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Review

L-carnitine's role in KAATSU training- induced neuromuscular fatigue

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ABSTRACT

KAATSU training at greatly reduced intensities has been proven to result in substantial increases in both muscle hypertrophy and strength. Nevertheless, this revolutionary training method (combined with the restriction of venous blood flow from the working muscle) may cause underlying hypoxia and neurotransmitter dysfunction, which are linked to neuromuscular fatigue. Hence, an exploration of KAATSU training-induced hypoxic and neurodegenerative events is of utmost importance before promoting this training mode, although KAATSU has been shown to result in numerous positive training adaptations. Furthermore, based on substantial evidence, L-carnitine supplementation exerts neuroprotective effects by attenuating hypoxic stress and neurotransmitter dysfunction. However, studies directly examining the effects of KAATSU exercise on both hypoxia and neurotransmitter dysfunction, which would aggravate the detrimental effects of neuromuscular fatigue, are lacking. In addition, an expansion of the applications of L-carnitine to a smaller-molecule field for treating KAATSU training-evoked neuromuscular fatigue requires further clarification. Therefore, this review aims to present the current evidence for the effectiveness of exogenous L-carnitine at reducing the amount of hypoxic damage and its neuroprotective effects mediated by increasing cerebral acetylcholine levels. Simply, L-carnitine administration may be an important contributor to the mechanisms curtailing KAATSU training-induced neuromuscular fatigue.

1. Introduction

KAATSU is derived from the combination of the Japanese words for 'additional' (ka) and 'pressure' (atsu). KAATSU training, also known as blood flow restriction (BFR) training or vascular occlusion (VO) training [1], is a patented training method that originated in Japan and was developed by Dr. Yoshiaki Sato during the 1960s [2]. KAATSU began to be practiced as a promising alternative to conventional training methods in the field of sports training in the 1970s and 1980s [3–6]. When KAATSU training is conducted, the blood flow to the exercising muscle is restricted or occluded by thin, computer-controlled, pressurized external constricting devices, such as pneumatic cuffs or inflated tourniquets, which are placed at the most proximal part of the arms or legs to reduce the amount of blood flowing back from the muscles in the extremities during a workout [3,5,7,8]. Within this framework of the training methodology, the participants, including professional athletes or even Olympic medalists who undergo KAATSU training combined with significantly lower intensity training protocols and short-term resistance exercise, are able to attain measurable increases in both skeletal muscle strength and hypertrophy that are comparable to the results of conventional heavy resistance training. Typically, standard conventional training conducted at an intensity of approximately 70–85% of one-repetition maximum (1-RM) is normally recommended to achieve a similar acute training stimulus [9]. Similarly, in the study by Fujita et al. [10], approximately 20 % of the 1RM intensity of KAATSU training compensated for 70-80% of the gains achieved with 1RM high-intensity traditional resistance training, which was assessed using magnetic resonance imaging (MRI).

As the implications of the effects of KAATSU training on muscle hypertrophy and strength gains with low-intensity loads and short-term training time among athletes are profound, this revolutionary training method is attracting increasing attention from fitness researchers and has been investigated in many studies. Notably, the restriction of blood flow is considered the potential cause of neuromuscular fatigue syndrome, which is linked to hypoxia [11] and neurotransmitter dysfunction [12], although organic neuromuscular fatigue is a complex phenomenon that may be caused by a combination of multiple factors. Thus, the effects of KAATSU resistance exercise on hypoxia and neuromuscular responses should be further researched prior to its actual application in sports training fields. Moreover, potential therapeutic

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strategies for the treatment of neuromuscular fatigue injury must be developed.

Based on the currently available scientific literature, the antioxidant capacity of increased carnosine levels in muscle tissue might be involved in its protective effects on neuromuscular fatigue [13]. Additionally, L-carnitine displays several antioxidant properties in the neuromuscular system that are similar to carnosine. Thus, we anticipated that L-carnitine may serve as an indirect alternative supplement, although not the exclusive supplement, to the nutritional intervention-based increase in muscle carnosine levels.

As shown in previous studies, dietary L-carnitine supplementation has successfully been used as an essential quaternary ammonium compound nutrient that exerts favorable effects on cellular energy metabolism and the processes involved in skeletal muscle remodeling [14–16]. Additionally, L-carnitine supplementation results in an increase in serum L-carnitine levels [17], and a significant positive correlation between increased serum L-carnitine concentrations and an alleviation of hypoxia-induced biochemical disruption has been reported in many trials [15,18]. Furthermore, we anticipate that increased serum L-carnitine concentrations may increase the transport of L-carnitine across the skeletal muscle and neuromuscular junction, an effect that might alleviate hypoxia and stimulate acetylcholine synthesis. Accordingly, data from early studies have indicated that the athletic population can benefit from L-carnitine intake due to the increased blood flow and oxygen supply to the muscle tissue, consequently reducing hypoxia-related disruptions [15]. For example, as reported by Karlic and Lohninger [19], treatment with L-carnitine attenuates the deleterious effects of high-intensity training by reducing the extent of hypoxic damage and exerts a favorable effect by accelerating recovery from exercise stress. In addition, dietary supplementation with L-carnitine has also shown promise in enhancing neuronal functionality, since the principal acetyl ester of L-carnitine, acetyl-L-carnitine (ALC) [14,20], exerts a wide spectrum of neuroprotective and neurotrophic effects on the nervous system [14], and ALC serves as a potential ergogenic aid to optimize neurotrophin signaling by increasing the energy supply and neuronal responses [14]. In particular, ALC increases neuroprotective properties by facilitating neurotransmitter biosynthesis in the brain [21], since ALC-derived acetyl-CoA serves as a promising alternative substrate for the synthesis of cerebral acetylcholine [22]. Therefore, L-carnitine may be recommended for conditions in which hypoxia and alternative neurotransmitter synthesis are indicated.

On the other hand, the potential hypoxia responses occurring following KAATSU training have been amply addressed, and practitioners have sought to identify the practical adaptations resulting from this training strategy for many years. Based on accumulating evidence, the effects of neurotransmitters on neuromuscular fatigue have already been confirmed by many investigators [23,24]. Nevertheless, the effects of both hypoxia and neurotransmitter dysfunction on the susceptibility to neuromuscular fatigue after KAATSU training remains an area of intense investigation. However, the specific neural responses explaining the causes of neuromuscular fatigue induced by hypoxia have not been extensively investigated. Finally, smaller-molecule investigations of the effects of L-carnitine on stimulating neuromuscular fatigue-associated neurotransmitter dysfunction have not yet been clarified in the existing literature.

Therefore, the purpose of this article is to provide evidence for the cause-and-effect association between KAATSU training and neuromuscular fatigue, to develop treatments, and to identify the adaptive considerations before incorporating KAATSU training in sports preparation. Importantly, this current study aimed to elucidate the particular neurometabolism of acetylcholine (a neurotransmitter released from the neuromuscular junction) to explain the neuromuscular fatigue observed after KAATSU training. Most notably, the favorable nutritional role of L-carnitine in ameliorating neuromuscular fatigue, which correlated with both hypoxia insults and acetylcholine dysfunction during the KAATSU training session, will also be reviewed.

2. KAATSU training induces neuromuscular fatigue

2.1. KAATSU training promotes hypoxia

KAATSU training is intimately linked with oxygen uptake and delivery, which are critically regulated by external pneumatic bands [8]. As the bands begin to tighten, blood pools and oxygen are depleted in the working limbs during KAATSU training, suggesting that the body or a region of the body is exposed to an inadequate oxygen supply at the tissue level [25]. When the supply of oxygen from the bloodstream does not meet the demand from the cells in the tissue, this supply-demand mismatch state is referred to as hypoxia [26].

As hypoxia can occur in both physiological and pathological conditions [26], the first challenge is to distinguish susceptibility and different thresholds of hypoxic responses in physiological states. In other words, we must appreciate the basics of the range of oxygen concentrations that is sufficiently low to induce hypoxic signaling and identify the corresponding hypoxia level during KAATSU training.

At the physiological level, an increased serum lactate level in tissues and blood traditionally accompanies tissue hypoxemia, poor blood flow or a combination of the two [27], and the presence of hypoxemia normally suggests hypoxia [28]. Thus, elevated serum lactate levels usually indicate tissue hypoxia [28]. Accordingly, both Nalos et al. [29] and Samuel and Franklin [28] noted that an increased serum lactate content conventionally characterizes tissue hypoxia. Likewise, Carpenter et al. [30] identified an association between glycolysis culminating in an increase in serum lactate levels with anaerobic metabolism, which is viewed as a manifestation of hypoxic stress. Moreover, Sato et al. [31] obtained direct evidence for the serum lactate level during KAATSU training-induced hypoxia, and reported that the serum lactate concentration was markedly increased and reached its maximum level when the blood pressure cuff was released after KAATSU training. Thus, a significant increase in serum lactate levels in response to prolonged hypoxia occurs during KAATSU training and the increased serum lactate level is a good 'marker' of hypoxia. Based on the association between the serum lactate concentration and prolonged hypoxia, we hypothesize that a high serum lactate level may be linked to more extreme levels of hypoxia. In other words, the continuous production of lactate in serum may be synonymous with the anaerobic metabolism-associated exaggerated hypoxia levels observed after KAATSU training.

2.2. Hypoxia causes neuromuscular fatigue

2.2.1. Defining neuromuscular fatigue

Angelo Mosso, an eminent Italian physiologist, first described neuromuscular fatigue as able to be elicited by 'central' and/or 'peripheral' mechanisms [32]. 'Central' fatigue is presumably due to a reduction in neurostimulation or a compromise in the capacity of the central nervous system to activate skeletal muscles. In addition, central fatigue does not simply define constant physical exhaustion; it also involves an impaired cognitive component, such as augmented perception of effort and an increased magnitude of mental inactivity [33]. "Peripheral' fatigue is potentially due to impaired mechanisms ranging from excitation to muscle contraction, particularly an attenuation of the maximal force-generating capacity caused predominantly by metabolic events within the muscle [34–37]. Furthermore, peripheral fatigue includes the function of the neuromuscular junction [38].

Within the framework of sports exercise, neuromuscular fatigue is a frequently occurring symptom, and the loss of the initial maximal force capacity is reversible by rest. However, an exercise-induced reduction in the ability of a locomotor muscle to develop force or power output would lead to a phenomenon characterized by difficulty performing the adequate voluntary activation of skeletal muscles at a given load over a period of time [39,40]. Neuromuscular fatigue simply represents any exercise-induced decrease in the capacity of a muscle to produce a desired voluntary force or power output, regardless of whether the task

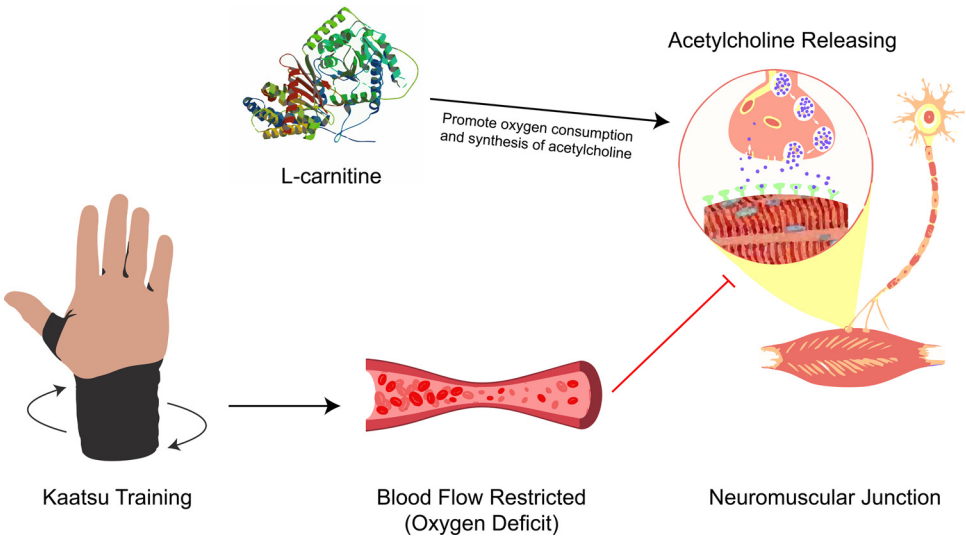

Fig. 1. L-carnitine mediates the adaptation to hypoxia-induced neuromuscular fatigue.
KAATSU training promotes a tissue oxygen deficit or hypoxia, and L-carnitine mediates the adaptation to KAATSU training-induced hypoxia by increasing mitochondrial oxygen consumption. Furthermore, KAATSU training-induced hypoxia triggers neuromuscular fatigue (a combination of peripheral and central fatigue), and peripheral fatigue involves alterations in acetylcholine transmission that occur at the neuromuscular junction. Briefly, hypoxia triggers the inadequate presynaptic release of acetylcholine, which is associated with neuromuscular fatigue, and L-carnitine plays a major modulatory role in the indirect transportation of acetyl groups for acetylcholine synthesis, thus attenuating neuromuscular fatigue.

is able to be sustained [38,41].

2.2.2. Relationship between the hypoxia level and neuromuscular fatigue

For the purpose of this review, a correlation between different hypoxia levels and the corresponding contributions of peripheral mechanisms of fatigue (the loss of peripheral muscle force and power) or (and) central nervous system (CNS) mechanisms of fatigue (a progressive failure to initiate or sustain voluntary drive to motor neurons by the CNS) must be identified [42]. Namely, this paper sought to obtain a better understanding of how neuromuscular fatigue is affected by different levels of hypoxia. Moreover, the associated magnitude of hypoxia, a critical threshold or 'sensory tolerance limit' magnitude, in which individual central and/or peripheral fatigue would become dominant must be determined.

Undoubtedly, the hypoxia condition indicates increased neural activation and greater muscular fatigue to sustain a particular task and the same total work output [43]. For example, Karabulut et al. [7,12] provided evidence that a low-intensity leg extension exercise (~20 % of 1RM) coupled with KAATSU training (under hypoxia conditions) produces greater postneuromuscular fatigue than non KAATSU training. More specifically, Fatela et al. [43] observed that KAATSU training elicits neuromuscular fatigue when performed at an 80 % vascular restriction level combined with 20 % 1RM; moreover, neuromuscular activation varies as a function of the KAATSU training relative pressure. Thus, at values greater than the vascular restriction level (80 % occlusion condition combined with 20 % 1RM), the associated hypoxia level may serve as an important threshold for neuromuscular fatigue development.

On the other hand, the potential mechanisms of neuromuscular fatigue are associated with both peripheral and central origins [32], and hypoxia levels range from mild to moderate and severe [11]. Moreover, the relative determinants of central and/or peripheral fatigue depend on the severity of hypoxia. Strikingly, as the severity of hypoxia increases, the predominant factor inhibiting neuromuscular activation and exercise performance switches from a peripheral to a central component of fatigue [11,34]. Notably, more specific information about the mechanisms underlying neuromuscular fatigue has been substantiated by Amann et al. [11], who recently observed a correlation between variable levels of hypoxia and corresponding components of the peripheral and/or central origins of fatigue among eight cyclists. Amann et al. [11] provided detailed evidence for a main association of decreases in athletic performance with peripheral determinants of fatigue when the severity of hypoxia switches from normoxia to a moderate level. In addition, as the severity of hypoxia increases from mild to moderate, impaired exercise performance is still primarily, but not exclusively, coupled with peripheral mechanisms of fatigue; in other words, this finding has been interpreted as involving a combination of peripheral and central determinants of fatigue. Subsequently, under severe hypoxia conditions, the major determinants of autonomic control and central motor drive are markedly associated with the central (cerebral) component of fatigue and are independent of peripheral somatosensory feedback mechanisms.

2.2.3. KAATSU training-induced hypoxia triggers a combination of peripheral and central fatigue

Strikingly, investigators have reached a consensus concerning the determinants of peripheral and central fatigue resulting from KAATSU training in some studies of neuromuscular fatigue. Some investigators verified that hypoxia (via KAATSU training) exacerbates the development of neuromuscular fatigue of both peripheral and central origins, rather than just one origin [44]. Similar to the results presented by Amann et al. [11], KAATSU training-induced fatigue might be due to a combination of peripheral (i.e., decreases in potentiated twitch) and central (i.e., decreases in the percentage of vascular restriction and electromyography amplitude) components of neuromuscular fatigue. As stated above, a combination of peripheral and central mechanisms of fatigue is activated in response to a moderate level of hypoxia [11]. Therefore, we postulate that the hypoxia level observed during KAATSU training may not be adequate to induce CNS fatigue alone, which is elicited by cerebral (severe) hypoxia and attributable to mechanisms susceptible to a severe insufficiency of oxygen delivery [11]. Thus, our hypothesis suggests that a 'moderate' level of hypoxia may occur during KAATSU training. Nevertheless, this 'moderate' level is not comparable to the exact hypoxia level suggested by Amann et al. [11]. However, much less is known about precise moderate levels of hypoxia occurring within the KAATSU training domain.

We conclude that with alterations in hypoxia levels, changes in the determinants of neuromuscular fatigue might simultaneously occur because of hypoxia-sensitive mechanisms. In addition, KAATSU training may provoke 'moderate' hypoxia and subsequently induce compromised neuromuscular excitability, which involves both central and peripheral origins of fatigue (Fig. 1). Nevertheless, researchers have not reached a consensus on whether the peripheral or central component plays a pivotal role in the genesis of the neuromuscular fatigue associated with KAATSU training.

3. Oral administration of L-carnitine may be recommended due to its efficacy

3.1. L-carnitine

L-carnitine (3-hydroxy-4-*N*-trimethylammonium-butyrate), also termed vitamin BT, was first detected in the mealworm [45,46]. As a chemical analog of choline, L-carnitine is an amino acid derivative and a micronutrient [47] that was first identified in muscle extracts in 1905 [48]. L-carnitine is acquired from both endogenous synthesis (25 %) and dietary administration (75 %) in the human body [49]. Regarding endogenous synthesis, L-carnitine is produced from the amino acids lysine and methionine. Additionally, the main tissue reservoirs of L-carnitine are naturally the heart and skeletal muscle tissues (containing an estimated 95 % of the total content) [15,19], and L-carnitine is synthesized in the brain, liver and kidney [47,50]. Regarding dietary L-carnitine, the major sources are food, such as red meat, fish, and dairy products, as well as the nutritional L-carnitine supplement with high purity [19,47,51].

3.2. Effect of L-carnitine supplementation on muscle carnosine concentrations

3.2.1. Muscle carnosine characteristics and its role in neuromuscular fatigue

Based on the currently available scientific literature, carnosine performs critical biological functions; in particular, the neuroprotective ability to suppress the deleterious effects of neurodegenerative disorders has been investigated in many studies [52–54].

In general, as a pluripotent dipeptide, more than 99 % of total carnosine content in the human body is present in skeletal muscle. According to Parkhouse and McKenzie [55], muscle carnosine loading is ergogenic in sprint-type exercise, since high muscle carnosine concentrations significantly alleviate oxidative stress, thereby preventing mitochondrial ATP depletion and improving anaerobic exercise performance [56]. Furthermore, according to Stout et al. [57], an increase in the muscle carnosine concentrations induced by the use of β-alanine supplements may increase the total muscular work performed and delay neuromuscular fatigue. More specifically, the antioxidant capacity of increased carnosine levels in muscle tissue might be involved in its protective effects on peripheral (rather than central) fatigue [13].

3.2.2. L-carnitine supplementation may alleviate the loss of the muscle carnosine content

In contrast, the carnosine content decreases in exercising muscle, and the loss of the dipeptide tends to be responsible for fatigue [58]. A potential implication of this finding is that the loss of the muscle carnosine content is closely associated with fatigue. Logically, KAATSU training may induce lower muscle carnosine contents and neuromuscular fatigue. A plausible speculation is that a decrease in the muscle carnosine content appears to be a promising neuromuscular fatigue biomarker during anaerobic training. Furthermore, L-carnitine is positively correlated with the attenuation of neuromuscular fatigue [22]. Therefore, we postulated that L-carnitine supplementation may alleviate the loss of carnosine in muscle tissue by attenuating neuromuscular fatigue.

Nevertheless, few studies published to date have investigated the direct effects of the anaerobic training status on muscle carnosine contents, and the link between muscle carnosine fractions during anaerobic exercise and neuromuscular fatigue is still poorly defined.

3.2.3. L-carnitine supplementation may represent an indirect alternative to the nutritional intervention-based increase in muscle carnosine concentrations

Because L-carnitine possesses several antioxidant properties in the neuromuscular system that are similar to that of carnosine, we anticipated that L-carnitine may serve as an indirect alternative supplement to the nutritional intervention-based increase in muscle carnosine concentrations that aims to suppress the deleterious effects of neuromuscular fatigue.

3.3. Intramuscular effects of L-carnitine on skeletal and respiratory muscles

L-carnitine plays vital roles in fatty acid metabolism and mitochondrial energy production in the skeletal muscles [17,59]. Additionally, L-carnitine may reduce intermittent hypoxia-induced skeletal muscle oxidative stress and thereby improve the strength, remodeling and fatigue recovery of skeletal muscle [14–16,60].

According to Gross et al. [61], the efficiency of ALC is approximately equivalent to L-carnitine. Based on the currently available data, an ALC treatment enhances skeletal muscle respiration by increasing the mitochondrial protein mass in old rats [62]. Thus, new research has provided insights into the importance of L-carnitine in the mechanism regulating biochemical metabolism in respiratory muscles.

Based on accumulating evidence, an L-carnitine intervention improves the strength of respiratory muscles, an important factor in respiratory functions, in patients with chronic obstructive pulmonary disease who developed various degrees of respiratory muscle fatigue [63–65]. Another important finding was that L-carnitine may protect against respiration dysfunction by preventing the accumulation of lactate [66].

Collectively, the properties of L-carnitine described above have prompted the hypothesis that the intramuscular effects of L-carnitine are similar on skeletal and respiratory muscles.

4. L-carnitine mediates the adaptation to KAATSU training-induced hypoxic stress

4.1. Evidence for KAATSU training-induced hypoxic stress

In the context of a normal oxygen tension, one predominant source of pyruvate is the degradation of glucose via glycolytic enzymes [67,68]. As a pivotal intermediate, pyruvate is used in the maintenance of the tricarboxylic acid (TCA) cycle flux [68], since pyruvate is oxidized to yield acetyl-CoA, subsequently feeding the TCA cycle and resulting in ATP production [26,69].

However, in the presence of a low oxygen tension, low levels of oxygen are available, and hypoxic cells shift to anaerobic metabolism [11]. This metabolic switch shunts glucose metabolites from the mitochondria to glycolysis to produce ATP, which may not be sufficient to meet the energy required by hypoxic cells [67]. Accordingly, under the hypoxic conditions occurring during KAATSU training, cells catabolize glucose to pyruvate through anaerobic glycolysis [68]. Moreover, the enzyme pyruvate dehydrogenase (PDH) represents the sole bridge between anaerobic and aerobic energy metabolism [15], and the loss of the PDH complex reaction occurs under prolonged hypoxic conditions [67], which are mainly characterized by hypoxia-associated anaerobic metabolism [11]. Thus, this inactivation of PDH would shunt pyruvate toward lactate production via lactate dehydrogenase A and thus block the conversion of pyruvate into acetyl-CoA. Nevertheless, the process still permits continued glycolysis and ATP production, which partially compensates for the energy required by hypoxic cells [67]. Collectively, the findings presented here show that hypoxia impairs ATP production in response to metabolic switching by increasing the conversion of glucose to pyruvate and subsequently to lactate. In other words, hypoxia leads to a suppression of the TCA cycle, which is associated with the inactivation of PDH and the blockade of the mitochondrial pyruvate to acetyl-CoA transformation, thereby attenuating ATP production (Fig. 2).

Furthermore, metabolic homeostasis is disrupted by hypoxic insults because of metabolic by-product accumulation [1]. Based on accumulating evidence, insufficient glucose and ATP production would

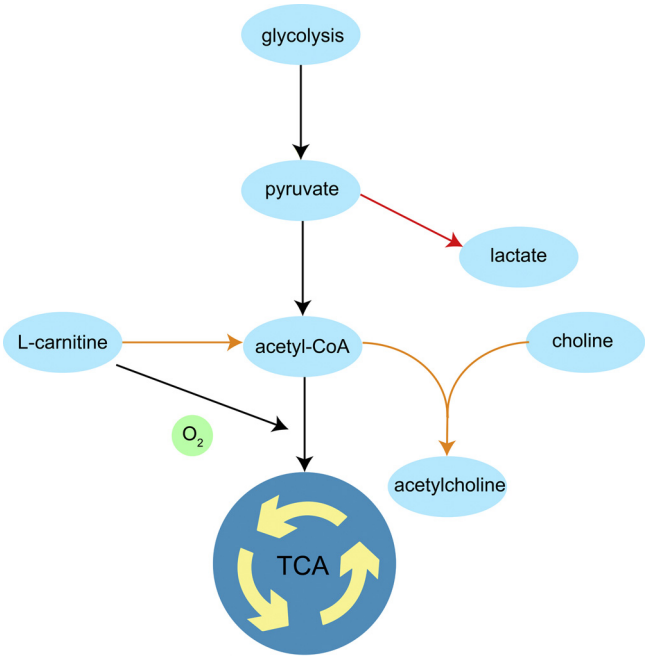

Fig. 2. L-carnitine promotes the synthesis of acetylcholine.
During hypoxia, during which the oxygen supply is reduced, cells catabolize glucose to pyruvate through anaerobic glycolysis, which subsequently shunts pyruvate into lactate and blocks the conversion of pyruvate into acetyl-CoA. Blockade of acetyl-CoA transport further leads to the suppression of the activity of the TCA cycle and blocks acetylcholine synthesis. On the other hand, L-carnitine ameliorates both hypoxic stress and neuromuscular transmission failure by stimulating the activity of the TCA cycle and increasing acetylcholine synthesis, respectively, since ALC-derived (the principal acetyl ester of L-carnitine) acetyl-CoA not only serves as an oxidizable substrate of the TCA cycle but also as a promising alternative substrate for the synthesis of acetylcholine.

increase both pyruvate and lactate levels [1,30,39]. In addition, hypoxia also exerts a highly sensitive effect on the accumulation of other metabolites, including hydrogen (H+) protons [1], which is coupled with a decrease in the pH [25] and an accumulation of inorganic phosphate (Pi) protons [1].

Collectively, the metabolic adaptation to hypoxia involves both the suppression of the TCA cycle and shunting of pyruvate to lactate, processes that are accompanied by impaired ATP formation in the glucose-to-pyruvate pathway, and the accompanying accumulation of metabolic by-products is presumed to be associated with the adaptation to hypoxic stress during KAATSU training.

4.2. Effect of L-carnitine administration on hypoxic stress

Numerous animal studies in the literature reported the efficacy of L-carnitine in reducing brain injury after hypoxia or hypoxia-ischemia in rats [60,70–73]. Therefore, a plausible speculation is that L-carnitine may reduce exercise- induced hypoxia in humans. Kraemer et al. [74–76] obtained compelling results in a series of human studies showing that L-carnitine intake exerts a favorable effect on attenuating exercise-induced hypoxic damage.

4.2.1. Important role of serum L-carnitine in biological functions

L-carnitine supplementation in healthy individuals increases the blood carnitine [77] or plasma free L-carnitine [64] concentrations compared to the normal levels, and an oral L-carnitine treatment increases the excretion of serum L-carnitine in rat models [17]. Briefly, L-carnitine supplementation results in an increase in serum L-carnitine levels in both animals and humans.

Moreover, increased serum L-carnitine concentrations have been reported to exert widespread systemic effects on metabolism, including increases in the blood flow and oxygen supply to the muscle tissue [15], a positive effect on post-exercise recovery mediated by the increased excretion of both free and esterified carnitine [78], and in particular, the attenuation of muscle soreness and hypoxia-induced metabolic stress [15,18] in many studies.

As stated above, hypoxia is invariably associated with the cascade of events occurring during metabolic switches. The most notable of these changes include decreases in oxygen consumption [79], the accumulation of metabolic by-products [1], and inadequate ATP synthesis [67]. Thus, serum L-carnitine concentrations may represent a potentially important signal for hypoxia, and researchers have attempted to evaluate the effects of L-carnitine supplementation on this hypoxic chain of events.

4.2.2. Important role of L-carnitine in the hypoxic chain of events

4.2.2.1. L-carnitine increases oxygen consumption. Prolonged exposure to an environment with reduced oxygen concentrations or hypoxic conditions indisputably leads to decreased oxygen consumption [79]. According to data from preliminary studies, an L-carnitine treatment, as a potential ergogenic aid, may increase mitochondrial oxygen consumption [79], thereby attenuating the deleterious effects of hypoxic training [19] (Fig. 1). Moreover, Kraemer et al. [74–76] obtained compelling results from a series of studies and showed that L-carnitine L-tartrate (a type of L-carnitine) supplementation represents a promising intervention for inducing increases in the muscle oxygen consumption rate, and the increased oxygen consumption might mediate membrane disruption and occlude oxygen delivery, thereby attenuating the magnitude of exercise-induced hypoxia (Fig. 2). Hence, an increase in oxygen consumption might explain the attenuation of hypoxic stress observed after L-carnitine supplementation.

4.2.2.2. L-carnitine mediates the accumulation of metabolic by-products. In theory, the physiological process of metabolite accumulation in muscle cells is referred to as metabolic stress by researchers [80]. Notably, Aureli et al. [81] used both adult and aged rat models to verify that an ALC treatment induces a corresponding reduction in the concentration of inorganic phosphate (Pi) protons and the brain lactic acid concentration. Moreover, treatment with dietary L-carnitine decreases the mitochondrial pyruvate content and plasma lactate level by stimulating the activity of the PDH complex and maintaining a favorable acetyl-CoA/CoA ratio [19,82,83].

Based on the results described above, evidence is available for the beneficial effect of L-carnitine supplementation, which may protect against metabolic stress resulting from hypoxic insults (Fig. 2).

4.2.2.3. L-carnitine increases ATP production. Originally, the obligatory function of carnitine in shuttling long-chain fatty acids across the inner mitochondrial membrane in the liver was first described by Fritz [50] in 1959. In addition, previous studies of the main mitochondrial metabolism and function of L-carnitine revealed that it is intrinsically involved in the beta-oxidation of free fatty acids by shuttling through the inner mitochondria membrane and into cells to be processed for energy (ATP) [84]. On the other hand, the hypoxic process is believed to contribute to the development of mitochondrial decay and inadequate ATP synthesis [85]. Thus, researchers attempted to verify that L-carnitine rescues hypoxia-induced energy failure and oxidative damage.

However, because mitochondrial membranes are impermeable to long-chain fatty acids, the transport of acetylated fatty acids into the mitochondrial matrix for their subsequent β-oxidation is not possible. However, the salient finding of the current investigation was that the use of ALC (an acetylated derivative and the principal acetyl ester of L-carnitine) as an energy substrate is reasonable since ALC provides an acetyl moiety oxidized for energy (ATP) production [86]. Specifically, the binding of L-carnitine to acetyl groups occurs via carnitine

acyltransferase to form a long-chain acetylcarnitine ester [15,87], the acetyl component of ALC that shuttles acetylated fatty acids into the mitochondrial matrix where they are processed by β-oxidation, and subsequently provides acetyl-CoA as an oxidizable substrate for the TCA cycle, resulting in the production of large amounts of ATP [15,21]. Obviously, ALC acts as an energy store. Zanelli et al. [22] presented evidence that the acetyl component of ALC is metabolized in the brain and stimulates a change in cerebral energy stores. Similarly, Susanna et al. [21] also tested the hypothesis that the immature rat forebrain is capable of utilizing the acetyl moiety of exogenous ALC for energy metabolism. Hence, ALC has an essential function in energy crises: it increases ATP formation by providing acetyl-CoA as a substrate for the TCA cycle under hypoxic conditions. Thus, dietary supplementation with L-carnitine plays a pivotal role in the generation of ATP (Fig. 2).

Taken together, the evidence described above presents a particularly intriguing possibility that L-carnitine is therapeutically effective at mediating the adaptation to hypoxia stress, for example by increasing oxygen consumption, mediating metabolic disruption, and increasing ATP production. However, further translational studies are needed to completely explore these potential effects, particularly regarding the potential use of L-carnitine by humans.

5. L-carnitine attenuates neuromuscular fatigue

Our previous reports revealed a correlation between KAATSU training and hypoxic stress, which is postulated to be a factor causing neuromuscular fatigue. Moreover, L-carnitine is capable of mediating the adaptation to hypoxic stress. Nevertheless, one of the other aims of the current study was to explore the more direct roles of L-carnitine in modulating neuroplasticity and reducing neuromuscular fatigue by expanding its applications to smaller molecules, including neurological responses to neurotransmitter concentrations.

Indeed, many hypotheses have been proposed to explain the alterations in the levels of neurotransmitters associated with neuromuscular fatigue in the brain, such as acetylcholine (ach), 5-hydroxytryptamine (5-HT), dopamine (DA), gamma-aminobutyric acid (GABA), cytokines, ammonia, glutamate, serotonin, and norepinephrine [23,24]. Notably, as a neuromuscular junction neurotransmitter, acetylcholine is the most ubiquitous transmitter [23] and has the highest intrinsic sensitivity among all neurotransmitters [24].

Thus, in addition to hypoxic stress, we proposed that the possible alterations in acetylcholine synthesis during KAATSU training may also be viewed as an explanation for neuromuscular fatigue.

5.1. Effect of L-carnitine administration on acetylcholine

Acetyl-CoA serves as a precursor for the cerebral synthesis of acetylcholine [86], and the conversion of blocked acetyl-CoA, which is derived from anaerobic glycolysis, is an alternative pathway that is activated upon supplementation with ALC [22]. In addition, based on accumulating evidence, ALC has the ability to cross the blood-brain barrier [88] and serves as a potential ergogenic aid to optimize the processes of skeletal muscle remodeling [16] and increase the neuroprotective properties by facilitating neurotransmitter biosynthesis in the brain [21]. Not surprisingly, the use of ALC-derived acetyl-CoA as a promising alternative precursor to the synthesis of cerebral acetylcholine is plausible [86].

On the other hand, the stores of acetylcholine in neurons are determined by both the availability of acetyl-CoA and choline [89]. Nevertheless, a small pool of choline located within cholinergic nerve endings is available for acetylcholine synthesis, even when the production of acetyl-CoA that is used for the synthesis of acetylcholine is not impaired [90]. Thus, ALC predominantly increases cerebral acetylcholine levels by increasing the acetyl-CoA content. Consistent with the findings described above, Szutowicz et al. [91] provided evidence that increases in acetyl-CoA levels readily stimulate acetylcholine synthesis in the brain by increasing the viability of cholinergic neurons and cholinergic activity. According to Gibson et al. [92], decreased synthesis of acetylcholine may be due to a lack of acetyl-CoA in brain slices and synaptosomes. Based on the currently available evidence, ALC-derived acetyl-CoA serves as a promising alternative substrate for the cerebral synthesis of acetylcholine, and an increase in the acetyl-CoA content concurrently increases acetylcholine formation. In other words, an alternative source of acetyl-CoA generated by dietary L-carnitine plays a major role in modulating the indirect transportation of acetyl groups for acetylcholine synthesis (Fig. 2).

5.2. Relationship between acetylcholine levels and neuromuscular fatigue

Continuous acetylcholine release or depletion from the nerve terminals during exercise is associated with neuromuscular fatigue [38,93,94]. For example, Sieck and Prakash [94] reported an association between reductions in the quantal release of acetylcholine and presynaptic sites of neuromuscular transmission failure that lead to peripheral muscle fatigue (neuromuscular fatigue), suggesting that inadequate presynaptic release of acetylcholine induces neuromuscular fatigue. Likewise, Boyas and Guével [38] noted that peripheral fatigue involves alterations in neuromuscular transmission, which occurs at the neuromuscular junction and is caused by reduced neurotransmitter release.

Inadequate presynaptic release of acetylcholine or a decrease in the quantity of acetylcholine released by each nerve ending is associated with neuromuscular fatigue. In contrast, corresponding increases in acetylcholine production may be an important determinant of the attenuation of neuromuscular fatigue. More precisely, an L-carnitine treatment is likely to exert favorable therapeutic effects on ameliorating neuromuscular fatigue by modulating the acetylation status through an increase in the acetyl-CoA content that concurrently increases acetylcholine production in the brain (Fig. 1).

6. Prospects and conclusion

Research on sport-specific hypoxia and neurotransmitter responses is crucial before incorporating KAATSU training in sports preparation to assist in the recovery from neuromuscular fatigue, although the effective practice of KAATSU exercise causes skeletal muscle hypertrophy and strength gains. Moreover, the neuroprotective effects induced by dietary L-carnitine on attenuating both hypoxia and neuromuscular fatigue appear feasible, and this hypothesis is of considerable heuristic value. Nevertheless, the precise mechanisms underlying the effects of L-carnitine on neuromuscular fatigue during KAATSU training are still poorly understood and more information is needed to thoroughly elucidate this adaptation.

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Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

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