Role of carnitine acetylation in skeletal muscle

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Received: March 27, 2014 / Accepted: April 3, 2014

Abstract Carnitine is known for its role in the transport of long-chain fatty acids into the mitochondrial matrix for subsequent β-oxidation. In addition, carnitine acts as an acceptor of excess acetyl-CoA and forms acetylcarnitine to relieve inhibition of pyruvate dehydrogenase. Recent studies have demonstrated that carnitine acetylation is essential for glucose homeostasis, and its dysfunction induces metabolic failure. Furthermore, it has been suggested that acetylcarnitine might be exported from skeletal muscle into the blood. Considering that acetylcarnitine is a bioactive molecule involved in glucose metabolism and neuroprotection, we expect that acetylcarnitine production is beneficial to the body. In this article, we reviewed recent knowledge on the role of carnitine acetylation in glucose metabolism within skeletal muscle. Furthermore, this article introduces acetylcarnitine as a physiologically active substance and discusses carnitine dynamics during exercise.

Keywords: acetylcarnitine, mitochondria, fat oxidation, muscle contraction, carnitine transporter

Introduction

Carnitine is an essential nutritional supplement that is required for the transport of long-chain fatty acids into the mitochondrial matrix for subsequent β-oxidation to produce energy. This has been the focus of numerous recent studies. Many researchers have proposed that carnitine supplementation improves skeletal muscle function and athletic performance in healthy individuals by an increase in fatty acid utilization. However, there is no convincing evidence showing that carnitine supplementation has an enhancing effect on human metabolism and exercise performance. Rather, recent reports show that carnitine supplementation has a therapeutic effect on skeletal muscle metabolism in diabetic patients. This effect is attributed to another role of carnitine, namely, buffering excess carbon substrate in the cells. In the mitochondrial matrix, carnitine acts as an acceptor of excess acetyl-CoA, which is a potent inhibitor of pyruvate dehydrogenase (PDH), in the form of acetylcarnitine. Interestingly, it is demonstrated that acetylcarnitine is exported from skeletal muscle cells and is a potential biological activator. In this manuscript, we have focused on the role of carnitine acetylation in skeletal muscle and reviewed recent publications on the subject.

Two major roles of carnitine in skeletal muscle metabolism

Transport of long-chain fatty acids into the mitochondria.

Long-chain acyl-CoA taken up from the blood plasma must be transported across the impermeable mitochondrial inner membrane for subsequent β-oxidation. Here, acyl-CoA is converted to acylcarnitine and translocated across the mitochondrial membrane via the carnitine shuttle (shown with green arrows in Fig. 1). Carnitine palmitoyltransferase 1 (CPT1), located in the outer mitochondrial membrane, catalyzes the reaction between carnitine and acyl-CoA to form acylcarnitine. Cytosolic acylcarnitine is then transported into the mitochondria by carnitine palmitoyltransferase 2 (CPT2). Acyl-CoA then undergoes β-oxidation to produce acetyl-CoA.

Regulation of the acetyl-CoA/free-CoA ratio by buffering excess acetyl-groups.

Another major role of carnitine in skeletal muscles is to regulate the acetyl-CoA/free CoA ratio by buffering excess acetyl groups. In cases where the rate of substrate catabolism during β-oxidation exceeds the capacity of acetyl-CoA utilization in the tricarboxylic acid (TCA) cycle, short-chain acyl-CoA intermediates accumulate in the muscle cell mitochondria, thereby depleting free CoA. Since free CoA serves as a substrate for fat and glucose oxidation, reduction of free CoA results in
the loss of the ability of continuous muscle contraction. This situation is reversed by carnitine acetyl-transferase (CrAT), present within the mitochondrial matrix. CrAT catalyzes the reversible conversion of acetyl-CoA and carnitine into acetylcarnitine and free CoA, thus creating a buffer system that maintains appropriate levels of acetyl-CoA and CoA in the mitochondrial matrix (indicated with red arrows in Fig. 1).

Carnitine acetylation contributes to glucose metabolism homeostasis

CrAT relieves inhibition of PDH induced by acetyl-CoA accumulation. In addition to maintenance of a free CoA pool, CrAT also contributes to metabolic flexibility by preventing acetyl-CoA accumulation. Although acetyl-CoA is an essential intermediate metabolite that enters the TCA cycle and is oxidized to yield energy, its accumulation inhibits glucose metabolism. Acetyl-CoA itself acts as a potent allosteric inhibitor of PDH, a rate-limiting...
enzyme for pyruvate entry into the TCA cycle\(^9\) (shown in blue in Fig. 1). Inhibition of PDH activity was presumed to slow down the glycolytic flux, resulting in negative feedback on glucose uptake. It occurs during increasing energy production, such as during high-intensity exercise, a change from fasting to feeding state, or in a chronically over-fed state\(^7\). In these situations, CrAT converts acetyl-CoA to membrane-permeable acetylcarnitine and controls mitochondrial acetyl-CoA levels. Produced acetylcarnitine is then transported to the cytosol via CACT activity.

Since deficiency of mitochondria is involved in insulin resistance in skeletal muscle, CrAT has gained popularity as a key regulator of glucose homeostasis in skeletal muscle\(^8\). Muoio et al. (2012) generated muscle-specific CrAT knockout mice and showed that CrAT regulates PDH activity and enhances insulin action by permitting efflux of excess acetyl-CoA from mitochondria\(^3\). In healthy skeletal muscle, excess acetyl-CoA is converted to membrane-permeable acetylcarnitine and exported into the bloodstream. However, in the CrAT-null muscle, acetyl-CoA is accumulated in mitochondria, and glucose oxidation is diminished by inhibition of PDH activity\(^3\). More recently, it was reported that CrAT activity diminished in muscles from obese and diabetic rodents, suggesting a pathophysiological role of CrAT\(^9\). Taken together, the data indicate that CrAT balances the supply and demand of energy in mitochondria, contributing to metabolic homeostasis.

**Carnitine treatment improves glucose homeostasis via CrAT function.** Many researchers have focused on the role of carnitine in the transfer of fatty acids into mitochondria and proposed that carnitine supplementation enhances fat oxidation following the increase in carnitine concentration within muscle cells. However, most studies showed that carnitine supplementation did not increase carnitine levels in skeletal muscle cells\(^2\,10\). Since the carnitine concentration in skeletal muscle (2–4 mmol kg\(^{-1}\) wet muscle weight) is approximately 100 times higher than that in the blood\(^9\), carnitine should be transported into muscle cells against a considerable concentration gradient. Carnitine uptake into skeletal muscle cells is mediated by a sodium-dependent high-affinity transporter—organic cation transporter system (OCTN2/SLC22A5)\(^12\). Thus, the increase in plasma carnitine delivery per se (i.e., increased blood flow) does not account for an increase in carnitine influx into skeletal muscles.

Although carnitine supplementation has no obvious effect on healthy humans and animals, it has been shown that carnitine treatment was beneficial in diabetic subjects\(^13\,11\). In diabetic mice, chronic carnitine supplementation increased insulin sensitivity despite having no effect on normal mice\(^13\). In human pilot experiments, 6 months of carnitine supplementation increased glucose homeostasis in the insulin-resistant group, whereas there was no effect in the insulin-sensitive group\(^3\). Since these therapeutic effects of carnitine were accompanied by PDH activity, it is assumed that supplemental carnitine could reverse lipid overload and glucose intolerance in diabetic muscle cells. It remains unclear however, as to why carnitine supplementation is effective in diabetic patients but not in healthy humans; further studies are required to clarify this question.

**Acetylated carnitine is exported from skeletal muscle cells**

It has been debated whether acetylcarnitine is transported across the skeletal muscle plasma membrane. Since acetylcarnitine could be reversibly converted to acetyl-CoA by CrAT, acetylcarnitine is often assumed to be stored in skeletal muscle as a readily available substrate for utilization in the TCA cycle\(^10\). However, early studies have shown that carnitine is released as acetylcarnitine in heart cells\(^9\,10\). In addition, Noland et al. (2009) reported direct evidence that acetylcarnitine was detected in the conditioned media from cultured muscle cells, indicating that acetylcarnitine is exported from skeletal muscle cells\(^3\). They also demonstrated that overexpression of CrAT increased acetylcarnitine production and efflux. Taken together, CrAT inhibits acetyl-CoA accumulation by regulating the export of acetyl-CoA as acetylcarnitine and contributes to the regulation of PDH activity.

Although carnitine uptake in skeletal muscle is well studied, it remains unclear how acetylcarnitine is transported across the muscle plasma membrane. The sarcolemmal membrane is impermeable to carnitine species, indicating that specific transport systems must exist. There are two candidate proteins for acetylcarnitine transporters\(^17\). Along with carnitine uptake, OCTN2 is able to transport acetylcarnitine\(^18\,20\), thus OCTN2 has the potential to interchange carnitine and acetylcarnitine. Monocarboxylate transporter 9 (MCT9) is also reported as a carnitine efflux transporter\(^21\). In this study, tritium-labeled carnitine was injected in oocytes, and carnitine efflux was found to be higher in transfected oocytes expressing MCT9 than in the non-transfected cells. However, there are no reports to show the expression of MCT9 in skeletal muscles; thus, the specific protein involved in carnitine export in skeletal muscle is unknown.

The physiological purpose of acetylcarnitine efflux from skeletal muscle is undefined. Acetylcarnitine itself is a biologically active substance. For instance, acetylcarnitine supplementation in diabetic patients resulted in increased glucose disposal and glucose storage in skeletal muscle\(^21\). Moreover, in cell culture experiments, acetylcarnitine inhibited tumor necrosis factor-α (TNF-α)-induced insulin resistance via the 5’AMP-activated kinase (AMPK) pathway\(^21\). These reports suggest that exported acetylcarnitine has autocrine or paracrine effects on skeletal muscle. In addition, acetylcarnitine is widely known to
have beneficial effects on the brain\textsuperscript{25}. Acetylcarnitine can readily cross the blood–brain barrier\textsuperscript{26}, therefore, plasma acetylcarnitine could have an effect on metabolism in the brain. Indeed, several studies have shown that acetylcarnitine treatment improves neurological health, possibly by normalizing energy metabolites in the brain\textsuperscript{27-29}. Moreover, acetylcarnitine has therapeutic potential in the treatment of various neurological disorders, including chronic fatigue syndrome\textsuperscript{30}, age-related memory loss\textsuperscript{31}, and Alzheimer’s disease\textsuperscript{27}. These observations imply that acetylcarnitine may have effects on organ systems apart from skeletal muscle via inter-organ transport of carnitine (Fig. 2).

Carnitine dynamics during muscle contraction

Carnitine metabolism during exercise (muscle contraction) has received attention, since carnitine plays an important role in energy production within contracting muscle. During high-intensity exercise, there is a rapid decrease in carnitine levels in the skeletal muscle, as carnitine is converted to acetylcarnitine\textsuperscript{14}. Such acetylation depletes the carnitine pool; thus, carnitine availability might be the rate-limiting factor for fat oxidation in skeletal muscles\textsuperscript{32-34}.

Although intracellular carnitine metabolism during exercise is well studied, its transport across the plasma membrane is not fully clarified. We have recently demonstrated that electrically induced muscle contraction amplified carnitine uptake into rat skeletal muscle \textit{in vivo}, but this uptake was independent of changes in blood flow\textsuperscript{35}. Since carnitine uptake is dependent on expression levels of OCTN2\textsuperscript{32}, the upregulation of carnitine transport during muscle contraction could be induced by redistribution of OCTN2 on the surface of skeletal muscles. As free carnitine availability in muscle cells becomes limited to CPT1 during exercise\textsuperscript{10}, the increase in carnitine turnover within contracting muscle is likely to maintain availability of free intracellular carnitine to sustain fatty acid oxidation in muscle.

Interestingly, some studies have revealed that plasma acetylcarnitine concentration increased during exercise, indicating an efflux of acetylcarnitine from the muscles\textsuperscript{33,36,37}. As described above, acetylcarnitine has shown biological activity in skeletal muscle and the brain. Thus, skeletal muscle may produce useful metabolites that have a positive effect on overall health. However, since mechanisms of carnitine export from the muscle cell during contraction have not been demonstrated, further investigations are required. These hypotheses are summarized in Fig. 2.

Acknowledgments

This review was supported by a Grant-in-Aid for JSPS Fellows (#24-173, YF) from the Japanese Ministry of Education, Science, Sports and Culture, and Funding Program for Next Generation World-Leading Researchers LS102 to NLF.

![Skeletal Muscle](image)

**Fig. 2** Carnitine dynamics between skeletal muscle and the brain during exercise (muscle contraction). Carnitine is imported by OCTN2 and used for fatty acid transport across the mitochondrial membrane. When energy production exceeds the capacity of mitochondrial utilization, carnitine is converted to acetylcarnitine by the mitochondrial enzyme CrAT. The acetylcarnitine produced is exported from skeletal muscle cell into the bloodstream. Muscle contraction increases both carnitine uptake and its acetylation. Although there is no direct evidence, plasma acetylcarnitine levels increase during exercise. Acetylcarnitine has beneficial effects, namely, glucose homeostasis in skeletal muscles and neuroprotection in the brain.
References


30) Vermeulen RCW. 2004. Exploratory open label, randomized


