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Research Communications

Tissue carnitine fluxes in vitamin C depleted-repleted guinea pigs

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The biosynthesis of carnitine requires vitamin C as a cofactor for two separate hydroxylation steps. The majority of body carnitine (approximately 98%) is located in muscle and less than 0.5% is present in plasma. We examined the physiologic dynamics of plasma free carnitine and muscle total acid-soluble carnitine in vitamin C-depleted guinea pigs repleted with increasing amounts of vitamin C. Animals were fed a vitamin C-deficient diet for 3 weeks at which time symptoms of scurvy were evident. Animals were repleted with increasing doses of vitamin C, from 0.5 to 10.0 mg vitamin C/100 g body weight daily. Muscle total acid-soluble carnitine concentrations tended to correlate directly with plasma vitamin C ($r = 0.41$, $P = 0.087$*) during the repletion phase of the study. Conversely, plasma free carnitine was inversely related to liver vitamin C (*$r = -0.54$, $P = 0.020$*) and to muscle total acid-soluble carnitine (*$r = -0.56$, $P = 0.015$*). Mean plasma free carnitine values fell 30% over the course of vitamin C repletion (*$P > 0.05$*) and mean muscle total acid-soluble carnitine rose by 30% (*$P > 0.05$*). These data suggest that elevated plasma free carnitine may indicate a low to marginal vitamin C status.* (J. Nutr. Biochem. 10:696–699, 1999)

Keywords: guinea pigs; vitamin C deficiency; plasma carnitine; muscle carnitine

Introduction

Carnitine is necessary for the transport of long-chain fatty acids into the mitochondria for oxidation to acetyl coenzyme A (CoA).[1,2] In addition, transfer of acetyl carnitine from intra- to extra-mitochondrial space increases the availability of mitochondrial free CoA thereby promoting the flux of substrates into the trichloroacetic acid (TCA) cycle.[3–5] Accordingly, carnitine plays an important role in substrate oxidation.[6] In well nourished adults the human requirement for carnitine can be met almost entirely from endogenous synthesis.[7,8]

Vitamin C is a required cofactor for the two hydroxylation steps in the biosynthesis of carnitine,[9–12] and vitamin C deficiency is associated with reduced total body carnitine. Hughes et al.[13] demonstrated that guinea pigs fed marginal amounts of vitamin C had 50% less carnitine in skeletal muscle relative to vitamin C-saturated animals. These investigators postulated that carnitine depletion was responsible for the early emergence of fatigue and lassitude noted in the initial phase of vitamin C deficiency. Similarly, Ciman et al.[14] and Nelson et al.[15] reported that scorbutic guinea pigs had 50% less carnitine in heart and skeletal muscle compared with pair-fed controls. Conversely, plasma free carnitine, which is easily accessed but represents less than 0.5% of total body carnitine, was elevated in individuals with poor vitamin C status.[15–17] Urinary carnitine was also elevated in vitamin C-depleted guinea pigs[18] and humans,[19] and carnitine excretion in urine has been directly related to plasma carnitine concentrations.[20] These data are perplexing because they indicate that plasma free carnitine and urinary carnitine concentrations are elevated while carnitine biosynthesis is depressed and muscle carnitine concentrations are reduced.

To better understand the relationship between plasma and muscle carnitine concentrations relative to vitamin C

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status, we examined fluctuations in plasma free carnitine and muscle total acid-soluble carnitine in vitamin C-depleted guinea pigs repleted with increasing dosages of vitamin C.

Materials and methods

Animals and study design

Thirty-six male guinea pigs weighing approximately 150 g each (Charles River Laboratories, Wilmington, MA USA) were housed individually in wire-meshed cages in a temperature-regulated (22°C), light-controlled (light from 7:00 AM to 7:00 PM) room. Throughout the study, all animals consumed a convenient scorbutogenic diet, a nonpurified diet designed for rabbits (Co-op Rabbit Family Pellets, Southwest Co-operative Wholesale, Phoenix, AZ USA), and water ad libitum. This plant-based diet contained only trace amounts of carnitine (approximately 3 μM). The study was approved by the Institutional Animal Care and Use Committee at Arizona State University, which follows the guidelines of the National Institutes of Health on the humane use of laboratory animals.

During the initial 3-day acclimation period, all animals received a single, oral dose of L-ascorbic acid (0.5 mg/100 g body weight daily) that was adequate for growth and the prevention of scurvy.[21] Following the acclimation period, vitamin C was withheld from all animals, and five animals were sacrificed weekly for tissue vitamin C and carnitine analyses. After 3 weeks of vitamin C restriction, animals were classified as scorbutic based on plasma and liver vitamin C analyses and physical appearance. All remaining animals were repleted for 4 weeks with a marginal dose of vitamin C (0.5 mg/100 g body weight daily) that was adequate to support growth. Three of these animals did not recover from scurvy and were euthanized; all other animals recovered fully from scurvy as indicated by weight gain and appearance, and five of these animals were sacrificed for tissue analyses. This depletion protocol assured a uniform vitamin C-depleted state among animals prior to the initiation of vitamin C repletion.

In the second 4-week period of repletion, the vitamin C dosage was increased to 2.0 mg/100 g body weight per day, a level that promotes odontoblast growth, wound healing, bone regeneration, and immune responsiveness.[21] Five animals were sacrificed for tissue analyses at the end of this period, and the remaining animals ($n = 8$) were divided into two dietary treatment groups and received an oral dose of either 6.0 or 10.0 mg vitamin C/100 g body weight daily. After 4 weeks of this dietary regimen, animals were sacrificed for tissue analyses.

Tissue analyses

All tissue samples were tested in duplicate. Plasma anticoagulated with ethylenediaminetetraacetic acid (EDTA) was deproteinized and stabilized in equal volumes of ice-cold 10% TCA, and freshly excised liver was homogenized in 9 volumes of ice-cold 6% TCA. Supernatants from these preparations were frozen (−45°C) and analyzed within 1 week for vitamin C using the dinitrophenylhydrazine reagent.[22] Plasma free carnitine in undiluted samples was measured radiochemically.[23,24] The entire gastrocnemius muscle was extracted from the hind leg, immediately frozen in liquid nitrogen, and stored at −45°C. Frozen muscle (0.25 mg) was homogenized in 1.5 mL ice-cold 3% perchloric acid. The supernatant, which contained all acid-soluble carnitine (free and short-chain acyl carnitines), was analyzed for carnitine following alkalinization and neutralization.

Statistical analyses

Data are reported as the mean ± SEM. Differences between means were determined by one-way analysis of variance and the post-hoc Tukey test. Pearson's correlation coefficients were calculated to examine the relationship between variables. A P-value of 0.05 was considered significant. The Statistical Package for the Social Sciences (SPSS/PC+, SPSS, Inc., Chicago, IL USA) was utilized for all statistical analyses.

Results

At the end of the 3-week depletion period, plasma vitamin C was not detectable, and the mean liver vitamin C concentration (0.143 ± 0.029 μmol/g) was indicative of scurvy.[25] Mean muscle total acid-soluble carnitine at the end of depletion (308.8 ± 61.9 nmol/g) was well below previously reported values for healthy guinea pigs (715–840 nmol/g),[9,26] and mean plasma carnitine was 55.3 ± 8.2 μmol/L.

Differences in plasma and liver vitamin C and in plasma free carnitine and muscle total acid-soluble carnitine as a function of the vitamin C repletion dosages (0.5–10 mg/100 g body weight) are depicted in *Figure 1*. Compared with vitamin C-depleted animals [i.e., animals consuming the minimal dosage of vitamin C for growth (0.5 mg/100 g body weight)], animals ingesting higher dosages of vitamin C (2–10 mg/100 g body weight) had significantly higher concentrations of vitamin C in plasma and liver (*Figure 1*). Liver and plasma vitamin C concentrations were significantly correlated ($r = 0.65$, $P = 0.004$).

Mean plasma free carnitine and muscle total acid-soluble carnitine did not vary significantly over the course of the study (*Figure 1*). Although muscle total acid-soluble carnitine tended to correlate with plasma vitamin C ($r = 0.41$, $P = 0.087$), plasma free carnitine concentrations were inversely associated, but not significantly, with plasma vitamin C concentrations ($r = -0.26$, $P = 0.30$). Plasma free carnitine was inversely related to liver vitamin C concentrations ($r = -0.54$, $P = 0.020$) and to muscle total acid soluble carnitine ($r = -0.56$, $P = 0.015$).

Discussion

The biosynthesis of carnitine requires vitamin C as a cofactor for two separate hydroxylation steps catalyzed by α-ketoglutarate-coupled dioxygenases: the hydroxylation of 6-N-trimethyl-L-lysine to 3-hydroxy-6-N-trimethyl-L-lysine and the hydroxylation of γ-butyrobetaine to L-carnitine. The data reported herein indicated that muscle total acid-soluble carnitine concentrations were directly related to vitamin C status in vitamin C-depleted–repleted guinea pigs. However, plasma free carnitine, derived from hepatic conversion of γ-butyrobetaine to L-carnitine, was inversely related to muscle total acid-soluble carnitine.

We previously demonstrated that mean plasma free carnitine concentrations were elevated in human subjects with marginal vitamin C status [58.0 ± 26.0 to 61.7 ± 15.8 μmol/L[16] (normal range: 21–53 μmol/L[27–29])]. The mean plasma free carnitine in these subjects did not normalize when diets were supplemented with 30 or 60 mg vitamin C per day[16]; however, mean plasma free carnitine did decrease

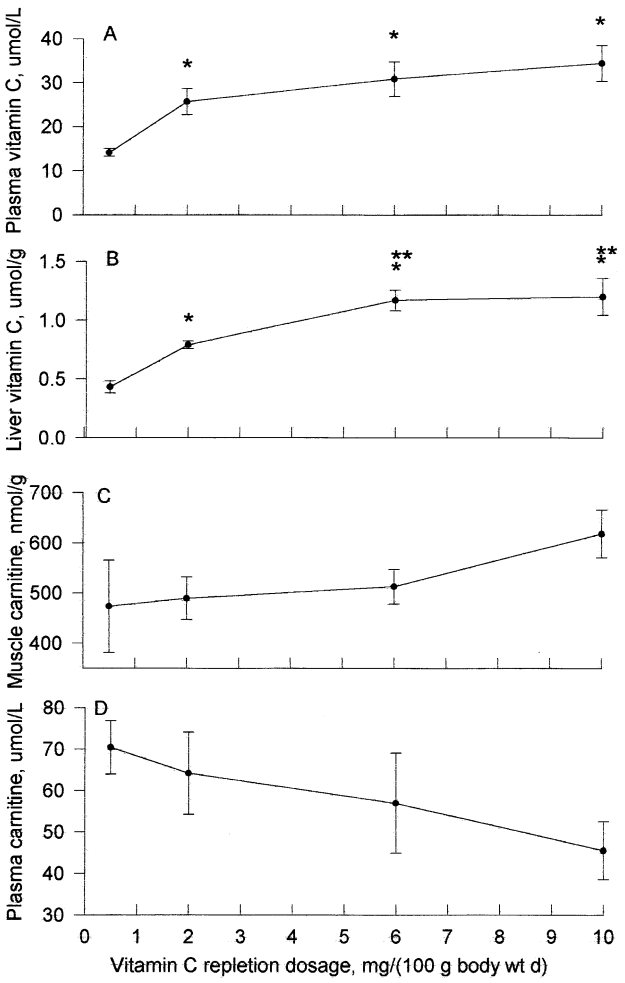

Figure 1 Relationship between *(A)* plasma vitamin C, *(B)* liver vitamin C, *(C)* muscle total acid-soluble carnitine, and *(D)* plasma free carnitine in vitamin C-depleted guinea pigs repleted with increasing doses of vitamin C (2, 6, and 10 mg/100 g body weight daily). Values are expressed as means ± SEM, $n = 4$ to 5 animals. *Significant differences versus the 0.5 mg dosage. **Significant differences versus the 2 mg dosage.

significantly when the diet was supplemented with 125 mg vitamin C daily. In a separate report, mean plasma free carnitine fell from 58.1 ± 4.7 to 47.2 ± 3.7 μmol/L ($P < 0.05$) in subjects with marginal vitamin C status following vitamin C repletion (500 mg vitamin C daily for 2 weeks).[17] Additionally, Nelson et al.[15] reported elevated plasma total carnitine in scorbutic guinea pigs versus controls; nonetheless, contradictory findings have been reported.[18]

The present data suggest that elevated plasma free carnitine may indicate a marginal vitamin C status, a subclinical condition short of scurvy. The mechanism for this phenomenon (elevated plasma free carnitine concurrent with reduced muscle carnitine) has not been explored. We speculate that transport of free carnitine into muscle is hindered in the vitamin C-depleted state as a consequence of γ-butyrobetaine build-up in muscle inhibiting carnitine transport into muscle.[9,30,31] This inverse relationship between plasma and muscle carnitine levels may not extend to a scorbutic state given the severity of the vitamin C-deficient state and the presence of confounding factors such as inanition.[7]

Interestingly, Jacob and Pianalto[19] observed no change in plasma *total* carnitine concentrations in human subjects depleted and repleted with vitamin C. Total carnitine encompasses acyl-carnitines formed mainly during muscle metabolism as well as free carnitine derived from hepatic synthesis. The acyl carnitine to free carnitine ratio in plasma is likely to be high when vitamin C status is sufficient because muscle carnitine would be available to transport acyl groups out of muscle. If vitamin C status is marginal, muscle carnitine is reduced as much as 50% and less acylcarnitine would be generated during muscle metabolism.[14] Hence, the decrease in free carnitine noted in vitamin C-depleted individuals repleted with vitamin C may be masked if only total carnitine was measured because acyl carnitines would rise in this instance.

As a further consideration, recent investigations indicated that vitamin C deficiency was associated with metabolic changes similar to those that occur in an acute phase response.[32,33] Alterations in carnitine metabolism, including a rise in urinary excretion of carnitine, occur during the acute phase response.[34,35] These changes in carnitine metabolism were associated with altered substrate oxidation (reduced fat oxidation), which is characteristic of the catabolic state of injury/immunologic stress.[34] Thus, vitamin C deficiency may impact carnitine homeostasis by two separate mechanisms: reduced carnitine biosynthesis and elevated carnitine excretion. Rebouche[18] reported that a fourfold increase in carnitine excretion in scorbutic guinea pigs corresponded to a 15% decrease (as percent of filtered load) in the rate of carnitine reabsorption.

Reduced muscle carnitine is associated with muscle weakness and, hence, may be responsible for the fatigue noted in vitamin C-depleted patients. We recently demonstrated that work efficiency during a submaximal exercise bout was significantly improved in vitamin C-depleted individuals following vitamin C supplementation (500 mg/d for 2 weeks).[17] Vitamin C repletion also was associated with a significant reduction in plasma free carnitine concentrations in these individuals.

In summary, it is unequivocal that muscle carnitine levels are reduced in vitamin C deficiency; however, fluxes in plasma carnitines as a function of vitamin C status are not well documented. The present report provides evidence that plasma free carnitine is inversely related to both muscle total acid soluble carnitine and vitamin C nutriture. Further research is necessary to delineate the physiologic mechanisms responsible for the elevation in plasma free carnitine, as well as the reported enhanced rate of carnitine excretion, in vitamin C-depleted subjects.

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