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ORIGINAL ARTICLE

Vegetarianism, female gender and increasing age, but not *CNDP1* genotype, are associated with reduced muscle carnosine levels in humans

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Abstract Carnosine is found in high concentrations in skeletal muscles, where it is involved in several physiological functions. The muscle carnosine content measured within a population can vary by a factor 4. The aim of this study was to further characterize suggested determinants of the muscle carnosine content (diet, gender and age) and to identify new determinants (plasma carnosinase activity and testosterone). We investigated a group of 149 healthy subjects, which consisted of 94 men (12 vegetarians) and 55 women. Muscle carnosine was quantified in M. soleus, gastrocnemius and tibialis anterior using magnetic resonance proton spectroscopy and blood samples were collected to determine *CNDP1* genotype, plasma carnosinase activity and testosterone concentrations. Compared to women, men have 36, 28 and 82% higher carnosine concentrations in M. soleus, gastrocnemius and tibialis anterior muscle, respectively, whereas circulating testosterone concentrations were unrelated to muscle carnosine levels in healthy men. The carnosine content of the M. soleus is negatively related to the subjects' age. Vegetarians have a lower carnosine content of 26% in gastrocnemius compared to omnivores. In contrast, there is no difference in muscle carnosine content between omnivores with a high or low ingestion of β-alanine. Muscle carnosine levels are not related to the polymorphism of the *CNDP1* gene or to the enzymatic activity of the plasma carnosinase. In conclusion, neither *CNDP1* genotype nor the normal variation in circulating testosterone levels affects the muscular carnosine content, whereas vegetarianism, female gender and increasing age are the factors associated with reduced muscle carnosine stores.

Keywords Carnosine · Gender · Age · Vegetarianism · *CNDP1* genotype · Androgens

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Introduction

Carnosine (β-alanyl-L-histidine) is a dipeptide found in high concentrations in skeletal muscles. Several of the physiological actions of carnosine are relevant to muscular function and homeostasis such as pH buffering (Abe 2000; Baguet et al. 2010; Bate Smith 1938; Boldyrev 2007), antioxidant effects (Boldyrev 2007; Kohen et al. 1988), increasing the Ca^{2+} sensitivity of the contractile apparatus (Dutka and Lamb 2004; Lamont and Miller 1992) and inhibiting protein glycation (Hipkiss et al. 1995), as recently reviewed (Boldyrev 2007; Derave et al. 2010; Sale et al. 2010). Interestingly, recent studies have shown that elevated muscle carnosine content is associated with attenuated fatigue and improved exercise performance in humans (Derave et al. 2007; Hill et al. 2007; Ponte et al. 2006; Stout et al. 2007, 2008; Suzuki et al. 2006; Van Thienen et al. 2009). The carnosine content in human muscles usually amounts to 20–30 mmol kg^{-1} in dry weight (5–8 mM in wet weight),

but can easily vary by a factor 3–4 between the highest and the lowest concentrations measured within a population. Yet, the muscle carnosine content is rather constant, as we showed that intra-individual variation in muscle carnosine content is only 9–15% over a 3-month period (Baguet et al. 2009). The MRS-based technique (Derave et al. 2007) gives us the opportunity to explore, without the need for muscle biopsy, existing and new determinants of the variation in muscle carnosine content within a large population.

The most established determinant of the muscle carnosine content is muscle fiber type. HPLC-based single fiber analysis in humans showed a 30–100% higher carnosine content in fast-twitch muscle fibers in comparison with slow-twitch (Harris et al. 1998; Hill et al. 2007; Kendrick et al. 2009). Indeed, Mannion et al. (1995) and Suzuki et al. (2002) showed a positive correlation between muscle fiber type and muscle carnosine content. Furthermore, elite sprinters who are characterized by a high proportion of fast-twitch muscle fibers have higher muscle carnosine content in comparison with marathon runners (Parkhouse et al. 1985).

The amount of food from animal sources is a likely determinant of muscle carnosine levels since β-alanine, the rate-limiting precursor in carnosine synthesis, is exclusively found in meat and fish. The ingestion of very high doses of β-alanine in pure form (4–6.4 g day^{-1} as a food supplement) for several weeks (4–10 weeks) results in 40–80% increases in muscle carnosine content (Baguet et al. 2009; Derave et al. 2007; Harris et al. 2006; Hill et al. 2007; Kendrick et al. 2009). Whether variations in daily meat intake within a normal omnivorous diet also affect muscular carnosine content remains to be established. The chronic and complete withdrawal of dietary β-alanine, such as in vegetarianism, supposedly results in decreased carnosine content, although the current evidence is scarce (Harris et al. 2007).

Men have been shown to have higher (21%) carnosine content in the quadriceps femoris when compared to women (Mannion et al. 1992). This sexual dimorphism is more pronounced in mice with a male/female ratio of approximately 3.5/1 (Penafiel et al. 2004), but absent in horses (Marlin et al. 1989). Concerning the effect of age, several studies on rodents demonstrated a decreasing muscle carnosine content of 35–50% with senescence (Derave et al. 2008; Johnson and Hammer 1992; Stuerenburg and Kunze 1999). To our knowledge, no longitudinal studies on humans are available, but there is cross-sectional evidence for a decreased muscle carnosine content of 33–60% in elderly people with specific pathologies (Stuerenburg and Kunze 1999; Tallon et al. 2007).

A possible explanation for lower muscle carnosine content amongst elderly people and women is their lower plasma (free) testosterone content. Both cross-sectional and longitudinal studies have confirmed an age-associated decline of plasma testosterone in aging men (reviewed in Kaufman and Vermeulen 2005). Penafiel et al. (2004) hypothesized that androgens might upregulate carnosine synthase based upon the findings that the muscle carnosine content was reduced by 40% in castrated mice, and that testosterone injections increased muscle carnosine content by 268% in female mice. To our knowledge, no study has investigated a possible connection between circulating testosterone and muscle carnosine content in eugonadal men.

Polymorphism in the enzymes involved in the synthesis (carnosine synthase) and hydrolysis (carnosinase) of the dipeptide could also contribute to the muscle carnosine content. Since the highest activity of carnosine synthase is found in skeletal muscles (Boldyrev 2007), polymorphisms of the gene encoding carnosine synthase are likely to clarify variations in carnosine. As the gene has only recently been identified (Drozak et al. 2010), there are no studies that have examined the effects. A leucine repeat in exon 2 of the *CNDP1* gene, coding for the serum carnosinase enzyme, has been shown to affect carnosinase activity (Janssen et al. 2005; Mooyaart et al. 2009) and likely the duration of the presence of carnosine in plasma. The enzyme carnosinase is supposedly not present and/or not active in skeletal muscles (Baguet et al. 2009), but it is reasonable to assume that the plasma carnosinase activity could indirectly affect the muscle carnosine content.

The aim of this study was to further characterize previously reported determinants (diet, gender and age) and to identify new potential determinants (plasma carnosinase activity, *CNDP1* genotype and testosterone) of the human muscle carnosine content. Carnosine was quantified using proton magnetic resonance spectroscopy (^{1}H-MRS), as previously described (Baguet et al. 2009; Derave et al. 2007; Ozdemir et al. 2007), in the slow-twitch soleus and tibialis anterior and the fast-twitch gastrocnemius muscle, in order to explore the possible interaction of these factors with the muscle fiber type.

Materials and methods

Subjects

As depicted in Fig. 1, a total of 149 healthy subjects volunteered to participate in this study. The muscle carnosine content of 12 male vegetarians was determined. Vegetarian subjects were either lacto-ovo or vegan, and were required to be vegetarian for a minimum of 8 years (mean ± SD 15 ± 9.5 years, range 8–36 years) prior to the study. Blood samples were obtained from all omnivores (82 males, 55 females) for determination of plasma carnosinase activity

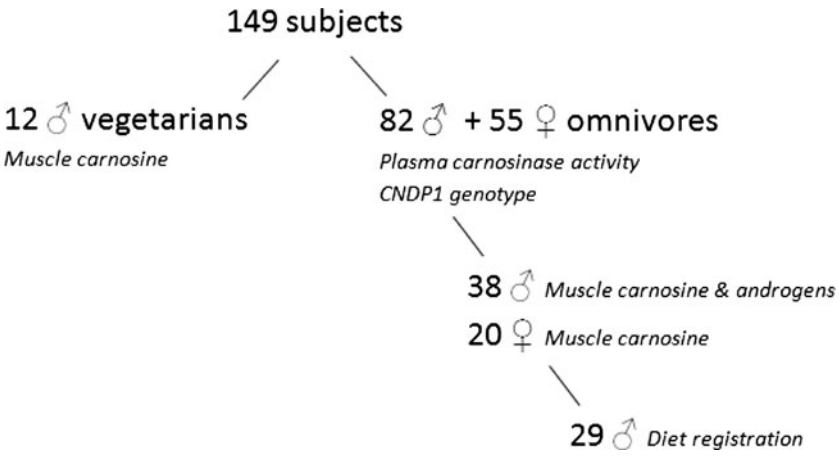

Fig. 1 Overview of subject population. Subjects with only plasma are for comparison of *CNDP1* activity between sexes and for relation between *CNDP1* genotype and plasma carnosinase activity

and *CNDP1* genotype (age: mean ± SD 23.9 ± 7.0, range 18–69 years). The muscle carnosine content (38 males, 20 females) and plasma concentration of androgens (38 males) were measured in a subgroup of omnivores, of which 29 male individuals registered their meat and fish consumption during 2 weeks. The mean (±SD) age of the male population (23.9 ± 7.2 years, range 19–47 years) is lower than the age of the vegetarians (31.3 ± 4.2 years, range 22–38 years) but not different from the female group (23.8 ± 6.7 years, range 19–46 years). The data of 19 male omnivore subjects were obtained from a previous study (Baguet et al. 2009). The study protocols were approved by the local ethical committee (Ghent University Hospital, Belgium) and written informed consent was obtained from all participants prior to the study.

^{1}H-MRS

The carnosine content of three skeletal muscles of the lower leg (soleus, gastrocnemius and tibialis anterior) of a subgroup of 70 subjects was measured using ^{1}H-MRS, as previously described (Baguet et al. 2009). The subjects lay 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 Tesla whole body MRI scanner (Siemens Trio, Erlangen) equipped with a knee-coil. Single voxel point-resolved spectroscopy 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 of the soleus, gastrocnemius lateralis and tibialis anterior was, respectively, 40 mm × 12 mm × 29 mm, 40 mm × 12 mm × 29 mm and 40 mm × 14 mm × 27 mm. Following shimming procedures, the linewidth of the water signal was on average 24.4, 25.5 and 27.6 Hz for soleus, gastrocnemius and tibialis anterior, respectively. A 500-ml spherical container filled with an aqueous solution of 20 mM carnosine (Sigma-Aldrich) was used as an external reference for absolute quantification. The following equation was used to determine the concentration of C2–H (at ~8 ppm) carnosine in vivo:

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

where $[C_m]$ is the carnosine concentration in vivo; $[C_r]$ is the concentration of the external reference phantom (20 mM); S_m and S_r are the estimated signal peak areas of the muscle and reference phantom, respectively, obtained by curve fitting performed in the frequency domain and were also corrected for differences in coil loading between phantom and the muscle; corrected for V_m and V_r, the volumes of the voxels in vivo and in the phantom, respectively; C_{T1m}, C_{T2m}, C_{T1r} and C_{T2r} are correction factors for the $T1$ and $T2$ relaxation times in vivo and in the phantom, respectively; T_m and T_r are the temperatures in vivo and in the phantom, respectively. The $T1$ and $T2$ relaxation times of in vitro carnosine were measured and were found to be 2,616 ± 20 and 250 ± 29 ms, respectively. The formulae used to calculate the correction factors are:

$$C_{T1} = (1 - e^{\wedge}(-\mathrm{TR}/T1))$$

$$C_{T2} = e^{\wedge}(-\mathrm{TE}/T2)$$

For the determination of $T1$ and $T2$ relaxation times in vivo, five healthy subjects (2 males and 3 females, age 21–26 years) were scanned for the soleus, five (3 males and 2 females, age 21–25 years) for the gastrocnemius and five for the tibialis anterior muscle (5 females, 22–26 years). $T1$ was measured using a TE of 30 ms and TR was set to 1,500, 2,000, 2,500, 3,000, 3,500, 4,000, 5,000 and 6,000 ms. $T2$ was measured using a TR of 4,000 ms and TE was set to 30, 60, 90, 120, 150 and 200 ms for soleus, 30, 45, 60, 75, 90 and 105 ms for gastrocnemius, and 30, 45, 50, 60, 70 and 75 for tibialis anterior muscle. For each measurement, 128 averages were acquired. The $T1$ relaxation times were shown to be 1,488 ± 377, 1,771 ± 225 and 1,692 ± 432 ms and $T2$ relaxation

times were 152 ± 28, 106 ± 50 and 64 ± 32 ms in soleus, gastrocnemius and tibialis anterior, respectively.

Venous blood sampling

For measurement of circulating androgens, plasma carnosinase activity and for *CNDP1* genotyping, blood of the antecubital vein was withdrawn in heparin tubes. Blood samples were centrifuged and the plasma and blood cells were stored separately at −80°C until further analyses.

Genotyping

A more detailed description of the *CNDP1* genotype determination is explained in the study of Mooyaart et al. (2009). In brief, a standard PCR protocol was used with primers 5-FAM-GCGGGGAGGGTGAGGAGAAC (forward) and GGTAACAGACCTTCTTGAGGAATT-TGG (reverse). The denaturing, annealing and extension temperatures were 94, 60 and 72°C, respectively. After PCR amplification, fragment analysis was performed on the ABI3130 analyzer (Perkin Elmer) to determine the fragment length corresponding to the different genotypes. Each peak corresponded with the number of leucine repeats on each allele. The 157, 160 and 163 base pair products corresponded with 5, 6 and 7 CTG codons encoding for 5, 6 and 7 leucine repeats, respectively. The 5–5 and the 5–6 *CNDP1* genotypes are widespread and each represents approximately 40% of the population. The 6–6 genotype is present in ±12% of the population while the 5–7 (±4%) and 6–7 (±4%) *CNDP1* genotypes are less common (Freedman et al. 2007; Janssen et al. 2005; Mooyaart et al. 2009).

Plasma carnosinase activity

Heparin plasma samples of 82 men and 55 women were used to quantify the plasma carnosinase activity. Plasma carnosinase activity was determined according to the method described by Teufel et al. (2003). Briefly, the reaction was initiated by addition of substrate (L-carnosine) to a plasma sample and stopped after 10 min of incubation at 37°C by adding 1% sulphate salicylic acid. The maximum increase was used for determining the maximum activity. Liberated histidine was derivatized with *o*-phtaldialdehyde (OPA). Fluorescence was measured by excitation at 360 nm and emission at 460 nm. The intra- and inter-assay variations were, respectively, 7 and 25%. The lowest carnosinase activity detectable was 0.117 μmol ml^{-1} h^{-1}.

Androgens

Heparin plasma samples of 38 men were analyzed using a commercial immunoassay kit to determine the plasma concentrations of testosterone (Orion Diagnostica, Espoo, Finland) and LH (ECLIA, Roche Diagnostics). The free fraction of testosterone was calculated from plasma testosterone, SHBG, and albumin concentrations using a previously validated equation (Vermeulen et al. 1999).

Dietary β-alanine intake

A subgroup of 29 male omnivore subjects completed a questionnaire about their meat and fish consumption for 2 weeks to quantify daily dietary β-alanine intake, as described in the study of Baguet et al. (2009).

Statistics

The *CNDP1* genotypes of exon 2 were categorized based on the leucine repeat length (5–5, 5–6, 5–7, 6–6, 6–7). Independent sample *t* tests were used to evaluate the effect of gender, age and *CNDP1* genotype on the muscle carnosine content. A univariate analysis of variance with age as covariate was used to verify the effect of vegetarianism on muscle carnosine content. The correlation between genotype and carnosinase activity was assessed by a univariate analysis of variance with carnosinase activity as dependent and both gender and *CNDP1* genotype as independent variables (post hoc: pairwise comparisons). The other possible determinants were analyzed by bivariate correlations. All statistical analyses were performed using SPSS 16.0 software (SPSS, Chicago, IL, USA) and a p value <0.05 was considered to be statistically significant.

Results

Gender

As illustrated in Fig. 2a, men had higher muscle carnosine content in comparison to women ($p \leq 0.004$), but the magnitude of this sex-related difference depends on the type of muscle. The soleus and gastrocnemius showed a 36 and 28% higher carnosine content in men, whereas the difference was 82% in tibialis anterior. The plasma carnosinase activity was significantly higher ($p < 0.001$) in women (7.20 ± 1.62 μmol ml^{-1} h^{-1}) in comparison with men (5.79 ± 1.58 μmol ml^{-1} h^{-1}; Fig. 2b).

Age

The carnosine concentration in the M. soleus ($n = 58$, $p = 0.049$, $r = -0.260$; Fig. 3) declines with age in the adult range of 19–47 years (M. gastrocnemius: $p = 0.112$, $r = -0.211$; M. tibialis anterior: $p = 0.482$, $r = -0.096$).

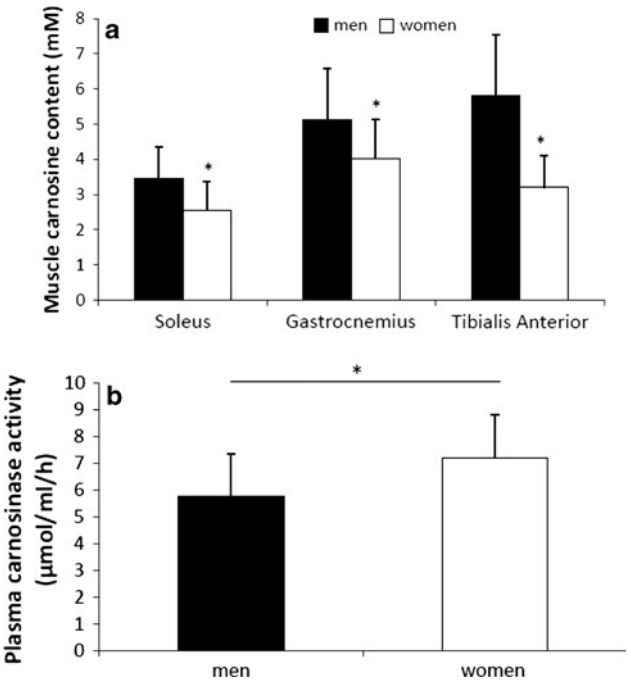

Fig. 2 **a** The carnosine content of men ($n = 38$) versus women ($n = 20$) in soleus, gastrocnemius and tibialis anterior, measured by proton magnetic resonance spectroscopy; * different from men ($p \leq 0.004$). **b** The plasma carnosinase activity of the female ($n = 55$) is 24.3% higher in comparison with the carnosinase activity of the male population ($n = 82$) ($p < 0.001$)

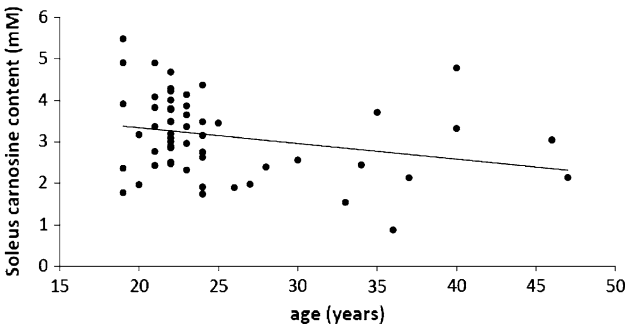

Fig. 3 Effect of age on muscle carnosine content in both male and female omnivores. There is a negative correlation of age and carnosine concentration in soleus ($p < 0.05$, $r = -0.260$, $n = 58$)

In the same group, age did not significantly correlate with plasma carnosinase activity ($p = 0.355$, $r = 0.124$).

Androgens

In order to elucidate the mechanisms of the age and gender related effects on the muscle carnosine content, we measured plasma testosterone and free testosterone concentrations. The mean (±SD) total testosterone and free testosterone plasma levels in the male reference population were, respectively, 538.6 ± 140.3 and 11.7 ± 3.5 ng dl^{-1}.

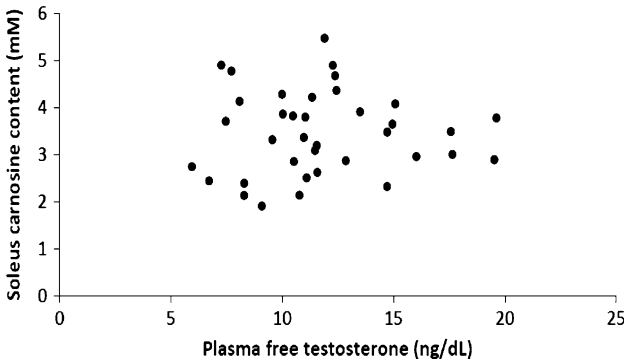

Fig. 4 The lack of correlation between plasma free testosterone and soleus carnosine content ($p = 0.921$). Similar results were obtained for gastrocnemius and tibialis anterior ($p = 0.851$ and $p = 0.794$, respectively) and for the correlation of the muscle carnosine content with testosterone, luteinizing hormone (LH) and sex hormone binding globulin (SHBG) ($p > 0.05$)

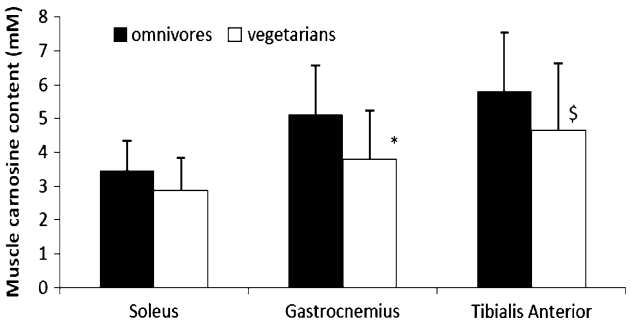

Fig. 5 Male vegetarians ($n = 12$) have a lower muscle carnosine content ($^{*}p < 0.05$ and $^{\$}p < 0.10$) in comparison with male omnivores ($n = 38$)

Neither of them correlated with muscle carnosine content (soleus, gastrocnemius and tibialis anterior) or with carnosinase activity ($0.238 \leq p \geq 0.921$). A scatter plot of plasma free testosterone content with M. soleus carnosine content is depicted in Fig. 4. The plasma total and free testosterone concentration is inversely related to the subjects' age ($p < 0.02$; $r = -0.410$, -0.402, respectively).

Dietary β-alanine intake

Long-term vegetarianism (>8 years) is associated with declined muscle carnosine stores (Fig. 5). Vegetarians have lower carnosine levels of 17% in M. soleus ($p = 0.05$) and 26% in M. gastrocnemius ($p = 0.009$) and a trend to a lower carnosine content (20%) in M. tibialis anterior ($p = 0.068$) compared to omnivores. However, the significance of the effect of vegetarianism on soleus carnosine content disappeared when the data were corrected for age ($p = 0.304$), whereas this was not the case in M. gastrocnemius or in tibialis anterior. The mean (±SD) daily average β-alanine ingestion by meat and fish consumption

in a subgroup of 29 omnivore male subjects was 0.332 ± 0.144 g. Within a normal Western omnivore diet, β-alanine intake by meat and fish consumption is not a determinant of the muscle carnosine content, as there is no correlation between β-alanine ingestion and muscle carnosine content ($0.671 \leq p \geq 0.885$), or a difference in muscle carnosine content between subjects with a low (<0.332 g day^{-1}) or a high (>0.332 g day^{-1}) intake of β-alanine ($0.296 \leq p \geq 0.562$).

Genotype and plasma carnosinase activity

The most common *CNDP1* genotypes were 5–5 (35.8%) and 5–6 (38%). The 5–7, 6–6 and 6–7 *CNDP1* genotypes were detected in, respectively, 7.3, 15.3 and 3.6% of the subjects. Figure 6a shows that the plasma carnosinase activity is dependent on the *CNDP1* genotype ($p = 0.054$). The plasma carnosinase activity of the 5–5 genotype is lower compared to the 5–6 ($p = 0.05$) and to the 6–6 genotype ($p = 0.025$). Also, the 6–7 alleles show a lower plasma carnosinase activity than the 6–6 alleles ($p = 0.035$). The relation between the most frequent *CNDP1* genotypes (5–5 and 5–6) and the muscle carnosine content is depicted in Fig. 6b. The muscle carnosine content of the individuals with the 5–5 *CNDP1* genotype is similar to the carnosine levels of the subjects with the 5–6 *CNDP1* genotype in M. soleus, gastrocnemius and tibialis anterior ($0.393 \leq p \leq 0.576$). Likewise, there is no correlation between muscle carnosine levels and the activity of the carnosine degrading enzyme in plasma ($0.154 < p < 0.744$).

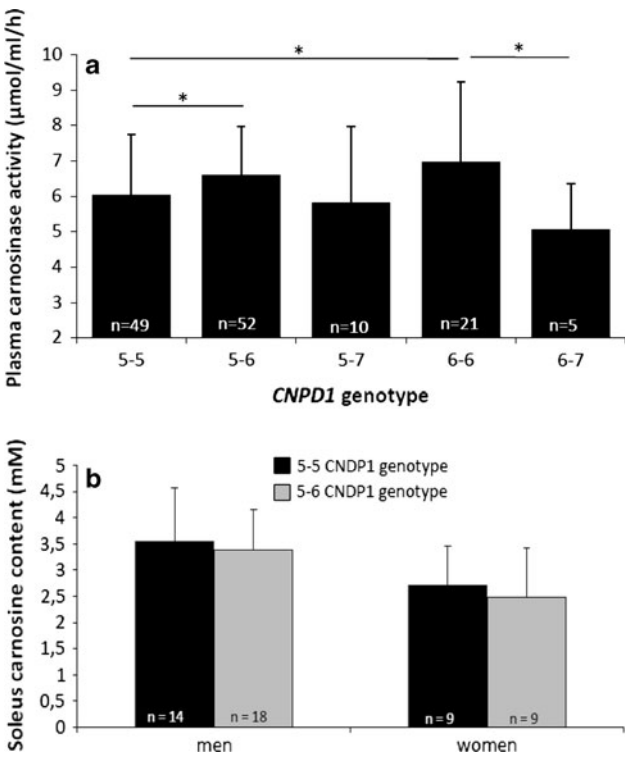

Fig. 6 **a** Mean (±SD) plasma carnosinase activity (µmol ml^{-1} h^{-1}) categorized by amount of leucine repeats in *CNDP1* gene of both 82 males and 55 females. **b** The carnosine content of individuals with the 5–5 *CNDP1* genotype in comparison with individuals with the 5–6 *CNDP1* genotype ($p > 0.05$)

Discussion

Carnosine is synthesized in skeletal muscles from histidine and β-alanine, with the latter being the rate-limiting precursor. It is demonstrated that an increased availability of β-alanine, by means of oral supplementation, results in higher muscle carnosine levels (for review see Derave et al. 2010; Sale et al. 2010). This is the first peer-reviewed study which shows that a complete and long-lasting restriction of β-alanine from the diet, as is the case in habitual vegetarians (lacto-ovo or vegan, >8 years), results in lower intramuscular carnosine concentrations as compared with omnivorous subjects. In a proceedings abstract by Harris et al. (2007), it was shown that female vegetarians have a 45% lower carnosine content in M. vastus lateralis compared with male sports science students. We observed a 26% lower carnosine content in the gastrocnemius of male vegetarians compared to male omnivores. The lower muscle carnosine levels in vegetarians implies that it may be important for vegetarian athletes, involved in high-intensity exercise, to compensate a possible shortage of muscle carnosine by means of β-alanine supplementation, as also recommended for creatine (Enette Larson-Meyer 2006). However, the decreased intramuscular total creatine content in vegetarians versus omnivores is less pronounced (11.1%, Burke et al. 2003). As there is no difference in muscle carnosine content between individuals with a higher or a lower β-alanine intake within a normal western omnivore diet, we can conclude that only the supplementation with very high doses of β-alanine, and the complete and prolonged restriction of β-alanine from the diet does influence the carnosine content.

This study confirms the higher muscle carnosine content in males versus females shown by Mannion et al. (1992), but in the currently studied muscles of the lower leg, the gender-based differences are even more pronounced (28–82%) compared to the differences in M. quadriceps femoris of the previous study (21%). To the best of our knowledge, no other metabolite in the muscle shows such a large gender-dependent difference. In that light, it is interesting that a sex-specific effect was seen in the relation between diabetic nephropathy and the *CNDP1* genotype (Mooyaart et al. 2010). Additionally, this gender difference could contribute to the lower high-intensity exercise capacities of women compared to men. Besides the effect

of gender on the muscle carnosine content, there is also a negative correlation between age and muscle carnosine content. Stuerenburg and Kunze (1999) described a correlation coefficient of −0.4 in patients with neuromuscular diseases ranging from 20 to 80 years. This study is the first that shows this negative correlation in a healthy adult population (19–47 years). Our cross-sectional data suggest a decline in soleus carnosine content of 1.2% per year. However, the majority of the subjects are less than 30 years of age, and the confirmation of these data in an older healthy adult population is recommended. A number of possible factors exist as to why the carnosine levels are affected by gender and age. We hypothesize that both muscle fiber type and circulating androgen levels could be responsible for this gender- and age-based distinction in muscle carnosine levels.

Despite the conflicting reports regarding the percentage muscle fiber type proportion amongst gender and age, it is generally acknowledged that women and elderly people are characterized with a smaller cross-sectional area of muscle fibers which is most pronounced in fast-twitch fibers (Simoneau and Bouchard 1989; Staron et al. 2000). Women have 56% smaller cross-sectional area (CSA) of type IIB/X muscle fibers in comparison with men, whereas this gender-induced difference is only 14% in slow-twitch muscle fibers (Simoneau and Bouchard 1989; Staron et al. 2000). As already mentioned in "Introduction", fast-twitch muscle fibers are characterized by 30–100% higher carnosine levels compared to slow-twitch muscle fibers (Harris et al. 1998; Hill et al. 2007; Kendrick et al. 2009). Thus, it is possible that women and elderly people have lower carnosine levels as a result of a smaller proportion of fast-twitch muscle fibers compared to young men.

The hypothesis that circulating androgen levels could affect the muscular carnosine levels is based on the study of Penafiel et al. (2004), in which they successfully manipulated the carnosine content of murine skeletal muscles by means of subcutaneous testosterone injections in female mice and by removing the testes in male mice. However, we found no correlation between the plasma (free) testosterone levels and muscle carnosine content within a healthy male population. Nevertheless, this does not exclude the possibility that more extreme variations in total or free testosterone such as overt hypogonadism or exogenous testosterone supplementation do influence muscle carnosine content. This argumentation is supported by the twofold higher muscle carnosine content of the bodybuilders in the study of Tallon et al. (2005), in which five of the six subjects reported to have taken anabolic steroids in the last 6 months.

The observed influence of vegetarianism, gender and age on carnosine content seems to depend on the muscle type under investigation. The gastrocnemius muscle is most affected by dietary β-alanine restriction; the tibialis anterior is the muscle that displays the largest sexual dimorphism, and the age-related effect on muscle carnosine content is only observed in soleus. Muscle-specific differences in expression profiles of, for e.g., enzymes of carnosine metabolism, amino acid transporters or androgen receptors could be hypothesized.

In line with previous reports, we observed that the *CNDP1* polymorphism affects the plasma carnosinase activity. The higher activity of the carnosine degrading enzyme for the 5–6 and the 6–6 *CNDP1* genotypes compared to the homozygosity for the Mannheim allele (5–5) is confirmed (Janssen et al. 2005; Mooyaart et al. 2009). However, it has to be noted that there is a high variation in plasma carnosinase activity within one genotype group (e.g. a range of 2–10 μmol ml^{-1} h^{-1} in 5–5 genotype). Remarkably, individuals with the 6–7 *CNDP1* genotype have a significantly lower carnosinase activity than individuals with the 6–6 variant. This is in contrast with the suggestion of Janssen et al. (2005) that both the 6 and 7 leucine alleles can be regarded as gain-of-function mutations associated with a higher enzyme activity and with the results of Riedl et al. (2007) that the percentage of secreted carnosinase increases with increasing length of the leucine repetitions in the signal peptide. Variations of natural inhibitors of carnosinase such as homocarnosine may account for additional fluctuations in enzyme activity (Peters et al. 2009). Furthermore, the results of this study reveal that females are characterized with a higher plasma carnosinase activity versus males, confirming and strengthening the non-significant gender-based differences in plasma carnosinase levels which were previously reported (Bando et al. 1984; Peters et al. 2009). Despite the combination of declined muscle carnosine levels and a higher activity of the plasma carnosinase enzyme in both women and elderly people (Peters et al. 2009), we shown no inverse relationship between both parameters in a gender—and age—mixed population. Similarly, the polymorphism of the *CNDP1* gene does not determine the muscle carnosine levels. A different compartmentation could underlie this finding, as carnosine is mainly present in the muscle and the enzyme carnosinase is present in the circulation but not (active) in the muscle. Additionally, muscle carnosine could be more sensitive to the carnosine synthetase activity than to the carnosinase activity. However, we are aware that our population is probably too small to completely exclude the possibility that muscle carnosine is related to *CNDP1* genotype. It is therefore necessary to confirm these data in a larger population with a relevant number of subjects with less common genotypes (5–7, 6–6, …).

In conclusion, the results of this study provide evidence for (1) a declined muscle carnosine content in vegetarians

which implies that it may be important for vegetarian athletes, involved in high-intensity exercise, to compensate a possible shortage of muscle carnosine by means of β-alanine supplementation, (2) a marked sexual dimorphism in muscle carnosine levels and plasma carnosinase activity, and (3) an independency of the muscle carnosine content to the polymorphism of the *CNDP1* gene and the enzymatic activity of plasma carnosinase.

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