Vegetarians have a reduced skeletal muscle carnitine transport capacity

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ABSTRACT

Background: Ninety-five percent of the body carnitine pool resides in skeletal muscle where it plays a vital role in fuel metabolism. However, vegetarians obtain negligible amounts of carnitine from their diet.

Objective: We tested the hypothesis that muscle carnitine uptake is elevated in vegetarians compared with that in nonvegatarians to maintain a normal tissue carnitine content.

Design: Forty-one young (aged ~22 y) vegetarian and nonvegetarian volunteers participated in 2 studies. The first study consisted of a 5-h intravenous infusion of L-carnitine while circulating insulin was maintained at a physiologically high concentration (~170 mU/L; to stimulate muscle carnitine uptake) or at a fasting concentration (~6 mU/L). The second study consisted of oral ingestion of 3 g L-carnitine.

Results: Basal plasma total carnitine (TC) concentration, 24-h urinary TC excretion, muscle TC content, and muscle carnitine transporter [organic cation transporter 2 (OCTN2)] messenger RNA and protein expressions were 16% (P < 0.01), 58% (P < 0.01), 17% (P < 0.05), 33% (P < 0.05), and 37% (P = 0.09) lower, respectively, in vegetarian volunteers. However, although nonvegetarians showed a 15% increase (P < 0.05) in muscle TC during L-carnitine infusion with hyperinsulinemia, L-carnitine infusion in the presence or absence of hyperinsulinemia had no effect on muscle TC content in vegetarians. Nevertheless, 24-h urinary TC excretion was 55% less in vegetarians after L-carnitine ingestion.

Conclusions: Vegetarians have a lower muscle TC and reduced capacity to transport carnitine into muscle than do nonvegetarians, possibly because of reduced muscle OCTN2 content. Thus, the greater whole-body carnitine retention observed after a single dose of L-carnitine in vegetarians was not attributable to increased muscle carnitine storage.


INTRODUCTION

Omnivorous humans obtain ~2–13 μmol L-carnitine/kg BM1 (0.3–2 mg/kg BM) per day from dietary meat sources, with ≤1 μmol L-carnitine · kg BM−1 · d−1 by endogenous synthesis from trimethyllysine, and excrete ~7 μmol L-carnitine/kg BM via the kidneys to tightly regulate carnitine homeostasis and maintain a total body content of ~2 mmol L-carnitine/kg BM (1). Conversely, because carnitine is mainly found in foods of animal origin, vegetarians (lactoovovegetarians and vegans) obtain negligible amounts of carnitine from their diet (~0.04–0.4 μmol · kg BM−1 · d−1) and therefore are thought to have a greater rate of endogenous synthesis and a more efficient renal reabsorption of carnitine to maintain a normal whole-body carnitine store (1, 2). However, plasma carnitine concentrations in vegetarians were reported to be ~20–30% lower than those in nonvegetarians (1, 3, 4), which suggests that tissue carnitine stores may also be reduced. In contrast, because the skeletal muscle carnitine content of vegetarians is unknown, tissue carnitine uptake capacity may be elevated in vegetarians to scavenge any available carnitine to maintain the tissue carnitine content for normal physiologic function and thereby result in the observed lower plasma carnitine concentration.

More than 95% of the body’s TC store is localized in skeletal muscle tissue where it plays an essential role in fat and carbohydrate metabolism (for review, see reference 5). Carnitine is transported into skeletal muscle against a considerable concentration gradient (>100-fold) via the Na+-dependent, high-affinity OCTN2 (6–8). However, carnitine uptake in skeletal muscle appears to be saturated under basal physiologic conditions (7) and relatively slow compared with that in other tissues [turnover of 190 h (9)]. Indeed, the maintenance of a supraphysiologic plasma TC concentration for 5 h in healthy nonvegetarians has no effect on the skeletal muscle TC content (10). Nevertheless, in line with the hypothesis that insulin will augment Na+-dependent muscle carnitine transport via OCTN2 (secondary to its action of increasing sarcolemmal Na+/K+ ATPase pump activity and therefore intracellular Na+ flux), we have previously shown that maintaining hyperinsulinemia (~170 mU/L) during 5 h of steady state hypercarnitinemia (~550 μmol/L) increased the skeletal muscle TC content by 15% in healthy human volunteers (10). Therefore, the aims of the current study were to

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5Abbreviations used: BM, body mass; dm, dry muscle; HMBS, hydroxymethylbilane synthase; mRNA, messenger RNA; OCTN2, organic cation transporter 2; TC, total carnitine.

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Subject characteristics are presented in Table 1. All values are means ± SEMs. TC, total carnitine; dm, dry muscle. **Significantly different from nonvegetarian value (2-tailed unpaired Student’s t test); *P < 0.05, **P < 0.01. ††Significantly different from male vegetarian value, P < 0.01 (2-tailed paired Student’s t test).
Sample collection and analysis

During each experimental visit in study A, 1 mL arterialized venous blood was obtained every 5 min for monitoring the blood glucose concentration (YSI 2300 STATPlus; Yellow Springs Instruments) and an additional 5 mL arterialized venous blood was obtained every hour. Five milliliters of venous blood was also obtained from nonvegetarian control subjects in study A and in study B immediately before and 24 h after 1-carntine consumption. Two milliliters of this blood were collected into lithium heparin containers, and after centrifugation, the plasma was removed and immediately frozen in liquid nitrogen. These samples, together with 1-mL aliquots from the 24-h urine collections in study B were stored at −80°C and analyzed at a later date for TC concentrations by using a radioenzymatic assay previously described by Cederblad et al (14). The remaining blood was allowed to clot, and after centrifugation, the serum was stored frozen at −80°C. Insulin was measured in these samples at a later date with a radioimmunoassay kit (Coat-a-Count Insulin; DPC).

Muscle biopsy samples were obtained in study A from the vastus lateralis muscle immediately before and after each insulin clamp in the experimental visits by using the percutaneous needle biopsy technique (15) and were snap frozen in liquid nitrogen <5 s after removal from the limb. One portion of the sample was subsequently freeze-dried and stored at −80°C, and the remainder of the sample was stored wet in liquid nitrogen. After the removal of visible blood and connective tissue, freeze-dried muscle samples were powdered, and free carnitine, acetylcarnitine, long-chain acylcarnitine contents were determined radioenzymatically by using a modified version of the radioenzymatic method of Cederblad et al (14). Values were subsequently summed to calculate muscle TC.

Total RNA was extracted from 30 mg of the wet muscle tissue by homogenizing in ice-cold TRIzol reagent (Invitrogen Ltd) according to the method of Chomczynski and Sacchi (16). After spectrophotometric quantification at 260 nm, first-strand complementary DNA was generated from 0.5 μg of the RNA by using Moloney murine leukemia virus reverse transcriptase and stored at −80°C. Subsequent analyses involved a predesigned Taqman primer and probes for OCTN2 (Hs00161895) obtained from Applied Biosystems Assays on Demand and a real-time reverse transcriptase polymerase chain reaction performed with Taqman primer and probes for OCTN2 (Hs00161895) obtained from Applied Biosystems Assays on Demand and a real-time reverse transcriptase polymerase chain reaction performed with the use of an ABI PRISM 7000 sequence detection system (Applied Biosystems). Briefly, fluorescent emission data were captured and triplicate mRNA concentrations were quantified from a relative standard curve and normalized with endogenous HMBS (Hs00069297) to compensate for variations in input RNA amounts and the efficiency of reverse transcription. Cycle threshold values for HMBS were unchanged across time points and between vegetarian and nonvegetarian volunteers (data not shown).

In addition, total muscle protein homogenates were extracted from −30 mg of wet muscle by homogenization in a 50 mmol/L Tris buffer (pH 7.5) in the presence of protease inhibitors (Sigma-Aldrich) as previously described (17). After quantification by using the Bradford assay, 30 μg protein per lane were loaded onto a 4–12% Bis-tris acrylamide gel (Invitrogen) at 200 V for 1 h and transferred onto a polyvinylidene difluoride membrane. Thereafter, blots were probed with polyclonal anti-OCTN2 antibody (1:1000; goat anti-rabbit; Santa Cruz Biotechnology) overnight and incubated for 1 h with a IRDye 800CW secondary antibody (1:10,000; donkey anti-goat; LI-COR Biosciences), respectively. The protein content was measured in accordance with the manufacturer’s instructions with an Odyssey scanner (Application software version 3.0; Licor Biotechnology Ltd) and normalized to α-actin (Sigma-Aldrich) control, the protein content of which did not change across time points and treatments (data not shown).

Statistical analysis and calculations

Male vegetarian volunteers were compared with male nonvegetarian volunteers for all baseline measurements of urinary, plasma, and muscle TC content and OCTN2 mRNA expression by using an unpaired Student’s t test. All other data obtained during the studies were analyzed by using a 2-factor ANOVA (time and treatment effects; GraphPad Prism 5.0; GraphPad Software Inc). When a significant main effect was detected, data were further analyzed with a 1-factor ANOVA or Student’s t test by using Bonferroni correction to avoid a type 1 error. Two of the male and one of the female vegetarian volunteers did not complete both experimental visits, and 7 of the nonvegetarian volunteers only had a basil biopsy taken for the additional mRNA and protein measurements and, thus, were not included in the paired analysis. Significance was declared at P < 0.05, and all values presented in the text, tables, and figures are means ± SEMs.

RESULTS

Basal skeletal muscle TC

The content of basal skeletal muscle TC was 17% lower in vegetarian volunteers than in nonvegetarian volunteers (P < 0.05; Table 1, Figure 1).

![Figure 1](image.png)

**Figure 1.** Individual skeletal muscle (vastus lateralis) total carnitine content for male and female vegetarian volunteers and male nonvegetarian control subjects. Largers represent the mean (±SEM) carnitine content and duration of the vegetarian diet (n = 14 male control subjects, 7 male vegetarians, and 4 female vegetarians). *The male vegetarian muscle carnitine content was significantly lower than the male nonvegetarian muscle carnitine content, P = 0.011 (2-tailed unpaired Student’s t test), dm, dry muscle.
Serum insulin

After the 1-h equilibration period, the euglycemic insulin clamp calibrated at 5-mU euglycemic insulin · m⁻² · min⁻¹ produced similar steady state (1–6 h) serum insulin concentrations of 6.7 ± 0.4 and 6.2 ± 0.5 mU/L, whereas the euglycemic hyperinsulinemic clamp calibrated at 105-mU euglycemic insulin · m⁻² · min⁻¹ produced similar steady state serum insulin concentrations of 163.2 ± 5.9 and 181.3 ± 9.7 mU/L for nonvegetarians and vegetarians, respectively.

Plasma carnitine

The basal plasma carnitine concentration was 16% lower in vegetarian volunteers than in nonvegetarian volunteers in both studies (43.0 ± 1.7 compared with 50.9 ± 1.6 μmol/L, respectively; P < 0.01).

In study A, from similar basal plasma TC concentrations in the 2 experimental visits for nonvegetarian volunteers of 53.1 ± 2.5 and 56.5 ± 3.4 μmol/L for carnitine and carnitine + insulin, respectively, the plasma TC concentration increased after the onset of L-carnitine infusion in both visits, but it remained at a lower steady state concentration in the presence of hyperinsulinemia (525.7 ± 24.1 compared with 472.3 ± 19.7 μmol/L, respectively; P < 0.05, 1-factor ANOVA; Figure 2). Similarly, the L-carnitine infusion in vegetarian volunteers increased the plasma TC concentration in both visits from similar basal concentrations (39.4 ± 4.7 and 40.4 ± 4.2 μmol/L for carnitine and carnitine + insulin, respectively). However, the steady state plasma TC concentration remained the same in the absence and presence of hyperinsulinemia (456.4 ± 39.1 compared with 472.0 ± 42.6 μmol/L, respectively).

In study B, the plasma TC concentration was 18% lower in vegetarian than in nonvegetarian volunteers (41.2 ± 2.4 compared with 50.0 ± 2.3 μmol/L; Figure 3). However, although the plasma TC concentration was not different than the basal concentration 24 h after the ingestion of 18.6 mmol L-carnitine in nonvegetarians (53.3 ± 1.8 μmol/L), the plasma TC concentration increased in vegetarian volunteers 24 h after the ingestion of L-carnitine such that the plasma TC concentration was 18% greater than the basal concentration (48.6 ± 1.2 μmol/L; P < 0.05) and was similar to the nonvegetarian value.

Urinary carnitine

In study B, the basal 24-h urinary TC excretion was 58% lower in vegetarians than in nonvegetarians (P < 0.01; Table 1, Figure 3). Furthermore, the day after ingestion of 18.6 mmol L-carnitine, the urinary TC excretion remained 55% lower in vegetarians than in nonvegetarians (1242.7 ± 177.8 compared with 555.8 ± 56.3 mmol, respectively; P < 0.01).

Skeletal muscle carnitine after L-carnitine infusion

The skeletal muscle TC content during the L-carnitine–infusion visits in study A is presented in Figure 4. Five hours of intravenous L-carnitine in the absence of hyperinsulinemia had no effect on the muscle carnitine pool in nonvegetarian volunteers (22.9 ± 0.9 compared with 23.2 ± 1.1 mmol carnitine/kg dm), whereas the intravenous infusion of L-carnitine in the presence of hyperinsulinemia increased the muscle carnitine content by 15% (22.8 ± 0.7 compared with 26.3 ± 1.3 mmol carnitine/kg dm). However, 5 h of intravenous carnitine or carnitine + insulin infusion did not affect the skeletal muscle TC pool in vegetarian volunteers (17.9 ± 1.5 compared with 17.8 ± 1.3 mmol TC/kg dm and 18.7 ± 1.0 compared with 17.9 ± 1.0 mmol TC/kg dm, respectively).

Muscle OCTN2 mRNA and protein expression

The basal skeletal muscle expression of OCTN2 mRNA relative to HMBS mRNA was 33% lower (P < 0.05) in male vegetarian volunteers than in male nonvegetarian volunteers (Figure 5A). The relative OCTN2 mRNA expression did not change after each experimental visit (1.4 ± 0.4- and 1.6 ± 0.3-fold change from the preinfusion value for Carnitine and Carnitine + Insulin, respectively), and there were no differences between infusion visits in vegetarian volunteers. Furthermore, there was a trend (P = 0.09) for the basal skeletal muscle protein content of OCTN2 mRNA relative to α-actin to be 37% lower in
DISCUSSION

The aim of the current study was to test the hypothesis that vegetarians (lactoovovegetarians and vegans) have an increased capacity to retain supplemented L-carnitine to maintain normal skeletal muscle carnitine content and that this response is attributable to an enhanced muscle carnitine–transport capacity compared with that in omnivores. However, in contrast to this theory, we have shown, for the first time to our knowledge, that vegetarian skeletal muscle has a reduced capacity to uptake carnitine as evidenced by a lower muscle TC content, no change in the muscle carnitine content after an l-carnitine infusion during fasting or hyperinsulinemic conditions, and a lower OCTN2 mRNA and protein expression than in nonvegetarians. Thus, this would suggest that the greater whole-body carnitine retention observed after a single oral dose of l-carnitine in vegetarian volunteers in the current study, which was directly in line with the more-efficient renal conservation of carnitine in vegetarians reported in the literature, did not reside within muscle, which contains the largest store of carnitine in the body.

Almost all of the carnitine excretion in nonpathologic states occurs through the kidneys (2). In contrast to the relatively slow turnover in other tissues, circulating carnitine is highly conserved by the kidney with >95% of filtered free carnitine being reabsorbed (18). It has been suggested that, because vegetarian adults maintain plasma carnitine concentrations slightly lower than do nonvegetarians but excrete carnitine at less than one-half the rate of omnivores, vegetarians have a more efficient renal reabsorption of carnitine to prevent overt deficiency and low plasma carnitine concentrations (1, 19). Indeed, the basal plasma TC concentration and 24-h urinary TC content were ~15% and 60% lower, respectively, for vegetarian volunteers than for nonvegetarian volunteers in the current study. Furthermore, by infusing l-carnitine at variable rates in vegetarian volunteers before and after 60 d of l-carnitine consumption (248 μmol l-carnitine/d), Rebouche et al (1) showed that TC reabsorption was less at a given plasma TC concentration over a high physiologic range (55–80 μmol/L), which suggested that the efficiency of renal carnitine reabsorption was greater when dietary carnitine was infused in nonvegetarian volunteers compared with that in vegetarian volunteers (Figure 5B).

FIGURE 3. Mean (±SEM) plasma (A) and 24-h urinary (B) total carnitine (TC) in male nonvegetarian and vegetarian volunteers before and 24 h after ingestion of 4.5 g l-carnitine l-tartrate (which contained 3 g l-carnitine), *P = 0.0032 and †P = 0.016 for plasma TC time and treatment effects, respectively (2-factor ANOVA). P = 0.036, P < 0.0001, and P < 0.0001 for urinary TC interaction, time, and treatment effects, respectively (2-factor ANOVA). *Significantly different from nonvegetarian values, †Significantly different from the corresponding pre-l-carnitine ingestion values, ‡Significantly different from the corresponding pre-l-carnitine ingestion values for days 1 and 2 (P = 0.0098 and P = 0.0012, respectively; post hoc 2-tailed unpaired Student’s t test with Bonferroni correction); ***Significantly different from the corresponding pre-l-carnitine ingestion values for nonvegetarians and vegetarians (P = 0.0008 and P = 0.0003, respectively; post hoc 2-tailed paired Student’s t test with Bonferroni correction).

FIGURE 4. Mean (±SEM) skeletal muscle total carnitine contents in 7 male nonvegetarian and 8 vegetarian (5 men, 3 women) volunteers before (Pre) and after (Post) a 5-h intravenous infusion of l-carnitine in the presence of fasting (Carnitine) or hyperinsulinemic (Carnitine + Insulin) serum insulin concentrations. *P = 0.047 and †P = 0.027 for nonvegetarian muscle total carnitine interaction and time effects, respectively (2-factor ANOVA). *The Carnitine + Insulin Post value was significantly greater than the corresponding Carnitine Post value (P = 0.021, post hoc 2-tailed paired Student’s t test with Bonferroni correction); †The Carnitine + Insulin Post value was significantly greater than the corresponding Carnitine + Insulin Pre value (P = 0.024, post hoc 2-tailed paired Student’s t test with Bonferroni correction). dm, dry muscle.
carnitine intake was less. Indeed, 24 h after the ingestion of 18.6 mmol l-carnitine in the current study, the urinary TC excretion was 55% less in vegetarian volunteers than in nonvegetarian volunteers, despite similar plasma TC concentrations. In agreement with these findings are the observations that chronic l-carnitine feeding in rats resulted in a diminished capacity for carnitine transport into brush border membrane vesicles (20) and reduced OCTN2 transporter protein content compared with controls (21). Taken together, these results suggest that the carnitine reabsorptive capacity of vegetarians adapted to a very low L-carnitine diet such that they were more efficient at retaining carnitine within the body. Thus, the following question remains: Why did we not observe greater muscle retention of TC after intravenous l-carnitine administration in vegetarian volunteers in the current study?

In contrast to low circulating plasma carnitine concentrations, under normal conditions the TC concentration in some tissues can be >100-fold more and appears to parallel the capacity of the tissue to metabolize fatty acids. Thus, dietary derived or endogenously synthesized carnitine is transported into skeletal muscle against a considerable concentration gradient via the saturable, Na⁺-dependent, high-affinity, active-transporter OCTN2 (6). In keeping with the hypothesis that insulin promotes Na⁺-dependent skeletal muscle carnitine transport secondary to its action of increasing the sarcomlemmal Na⁺/K⁺ ATPase pump activity, and thereby the intracellular Na⁺ flux, 5 h of hypercarnitinemia (>500 µmol/L) combined with hyperinsulinemia (∼160 mU/L) in the current and previous studies (21) increased the skeletal muscle TC content by ~15% in healthy nonvegetarian volunteers. Furthermore, the increase in muscle TC content observed after hypercarnitinemia combined with hyperinsulinemia in the current and previous studies (22) was associated with a lower steady state plasma TC concentration than that after hypercarnitinemia alone [ie, which showed that the plasma carnitine clearance into skeletal muscle was greater (23)]. However, although the intravenous infusion of l-carnitine produced hypercarnitinemia in vegetarian volunteers in the current study, it had no effect on the muscle TC content in the absence or presence of hyperinsulinemia, and there was no difference in the steady state plasma TC concentration between the carnitine and carnitine + insulin visits. Thus, if vegetarians are more efficient at conserving supplemented l-carnitine within the body, then the retention must reside in body tissues other than in muscle because it appears that vegetarian skeletal muscle is insensitive to an acute (5 h) elevation in circulating carnitine. In addition, because vegetarians have a greater rate of endogenous carnitine synthesis (1, 2), one could speculate that carnitine synthesizing tissues such as the liver and kidney (24) would have an adequate store, and thus, carnitine would not be diverted to these tissues.

Another finding of the current study was that the lack of uptake of l-carnitine into the skeletal muscle of vegetarian volunteers was possibly due to a reduced muscle content of OCTN2 as evidenced by 33% and 37% lower OCTN2 mRNA and protein expression than the expression in nonvegetarian volunteers. If so, this would be in agreement with the recent finding that the l-carnitine uptake capacity of rat skeletal muscle was directly proportional to the amount of muscle OCTN2 protein (8). We have previously observed in nonvegetarian volunteers (10) that the OCTN2 mRNA expression was increased 2.3-fold during l-carnitine infusion in the presence of hyperinsulinemia where the muscle TC content increased by 15%. The lack of an effect of l-carnitine infusion during hyperinsulinemia on the muscle TC content and OCTN2 mRNA expression in vegetarian volunteers in the current study perhaps suggested that the intracellular carnitine concentration regulated the OCTN2 mRNA expression. However, whether this was the case in other tissues requires additional investigation, particularly because the OCTN2 content has been reported to be greater in the kidneys of rats that were chronically fed a low compared with high l-carnitine diet (21). Furthermore, if the body’s adaptation to a low l-carnitine diet is to divert carnitine away from muscle to meet the carnitine requirements of noncarnitine synthesizing vital organs such as the heart, then such tissues may also have a greater OCTN2 content. Indeed, steady state plasma TC concentrations during both infusion visits in vegetarian volunteers were similar to those observed in nonvegetarian volunteers during hyperinsulinemia (ie, lower than the steady state concentrations observed during the nonvegetarian carnitine visit), which may support the premise that carnitine was retained in...
a tissue other than in skeletal muscle in vegetarian volunteers. This possibility could also explain why plasma TC concentrations were not different the day after L-carnitine ingestion in vegetarian and nonvegetarian volunteers, despite a 55% lower urinary carnitine excretion.

In conclusion, in contrast to our hypothesis, it appeared that healthy vegetarian volunteers had a reduced capacity to uptake carnitine into the skeletal muscle (which is the main store of carnitine within the body) perhaps because of an adaptation of OCTN2 to lower muscle carnitine stores to conserve carnitine for other tissues. These findings could have important implications for patients on long-term carnitine-free parenteral nutrition or hemodialysis treatment who become carnitine deficient over time (25–27). With this in mind, one of the participants in the current study returned to an omnivorous diet after 11 y as a vegetarian and his plasma and muscle TC values had returned to normal after 6 mo. However, whether reduced muscle carnitine content in vegetarian volunteers has an effect on physiologic functions requires additional investigation, particularly because the muscle carnitine availability is rate limiting for fat oxidation and carbohydrate flux during exercise (5).

The authors’ responsibilities were as follows—FBS: planned and designed both studies; conducted study A; collected, analyzed, and interpreted data from both studies; and drafted the manuscript; MK: conducted, collected, and analyzed data in study B; and critically revised the manuscript. None of the authors had a conflict of interest.

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