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ORIGINAL ARTICLE

Comparison of the carnosine and taurine contents of vastus lateralis of elderly Korean males, with impaired glucose tolerance, and young elite Korean swimmers

Hyo-Jeong Kim

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Abstract The carnosine and taurine contents of the vastus lateralis of two diverse groups of Korean male subjects (elderly and impaired glucose-tolerant (IGT) subjects and young elite swimmers at a national sport university) having a similar national diet, were examined. Despite marked differences in age, fitness and clinical status the two groups showed almost identical muscle carnosine and taurine contents. In the case of carnosine, the results suggest a similar contribution to intracellular buffering capacity in the two groups of subjects, with no evidence of a reduction of this in elderly IGT subjects. In addition, both groups showed the same inverse relationship between the muscle carnosine and taurine contents; the spread of values between subjects, within-groups, most likely reflect variations in the type I (low carnosine, high taurine) or type II (high carnosine, low taurine) composition of the vastus lateralis. The relationship is consistent with a role of taurine in osmoregulation, compensating for variations between fibre types in the carnosine content.

Keywords Carnosine · Taurine · Korean males · Swimmers · Impaired glucose tolerance · Ageing

Introduction

Carnosine is the sole dipeptide within the β-alanylhistidine family, found in human skeletal muscle. With a pKa of 6.8 for the imidazole ring of the histidine residue, together with a high concentration in muscle, carnosine contributes significantly to the physical chemical buffering of protons in muscle.

Two studies have suggested lower muscle contents of carnosine in older subjects (Stuerenburg and Kunze 1999; Tallon et al. 2007). In the study of Tallon et al. (2007) this involved a halving of the normal carnosine content in type II muscle fibres, with little or no change in type I fibres. The decrease in muscle content with age appeared to confirm earlier observations in ageing rats (Johnson and Hammer 1993), and also more recently in senescence-accelerated mice (Derave et al. 2008). However, in Tallon et al. (2007) subjects were both elderly and diagnosed with osteoarthritis of the knee, and in Stuerenburg and Kunze (1999) were diagnosed with motor neuron disease. The clinical conditions of the subjects in these two studies could well have been a factor contributing to the changes in muscle carnosine insofar as the physical activity levels would have been reduced. In addition, no information was collected at that time on the diet of the subjects studied, and specifically of the intake of meat as a source of dietary β-alanine, which again could influence the muscle content. A reduced content in muscle of elderly subjects, for whatever reason, would affect intracellular buffering capacity as well as possible defences to intracellular glycation and carbonyl cross-linking of proteins (Hipkiss 2000; Hipkiss et al. 1995, 2001) and free radical damage (Boldyrev et al. 1993).

Synthesis of carnosine occurs locally in muscle fibres (Bakardjiev and Bauer 1994; Bauer and Schulz 1994) and is limited by the availability of β-alanine (Harris et al. 2006), which may be obtained either from the degradation of uracil in liver (the only significant source available to vegetarians) or additionally from the hydrolysis of β-alanylhistidine dipeptides with ingestion of meat. Variations

H.-J. Kim (✉)
Human Physiology, Korea National Sport University,
88-15 Oryun-dong, Songpagu, Seoul, South Korea
e-mail: jasminhj@yahoo.co.kr

H.-J. Kim
Sports Medical Center, Yonsesarang Hospital, 478-5 Seocho-gu,
Bangbae-dong, Seoul, South Korea

in meat intake, which may in part be reflected in national diets, could well be a factor for determining the synthesis of carnosine in the human muscle. Superimposed on this, type II fibres intrinsically have a higher carnosine content than type I fibres in all species investigated (Dunnett and Harris 1995, 1997; Dunnett et al. 1997; Harris et al. 1998; Hill et al. 2007; Kendrick et al. 2008). Transport of β-alanine into muscle cells involves the inward co-transport of sodium and chloride ions (Bakardjiev and Bauer 1994; Komura et al. 1996) and as such could be affected by the electrochemical Na^+ gradient across the sarcolemma. By analogy to the effect of insulin on the uptake of creatine (Green et al. 1996; Steenge et al. 1998) and carnitine (Stephens et al. 2006), it is possible that differences in insulin sensitivity affecting Na^+/K^+ exchange pump activity (Ewart and Klip 1995; Hundal et al. 1992; Marette et al. 1993; Sweeney and Klip 1998) could influence β-alanine uptake and thus also carnosine synthesis.

Taurine, a beta-sulphonic non-proteogenic amino acid, is not involved in carnosine synthesis but shares the same transporter as β-alanine for uptake into muscle (Komura et al. 1996). Taurine is found in equally high concentrations as carnosine in mammalian skeletal muscle, but shows the opposite distribution in type I and II muscle fibres (Dunnett and Harris 1995; Harris et al. 1998; Kendrick et al. 2008), with 1.3–2 times higher concentrations in type I fibres in humans. The reason for the opposite distribution is unknown, but may reflect a role for taurine in osmoregulation (Cuisiner et al. 2002), compensating for variations in muscle carnosine.

The aim of the present investigation was to compare the muscle carnosine and taurine contents of two radically different groups, albeit of the same gender and maintained on a similar national (Korean) diet.

Materials and methods

Eight of ten non-obese elderly Korean men (age range 58–67 years) with IGT, recruited to a previously reported study to investigate the effect of training on glucose transporter 4 protein and intramuscular lipid (Kim et al. 2004), were investigated. Only samples obtained before training were analysed for carnosine and taurine. Glucose tolerance was assessed during 2 h of an oral glucose tolerance test (OGTT). For each subject a medical history was obtained, and physical examination was performed. Information was collected on the dietary habits of the subjects, and in particular whether they were vegetarians. None of the subjects had engaged in regular exercise or any diet program for weight reduction within at least a year of the study and they had maintained their current body weight for more than 3 months.

Ten fit Korean male students at the Korea National Sport University (age range 23–25 years), all of whom were elite swimmers and at the start of their training season, and who had been recruited to investigate the effects of training on muscle carnosine, were included as a contrast group. Only the results from samples obtained prior to the start of training are reported here. The investigation of the two groups of subjects was first approved by the ethics committee of the KNSU and the nature of the primary study in each case, including the procedures to be used, were explained to each of the subjects before agreement to participate was obtained.

From each of the 18 subjects, a single percutaneous muscle biopsy was obtained from the lateral portion of the vastus lateralis using a 5 mm Bergström needle (Bergström 1962), which was frozen in iso-pentane pre-cooled with liquid nitrogen to its melting point, and later freeze-dried. Neutralised perchloric acid extracts of 2–4 mg of powdered freeze-dried muscle, dissected free of obvious blood and connective tissue, were prepared with a muscle to extract ratio of 10 mg/1ml (Harris et al. 1974). A volume of 10 μL of extract was further diluted with 1 ml of 0.05 M borate, pH 9.5, filtered through 0.4 μm centrifugal-filter (Pall, Portsmouth, UK) and analysed by HPLC for carnosine and taurine (Dunnett and Harris 1997) using 25 mM sodium phosphate buffer, pH 6.8, in place of 12.5 mM sodium acetate buffer. Balenine was added to diluted extracts as an internal standard.

Results

Mean characteristics of all subjects are shown in Table 1. None of the subjects were vegetarians and all ate a typical Korean diet. This was determined to include chicken, pork and beef meat, as well as fish, all of which are potential dietary sources of β-alanine from the ingestion of carnosine and related methyl derivatives (anserine and balenine). Korean cooking is typically done "in the pot" or by barbeque with the result that loss of dipeptides is minimal. Carnosine, and related peptides, are stable to prolonged heating and are mainly lost when cooking stock is discarded.

Despite the marked differences in ages of the two groups, as well as their activity and clinical history, mean carnosine and taurine contents of the vastus lateralis were

Table 1 Mean characteristics of elderly Korean subjects characterised as IGT, and younger active Korean swimmers

	Elderly IGT	Swimmers
Age (years)	60.7 (5.1)	24.4 (1.6)
Body mass (kg)	60.6 (4.6)	76.4 (4.2)
BMI (kg/m^2)	23.4 (2.4)	23.9 (2.6)

Table 2 Mean carnosine and taurine contents of vastus lateralis of elderly Korean subjects characterised as IGT and younger active Korean swimmers

	Carnosine (mmol kg^{-1} dm)	Taurine (mmol kg^{-1} dm)
Elderley IGT		
Mean	21.46	44.87
SD	3.75	11.99
n	8	8
Swimmers		
Mean	22.05	42.76
SD	2.75	10.34
n	10	10

almost identical (Table 2) as were the distributions of individual values (Fig. 1). In the case of both groups, values for carnosine and taurine were normally distributed about the mean with no evidence of outlier values influencing the estimated variance. In both groups of subjects, carnosine showed an almost identical inverse relationship to taurine (Fig. 2), and when the data for both groups were combined was significant ($P < 0.034$).

Discussion

The muscle carnosine and taurine contents of the elite swimmers (and indeed of both subject groups) were similar to those previously observed in UK and Vietnamese sports science students (Harris et al. 2006; Hill et al. 2007 and Kendrick et al. 2008). Higher muscle carnosine contents have been reported in chronically trained body builders

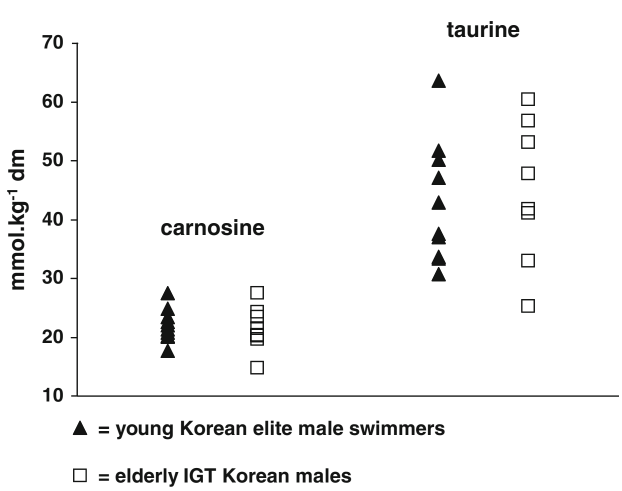

Fig. 1 Individual values of carnosine and taurine determined in biopsy samples of vastus lateralis of young Korean elite male swimmers ($n = 10$) and elderly Korean male subjects with impaired glucose tolerance ($n = 8$)

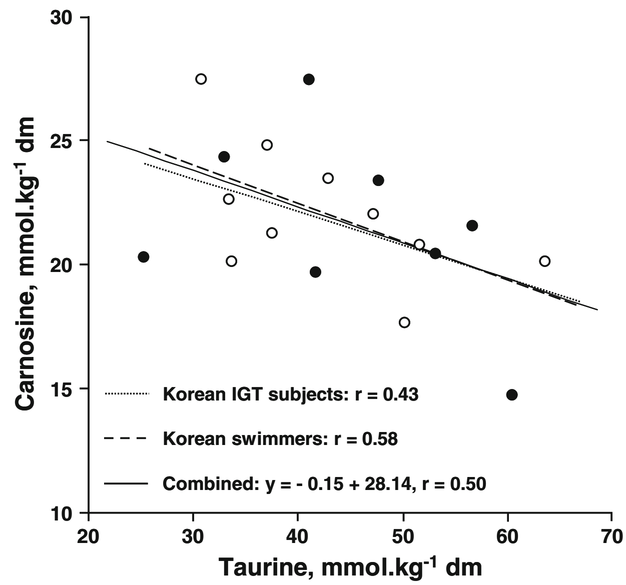

Fig. 2 Comparison of the muscle carnosine content with the muscle taurine content in vastus lateralis in elderly Korean IGT subjects and young Korean swimmers. On combining all data, the comparison of muscle carnosine with muscle taurine ($r = 0.50$) was significant ($P < 0.034$; Pearson product moment correlation)

(Tallon et al. 2005), all of whom noted current or previous use of anabolic steroids, as well as Korean speed skaters (Kim et al. 2005). On the other hand, acute training of 10–16 weeks duration (Mannion et al. 1994; Kendrick et al. 2008) appears to have no effect upon the muscle carnosine content unless subjects are additionally supplemented with β-alanine. Even then, however, the increase in muscle carnosine was no greater than that found in untrained subjects supplemented with β-alanine (Harris et al. 2006; Hill et al. 2007). Lower than "normal" muscle carnosine contents have recently been reported in a small study of vegetarians (Harris et al. 2007), where the only significant supply of β-alanine is from the degradation of uracil in the liver. Taken together these results suggest that the dietary supply of β-alanine is an important determinant of the carnosine content in muscle, in addition to the muscle fibre composition. The observations made in Tallon et al. (2005) and Kim et al. (2005) on chronically trained athletes can most probably be explained on the basis of an increased proportion of type II muscle fibres in what are essentially strength or sprint trained athletes, although long-term changes in dietary patterns may also have been a factor in "maximizing" the carnosine content of all fibre types. Swimmers, in comparison, are more likely to have a higher proportion of slow-twitch or type I fibres than strength-trained athletes (Gollnick et al. 1972).

The remarkable similarity in the muscle carnosine and taurine contents between the elderly subjects with IGT, and elite swimmers, suggests that altered insulin sensitivity is not a factor in determining the level of either. Neither did age in the current group of non-obese Korean omnivorous males (mean age 60.7 (SD 5.1) years), eating a Korean national diet, appear to be a factor. It is unlikely that there was anything of special consideration in the diet itself, other than that the amounts of meat and fish ingested were sufficient to supply adequate levels of β-alanine for muscle carnosine synthesis. As reported by Kim et al. (2004), the elderly IGT subjects had a mean type I fibre composition of 50.7 (SD 4.6) %) within the range found in vastus lateralis encompassing younger subjects and of different levels of training (Gollnick et al. 1972; Saltin et al. 1977). Given the similarity in the muscle contents, it seems unlikely that major differences were present in the mean carnosine and taurine contents of similar muscle fibres (types I and II) in the two diverse subject groups. At a functional level the results suggest a similar contribution of carnosine to intracellular buffering in the two groups of subjects, with no evidence of a reduction of this in elderly IGT subjects.

Despite there appearing to be no effect of age in this study, progressive changes in type II muscle fibre characteristics, including composition and reduction in cross-sectional area (Aniansson et al. 1986), due to the effects of disuse, ageing and denervation (Essen-Gustavsson and Borges 1986) would eventually favour a reduction in carnosine and increase in taurine at the muscle level. This would be still more apparent if, in addition to a decrease in the type II fibre composition, the intrinsic carnosine content of type II fibres also decreased as observed by Tallon et al. (2007). However, whether the changes in type II fibres observed by Tallon et al. (2007) were a direct result of sarcopenia, or the result of reduced activity and long-term changes in dietary patterns, is not known. Variations in diet with ageing is influenced by national traditions: the trend with advancing age being, for the most part, a reduction in meat and fish consumption. As already noted, it seems on the basis of observations made in vegetarians that β-alanine availability through meat ingestion is critical to the synthesis and maintenance of normal muscle carnosine concentrations.

Finally in this and other studies, carnosine again showed an inverse relation with taurine. For the most part, this can be explained on the basis of the lower carnosine/higher taurine content of type I fibres compared to type II, and the variance in fibre compositions between individual muscle samples. However, this does not explain the opposite distributions of carnosine and taurine in different fibre types. One possibility is that this reflects a role of taurine in intracellular osmoregulation (Cuisinier et al. 2002), in this case taurine compensating for the differences between fibre types in carnosine. But if that were the case, then this would suggest that further increases in carnosine with β-alanine supplementation, or increases in other osmolytes, e.g. creatine with "creatine loading", would depress the muscle taurine content still further. To date no such observations on this have been reported, but remains a hypothesis that could be tested in the future.

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