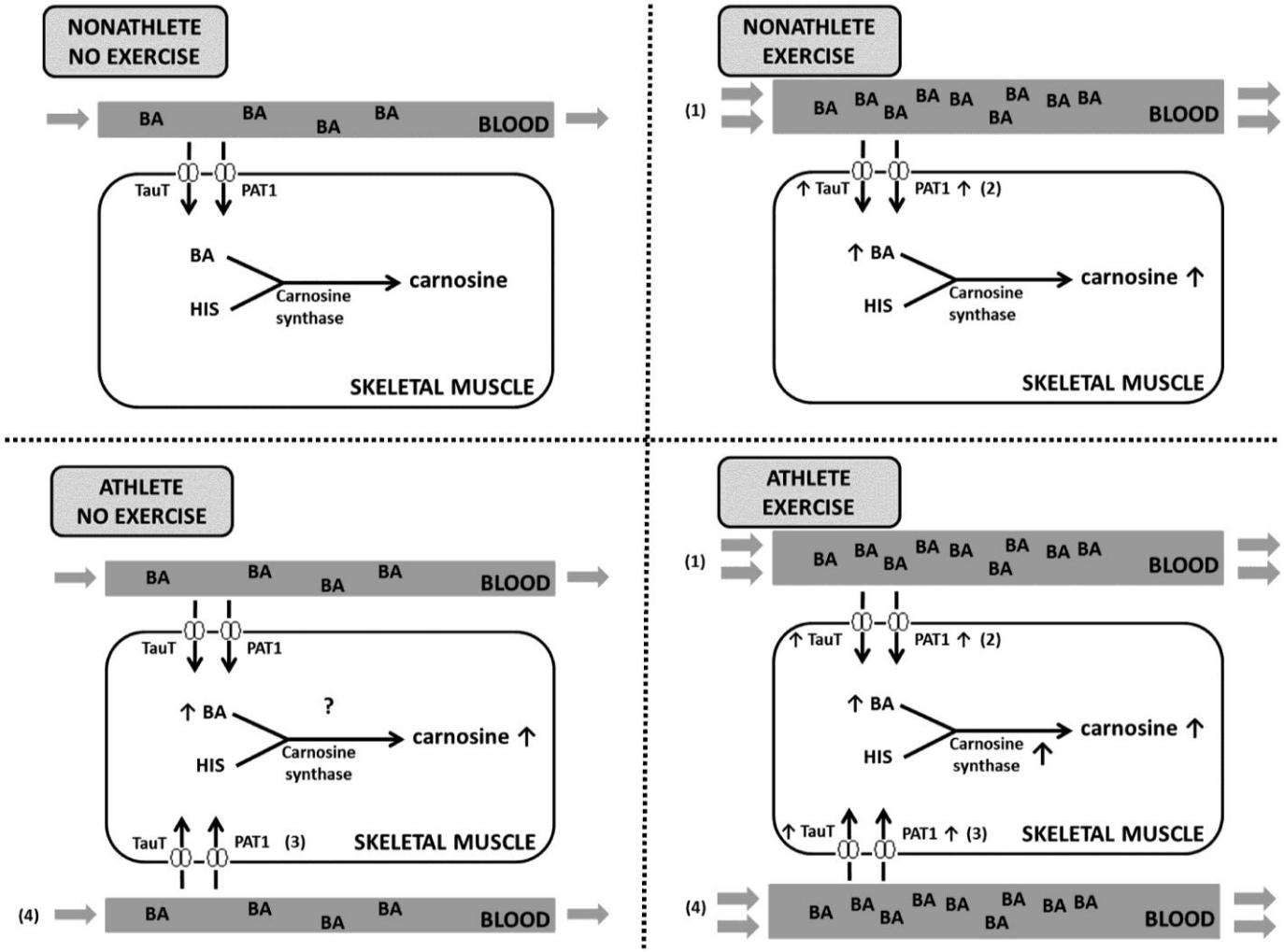

Figure 1. The effect of acute and/or chronic exercise on BA-induced carnosine loading, illustrated with 4 different mechanisms: (1) increase in blood flow, (2) translocation of transporters, (3) higher capillary density and (4) higher expression of BA transporters and/or carnosine synthase enzyme

1.3. Practical applications for athletes

During the past years, BA is a very popular supplement among athletes to increase their performances at the highest level. Based on these new studies of exercise training on BA-induced carnosine loading, it could be concluded that this parameter should be integrated in the supplementation protocol, together with meal coingestion (Fig 2). This will ensure the highest possible increases in carnosine concentrations.

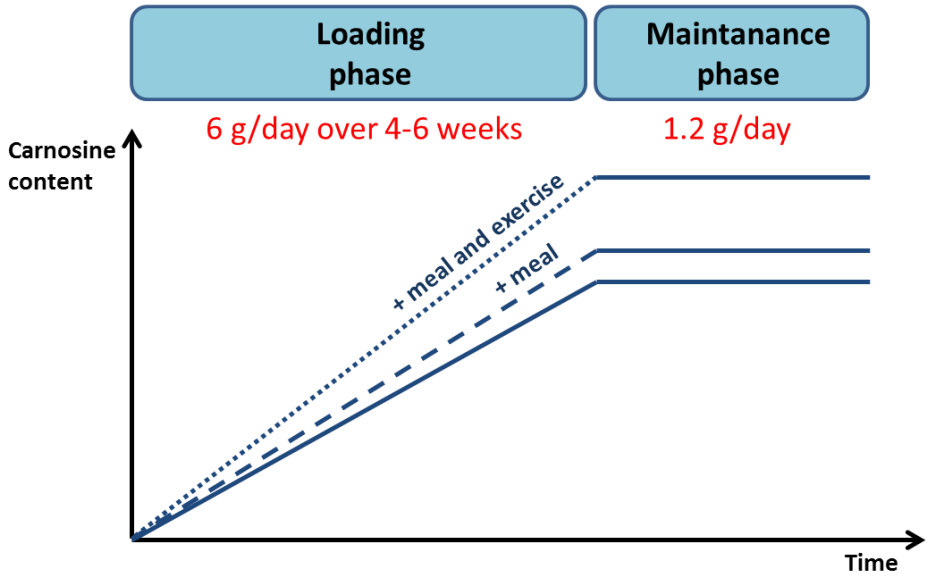

Figure 2. Loading and maintaining phase of BA supplementation, with a higher increase in carnosine concentrations after loading phase due to the combination of BA intake and meal and/or exercise training.

Some **guidelines** to take this supplement.

- Loading protocol:
  - 4 – 6 g/day, spread into 4 doses per day
  - Intake together with meals and/or training sessions
  - Supplementation during a training period
  - 4 – 6 weeks of supplementation
- Maintenance protocol: ~ 1.2 g/day during race season
- Most evidence for exercises between 60 – 240 s

Example of 1500m runner taking this supplement.

Goal: World Championship (Series: 27/08, Semi-Final: 28/08, Final: 30/08)

- Start BA supplementation: 16/07 – 13/08:
  - 6 g/day, spread into 4 doses of 1.5 g slow-release (SR) BA tablets
  - 3 doses before meal (breakfast, lunch, dinner) and 1 dose before training session
- Maintenance phase: 13/08 – 30/08:
  - 1 dose of 1.5 g SR BA tablets before training session or before meal

2. Muscle fiber type composition and influence of movement frequency

As seen in the introduction, there are large inter-individual differences in MFTC between people. Knowing athletes' MFTC can be valuable for sport scientific guidance, mainly to optimize training advice, to identify talents and potentially to prevent injury risk. In track-and-field, the importance of having the right MFTC to excel in a certain discipline has already been suggested by biopsy studies in the seventies (Gollnick et al 1972; Costill et al 1976a; Tesch and Karlsson 1985). In other sports, the literature is quite limited and an overall distribution of differences in MFTC in athletes is not available. Due to the new methodology to estimate MFTC based on the amount of muscle carnosine by ^{1}H-MRS (Baguet et al 2011b), a bigger database of estimated MFTC in athletes of different sports could be collected and analyzed. In this thesis, ^{1}H-MRS measurement of carnosine was optimized in the arm muscles (see part 5 of the general discussion: NMR methodology) and therefore this method could be applied in different athlete populations, other than track-and-field. **Study 3** combined this new data with earlier published literature to make a round up of all these data.

2.1. Distribution of MFTC in different cyclic sports

It could be confirmed that explosive athletes contain a high proportion of FT fibers in the different muscles of the body. Depending on the discipline in which athletes succeed, specific properties of muscle were present. As there was almost no information on specific subdisciplines in cycling, swimming and kayaking, this information is quite new. Figure 3 represents the values in gastrocnemius muscle and figure 4 shows the data of deltoideus muscle. This figures show that, *within* sports, a clear distinction in MFTC is present between explosive and endurance athletes.

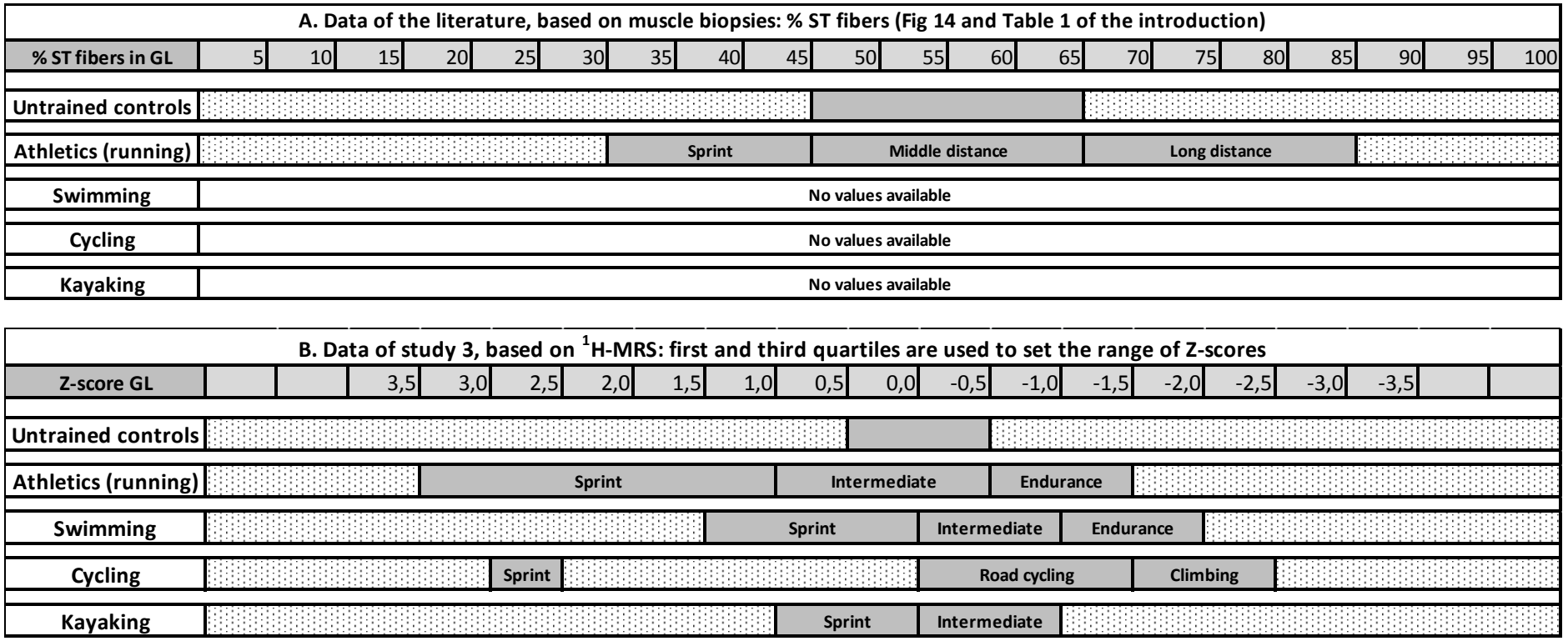

Figure 3. Schematic overview of distribution in muscle fiber type composition in gastrocnemius lateralis (GL) in different cyclic sports: comparison of available literature (A) and new data of study 3 (B).

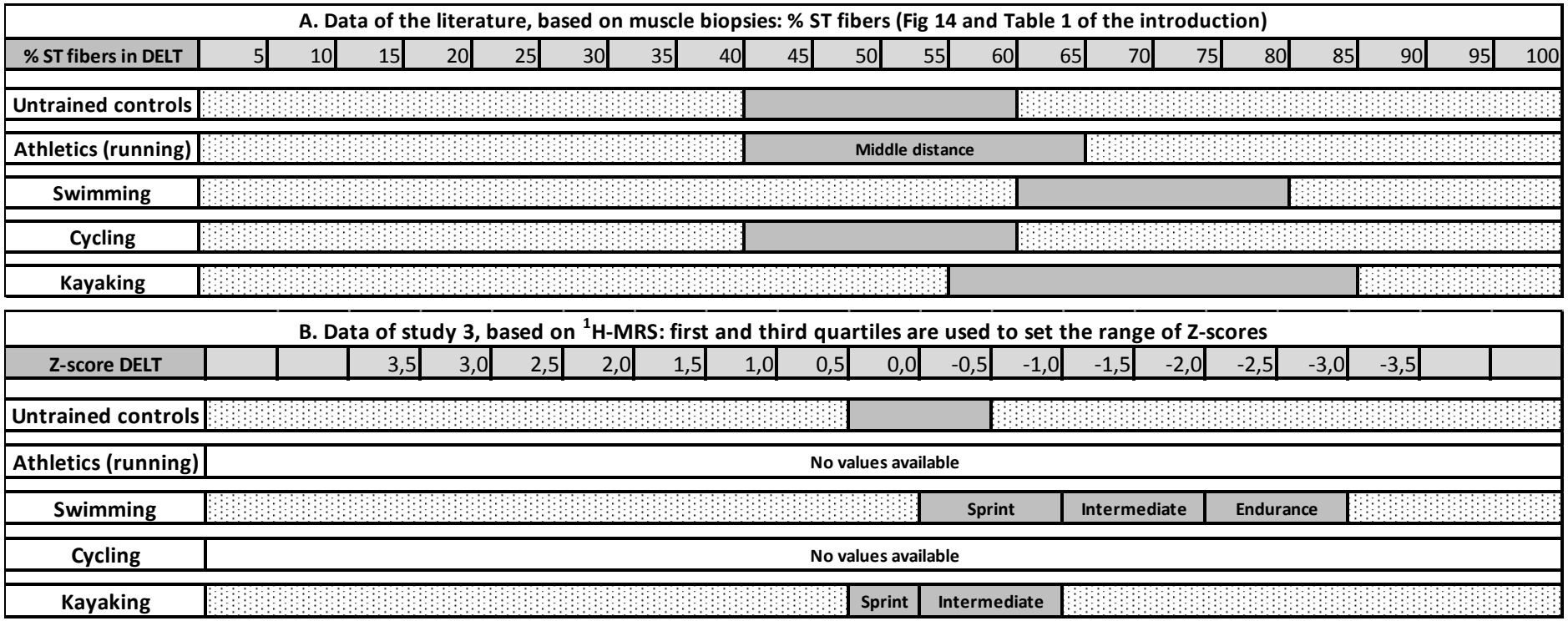

Figure 4. Schematic overview of distribution in muscle fiber type composition in deltoideus (DELT) in different cyclic sports: comparison of available literature (A) and new data of study 3 (B).

2.2. Carnosine content as estimated MFTC is linked to cyclic movement frequency

Study 3 gives the possibility to compare MFTC *between* different sports. In this way, specific requirements regarding different sports and their disciplines could be investigated. To start with, the specific disciplines in each sport were analyzed regarding exercise duration and movement frequency of the cyclic sports. Linking the information to muscle carnosine concentration of the athletes (and thus indirect their MFTC) shows that movement frequency is a better prediction for indirect MFTC compared to exercise duration. It seems that the higher shortening velocity of FT muscle fibers underlies this mechanism, suggesting the need for high percentage of FT fibers in cyclic sports with high movement frequency.

Taking a closer look to cycling, the study of Kohler and Boutellier (2005) analyzed the most powerful pedaling rate. In this study, it was calculated that in mixed muscles, consisting of 50 % of both ST and FT fibers, a pedaling rate between 80 and 115 rpm is the most powerful. However, in track cycling pedaling rates over the 150 rpm were reached (Dorel et al 2005) and as seen in figure 5, ST fibers were unable to deliver power at such high pedaling cadence (and thus such high shortening velocities). Muscle shortening velocity is proportional to pedaling rate (assuming cycling as a one-muscle movement). Therefore, athletes with very high proportions of FT fibers are required to deal with those high pedaling rates.

Road cycling is characterized by pedaling rates around 80 to 105 rpm and climbing between 62 and 85 rpm (Chavarren and Calbet 1999; Lucia et al 2001; Lucía et al 2001; Vogt et al 2007). Due to the long duration of these exercises, a high percentage of ST fibers is required. However, to win in a flat stage, cyclists should be able to deliver a final sprint with pedaling rates up to 120 rpm. To deal with this, a trade-off should be made between proportion of ST and FT fibers. Based on their MFTC, cyclists with more FT fibers could be seen as specialists on flat races ending with a final sprint. In mountain stages, power delivering will be less important and a more efficient pedaling cadence will induce lower oxygen consumption and thus higher economy and therefore will be more useful. As a higher proportion ST fibers will cause higher peak efficiencies compared to more FT

fibers, specialists in mountain stages will be characterized by a high percentage of ST muscle fibers.

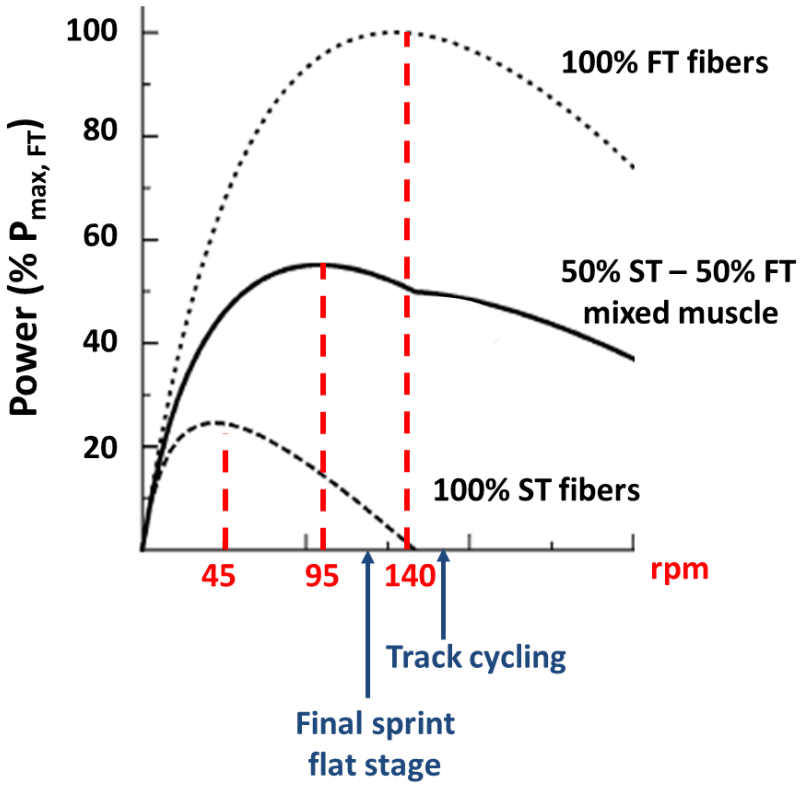

Figure 5. Power-pedaling-rate relationship. *Dashed line* is a hypothetical muscle composed exclusively of ST fibers; *dotted line* is a muscle composed exclusively of FT fibers; *continuous line* is a muscle with equal cross-sectional area of both fiber types. The most powerful pedaling rate is 45 rpm for exclusively ST muscles, 95 rpm for mixed muscles and 140 for exclusively FT muscles, identified as the red dashed lines (figure adapted from Kohler and Boutellier (2005)).

2.3. Level of athletes in relation to estimated MFTC

Another question dealing with MFTC is whether a higher level of runners goes together with having a more extreme MFTC. Based on the literature, it was hypothesized that more successful sprint runners should possess a greater percentage of FT fibers than less successful counterparts, while it was expected that this was the other way around for endurance runners with more ST fibers in the better endurance runners. For sprint running performance, this idea made sense and **study 3** showed that the better sprint runners were characterized by even higher carnosine levels and thus greater estimated area of FT fibers. This was not the case within endurance running, where the same proportion of ST fibers was found in the two subclasses of endurance runners. This is in

accordance with the relatively high movement frequency during endurance running events, especially during the final lap(s) in 5K and 10K running, suggesting that endurance runners need a proportion of FT fibers to reach world level (Fig 6).

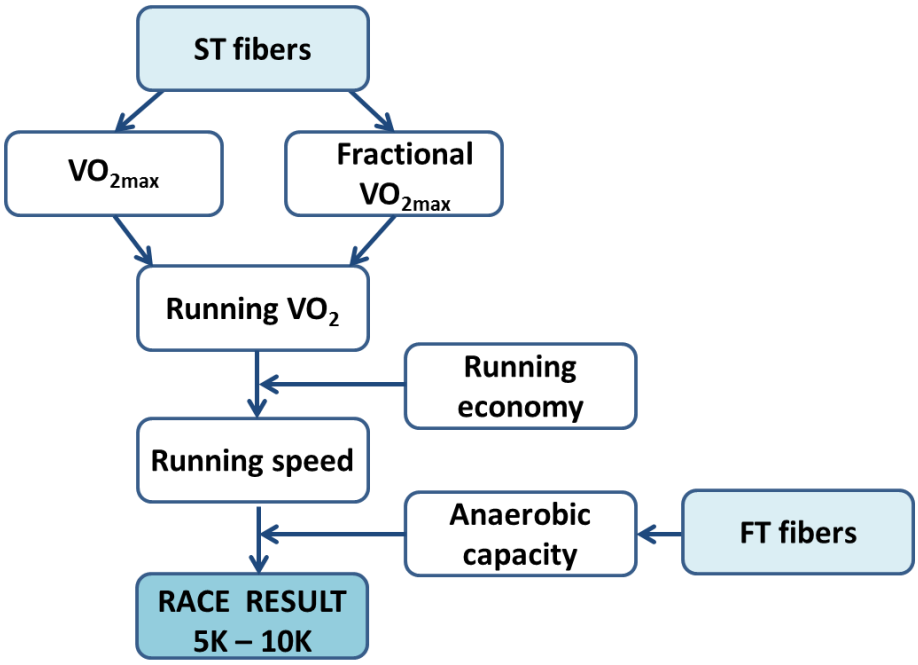

Figure 6. Schematic illustration how different muscle fiber types could influence the race results in endurance events.

All this new information can be **summarized** by the fact that analyzing movement frequency of a subdiscipline in a specific sport could deliver information about the properties of muscles (e.g. specific MFTC) of athletes needed for elite performance. Measuring carnosine concentrations could, in this way, be used to guide athletes to their best discipline in a specific sport, based on the indirect estimation of MFTC. As it has been shown that MFTC is partly influenced by training (comparing arm and legs of swimmers), but mostly genetically determined, the measurement of muscle carnosine could be used for talent identification. Therefore, this new method to estimate MFTC is called the "Muscle Talent Scan". However, as seen in the introduction, carnosine levels can undergo changes in puberty, especially in boys, and can be influenced by BA supplementation. Therefore, we advice to measure athletes after their puberty and at least after a 3 month BA supplementation free period.

3. Musculo-skeletal characteristics of Caucasian and East-African endurance runners – Unpublished work (Study 5)

3.1. Background

Runners of East-African origin, particularly those from Kenya, have dominated the world top 100-lists of 5 and 10K races for many years. It is observed that a vast majority of the Kenyan endurance runners originate from a single tribe, the Kalenjin. The Kalenjin homeland is located in the Great Rift province at altitudes of over 2000 m above sea level (Onywera et al 2006; Wilber and Pitsiladis 2012). Furthermore, a subtribe of the Kalenjin, known as the Nandi, represents almost half of Kenya's international runners (Larsen 2003; Tucker et al 2013). Literature on this topic is widespread, although clear explanations for this phenomenon are not yet given. To explain success of East-African runners, differences in body morphology between East-African and Caucasian runners should be directly linked to physiological and biomechanical parameters. Up until now, most studies investigated only a small part of the success of East-African runners with some hypotheses or suggestions, but with no clear proofs. Therefore, a complete overview of both musculo-skeletal characteristics as functional physiological and biomechanical measurements on East-African runners and Caucasian counterparts is required. In this thesis, analysis has been initiated on the leg morphology of endurance runners with the same methodology as used in study 4. Some preliminary results will be presented here, but the sample size will be expanded in the future. After collecting all the morphological data, athletes will return for physiological and biomechanical analyses. This comprehensive study will hopefully lead to new insights in the superiority of East-African runners in endurance performance. Until now, 3 East-African runners and 4 Caucasian runners from international level have been measured to determine their musculo-skeletal characteristics.

3.2. Preliminary results and conclusions

Are skeletal properties of East-African endurance runners comparable to those of their Caucasian counterparts?

It is known that Africans have longer extremities than Caucasians, suggesting the existence of racial differences (Wagner and Heyward 2000). Indeed, some earlier studies already suggested East-African runners having longer shank and thigh lengths compared to endurance runners of other ethnicities (Lucia et al 2006; Kunimasa et al 2014; Sano et al 2015). In this preliminary study, data was available on 3 East-African and 4 Caucasian endurance runners from the same performance level. Two of the three East-Africans were Kalenjin and one is from Somali ethnicity. The endurance runners had a personal best on 1500m between 3'34"49 and 3'39"73 (3 of them) and on 5K between 13'06"10 and 13'37"48 (4 of them). The mean running performance, expressed in IAAF scores, was 1127.5 ± 51.4 for the Caucasians and 1144.0 ± 39.3 for the East-African runners. Table 1 shows data of the body segments and the ratios of different body segments upon each other, based on our MRI-data. These data could confirm that East-African runners have longer legs which is mainly due to longer shank lengths. Leg length might be in relationship with stride length and some suggestions were made that leg length can influence running economy (Anderson 1996). Until now, this assumption is only indirectly investigated and therefore, the follow-up of this study will try to establish leg length and the link with RE in a direct way.

Table 1: Body segments and ratio of different body segments.

	Caucasian (n = 4)	East-African (n = 3)
Height (cm)	182.7 ± 1.7	176.0 ± 8.3
Leg length (cm)	88.3 ± 2.6	90.5 ± 4.2
Length femur (cm)	48.2 ± 1.6	48.5 ± 2.6
Length tibia (cm)	40.4 ± 1.4	42.4 ± 1.8
Ratio leg length/height	0.483 ±0.017	0.517 ±0.021 *
Ratio length femur/height	0.264 ±0.008	0.276 ±0.008 $
Ratio length tibia/height	0.221 ±0.009	0.241 ± 0.010 *

Data are means ± SD. * $p < 0.05$ versus Caucasian. $ p = between 0.05 - 0.10 versus Caucasian (Statistical Test: non-parametrical Kruskal-Wallis)

Is muscle fiber type composition different between elite Caucasian and elite East-African endurance runners?

A high percentage of slow-twitch (ST) muscle fibers is required for endurance performances. However, based on the literature (Coetzer et al 1993; Saltin et al 1995a; Weston et al 2000), no differences are expected between endurance runners of the same performance level regarding muscle fiber type composition (MFTC). Carnosine levels, measured by ^{1}H-MRS, were used to indirectly estimate MFTC in this study. Our results found no significant differences in Z-score of the soleus muscle (-1.02 ± 0.70 vs. -1.44 ± 0.80) and a tendency to a lower Z-score of the gastrocnemius muscle (-0.28 ± 0.47 vs. -1.56 ± 0.78) in Caucasians versus East-Africans, respectively. These results suggested that Caucasian and East-African endurance runners of the same level have an equal proportion of ST fibers.

Do East-African endurance runners have slender limbs with lower masses compared to Caucasian runners?

Adding a mass to the legs has a negative effect on the running economy by requiring a higher energy cost during running (Myers and Steudel 1985). A common hypothesis is that East-African endurance runners are characterized by slim limbs, causing a low moment of inertia. Some studies (Saltin 2003; Lucia et al 2006; Kong and de Heer 2008) showed already a lower leg circumference in African runners, although a direct relationship between leg mass and RE has not yet been investigated. In this study, a detailed analysis was started on the muscle volumes and muscle distribution of the leg between endurance runners of different origin. A surprising result was that no differences were present in the upper leg concerning muscle volumes, muscle distribution and center of mass of the different muscles and muscle groups (Table 2A). Taking a closer look to the lower leg, East-Africans showed significant lower total leg volumes compared to Caucasians, especially gastrocnemius lateralis and medialis were much smaller (Table 2B).

Table 2: Muscle volumes, ratios and center of mass (COM) of upper leg (A) en lower leg (B). Ratios were expressed in percentage of the total analyzed thigh volume (RF, VL + VI, VM and HAMSTR). COM was defined in the direction of gravity and expressed in percentage of the total leg length.

A	Upper leg	Caucasian (n = 4)	East-African (n = 3)
Volume (cm^3)	RF	304.90 ± 49.41	328.45 ± 103.84
	VL + VI	1171.29 ± 170.00	1263.54 ± 186.93
	VM	438.37 ± 59.89	440.26 ± 85.27
	HAMSTR	828.63 ± 67.48	898.70 ± 160.10
	Total volume	6180.00 ± 639.47	6341.36 ± 774.76
Ratio (%)	RF	11.25 ± 2.67	11.12 ± 2.46
	VL + VI	42.55 ± 3.03	43.07 ± 0.48
	VM	15.93 ± 1.10	15.03 ± 1.95
	HAMSTR	30.27 ± 2.33	30.77 ± 4.69
COM (%)	RF	21.54 ± 1.69	21.14 ± 1.75
	VL + VI	29.35 ± 0.78	27.83 ± 2.02
	VM	34.17 ± 1.29	33.10 ± 1.80
	HAMSTR	35.74 ± 0.69	33.97 ± 3.60
	Total volume	35.68 ± 0.83	34.46 ± 3.11

B	Lower leg	Caucasian (n = 4)	East-African (n = 3)
Volume (cm^3)	SOL	446.62 ± 57.69	371.76 ± 48.93
	GM	273.76 ± 52.64	194.15 ± 18.60 *
	GL	177.14 ± 25.21	119.22 ± 13.17 *
	Total volume	2662.77 ± 196.79	2315.86 ± 27.54 *
Ratio (%)	SOL	49.82 ± 2.03	54.14 ± 4.38
	GM	30.42 ± 2.84	28.34 ± 1.91
	GL	19.77 ± 1.72	17.52 ± 2.95
COM (%)	SOL	82.14 ± 1.31	81.98 ± 2.01
	GM	69.65 ± 2.19	65.49 ± 2.08 $^{\$}$
	GL	67.35 ± 1.42	65.92 ± 2.29
	Total volume	83.20 ± 1.64	83.34 ± 1.33

Data are means ± SD. * $p < 0.05$ versus Caucasian. $^{\$}$ p = between 0.05 - 0.10 versus Caucasian. (Statistical Test: non-parametrical Kruskal-Wallis)

Figure 1 shows the center of mass of the different muscles in percentage of their leg length. Only a tendency to a more proximal center of mass was found in the gastrocnemius medialis muscle for East-African runners compared to the Caucasians.

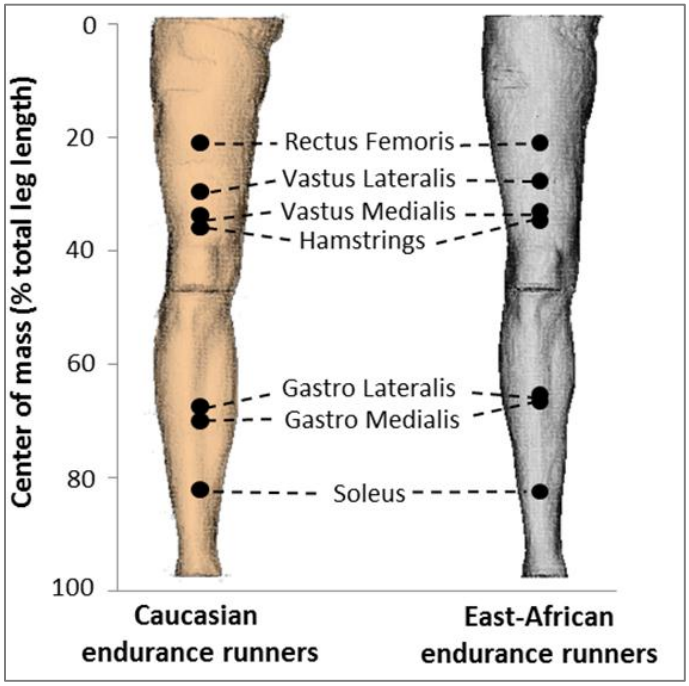

Figure 1: Center of mass expressed in percentage of total leg length for the different muscles in Caucasian and East-African endurance runners.

Based on the results of the volumes and center of mass of the lower leg muscles, it could be expected that inertia moments would be smaller especially for the lower leg at knee joint. MRI-data were used to determine mass distribution within the limb segments accurately. No differences were found in transverse moment of inertia of the upper leg at hip joint or the lower leg at knee joint (Fig 2). A possible explanation for finding no effect on inertia moment is the longer shank lengths of East-African runners.

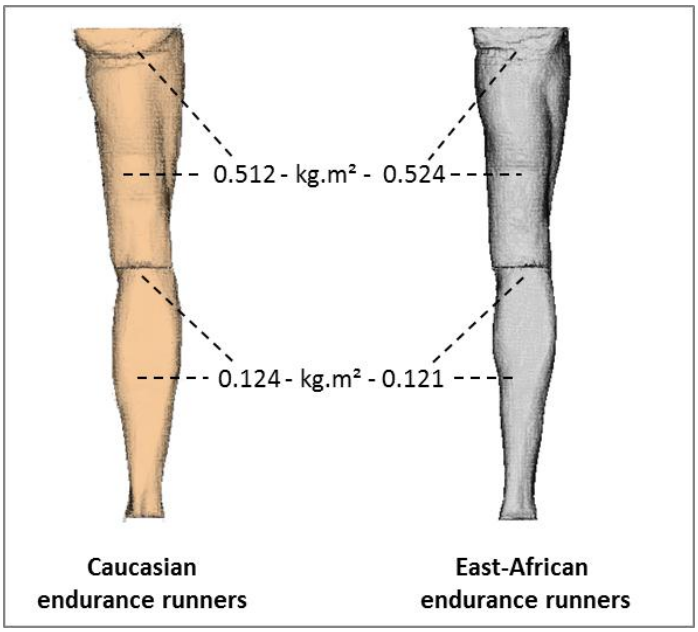

Figure 2: Transverse moment of inertia for the upper leg at hip joint and the lower leg at knee joint in both Caucasian en East-African endurance runners.

Summarizing these preliminary data on the leg morphology of endurance runners: (1) East-Africans have longer legs compared to Caucasian counterparts, which is especially due to the longer shank lengths, (2) no clear distinction was present in MFTC between the two groups of endurance runners, and (3) muscle volume was smaller in the lower leg, but not in the upper leg for East-African endurance runners compared to endurance runners of Caucasian origin. How these musculo-skeletal characteristics can contribute to better performance in endurance running is still an open question and will be investigated in the follow-up of this study. Furthermore, the number of subjects should be expanded to prove significant differences between East-African and Caucasian endurance runners.

4. Musculo-skeletal characteristics of sprinters and endurance runners: an overview

As seen in study 3, sprint and endurance runners show great differences in MFTC with sprinters having more FT fibers compared to endurance runners (Gollnick et al 1972; Costill et al 1976a). This can be seen as one of the most important differences between these two groups of athletes. However, besides this discriminant, the question remains what other musculo-skeletal characteristics of the leg differ between sprinters and endurance runners. In **study 4**, a complete investigation of the leg morphology has been performed for sprint and endurance runners of Caucasian ethnicity. First of all, no differences were found in skeletal characteristics (e.g. upper leg length, total leg length) between both athlete populations. Moving to muscle properties, a detailed analysis was made of the distribution of muscle volumes in upper and lower leg. These analyses showed that sprinters have more pronounced muscle volumes especially in the proximal and lateral leg muscles, compared to endurance runners. In turn, for **study 5**, differences in leg morphology were analyzed in the same conduct as study 4 but this time between endurance runners from various ethnicity. The results showed divergent skeletal characteristics (longer leg and shank length for East-Africans) and muscle volumes (lower muscle volumes in lower leg for East-Africans).

In figure 7, the results of study 4 and 5 have been combined. The percentage differences in muscle volumes and skeletal lengths were plotted between Caucasian sprinters vs. endurance runners (data of study 4) and East-African vs. Caucasian endurance runners (data of study 5). First of all, it shows that Caucasian sprinters have higher muscle volumes in both upper (21 % more volume) and lower leg (15 % more volume) compared to endurance runners, which could be attributed to muscle hypertrophy. Furthermore, figure 7 clearly shows lower volumes in lower leg muscles for East-African endurance runners compared to Caucasian endurance runners (up to 25 % less volume). This contrasts with the upper leg, where no differences are present between these groups of athletes. Concerning skeletal lengths, no differences were found for thigh or shank length for Caucasian sprint and endurance runners. However, East-African endurance runners showed especially longer shank lengths compared to Caucasian endurance runners.

These results suggest that the variation in lower leg muscle volume and shank lengths between runners of the same ethnicity participating in different events seems to be smaller compared to runners of different origin competing in the same discipline (endurance running). This is quite surprising as a bigger difference could be expected between a sprinter and endurance runner as a result of the specific requirements for either running performance. Based on these data, it could be speculated that the differences between East-African and Caucasian endurance runners are due to racial variations rather than to an effect of training.

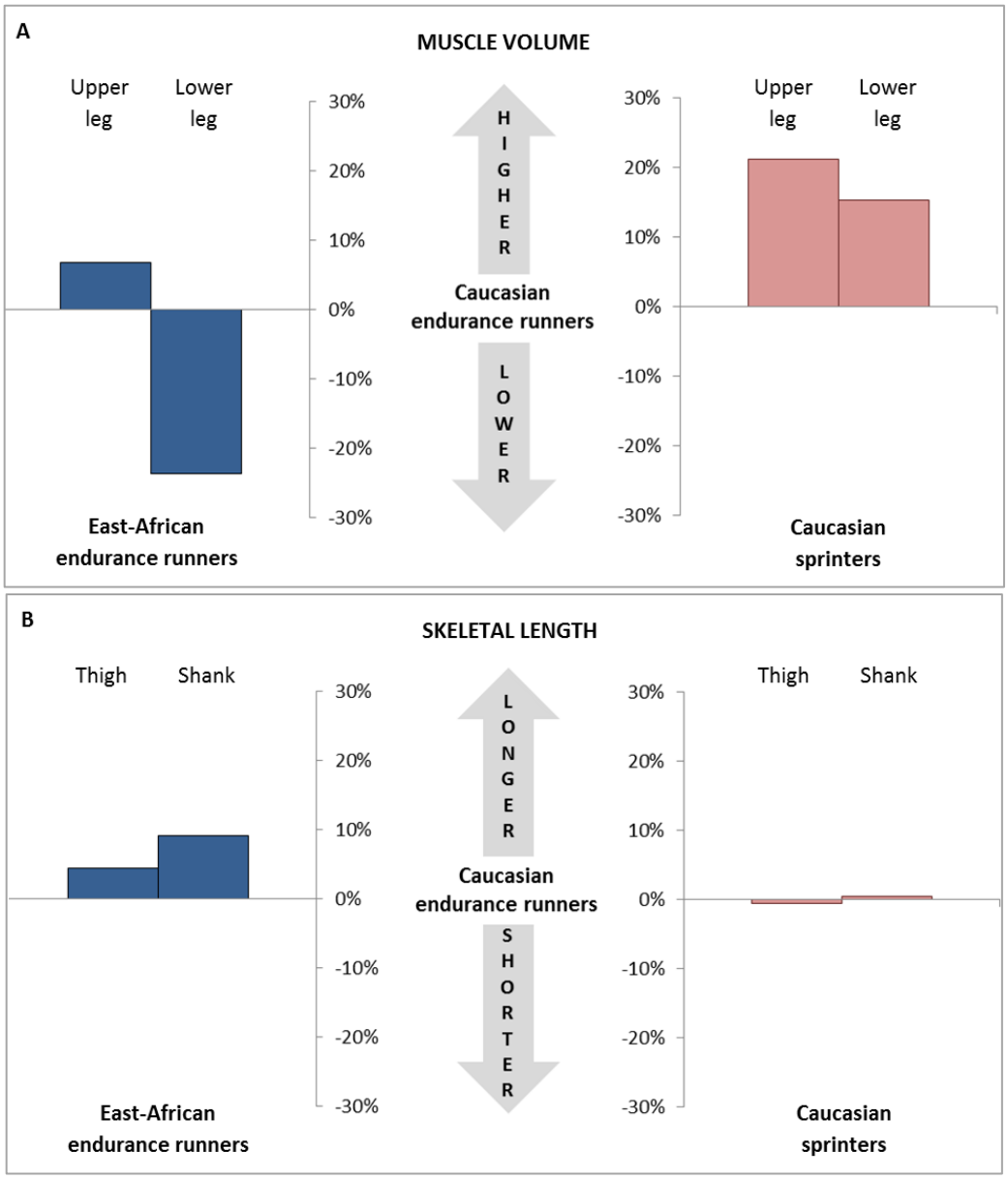

Figure 7. Percentage difference in muscle volumes (A) and skeletal lengths (B). *Blue bars* represent percentage difference in East-African endurance runners compared to Caucasian endurance runners and *red bars* represent percentage difference in Caucasian sprinters compared to Caucasian endurance runners.

Table 2 shows the calculated total mass of upper and lower leg and the transverse inertia moments. Starting with the upper leg, Caucasian sprinters showed the highest mass (around 8 kg) compared to endurance runners (around 7 kg in both Caucasians and East-Africans). Despite the higher mass, no differences were found in transverse moments of inertia of the upper leg at the hip joint. This could be due to the high variability between the subjects (can be seen at the quite large standard deviations).

Analysis of the data of the lower leg shows that the lower leg of East-African endurance runners had only 2.6 kg mass, while all Caucasians (both sprinters and endurance runners) had a lower leg mass around 3 kg. Although a lower mass of the lower leg was found, this was not reflected in a lower moment of inertia of the lower leg at knee joint. This could be attributed to the longer shank lengths in East-African runners compared to Caucasian endurance runners.

Table 2. Summarizing table of study 4 and 5 with masses and transverse inertia moments of upper and lower leg.

	STUDY 4		**STUDY 5**	
	Caucasians		**Caucasians (4)**	**East-Africans (3)**
	Sprint (8)	**Endurance (11)**	**Endurance**	
Mass upper leg (kg)	8.12 ± 0.73	6.94 ± 0.63	6.81 ± 0.70	6.99 ± 0.86
Mass lower leg (kg)	3.22 ± 0.25	2.98 ± 0.38	3.03 ± 0.22	2.64 ± 0.03
Inertia moment upper leg (kg.m²)	0.585 ± 0.10	0.525 ± 0.07	0.512 ± 0.08	0.524 ± 0.10
Inertia moment lower leg (kg.m²)	0.126 ± 0.02	0.117 ± 0.02	0.124 ± 0.01	0.121 ± 0.01

Although no differences were found in moment of inertia, it can be speculated that the longer legs and lower mass of the lower leg might have an effect on the physiological factors, especially on running economy (RE). As seen in the introduction of my thesis, a better RE in African endurance runners was found compared to Caucasian runners and the suggestion was made that RE can be the key factor in endurance running success. Figure 8 shows where morphological properties could play in a role in determining long distance race results.

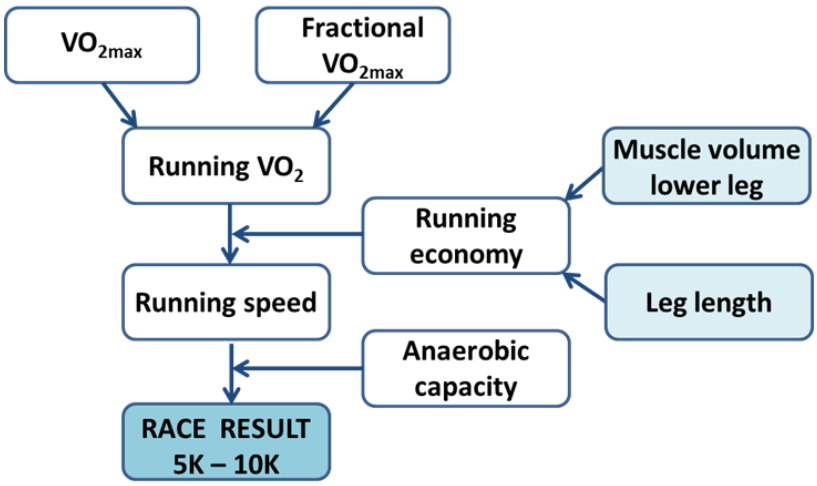

Figure 8. Schematic illustration of parameters influencing race results in endurance events with a speculation in which way muscle volumes and leg length could play a role.

In summary, the main discrepancy between sprint and endurance runners of Caucasian ethnicity is still the amount of FT and ST fibers in their muscles. Greater muscle volumes in the proximal and lateral leg muscles in sprinters compared to endurance runners were found, but these differences were rather small. Runners from different ethnicity, competing in the same discipline (namely endurance running), show greater differences in muscle volumes of the lower leg. Furthermore, skeletal characteristics (e.g. longer leg and shank length) differ between East-African and Caucasian endurance runners.

Together, these data suggest that differences In musculo-skeletal properties of runners from the same ethnicity competing in different disciplines were rather small and mainly influenced by training. Runners from different ethnicity both competing in endurance running were more distinctive at both skeletal level as lower leg muscle volume. The question then remains whether the combination of longer legs with lower masses could explain the superiority of East-African runners in endurance performance.

5. NMR methodology

The overall methodology in this thesis is the NMR-scanner. In the first three studies, carnosine concentrations were quantified by ^{1}H-MRS in different muscles. As this is a quite novel method, the methodology should be optimized. This thesis contributed to the development of measuring carnosine concentrations by ^{1}H-MRS in the upper body. To deal with some methodological problems of this method, specific calculations were used to allow comparison between specific muscles of the body and between different athlete populations.

5.1. Carnosine quantification by ^{1}H-MRS in different muscles

NMR equipment is very applicable for athlete populations and therefore used in all studies of this thesis. Until now, ^{1}H-MRS-based carnosine quantification in a single muscle of the upper body was not examined. Measuring metabolites in different muscles gives us the opportunity to compare supplementation-induced changes in carnosine content within one individual in different parts of their body. In **study 1**, carnosine was measured for the first time in deltoid and triceps brachii muscles. Voxel placement was quite easy in deltoid, but more difficult in triceps brachii, especially in subjects with low muscularity. Both in deltoid and triceps brachii, the spectra were large enough to allow quantification. Table 3 summarizes all the coefficients of variation (CV) of the different muscles. The CV within the same day was 6.6 % for deltoid and 9.2 % for triceps brachii. This was in accordance to soleus and gastrocnemius medialis, which was published by Ozdemir et al (2007). The CV increased to 13.3 % in deltoid muscle, measuring several weeks apart, which was also the case for soleus and gastrocnemius muscles (Baguet et al 2009). Due to the methodological problems (e.g. voxel placement) and higher CV in triceps brachii, this muscle was excluded in further research.

Table 3. Coefficients of variation (CV) of measuring carnosine by ^{1}H-MRS within the same day and over several weeks in the different muscles (based on data of Ozdemir et al (2007), Baguet et al (2009) and study 1 of this thesis).

	Methodological variation (%) (within the same day)	Biological variation (%) (several weeks apart)
Soleus	4.3	9.8
Gastrocnemius medialis	7.6	14.2
Deltoid	6.6	13.3
Triceps brachii	9.2	/
Average soleus and gastrocnemius	4.7	9.5

5.2. Absolute and relative carnosine concentrations

Using the MRS spectra, carnosine concentrations can be expressed in absolute (mM) or relative (to water signal) terms. Absolute quantification of carnosine is based on an external phantom with a well-known concentration and the use of transmit/receive coils. This method gives the possibility to calculate the amount of carnosine in millimolar concentrations by following equation (Baguet et al 2010a):

$$[\boldsymbol{C_m}] = [\boldsymbol{C_r}] \times \frac{(\boldsymbol{S_m} \times \boldsymbol{V_r} \times \boldsymbol{C_{T1r}} \times \boldsymbol{C_{T2r}} \times \boldsymbol{T_m})}{(\boldsymbol{S_r} \times \boldsymbol{V_m} \times \boldsymbol{C_{T1m}} \times \boldsymbol{C_{T2m}} \times \boldsymbol{T_r})}$$

$[C_m]$ = carnosine concentration in vivo (muscle)

$[C_r]$ = concentration of the external reference phantom (20 mM)

$S_m \; and \; S_r$ = estimated signal peak areas of the specific muscle and phantom

$V_m \; and \; V_r$ = volumes of the voxels in vivo and phantom

$C_{T1m}, C_{T2m}, C_{T1r} \; and \; C_{T2r}$ = correction factors for T1 and T2 relaxation times in vivo and phantom

$T_m \; and \; T_r$ = temperatures in vivo and phantom

The phantom method is not applicable with receive-only coils (like shoulder coil). Therefore, relative carnosine concentrations were used instead of absolute concentrations. Relative carnosine concentrations were calculated by the integral of the C2-H peak (at ~8 ppm) relative to integral of the water peak (x 1000) and were expressed

in arbitrary units. It has been shown that muscle water content was not changed pre- and postsupplementation. The following equation was used for relative expression:

$$\boldsymbol{C_{m\,(rel)} = \frac{(S_m)}{(S_w)} \times 1000}$$

$C_{m(rel)}$ = relative carnosine concentration in vivo (muscle)

$S_m \; and \; S_w$ = estimated signal peak areas of the specific muscle and water

In **study 1**, all values were expressed in relative terms to allow comparison between arm and leg muscles. In **study 2**, carnosine concentrations were expressed in millimolar concentrations as there were no measurements in the deltoid muscle. **Study 3 to 5** used muscle-specific Z-scores by converting all the carnosine concentrations to Z-scores. The mean and standard deviation for the control population was computed for each muscle type and sex to allow calculating athletes their individual Z-score.

$$\boldsymbol{Z_m = \frac{(C_m - \mu_c)}{(SD_c)}}$$

Z_m = muscle-specific Z-score

C_m = carnosine concentration in vivo (muscle)

μ_c = mean carnosine concentration of the control population

SD_c = standard deviation of the carnosine concentration of the control population

6. Limitations

In this thesis, some new applications of NMR in elite sports performance were revealed. However, some limitations came up based on the results of my studies.

- The main limitation across the different studies was the small sample size. Therefore, the power of some studies was quite low. In study 3 and 5 some subgroups were quite small due to limited availability of elite athletes in Belgium (i.e. kayakers, East-African endurance runners). Furthermore, BA supplementation in elite athletes, especially in swimmers and cyclists, was one of the main reasons to exclude participants from the study.

- The ^{1}H-MRS methodology was validated by Baguet et al (2011b) in the calf muscles. In study 1, this technique was optimized to measure carnosine also in the deltoid muscle. However, we did not relate muscle carnosine concentrations in the arm muscles to MFTC measured by the muscle biopsy. Therefore, we could not directly link the amount of carnosine to the percentage area occupied by FT fibers in the deltoid muscle. Therefore, the results of study 3 were expressed in Z-scores of carnosine content instead of area of FT fibers.

- From a mechanistic point-of-view, it would have been better to have taken biopsies in study 2 before and after the exercise bout. Some speculations were made towards higher expression of transporters of BA into the muscle cell, but this possible mechanism could not be examined without biopsies.

- In study 4 and 5, a great amount of muscle and bone parameters was already measured in sprinters and endurance runners, but additional data on tendon characteristics would be interesting to have a complete overview. Furthermore, other muscles in lower and upper leg could be analyzed to give more details of muscle and muscle groups in different running populations. As it was already shown that the length of the Achilles tendon moment arm differs in sprinters compared endurance runners (Lee and Piazza 2009), it would be interesting to include this parameter as well. Also measuring foot morphology, e.g. length of the toe, should be considered (Baxter et al 2011).

7. Future directions

Overall, this thesis contributed to the elaboration of various applications of the NMR methodology in healthy, sporty populations. The specific studies in this thesis investigated (1) the determinant training on the carnosine loading efficiency and (2) the musculo-skeletal characteristics of different athlete populations. As a result, these studies have created some new interests for further research.

- BA supplementation over 4 – 8 weeks resulted in an increase of muscle carnosine concentrations by 40 up to 100 %. Despite the high total amount of ingested BA, only a small part is actually incorporated into muscle carnosine. This thesis contributed to a steeper increase in the carnosine loading efficiency, although carnosine loading remains a very slow proces. Future research should try to investigate the unknown metabolic fate of the ingested BA. In understanding this, new strategies could be revealed to increase the loading efficiency and therefore shorten the loading protocol.

- Measurement of muscle carnosine by ^{1}H-MRS enables indirect estimation of MFTC. Knowing an athlete's MFTC could imply applications towards talent identification and training guidance. Until now, little or no literature is available on MFTC and the influence on risk of injury. However, it has already been suggested that the proportion of FT fibers could be related to strain injury. Therefore, the non-invasive ^{1}H-MRS technique gives the ability to directly investigate whether athletes with predominantly FT muscle fibers will be more sensitive to strain injury than athletes with mostly ST fibers. Additionally, this method could be used to examine whether recovery from training will be different for subjects with diverse MFTC.

- The non-invasive estimation of MFTC by ^{1}H-MRS already gives useful insights in different sports (e.g. track-and-field, swimming, kayaking and cycling). The question remains whether this method could also be applied in team sports (e.g. football, rugby, basketball etc.) in order to determine specific MFTC requirements in these disciplines and maybe to use this information to differentiate training load and recovery at individual level.

- MRS can also be used in the sport to control training and evaluate specific damage after concentric or eccentric exercise. Analyzing specific metabolites as glycogen, adenosine triphosphate, lactate, intramyocellular lipids can deliver interesting information towards training and recovery of athletes. Besides the medical applications, the NMR scanner is more and more accessible in other fields and thus a promising future towards sport scientific guidance is coming.

- Predict the adult height in children and young adults could be useful for sports like volleyball, basketball, gymnastics… Today, X-ray is the most commonly used method to determine the adult height prediction, however the NMR scanner could be developed as a more sensitive and radiation-free medical imaging alternative.

- In track-and-field, cycling and swimming, ^{1}H-MRS quantification of carnosine could be used to direct talented individuals towards their most promising discipline based on their estimated MFTC. Additionally, a longitudinal follow-up study in which athletes are analyzed during their career in relation to their estimated MFTC could be of extremely high value.

- Based on the extensive morphological comparison between endurance runners of different ethnic groups, potential differences at skeletal and muscle level between East-African and Caucasian runners were discovered. A first step is to expand the number of subjects to strengthen the data. If the results seems strong enough, than future research is needed to directly link these results to some physiological and biomechanical parameters to clarify excellence of East-African runners in endurance performance.

8. General conclusions

Take-home messages derived from this thesis:

- **Carnosine loading efficiency is enhanced by exercise training.** Higher increases in muscle carnosine concentrations were found after BA supplementation in trained muscles compared to untrained muscles (Study 1). Furthermore, carnosine loading effectiveness was improved by implementing high-volume and high-intensity training protocols in combination with BA in non-specifically trained subjects (Study 2). Based on these studies, it could be concluded that muscle carnosine loading efficiency is positively affected by an acute response of exercise training, with a possible additive effect on trained muscles.

- **Explosive athletes have higher muscle carnosine concentrations and thus higher estimated area FT fibers compared to endurance athletes.** To reach a certain performance level, different muscle characteristics (e.g. power output, fatigue profile) are required in sprint and endurance events. Therefore, explosive athletes should posses a high proportion of FT fibers, while endurance athletes need mostly ST fibers. This statement is already confirmed within different cyclic sports, namely running, cycling, swimming and kayaking (Study 3).

- **Cyclic movement frequency, rather than movement duration determines estimated MFTC across different sports.** High proportions of FT fibers are required for athletes participating in cyclic sports with high movement frequency (especially sprint running and track cycling) and less in disciplines of sports with lower frequency (e.g. swimming). Exercise duration seems to be less important than movement frequency, although earlier literature suggested duration as the most determining factor for defining MFTC in athletes (Study 3).

- **Sprint and endurance runners of the same ethnicity differ mainly in muscle fiber type and muscle volumes, with specifically greater lateral and proximal leg muscles in sprinters compared to endurance runners.** No differences were found in skeletal characteristics, muscle shape or muscle distribution. Furthermore,

longer muscle fascicle lengths should be favorable for sprinters, but this was not confirmed in our study (Study 4).

- **Endurance runners of different origins have distinctive characteristics at both skeletal as lower leg muscle level.** Longer shanks and longer total legs were found in East-African compared to Caucasian endurance runners. Furthermore, muscle mass of the lower leg was smaller in the Africans compared to their Caucasian counterparts and no differences were found in the upper leg (Study 5).

- **Differences in musculo-skeletal characteristics between sprinters and endurance runners of same ethnicity were rather limited and were mainly due to various training stimuli causing hypertrophy in sprint specific muscles. Runners of different ethnicity both specialized in endurance running show greater distinctions at skeletal lengths and lower leg muscle volumes, suggesting the presence of genetic predisposition.**

IV

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V

Publications

A1

Bex T, Iannaccone F, Stautemas J, Baguet A, De Beule M, Verhegghe B, Aerts P, De Clercq D, Derave W. Discriminant musculo-skeletal leg characteristics between sprint and endurance elite Caucasian Runners. *Scan J Med Sci Sport* (submitted).

Bex T, Baguet A, Achten E, Aerts P, De Clercq D, Derave W. Cyclic movement frequency dictates optimal muscle fiber type composition in athletes. *Scan J Med Sci Sport* (submitted).

Bex T, Chung W, Baguet A, Achten E & Derave W. Exercise training and beta-alanine-induced muscle carnosine loading. *Frontiers in Nutrition* 2: 13, 2015.

Bex T, Baguet A, Chung W, Stegen S, Stautemas J, Achten E, Derave W. Muscle carnosine loading by beta-alanine supplementation is more pronounced in trained vs. untrained muscles*. J Appl Physiol* 116(2):204-9, 2014.

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Stegen S, Blancquaert L, Everaert I, **Bex T**, Taes Y, Calders P, Achten E, Derave W. Meal and beta-alanine coingestion enhances muscle carnosine loading. *Med Sci Sports Exerc* 45(8):1478-85, 2013.

C1-C3

20th annual European College of Sport Sciences Congress – Malmö, Sweden – 24-27 June, 2015

Bex T, Aerts P, De Clercq D, Derave W. Discriminant musculo-skeletal leg characteristics between elite Caucasian sprint and endurance runners.

(oral presentation)

Carnosine Symposium – Gabicce Mare, Italy – 7-8 June, 2015

Bex T, Baguet A, Derave W. The use of carnosine content as indirect estimation of muscle fiber type composition.

(oral presentation)

19th annual European College of Sport Sciences Congress – Amsterdam, Netherlands – 2-5 July, 2014

Bex T, Baguet A, Derave W. Non-invasive estimation of muscle fiber type composition in swimmers.

(poster)

Aspire Talent Identificaton Conference – Doha, Qatar – 2-4 April, 2014

Bex T, Baguet A, Derave W. Non-invasive talent ID by MRS-based estimation of muscle fiber type composition.

(oral presentation)

18th*Symposium of the Flemish Society for Kinesiology – Leuven, Belgium – 13 December, 2013*

Bex T, Chung W, Baguet A, Stegen S, Stautemas J, Derave W. Muscle carnosine loading by beta-alanine supplementation is more pronounced in trained vs. untrained muscles.

(oral presentation)

18th annual European College of Sport Sciences Congress – Barcelona, Spain – 26-29 June, 2013

Bex T, Chung W, Baguet A, Stegen S, Derave W. Training increases muscle carnosine loading by beta-alanine supplementation.

(oral presentation)