L-Citrulline Supports Vascular and Muscular Benefits of Exercise Training in Older Adults

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FIGUEROA, A., S.J. JAIME, M. MORITA, J.U. GONZALES, and C. MOINARD. L-Citrulline supports vascular and muscular benefits of exercise training in older adults. *Exerc. Sport Sci. Rev.*, Vol. 48, No. 3, pp. 133–139, 2020. *Age-associated reduction in endothelial nitric oxide (NO) synthesis contributes to the development of cardiovascular diseases and sarcopenia. L-Citrulline is a precursor of NO with the ability to improve vascular function and muscle protein synthesis. We hypothesize that vascular and muscular benefits associated with oral L-citrulline supplementation might be augmented by concomitant supplementation with exercise training in older adults.* **Key Words:** arterial aging, vascular dysfunction, sarcopenia, oral L-citrulline, nitric oxide, exercise performance, muscle protein synthesis

Key Points

- Aging is associated with hypertension and reduced muscle mass and strength (quality) that may be mediated by impaired nitric oxide bioavailability.
- Oral L-citrulline increases plasma L-arginine and nitric oxide production.
- L-Citrulline supplementation improves muscle protein synthesis, muscle mass, and oxygen delivery that may benefit exercise performance.
- Combined with exercise training, L-citrulline may benefit vascular and muscular function via improvements in nitric oxide bioavailability and muscle protein synthesis in older adults.

INTRODUCTION

Advanced aging is associated with a progressive increase in risk factors for cardiovascular diseases (CVD) (1,2). Adverse structural and functional changes in the arterial wall such as arterial stiffness and endothelial dysfunction may contribute to the development of CVD with aging (1,2). In addition, the age-related loss of muscle mass and function, known as sarcopenia (3), is attributed, in part, to endothelial dysfunction (4,5). The age-related reduction in nitric oxide (NO) bioavailability may contribute to sarcopenia via reduced blood flow and delivery of amino acids to skeletal muscles (5,6). Evidence suggests that oral L-citrulline (L-CIT) supplementation may positively affect arterial and muscular function by increasing NO bioavailability (7,8). Given that L-CIT is an effective precursor of L-arginine (L-ARG) (9), the substrate for NO production, L-CIT supplementation may improve muscle mass (10) and function (11,12). Moreover, recent evidence suggests that L-CIT supplementation combined with exercise training may have additive benefits on arterial and muscular function and structure in older adults (13–15).

This article briefly summarizes the current literature on the impact of dietary L-CIT alone or combined with exercise training on vascular function and skeletal muscle structure and function (*e.g.*, protein synthesis, mass, strength, exercise performance, and mechanism of action). We hypothesize that vascular and muscular benefits associated with oral L-CIT supplementation might be augmented by concomitant supplementation with exercise training in older adults. Emphasis will be given to the potential effect of L-CIT alone and the combined effects of L-CIT supplementation and exercise training on vascular and muscular function in older adults.

Endothelial Function and Dysfunction

The endothelium is a single inner layer of cells in the arterial wall. Endothelial cells have a major role in blood pressure and organ blood flow regulation by producing NO, a potent substance that causes vascular smooth muscle relaxation and subsequent dilation. Endogenous NO is produced by a group of NO synthase (NOS) enzymes including endothelial (eNOS), inducible, and neuronal (nNOS) isoforms (16). The amino acid,

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L-ARG, is catabolized by NOS enzymes to NO and L-CIT. This reaction requires nicotinamide adenine dinucleotide phosphate (NADPH) and oxygen as cosubstrates, and tetrahydrobiopterin (BH_4) and flavin adenine dinucleotide as cofactors (16). Once produced, NO rapidly diffuses to the media layer to relax vascular smooth muscle cells. This process involves activation of soluble guanylate cyclase and production of cyclic guanosine monophosphate (cGMP) from guanosine-5′-triphosphate (GTP), which by decreasing sarcoplasmic calcium levels leads to smooth muscle relaxation and vasodilation.

Mechanisms of age-related endothelial dysfunction include low L-ARG availability, upregulation of arginase, increased oxidative stress, and BH_4 deficiency (2,17–19) (Fig. 1). Low L-ARG availability for eNOS could be attributed to catabolism of L-ARG to L-ornithine by the enzyme arginase, which competes with eNOS for their common substrate L-ARG. Aging-related oxidative stress is the result of the imbalance between increased production of reactive oxygen species, including superoxide anion and hydrogen peroxide by increased NADPH oxidase activity, and the inability to increase antioxidant defenses (2,16). The reaction of superoxide anion with NO produces peroxynitrite, leading to oxidation and decrease in BH_4 (converted to BH_2) and eNOS uncoupling (2). Uncoupled eNOS produces more superoxide and peroxynitrite instead of NO. Thus, increased arginase activity or expression and oxidative stress-associated eNOS uncoupling result in a further decrease in NO bioavailability and impaired endothelium-dependent vasodilation (16,18). In addition to endothelial dysfunction, conversion of L-ARG to L-ornithine by arginase contributes to arterial stiffening via promotion of polyamine- and proline-dependent collagen synthesis and cell proliferation (18). The role of arginase in age-associated arterial aging is supported by restoration of eNOS coupling and decrease in arterial stiffness after pharmacological arginase inhibition in old rats (18). Therefore, arginase inhibition and increased L-ARG bioavailability via oral L-ARG or L-CIT supplementation may improve endothelial function with aging.

L-CIT and Vascular Function

Evidence indicates that even though oral L-ARG supplementation effectively increases L-ARG bioavailability, improvement in endothelial-mediated vasodilation is not observed in healthy middle-aged adults (20). Long-term L-ARG supplementation becomes ineffective due to increased catabolism by arginase enzyme activation (19). Alternatively, the nonessential amino acid L-CIT is better absorbed when ingested as compared with a similar dose of oral L-ARG (19). The higher circulating level of L-ARG after L-CIT ingestion is the result of lack of catabolism by arginase and hepatic uptake (19). Part of this efficiency is attributed to the ability of L-CIT to inhibit arginase activity in humans and animals (11,21). Circulating L-CIT is converted to L-ARG (*de novo* synthesis) and released from the kidneys into the systemic circulation (22). The greater efficacy of acute oral L-CIT as a precursor of plasma L-ARG than an equimolar dose of oral L-ARG was demonstrated by Moinard *et al.* (9) in older adults. We have shown increases in plasma L-ARG or NO metabolites after 1 wk (7), 2 wk (23), and 8 wk (14) of oral L-CIT in older adults. Therefore, L-CIT supplementation may have the potential to improve age-related vascular dysfunction by increasing L-ARG bioavailability for eNOS via *de novo* synthesis and arginase inhibition.

We are aware of only three randomized, placebo-controlled trials that have been conducted to test the effect of L-CIT supplementation on endothelial NO production in humans. Two studies tested the acute effect of L-CIT (24,25), whereas one study tested the effect of short-term supplementation with L-CIT (20). Kim *et al.* (24) showed that acute ingestion of L-CIT, dissolved in distilled water until a total dose of 3 g

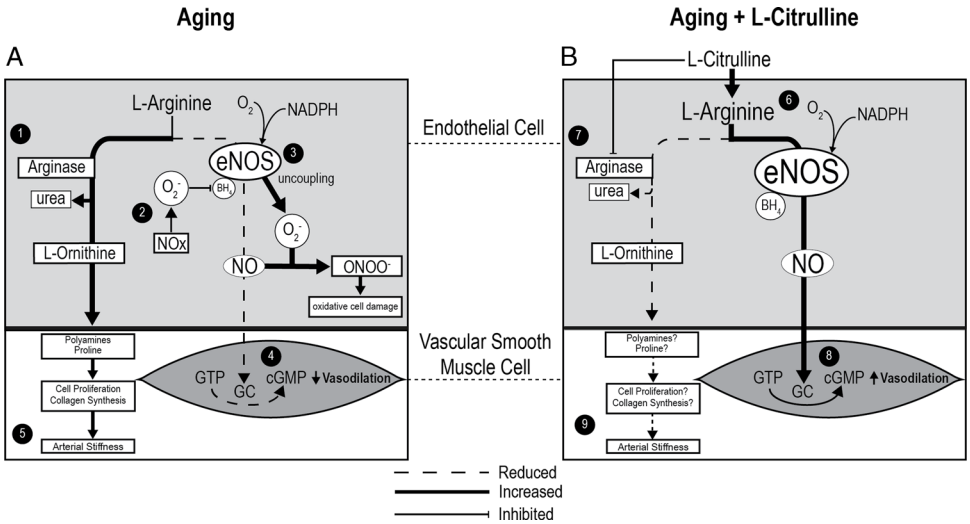

Figure 1. Potential mechanisms by which aging impairs vascular function (A), and oral L-CIT may improve age-related vascular dysfunction (B). L-ARG is catabolized by the enzyme arginase into urea and L-ornithine (1). Age-related increased NADPH oxidase (NOx) activity increases superoxide anion (O_2^-) production (2) and contributes to decrease BH_4, leading to eNOS uncoupling (3). Uncoupled eNOS produces O_2^-, which reacts with NO to produce peroxynitrite ($ONOO^-$) (3), a substance that causes oxidative cell damage. The resultant low NO fails to properly activate the guanylate cyclase (GC)–GTP–cGMP signal transduction pathway, leading to impaired vasodilation (4). L-Ornithine produced by arginase increases the production of polyamine and proline, resulting in collagen synthesis and cell proliferation contributing to arterial stiffness (5). Oral L-CIT supplementation increases L-ARG bioavailability for eNOS by greater *de novo* synthesis (6) and inhibition of arginase activity (7), resulting in improved NO-mediated vasodilation (8) and decreased arterial stiffness (9). The precise mechanism by which L-CIT may decrease arterial stiffness is yet unknown.

was reached, increased *de novo* L-ARG and whole-body NO synthesis rate through the use of isotope tracers in healthy young adults and older adults with heart failure (24). The increase in NO synthesis above a fasted baseline state was greater for young than for older adults. In a separate experiment, the investigators had the same subjects ingest 10 g of L-CIT or 10 g of mixed amino acid placebo, both dissolved in plain water, on two separate days separated by at least 48 h that served as a washout period. Peak reactive hyperemia measured using strain-gauge plethysmography was not different between L-CIT and placebo when assessed every 20 min up to 1 h after ingestion. However, it should be noted that the occlusion period was only 3 min in this study, which is much lower than the 10-min period of ischemia shown to be an effective stimulus for producing maximal arteriolar dilation in skeletal muscle (26). In addition, the last measurement occurred 1 h after consuming L-CIT, whereas Moinard *et al.* have shown that peak plasma L-ARG levels are reached around 1–1.5 h after L-CIT ingestion (9).

In another randomized crossover study, Cutrufello *et al.* (25) had young healthy adults consume 710 mL of a sucrose solution with 6 g of L-CIT or 710 mL of sucrose solution without L-CIT (placebo), each after 1-wk washout periods. Endothelial function was assessed using brachial artery flow-mediated dilation (baFMD) measured at 1 or 2 h after ingestion of the study solutions. Irrespective of the postingestion time of testing, baFMD was not different between conditions, although it should be noted that the average flow-mediated dilation for each condition was ~12%, suggesting that the young adults examined in this study had no endothelial dysfunction to correct with L-CIT supplementation. Thus, more information is needed to know whether acute L-CIT improves endothelial-dependent dilation in young and older adults with endothelial dysfunction using an appropriately timed protocol to test the capacity of endothelial cells to produce NO.

Another potential explanation for the absence of improved endothelial NO production after L-CIT is that longer L-CIT supplementation may be required for an effective improvement in endothelial function (24). Evidence to support this notion comes from a randomized, placebo-controlled, crossover design study conducted by Schwedhelm *et al.* (20). In this study, apparently healthy middle-aged adults with an average baseline baFMD of 6% consumed multiple doses of L-CIT for 7 d each with 1-wk washout periods between conditions. The highest dose of L-CIT was 6 $g \cdot d^{-1}$, which was shown to increase urinary nitrate levels (20). This study did not find baFMD to differ from baseline for any dose of L-CIT or placebo, but the investigators did observe a significant positive correlation with the change in baFMD after L-CIT and the change in the ratio of L-ARG to the competitive eNOS inhibitor asymmetric-dimethyl-L-arginine (ADMA) when data for all doses of L-CIT were pooled together (20). This ratio reflects greater L-ARG availability for eNOS (17), suggesting that L-CIT may improve vascular function when L-ARG bioavailability for eNOS is improved. Longer-term L-CIT supplementation may be required to increase L-ARG bioavailability. For instance, we have shown that 8 wk of L-CIT supplementation at 800 $mg \cdot d^{-1}$ reduced plasma ADMA levels in middle-aged patients with vasospastic angina and improved baFMD (27). These patients had a low baFMD at baseline (~3%), supporting the notion that L-CIT may have a positive impact on endothelial-dependent dilation in adults with endothelial dysfunction. However, this study by Morita *et al.* (27) was not randomized nor placebo controlled; thus, its results should be interpreted with caution. In summary, more information is needed about the potential for long-term L-CIT supplementation to improve endothelial NO production in middle-aged and older adults with or without endothelial dysfunction.

Another mechanism by which L-CIT may improve vascular function is via NO bioactivity within skeletal muscle. For instance, a splice variant of nNOS, nNOSμ, is anchored to the sarcolemma of skeletal muscle (28). NO produced at this site can decrease α-adrenergic vasoconstriction to match muscle blood flow to increase metabolic demand (28). This may partly explain the improved muscle oxygenation patterns at the onset of leg exercise in young men (11), and improved leg vascular conductance during exercise in older men that we observed (23) after chronic L-CIT supplementation. Support for the theory that L-CIT can increase NO production in skeletal muscle comes from recent work that showed 2 wk of swimming training in mice combined with L-CIT resulted in significantly elevated expression of skeletal muscle peroxisome proliferator-activated receptor-gamma coactivator-1α (PGC-1α) as compared with swimming training alone (29) (Fig. 2). PGC-1α is a transcriptional coactivator important for the regulation of mitochondrial respiration. NO can stimulate the expression of PGC-1α in skeletal muscle through multiple mechanisms including activation of 5' adenosine monophosphate-activated protein kinase, Ca^{2+}-mediated signaling, and phosphorylation of cyclic adenosine monophosphate response element-binding protein (30). Interestingly, inhibition of NOS in mouse C2C12 myotubes using L-NAME abolishes the increase in PGC-1α expression by L-CIT, indicating the ability of L-CIT to increase NO production in skeletal muscle (29). Thus, improved NO-mediated regulation of mitochondrial function after L-CIT supplementation may explain prolonged swimming time to exhaustion observed in rodents (29,31). Although evidence is emerging in animals to support L-CIT improving NO bioactivity within skeletal muscle, it is unknown what NOS isoform (endothelial or neuronal) is involved and whether these changes translate to improved vascular function, particularly in humans.

L-CIT and Exercise Performance

The influence of oral L-CIT on muscle oxygen delivery/utilization was found by Bailey *et al.* (11). They revealed that 6 $g \cdot d^{-1}$ of L-CIT for 7 d led to lower muscle deoxyhemoglobin patterns and higher muscle oxygenation index during high-intensity cycling in young healthy adults (11), suggesting that improved O_2 availability/distribution within the muscle microvasculature enhanced exercise performance. Our recent work (32) found that 2.4 $g \cdot d^{-1}$ of L-CIT for 7 d reduced time to complete the 4-km cycling time trial and increased power output in healthy trained men, despite no significant difference in O_2 consumption between placebo and L-CIT. In agreement with previous findings, Terasawa and Nakada (12) noted improved pedaling speed and mean power output after 3 $g \cdot d^{-1}$ of L-CIT for 7 d in male athletes. In recent studies by us and others, antimuscular fatigue effects of L-CIT have been elucidated during high-intensity exercise (12,32). The benefits of L-CIT on exercise performance could be due to improvement of peripheral vasodilation/perfusion and subsequent muscle oxygen utilization by increasing NO bioavailability in active skeletal

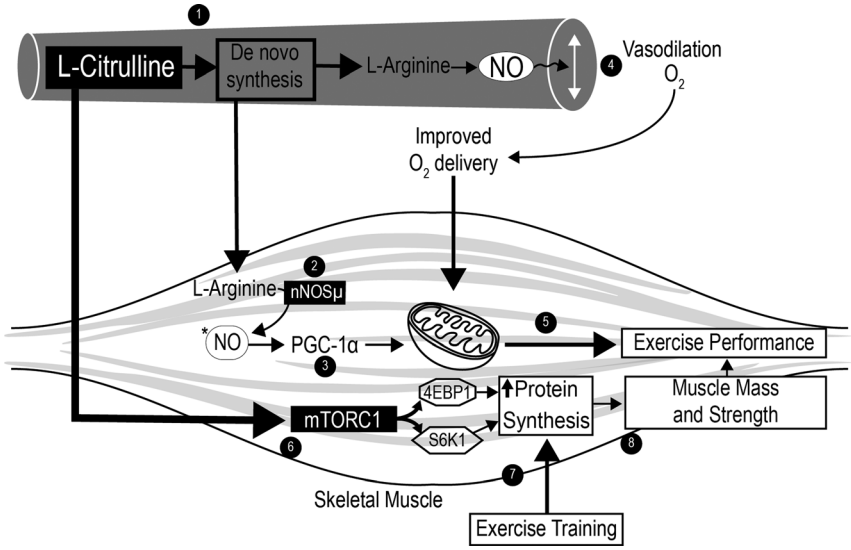

Figure 2. Purported mechanisms by which L-CIT improves exercise performance alone and combined with exercise training. *De novo* synthesis of L-ARG (1) from circulating L-CIT increases nNOS (nNOSμ) activity in the skeletal muscle (2). NO produced by nNOSμ stimulates expression of PGC-1α (3), a regulator of mitochondrial respiration. Improved peripheral NO-mediated vasodilation and subsequent muscle oxygen utilization in active skeletal muscles contribute to enhance exercise performance (4). An enhanced mitochondrial respiratory capacity from the combined effect of PGC-1α expression and O_2 availability from vasodilation contributes to improve exercise performance (5). Moreover, L-CIT directly promotes muscle protein synthesis by stimulation of the mTORC1 pathway, which involves increased phosphorylation of S6K1 and 4E-BP1 (6). This effect of L-CIT on protein synthesis may improve muscle mass or strength when combined with exercise training in obese older adults (7 and 8). Enhanced mitochondrial metabolism and skeletal muscle function may contribute to improve exercise performance. *NO produced by nNOS may contribute to vasodilation after L-CIT supplementation.

muscles, since an early study indicated that this is an important source of NO, which may influence muscle function and mass (33) (Fig. 2). Moreover, L-CIT supplementation could prolong time to exhaustion by attenuation of exercise-induced blood ammonia elevation in mice (31). Although the exact mechanism is unclear, as L-CIT and L-ARG are components of the urea cycle, L-CIT supplementation may promote ammonia elimination via the urea cycle. As a result of intense exercise, ammonia accumulation is linked to a variety of functional and metabolic neurological disturbances other than exercise and fatigue and is associated with inhibition of the oxidation of pyruvate to acetyl CoA in the mitochondria, which could result in a decrease in ATP production through the Krebs cycle and contribute to muscle fatigue (31,34).

L-CIT combined with L-ARG may have additive effects. We found that oral supplementation with L-CIT and L-ARG caused a greater increase in plasma L-ARG, NO metabolites, and cGMP levels than the single amino acid alone in animals (21). These effects of L-CIT plus L-ARG were associated with enhanced peripheral blood flow. The upregulation of the L-ARG-NO-cGMP pathway by the combined amino acid supplementation was likely via the effect of L-CIT on arginase activity, acting as a strong allosteric inhibitor (19). To test for the clinical and applied utility of this combined supplementation, Suzuki *et al.* (35) assessed performance on a bicycle ergometer in male athletes and demonstrated that 7 d of oral L-CIT ($1.2\ g{\cdot}d^{-1}$) and L-ARG ($1.2\ g{\cdot}d^{-1}$) improved 10-min full-power cycling test performance and postexercise subjective perception related to muscle fatigue. Although the L-CIT plus L-ARG combination may provide superior efficacy leading to cardiovascular benefits by promoting greater L-ARG-NO-cGMP bioavailability than each amino acid alone, further studies are needed to elucidate the effectiveness on exercise performance or tolerance in older adults and clinical populations. These findings suggest that L-CIT improves local muscle oxygen kinetics and cycling performance despite no changes in oxygen consumption, likely due to enhanced microvascular function and reduced ammonia accumulation. These effects may be more pronounced when combining L-CIT and L-ARG.

L-CIT and Regulation of Muscle Protein Synthesis

For decades, L-CIT was considered as an intermediate of the urea cycle with a limited interest. However, metabolic properties of this amino acid were subsequently demonstrated as an important modulator of nitrogen homeostasis.

The impact of L-CIT on muscle protein synthesis has been reported by Moinard and collaborators (8,22,36–43). In a pioneering article, Osowska *et al.* (22) demonstrated in a short bowel syndrome model that L-CIT improved nitrogen balance. Therefore, it was proposed that L-CIT would be a regulator of muscle protein synthesis. In 2006, the same authors demonstrated the stimulatory effect of L-CIT on muscle protein synthesis (36) in aged undernourished rats, which increased muscle protein synthesis by 80% after receiving L-CIT–enriched diet. This effect was also demonstrated in adult rats with protein-energy deficiency via short fasting (fasted for 18 h), resulting in decreased muscle protein synthesis (−40%) that was fully restored by an oral bolus of L-CIT. Similarly, Ventura *et al.* (37) reported in adult female rats supplemented with L-CIT and moderately feed restricted (60% of their spontaneous food intake) that L-CIT increased the synthesis of myofibrillar proteins. It seems that the positive effect of L-CIT on muscular gain was maintained over time. Hence, L-CIT supplementation for 3 months in "healthy" aged rats enabled an average 25% muscle gain specifically related to protein accretion and to an increase in muscle fiber size (38). It also was observed that protein accretion associated with L-CIT intake was accompanied by an improvement of muscular function, thus establishing a

continuum between metabolic action and clinical repercussions. In the protein-energy restriction model in aged rats, L-CIT increased muscle strength and mass (8). Muscular strength was also preserved by L-CIT in a food-restricted adult female rat model (37). The benefits of L-CIT were also observed in healthy conditions. Hence, Goron *et al.* (39) overcame this lack of data in a recent study where healthy adult rats received L-CIT supplementation (1 $g \cdot kg^{-1} \cdot d^{-1}$) or an isonitrogenous diet during 4 wk. As observed in catabolic conditions, L-CIT increased muscle protein synthesis (+33%), but without any effect on muscle mass and muscle protein content. Interestingly, despite this lack of anabolic effect on muscle protein, the authors observed that L-CIT supplementation improved exercise performance (running time increased by 14%). The accumulation of experimental data has made it possible to transpose the results obtained into clinical practice.

A prerequisite is to determine the safety and the optimal dose of L-CIT to use in humans. In young adults, consumption of 2, 5, 10, or 15 g of L-CIT was well tolerated and resulted in a dose-dependent increase in circulating L-ARG and nitrogen balance (44). Moreover, the dosage of 10 $g \cdot d^{-1}$ seems to be the most appropriate as it is not further metabolized and its urinary excretion increases. These results have been confirmed in elderly adults (9). Oral supplementation with L-CIT (10 g) improved muscle protein synthesis by 25% in healthy adults under a low-protein diet (8%) for 3 d (40). In addition, Bouillanne *et al.* (10) demonstrated that 21-d oral supplementation with L-CIT (10 $g \cdot d^{-1}$) in malnourished patients increased lean body mass (5%–10%) and reduced fat mass (−9% in women). However, the effect of L-CIT on protein synthesis seems to be muscle specific, as L-CIT supplementation has been unable to show a positive effect on whole-body protein metabolism (40).

Importantly, is an increase in protein turnover associated with improved performance? Clearly, an improvement of muscle protein synthesis may have major repercussions in muscle functionality. Hence, proteins are highly exposed to several forms of damage (*e.g.*, oxidative stress, carbonylation) that alter their biological roles (oxidized or carbonylated proteins are no longer functional) (45). In such conditions, any increase in protein turnover improves the continuous remodeling of muscular proteins, maintaining their quality and their functional properties.

L-CIT and Muscle Protein Synthesis: The Mechanism

Although the capacity of L-CIT to modulate muscle protein synthesis has been well demonstrated in several conditions, the precise mechanisms of action involved were still unclear. Recently, an article remedied this deficit. Le Plénier *et al.* (41) showed, in isolated incubated rat limb muscle, that muscle protein synthesis was higher with L-CIT, demonstrating a direct action. Moreover, this increase in muscle protein synthesis could be linked to stimulation of the mammalian target of rapamycin complex 1 (mTORC1) pathway. In this same *in vitro* study, the addition of rapamycin (mTORC1 inhibitor) blunted the positive effect of L-CIT on muscle protein synthesis. Moreover, the stimulation of mTORC1 by L-CIT was confirmed *in vivo* in a model of aged malnourished rats (41). These data were confirmed by Goron *et al.* (39), who demonstrated that L-CIT supplementation for 4 wk increases phosphorylation of S6 kinase 1 (S6K1) (+57%) and 4E-binding protein 1 (4E-BP1) (+46%) in muscle of adult healthy rats. These results seem to connect the ability of L-CIT to increase muscle protein synthesis via stimulation of the mTORC1 pathway (Fig. 2). In addition, this effect is partially Akt/PI3K independent, as L-CIT maintains 4E-BP1 activation by phosphorylation even when the Akt/PI3K pathway, upstream of mTORC1, is inhibited (41). However, the regulation of muscle protein metabolism by L-CIT seems to be more complex. Indeed, L-CIT is able to stimulate the expression of specific muscle proteins and inhibit the expression of others. Specifically, L-CIT stimulates the expression of myofibrillar proteins and modulates enzymes that drive energy metabolism. In the model of protein-energy malnutrition described earlier, a differential proteomics approach showed that L-CIT leads to overexpression of enzymes involved in glucose catabolism (*i.e.*, glycogenolysis and glycolysis) and lower expression of some enzymes of the Krebs cycle (*i.e.*, isocitrate dehydrogenase and succinate dehydrogenase) and the mitochondrial respiratory chain (*i.e.*, NADH dehydrogenase complex, NADH ubiquinone oxidoreductase, and ATP synthase) in skeletal muscle (42). Another proteomics study in "healthy" aged rats showed that L-CIT supplementation stimulates the expression of mitochondrial biogenesis factor (mitochondrial transcription factor A) as well as mitochondrial complex I activity (38). A more recent study has confirmed the effect of L-CIT supplementation on energy metabolism. Goron *et al.* (39) showed that L-CIT supplementation for 4 wk stimulates several enzymes involved in the pathways generating acetyl-CoA in adult rats. These properties of L-CIT were further confirmed *in vitro*. In myotubes derived from primary myoblasts, deprived of amino acids and serum during 16 h, L-CIT (5 mM) is able to stimulate protein synthesis (43). Thus, the stimulation of muscle protein synthesis by L-CIT could have contributed to the increase in lean mass observed in malnourished older adults (10). However, the precise mechanisms remain to be established.

L-CIT and Exercise Training

The American College of Sports Medicine recommends resistance training (RT) at 65%–85% of one-repetition maximum to augment muscle quality (*e.g.*, mass and strength) (46). High-intensity RT (≥80% of one-repetition maximum) is the most efficacious form of exercise training and intensity to induce muscular hypertrophy and strength gains (46). However, because of the increased arterial blood pressure responses to high-intensity RT, it may not be an appropriate intensity for all populations, especially older adults with hypertension and CVD (47). Although some studies have reported increases in arterial stiffness with high-intensity RT, the majority of data suggest little to no impact of RT at any intensity on vascular structure in young adults, but there are limited data in older adults (47). Given that the potential for adverse vascular effects of high-intensity RT exists, we suggested that low-intensity RT may be preferred for middle-aged and older adults (47). Alternatively, whole-body vibration training (WBVT) consisting of dynamic low-intensity leg exercises on a vibrating platform augmenting muscle activation has shown effectiveness as a strength modality with important vascular benefits. We reported similar improvements on muscle strength and endothelial function (baFMD) after 12 wk of low-intensity RT and WBVT in postmenopausal women (48), suggesting these modalities are effective to benefit both skeletal muscle and vascular function. On the basis of positive findings of our studies (39,47,48), the potential

additive effects of exercise training and L-CIT supplementation to enhance exercise performance is a reasonable hypothesis for future research.

Evidence provided by our studies has demonstrated that L-CIT for 1 and 2 wk reduced systemic arterial stiffness [brachial-ankle pulse wave velocity (baPWV)] at rest in middle-aged adults (7) and during low-intensity exercise in young healthy men (49). Although carotid-femoral PWV is the gold standard measure of aortic stiffening, baPWV is an independent predictor of cardiovascular risk because this large arterial segment includes the aorta as the main component (47). Given the positive impact on vascular function, including improvements in arterial stiffness (baPWV) (7,13) and endothelial function (baFMD) (27) via upregulation of NO synthesis (7,14) demonstrated in our studies, it remains a logical hypothesis that chronic, but not acute, L-CIT may alleviate or prevent potential arterial stiffening in response to RT. The additive effect of a dietary arginase inhibitor (L-CIT) (19) and exercise training on muscle mass and function was recently examined in obese older adults. We conducted the first study to examine the combined effect of L-CIT and exercise training (13,14). In this study, WBVT and L-CIT for 8 wk elicited positive effects on brachial and aortic blood pressure, independently and combined, in obese postmenopausal women (13,14). Although WBVT significantly reduced augmentation index adjusted for heart rate, the reduction was greater when exercise training was combined with L-CIT compared with L-CIT alone (14). The effect of this combined intervention may be attributable to the observed reduction in aortic stiffness (carotid-femoral PWV), which was not evident with each intervention alone. Similarly, an additive effect of L-CIT and WBVT on muscle mass may have important clinical implication for the prevention of sarcopenia in older women (13,14). A key mechanism underlying the vascular and muscular benefits of WBVT and L-CIT may be improved endothelial vasodilatory function (baFMD) (7,27,48) (Fig. 2). Because vascular and muscular adaptations to WBVT and low-intensity RT were comparable in our previous study (48), there is great potential for future research investigating the combination of L-CIT and RT, whether it be traditional or nontraditional strength exercises, on arterial and muscular function in older adults.

Although not RT, 12 wk of L-CIT combined with all-extremity high-intensity interval training using an elliptical trainer produced additional increases in handgrip strength and walking speed compared with training alone in obese older adults with dynapenia (age-related loss of muscle strength) (15,50). Therefore, 10 g of L-CIT per day combined with intense aerobic training was more effective for increasing muscle strength and function than training alone in dynapenic obese older adults (15). In a more heterogeneous senior population, it was shown that this combination of L-CIT and exercise training was more beneficial to people who consumed less than 1 $g \cdot kg^{-1} \cdot d^{-1}$ of protein than those who consumed more than 1 $g \cdot kg^{-1} \cdot d^{-1}$ of protein (50). In the previous studies, all characteristics of sarcopenia (muscle mass, strength, and exercise performance) were improved in obese older adults by L-CIT added to exercise training (Fig. 2). However, further studies are needed to investigate the effects of L-CIT supplementation and RT on skeletal muscle protein synthesis and vascular function in older adults with or at risk for CVD and sarcopenia. In summary, although high-intensity RT is the most recognized modality to augment skeletal muscle mass and strength, low-intensity RT and WBVT have the capacity to induce important muscular and vascular benefits (47,48). When WBVT is combined with L-CIT, the resultant benefits are additive in both skeletal muscle and vascular function (13,14), likely due to increases in muscle perfusion via improved endothelial function (48). The addition of L-CIT to high-intensity aerobic interval training also benefits strength in older obese dynapenic adults. However, the potential vascular and muscular effects of RT combined with L-CIT have not been examined.

SUMMARY

Evidence continues to emerge on the positive impact of chronic L-CIT on vascular and skeletal muscle function in older adults. L-CIT may improve exercise capacity by upregulating muscular perfusion and subsequent oxygen utilization. Evidence suggests that increased L-ARG and NO availability in skeletal muscle may be a crucial factor in promoting muscle function via PGC-1α stimulation of mitochondrial respiratory capacity. In addition, the action of L-CIT on muscle function also is related to its ability to promote muscle protein synthesis. Indeed, there are many studies that establish a continuum between experimental and clinical research that confirm its action. Although animal research is revealing the mechanistic insight upon how L-CIT positively alters the regulation of these physiological systems, human research is highlighting the effect of L-CIT on exercise performance and adaptations with exercise training. Future research is still needed to decipher the contribution of endothelial versus neuronal NO production to the vascular and muscular benefits observed after L-CIT supplementation to fully understand the ergogenic and therapeutic potential of this amino acid. More studies will be needed to investigate the additive clinical benefits of L-CIT supplementation and exercise training on arterial and muscle functions in older adults.

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