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ORIGINAL ARTICLE

The influence of sex, age and heritability on human skeletal muscle carnosine content

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Abstract The dipeptide carnosine is found in high concentrations in human skeletal muscle and shows large inter-individual differences. Sex and age are determining factors, however, systematic studies investigating the sex effects on muscle carnosine content throughout the human lifespan are lacking. Despite the large inter-individual variation, the intra-individual variation is limited. The question may be asked whether the carnosine content is a muscle characteristic which may be largely genetically determined. A total of 263 healthy male and female subjects of 9–83 years were divided into five different age groups: prepubertal children (PC), adolescents (A), young adults (YA), middle adults (MA) and elderly (E). We included 25 monozygotic and 22 dizygotic twin pairs among the entire study population to study the heritability. The carnosine content was measured non-invasively in the gastrocnemius medialis and soleus by proton magnetic resonance spectroscopy (^{1}H-MRS). In boys, carnosine content was significantly higher (gastrocnemius 22.9%; soleus 44.6%) in A compared to PC, while it did not differ in girls. A decrease ($\sim$16%) was observed both in males and females from YA to MA. However, elderly did not have lower carnosine levels in comparison with MA. Higher correlations were found in monozygotic ($r = 0.86$) compared to dizygotic ($r = 0.51$) twins, in soleus muscle, but not in gastrocnemius. In conclusion, this study found an effect of puberty on muscle carnosine content in males, but not in females. Muscle carnosine decreased mainly during early adulthood and hardly from adulthood to elderly. High intra-twin correlations were observed, but muscle-dependent differences preclude clear conclusions toward heritability.

Keywords Muscle carnosine · Puberty · Aging · Heritability · ^{1}H-MRS

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Introduction

Within a population there are large inter-individual differences in muscle carnosine concentration. Carnosine is a dipeptide found in high concentrations in human skeletal muscle, synthesized from the precursors beta-alanine and L-histidine. It has recently been shown that high levels of muscle carnosine can be beneficial in relation to sport performance (Suzuki et al. 2002; Baguet et al. 2010; Derave et al. 2007; Van Thienen et al. 2009; Hill et al. 2007 Stout et al. 2007). Due to its biochemical properties of anti-glycation, anti-oxidation or metal chelation, the therapeutic potential of carnosine is currently investigated in relation to aging (Gallant et al. 2000), diabetic complications (Janssen et al. 2005) and cataract (Quinn et al. 1992).

Since carnosine has many applications in sports and health and because of the large inter-individual variation, it would be useful to get a better insight into its metabolism and determinants. To date several determinants of muscle carnosine are known, i.e., dietary intake of beta-alanine, muscle fiber-type composition, sex and age. The manipulation of beta-alanine intake through diet or supplements

(extremes on both sides of the continuum), showed a marked influence on muscle carnosine. Beta-alanine supplementation can increase muscle carnosine both in untrained (Harris et al. 2006) and trained subjects (Derave et al. 2007). Carnosine in the diet can be found in red meat (e.g., beef), white meat (e.g., poultry) and fish (e.g., salmon) (Abe 2000). Recently, Everaert et al. (2010) have shown that chronic vegetarianism (>8 years) reduces muscle carnosine by approximately 20%. To become a vegetarian for a short period (several weeks) however, would probably have little or no effect on carnosine levels (Baguet et al. 2011a). Interesting to note is that there is no correlation between dietary beta-alanine ingestion (within the normal range ~200–400 mg per day (Everaert et al. 2010)) and initial pre-supplementation muscle carnosine content (Everaert et al. 2010; Stellingwerff et al. 2011; Baguet et al. 2009). A second determinant, which is often cited, is muscle fiber-type composition. Carnosine is probably one of the only metabolites in humans in which there is a robust fiber-type disparity. In a study by Harris et al. (1998), using the single fiber technique, it has been shown that human fast type II fibers contain twice as much carnosine as slow type I fibers. In animals as well, a greater intramuscular content of carnosine has been found in type II fibers compared with type I fibers (Dunnett et al. 1997; Harris et al. 1990; Sewell et al. 1992). Previous findings are supported by several papers demonstrating a positive correlation between muscle carnosine content and the fraction of type II muscle fibers in humans (Baguet et al. 2011b; Mannion et al. 1995; Suzuki et al. 2002).

The last two known determinants, i.e., sex and age, will be discussed together. It has already several times been established that men have higher (22–82%) muscle carnosine levels compared to women (Mannion et al. 1992; Everaert et al. 2010). The size of this sexual dimorphism appears to be dependent on muscle type (Everaert et al. 2010). It should however be noted that all the studies were conducted in adults (age range 18–47 years). Concerning the age effect, several cross-sectional studies demonstrated a decrease in muscle carnosine with age. Everaert et al. (2010) found a decrease in muscle carnosine of 1.2% per year in healthy subjects between 19 and 47 years. Two other studies also found a negative correlation between age and carnosine levels in patients with specific pathologies (20–80 years) such as osteoarthritis (Tallon et al. 2007) and neuromuscular disease (Stuerenburg and Kunze 1999). To our knowledge, there are no studies available which systematically compared the muscle carnosine content in children, adolescents, adults and healthy elderly subjects. It requires large samples representing all age groups of both sexes in order to obtain a clear understanding of the impact of puberty and aging on the human muscle carnosine concentration. From animal studies there are some indications that sex steroid hormones could influence muscle carnosine content (Penafiel et al. 2004). The current study will investigate if similar conclusions can be drawn in humans.

Finally, this paper will quantify the role of genetic and environmental sources of variation in inter-individual carnosine variability. Recent observations of Baguet et al. (2011b) showing that carnosine levels of ex-sprinters remained significantly higher compared to ex-endurance athletes, although they had discontinued training for many years already, suggest that carnosine levels are probably largely genetically determined. Carnosine levels have not been studied in twin or family studies, therefore estimates of heritability of this muscle characteristic are not available. Previous twin studies already demonstrated that monozygotic twins are quite similar with regard to muscle fiber composition (Komi et al. 1977), maximal muscle strength (Thomis et al. 1997) and neuromuscular coordination (Missitzi et al. 2004). Genetic factors probably account for about 45% of variability in type I fiber distribution in Caucasian populations (Simoneau and Bouchard 1995).

This cross-sectional study will investigate the effect of puberty and aging on muscle carnosine levels. A second aim was to find out if muscle carnosine is genetically determined, studying mono- and dizygotic twins.

Materials and methods

Subjects

A total of 263 healthy subjects (118 males and 145 females) volunteered to participate in this cross-sectional study. They were divided in five different age groups: prepubertal children (PC), adolescents (A), young adults (YA), middle adults (MA) and elderly (E). Table 1 shows the numbers, mean age and age range per group. All the subjects were moderately to highly physically active. In the total study population of 263 subjects, we included 25 monozygotic (MZ) (10 males and 15 females) and 22 dizygotic (DZ) twins (8 males and 14 females). Twin zygosity was known for most twins as they were members of the East Flanders Prospective Twins Survey, for which zygosity determination is based on the placental information and chorionicity at the time of birth, and polymorphic DNA-markers analysis. Other twins rated their similarity based on a zygosity questionnaire (Peeters et al. 1998). Within the twin group age ranged from 9–60 years. Exclusion criteria were vegetarian diet, diabetes and beta-alanine supplementation 3 months before the start of the study. All the subjects gave their written informed consent,

Table 1 Characteristics (numbers, mean age and age range) of the study population

	PC	A	YA	MA	E
Number of males	23	17	47	19	12
Number of females	22	47	44	20	12
Total	45	64	91	39	24
Mean age of males (years)	10.0 ± 0.9	16.5 ± 1.5	23.3 ± 2.4	43.1 ± 4.6	67.8 ± 5.2
Mean age of females (years)	9.6 ± 0.8	15.2 ± 1.8	23.3 ± 3.2	42.1 ± 4.3	67.9 ± 7.5
Age range (years)	8–11	Male (15–20) Female (13–18)	Male (21–30) Female (19–30)	31–50	60–83

and the study was approved by the local ethics committee (Ghent University Hospital, Belgium).

Muscle carnosine content

The carnosine content of all the subjects was measured by proton magnetic resonance spectroscopy (^{1}H-MRS) in soleus and gastrocnemius muscles, as previously described (Baguet et al. 2010). The subjects were lying in supine position on their back and the lower leg was fixed in a holder with the angle of the ankle at 20° plantar flexion. All the MRS measurements were performed on a 3-T whole body MRI scanner (Siemens Trio, Erlangen) equipped with a spherical knee-coil. 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 was 40 mm × 10 mm × 25 mm and the line width of the water signal was on average 27.1 Hz, following shimming procedures. The absolute carnosine content (in millimolar; mM) was calculated as described before by Baguet et al. (2010). From the total population three outliers were excluded for soleus and nine for gastrocnemius. Outliers were detected per age group. A value was considered as outlier when greater than (Q3 + 1.5 IQR) (Q: quartile; IQR: interquartile range) or smaller than (Q1 – 1.5 IQR).

Statistics

Effect of sex and age

The carnosine content of soleus and gastrocnemius muscles was analyzed using a two-way ANOVA analysis, with between-subjects factors sex and age group. In case of significance, two one-way ANOVA's were performed for males and females separately. When the ANOVA test differed significantly, a post hoc analysis with independent sample *t* tests was performed where each age group was compared with the previous age group within sex (SPSS statistical software, SPSS 17.0, Chicago, IL, USA).

Effect of heritability

Birth-order effects and differences in means or variances between MZ and DZ twins were tested with *t* tests and *F* tests, respectively. Pearson correlations between first- and second-born twins were computed for MZ and DZ twins. The biometric approach using path-analytic models was applied to determine the relative contribution of genetic and environmental factors to the observed variation in carnosine content in soleus and gastrocnemius muscle. MZ twins are genetically identical, whereas on average, DZ twins share 50% of their genes identical by descent. Additive (A) genetic variation is correlated 1 in MZ pairs and 0.5 within DZ pairs, whereas dominance (D) genetic effects have a correlation of 0.25 in DZ pairs and 1 in MZ pairs. The additive genetic effect represents the sum of all allelic effects on carnosine levels, while dominance genetic variation refers to the interaction effects between alleles at the same locus. Environmental causes of variation can be shared by twins or family members that are reared in the same family (C, common environment e.g., shared diet habits, socio economic status) and can be non-shared (E, specific environmental factors also includes random measurement error). The contribution of genes and environment to the total variance is reported in the standardized form, by dividing the specific variance component by the total phenotypic variance. In this model, it is also assumed that genetic and environmental factors do not correlate or interact, and that there is no significant parental correlation for these characteristics. Furthermore, in the classical twin design with twins reared together, C and D cannot be tested simultaneously in one model. The full information maximum likelihood estimation of sources A (C or D), and E was assessed using Mx (Neale et al. 2003). A saturated model with free estimation of means and twin (co-)variances was taken as baseline model. Significant contributions of the different sources of variance were tested by model comparisons. A parameter, e.g., A is dropped from a

model (comparing CE to ACE) and the change in −2 log likelihood between the ACE model and the nested submodel is tested using a likelihood ratio χ^2 test. A significant change in χ^2 ($P < 0.05$, for 1df, if 1 parameter is dropped) shows a significant decrease in fit of the model which indicates that the parameter should be included in the model. Akaike's information criterion is used to compare models with equal degrees of freedom (ACE and ADE), with lower values indicating better fit. Values are presented as means ± SD and significance was assumed at $p \leq 0.05$.

Results

Effect of puberty and aging

To investigate if puberty and aging had a sex-dependent effect on muscle carnosine content, carnosine was measured in soleus and gastrocnemius muscle by ^{1}H-MRS in PC, A, YA, MA and E (Fig. 1). A two-way ANOVA interaction analysis (sex x age group) was performed for soleus ($p = 0.020$) and gastrocnemius ($p = 0.067$). The one-way ANOVA test demonstrated that both in males (soleus $p < 0.001$; gastrocnemius $p = 0.002$) and females (soleus $p < 0.001$; gastrocnemius $p < 0.001$) carnosine was significantly different between different age groups.

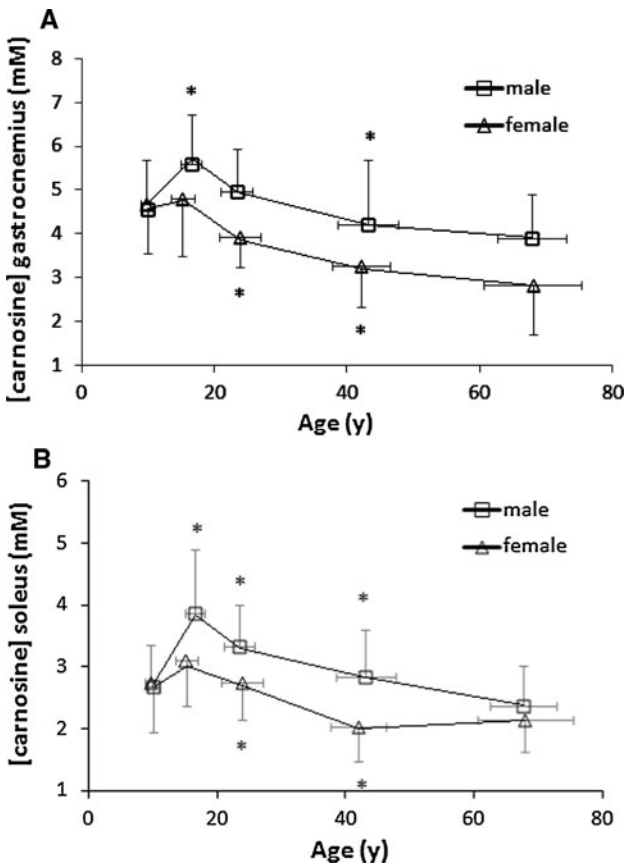

Fig. 1 Muscle carnosine concentration (mM) in gastrocnemius (**a**) and soleus (**b**) muscle in prepubertal children ($n = 45$), adolescents ($n = 64$), young adults ($n = 91$), middle adults ($n = 39$) and eldery ($n = 24$). Data are means ± SD. *Asterisk* indicates difference from the previous age group ($p < 0.05$)

From PC to A

In boys, gastrocnemius carnosine content (Fig. 1a) was significantly lower (−1.04 mM; −22.9%) in PC (4.55 ± 1.36 mM) compared to A (5.59 ± 1.13 mM) ($p = 0.02$), while no differences were found ($p = 0.73$) in girls (4.68 ± 1.20 mM vs. 4.79 ± 1.32 mM). A similar pattern was found in the soleus muscle (Fig. 1b); 44.6% higher ($p < 0.001$) carnosine content in A boys compared to PC and no significant change ($p = 0.06$) in girls (PC: 2.74 ± 0.61 mM vs. A: 3.10 ± 0.73 mM).

From A to YA

Figure 1a and b shows both in males (gastrocnemius, $p = 0.07$; difference of 11.4% and soleus, $p = 0.02$; difference of 14.0%) and females (gastrocnemius, $p < 0.001$; difference of 18.2% and soleus, $p = 0.02$; difference of 11.3%), A had more carnosine than YA.

From YA over MA to E: effect of aging

MA muscle carnosine levels were lower in comparison to YA both in males [−15.2% in gastrocnemius ($p = 0.03$) and −14.8% in soleus ($p = 0.01$)] and females [−16.3% in gastrocnemius ($p < 0.001$) and −26.2% in soleus ($p < 0.001$)]. Carnosine contents of elderly males (gastrocnemius 3.89 ± 0.80 mM; soleus 2.36 ± 0.64 mM) and females (gastrocnemius 2.82 ± 1.11 mM; soleus 2.15 ± 0.52 mM) were not significantly different (males, $p = 0.5$ in gastrocnemius and $p = 0.09$ in soleus; females, $p = 0.22$ in gastrocnemius and $p = 0.57$ in soleus) from MA.

Figure 2 a and b shows that the age-related carnosine decrease mainly took place between A, YA and MA in both muscles.

Sex differences

Figure 3 demonstrates the male to female (M/F) carnosine ratio for soleus and gastrocnemius muscles in five different age groups. The carnosine concentration was similar in boys and girls of the PC group, for soleus ($p = 0.721$; M/F = 0.97) and gastrocnemius ($p = 0.743$; M/F = 0.97). Within A (soleus $p = 0.002$, M/F = 1.24 and gastrocnemius $p = 0.045$, M/F = 1.17), YA (soleus $p < 0.001$, M/F = 1.21 and gastrocnemius $p < 0.001$, M/F = 1.26)

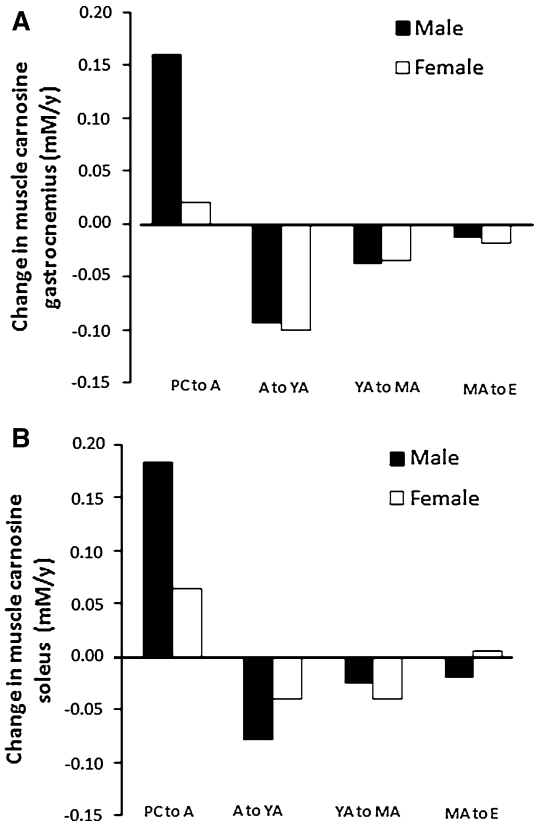

Fig. 2 Relative change in muscle carnosine (mM per year) in gastrocnemius (**a**) and soleus (**b**) muscle for males (*black bars*) and females (*open bars*) in five different age groups [pre-pubertal children (PC), adolescents (A), young adults (YA), middle adults (MA) and elderly (E)]. The relative change in muscle carnosine (expressed in mM/years) was calculated using the following calculation: (mean carnosine concentrations of age group X − mean carnosine concentration of age group Y)/(the mean age of group X − mean age of group Y)

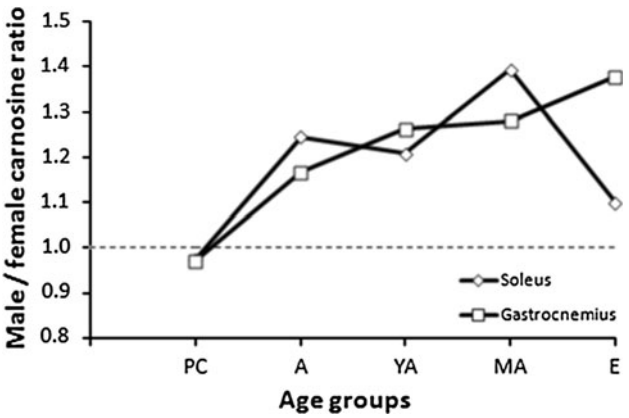

Fig. 3 Male-to-female ratio of carnosine content of gastrocnemius (*squares*) and soleus (*diamonds*) muscle for five different age groups [prepubertal children (PC), adolescents (A), young adults (YA), middle adults (MA) and elderly (E)]

and MA (soleus $p < 0.001$, M/F = 1.39 and gastrocnemius $p = 0.02$, M/F = 1.28) the carnosine content was significantly higher in males compared to females. As can be seen in Fig. 3, carnosine levels in E were higher in males compared to females ($p = 0.01$) in gastrocnemius muscle (M/F = 1.38), but did not differ in soleus ($p = 0.40$; M/F = 1.10).

Heritability

The carnosine content of 25 MZ and 22 DZ twins was measured to find out if carnosine is genetically determined. Figure 4 demonstrates the individual carnosine content of twin A (=first subject of twin pair) and twin B (=second subject of twin pair) in comparison to the line of identity for gastrocnemius (Fig. 4a) and soleus (Fig. 4b). Pearson correlations were higher for MZ twins ($r = 0.86$; $p < 0.001$) compared to DZ twins ($r = 0.51$; $p = 0.015$) for carnosine content in soleus muscle, indicating the role of genetic factors in this trait. However, for variation in carnosine content in gastrocnemius, the observed similarity in DZ twins was higher ($r = 0.83$; $p < 0.001$) than that in MZ twins ($r = 0.70$; $p < 0.001$), which is compatible with a strong influence of shared environmental factors without evidence for a genetic factor. Means and variances for first- or second-born twins or MZ and DZ twins did not differ ($p > 0.05$). Genetic model-fitting (Table 2) resulted in the AE model to be retained as best-fitting model for the carnosine content in soleus. Heritability estimate for soleus carnosine content was 85% (95% CI 0.71–0.91), while unique environmental factors accounted for the rest of the variation (15%) (95% CI 0.08–0.28). There was no evidence for genetic factors to be involved in individual variation in carnosine content in gastrocnemius muscle as indicated by the CE model as most parsimonious and best-fitting model. The contribution of shared environmental factors is estimated at 76% (95% CI 0.61–0.85), unique environmental variation contributes 24% (95% CI 0.14–0.38).

Discussion

A main novel finding of this study was that puberty had a positive influence on muscle carnosine levels in boys. In boys, the carnosine content increased significantly (22.9% in gastrocnemius and 44.6% in soleus) from pre-puberty to adolescence, while in girls it did not increase in the gastrocnemius or only tended ($p = 0.06$) to increase in the soleus during the same time period. This is in line with the findings of Penafiel et al. (2004) demonstrating that, from pre-pubertal to adult age, carnosine levels increased 3–7 times in male mice and did not change in female mice (Penafiel et al. 2004).

The sexual dimorphism for muscle carnosine content, which is present at adult age (Mannion et al. 1992; Everaert

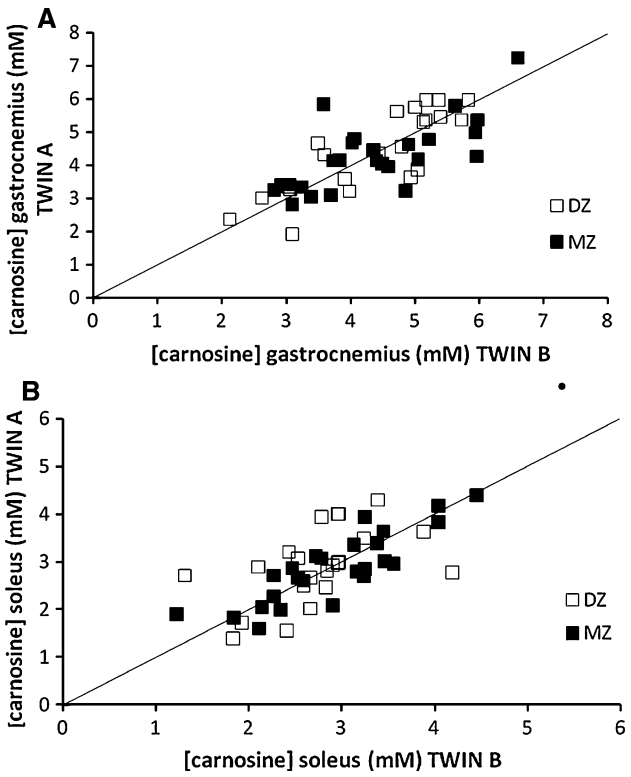

Fig. 4 Muscle carnosine content of gastrocnemius (**a**) and soleus (**b**) muscle in 25 monozygotic (MZ; *black squares*) and 22 dizygotic (DZ; *open squares*) twins. The *solid line* is the line of identity

et al. 2010), is not noticeable before puberty. The same trend can be seen for testosterone; no difference between boys and girls before puberty (Ramos et al. 1998) and clear differences (ten-fold) at adult age (Vingren et al. 2010). Together, these findings suggest that androgens could be important in the regulation of muscle carnosine. But there are some arguments which contradict this hypothesis. First, previous research did not find a correlation between muscle carnosine and (free) testosterone levels within a group of eugonadal young men. According to Everaert et al. (2010) this can probably be explained by the relatively small (a 3–4 fold difference) variation of testosterone levels within eugonadal men (Everaert et al. 2010). Second, the evolution of androgens (testosterone) throughout life (Vingren et al. 2010; Starka et al. 2009; Kaufman and Vermeulen 2005) does not match with the changes of muscle carnosine.

Regarding the age-related effect on muscle carnosine, our findings support previous research demonstrating that carnosine levels decline during aging, within the age range of 20–50 years (Stuerenburg and Kunze 1999; Tallon et al. 2007; Everaert et al. 2010). A remarkable, new finding is that the lower carnosine contents of elderly mainly result from a decrease that occurs during adulthood, even shortly after puberty, while this decrease is much less or even non-existing from adulthood to elderly. Given the early decline

Table 2 Model comparison for different sources of variance in gastrocnemius and soleus carnosine content

Soleus									
Model#		−2 Log likelihood	Param	df	AIC	Compare	$\Delta\ \chi^2$	Δ df	*p* value
S	Sat	164.30	10	84					
1	ACE	166.04	7	87	−7.96	1 with S	1.74	3	0.62
2	ADE	166.04	7	87	−7.96	2 with S	1.74	3	0.62
3	AE	166.04	6	88	−9.96	3 with 1	0	1	1
4	E	205.11	5	89	27.11	4 with 3	39.07	1	$4.0*E^{-10}$
5	CE	173.73	6	88	−2.27	5 with 1	7.69	1	0.005
Model 3: V_A: 0.85, V_E: 0.15									
Gastrocnemius									
Model#		−2 Log likelihood	Param	df	AIC	Compare	$\Delta\ \chi^2$	Δ df	*p* value
S	Sat	238.60	10	84					
1	ACE	241.17	7	87	67.17	1 with S	2.57	3	0.46
2	ADE	282.11	7	87	108.11	2 with S	40.97	3	$6.7\ *E^{-10}$
3	AE	252.54	6	88	76.54	3 with 1	11.37	1	0.0007
4	E	282.12	5	89	104.12	4 with 3	29.58	1	$5.3*E^{-08}$
5	CE	241.17	6	88	65.17	5 with 1	0	1	1
Model 5: V_C:0.76, $V_E = 0.24$									

All models include estimates for means for T1 and T2 in MZ and DZ twins

A additive genetic factors, *D* dominance genetic factors, *C* common environment, *E* unique environment, *AIC* Akaike's information criterion, *df* degrees of freedom

in muscle carnosine, compared with the much slower and later decline in testosterone, it is not possible to conclude that testosterone is the only regulator involved in the observed sex and age profile of muscle carnosine.

Muscle carnosine is related to anaerobic energy metabolism as it is mainly expressed in fast-twitch fibers and as it co-evolved in animals with a highly developed anaerobic energy delivery system, probably because it functions as a pH buffer during lactic acid generation. The finding that muscle carnosine already begins to decline in early adulthood is in line with the hypothesis of Kostka et al. (2009), showing that anaerobic capacity would decline faster than aerobic power. They found that the maximal power output per kg body weight decreased already from the age of 30–40 years, in contrast to aerobic power which was only reduced in 50-, 60- and 70-year-old people. This obviously has implications for the age at which performance is maximized. According to Horwill (2003), an 800 and 1500 m runner is almost likely to run his fastest time at around the age of 25, while for the 10 k runner it will be at $\sim$30 years (Horwill 2003). We hereby propose that muscle carnosine content is either an indirect marker of this early decline of anaerobic capacity, or even a direct contributing cause of it.

Finally, based on the current study of twins, it was examined if human muscle carnosine is genetically determined. This could be hypothesized because muscle carnosine is related to muscle fiber-type composition (Mannion et al. 1995; Suzuki et al. 2002; Baguet et al. 2011b). However, heritability estimates for fiber-type composition vary considerably, with an average estimate of 45% heritability for variability in % type I fibers (Simoneau and Bouchard 1995). To our knowledge, this is the first study which investigated a MRS-measureable muscle metabolite in a twin cohort (47 pairs). To date most twin studies were not involved measuring the muscle metabolite concentrations, but focused mainly on muscle enzyme concentrations and histology using muscle biopsies (Bouchard et al. 1986; Komi et al. 1977). This is probably because carnosine is unique, being relatively stable (Baguet et al. 2009) unlike other metabolites such as glycogen (Bergstrom et al. 1967), IMTG (Starling et al. 1997; Kiens et al. 1987), phosphocreatine (Sahlin et al. 1997) and it has also a large inter-subject variability, in contrast to for example ATP. In soleus muscle intra-twin similarities were higher for MZ twins compared to DZ twins, suggesting that muscle carnosine is probably largely genetically determined (AE model $h^2 = 85\%$). This has the consequence that, as suggested by Baguet et al. (2011b), muscle carnosine can in future be used to (re)orient someone to a particular sports or discipline (Baguet et al. 2011b). It should however be noted that in the current study in gastrocnemius the heredity factor could not be calculated, because correlations in dizygotic twins were larger than in monozygotic twins, although biologically not expected. The reason why the gastrocnemius is not in line with the soleus is not clear.

In conclusion, (1) muscle carnosine levels were influenced by puberty in males, but not in females, (2) an age-related decrease in muscle carnosine was found, mainly during early adulthood and not from adulthood to elderly, (3) once sex differences are established following puberty, they remain present throughout life, (4) the conclusion that muscle carnosine content is largely genetically determined could only be supported by the findings in soleus but not in gastrocnemius.

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Conflict of interest The authors declare that they have no conflict of interest.

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