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REVIEW

Carnosine as a potential therapeutic for the management of peripheral vascular disease

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Abstract *Aims:* To evaluate the potential role of carnosine in the management of peripheral vascular disease.
Data synthesis: Peripheral vascular disease is growing in its burden and impact; however it is currently under researched, and there are a lack of strong, non-invasive therapeutic options for the clinicians. Carnosine is a dipeptide stored particularly in muscle and brain tissue, which exhibits a wide range of physiological activities, which may be beneficial as an adjunct treatment for peripheral vascular disease. Carnosine's strong anti-inflammatory, antioxidant and antiglycating actions may aid in the prevention of plaque formation, through protective actions on the vascular endothelium, and the inhibition of foam cells. Carnosine may also improve angiogenesis, exercise performance and vasodilatory response, while protecting from ischemic tissue injury.
Conclusions: Carnosine may have a role as an adjunct treatment for peripheral vascular disease alongside typical exercise and surgical interventions, and may be used in high risk individuals to aid in the prevention of atherogenesis.
Clinical recommendation: This review identifies a beneficial role for carnosine supplementation in the management of patients with peripheral vascular disease, in conjunction with exercise and revascularization. Carnosine as a supplement is safe, and associated with a host of beneficial effects in peripheral vascular disease and its key risk factors.

1. Introduction

Peripheral vascular disease (PVD) is a growing concern for healthcare globally, in the face of an aging population and, with rising prevalence of cardiometabolic risk factors. PVD, also known as peripheral artery disease, is characterized by progressive occlusion of arteries due to atherosclerosis, leading to reduced blood flow in the limbs. This causes

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pain with physical activity, known as intermittent claudication, and progressive limb ischemia. The prevalence of PVD is challenging to assess and is therefore underreported, with more than 50% of cases being asymptomatic [1]. The rates vary drastically across region and socioeconomic groups due to differences in prevalence rates of cardiometabolic risk factors [2]. The global prevalence of PVD in those aged 25 and older is 5.56%, however it rises sharply with age affecting more than 18% of those over 85 [3]. Regardless of the presence of symptoms, PVD is associated with increased mortality, significant disability, and reduced quality of life [4,5]. Despite this significant burden, PVD receives significantly less research and healthcare attention than other cardiovascular diseases.

Presently very few effective treatments are available for PVD. Currently, two drugs, pentoxifyline and cilostazol, are approved for treating PVD. However, the effectiveness of these drugs is limited, hence they are not routinely used in clinical practice and are not recommended for use in Australia. Structured exercise programs, such as treadmill walking are effective in alleviating PVD symptoms, increase walking endurance, and improve a number of cardiometabolic risk factors [6]. However, exercise is limited by the intermittent claudication associated with physical activity [7]. Open surgical revascularization is the most invasive option, and is also effective in reducing symptoms and increasing exercise tolerance [8], particularly when combined with exercise [9]. Operative approaches are limited by cost, type of disease (not appropriate for diffuse disease), invasiveness and general surgical risk, and therefore are considered inappropriate in the early stages of the disease. Endovascular revascularisation, such as angioplasty, is a minimally invasive alternative to open surgery which is now performed more commonly than open bypass for peripheral vascular disease [10]. Given the negative cycle of PVD limiting physical activity, which subsequently worsens the disease, new treatments are required to help manage symptoms and prevent disease progression.

Carnosine is a naturally occurring dipeptide, with potent antioxidant, antiglycating and geroprotective effects [11,12]. This dipeptide is synthesised *in vivo* from the amino acids, β-alanine and histidine by the enzyme carnosine synthase (CARNS) [13]. Carnosine is present in the meat and in particular red meat, is a rich dietary source of carnosine [14]. β-alanine is the precursor amino-acid, which is not incorporated into proteins. Numerous studies show that carnosine concentrations can be increased through supplementation of β-alanine alone [15,16], increasing its concentrations in skeletal muscle, cardiac and brain tissues [11,17]. The chemical nature of carnosine offers significant physiological activity (Fig. 1). The imidazole ring derived from histidine imparts a high proton buffering capacity, while its nucleophilic structure helps it to form conjugates with reactive aldehydes, such as acrolien [18,19]. Reactive aldehydes are the downstream effectors of reactive oxygen species, which are formed due to decreased antioxidant ability in the face of oxidative stress [20]. They are generated by cytotoxic conversion of macromolecules to form advanced glycation end products (AGEs), such as methylglyoxal and advanced lipoxidation end products (ALEs), such as 4 hydroxynonenal. These cytotoxic products of oxidative stress cause changes to carbohydrate and lipid metabolism, acting as secondary signaling messengers of cytotoxic metabolic events [12,20]. The single nitrogen of the histidine also forms complexes with divalent metal ions allowing chelation [21], and the synergistic effect of the imidazole ring and the free amino group of β-alanine aid in inhibiting formation of harmful advanced glycation end products [22]. While carnosine is yet to be widely evaluated in atherosclerotic disease, these mechanisms have a naturally synergistic potential as an intervention in PVD, targeting a number of key processes underpinning PVD development, progression and symptoms.

2. Current therapeutic approaches for PVD

The current paradigm of medical management for PVD is centred on three key approaches: exercise prescription, pharmacotherapy, and revascularization surgery. While combinations of these treatments are effective in reducing the symptoms associated with PVD, however each of the interventions have limitations to their use.

Therapeutic exercise prescription is typically recommended as a first line treatment for symptomatic PVD [23]. As exercise both directly improves peripheral muscular and vascular function, as well as important risk factors such as obesity, hyperglycaemia and hyperlipidaemia, it plays a central role in management of the disease. The likely mechanisms underpinning these effects are increased capillary density in the lower leg, decreased inflammation, and improved vasodilatory response with exercise training [8]. The majority of research on exercise prescription for PVD is centred on supervised treadmill walking programs, with the most recent evidence confirming beneficial effects on aerobic capacity, plantar flexor muscle strength, peak walking distance and initial claudication time [8,24]. A smaller number of studies have evaluated resistance training as an interventions, which has been shown to have similar outcomes in muscular strength, walking capacity and claudication time [24]. While exercise has a number of benefits for these patients, there are significant challenges to its effective utilization clinically. The characteristic claudication of PVD causes significant pain on exercise, leading to challenges with compliance, as well as difficulty in acquiring sufficient training volume for benefit. Patients are commonly unable to sustain walking effort for longer durations, particularly if they are older, and more deconditioned due to comorbidity and inactivity. General compliance to exercise programs is often poor, while supervised exercise is more expensive, and may be inaccessible to many patients. PVD also commonly co-exists with a host of other factors, which may limit exercise participation, such as diabetes, cardiovascular disease, musculoskeletal disease and obesity, and while these are all likewise improved by exercise, they also reduce compliance and the ability to perform exercise effectively.

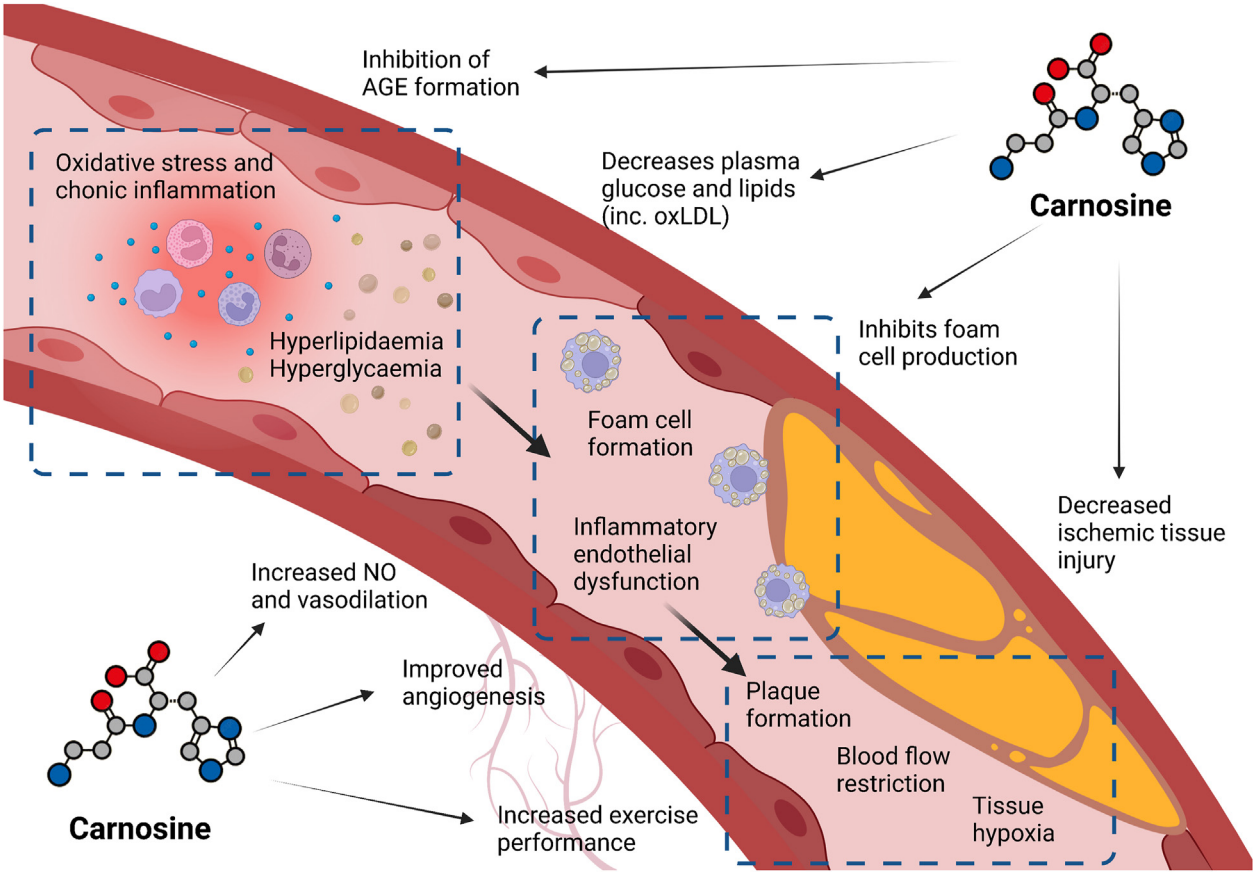

Figure 1 Proposed therapeutic mechanisms for carnosine in PVD. okLDL: oxidized low density lipoprotein, NO: Nitric oxide.

Pharmacological treatments for PVD rely on two major approaches, antiplatelet and hemorheological therapies. There are two approved medications for the specific treatment of PVD, the antiplatelet medication Cilostazol, and the hemorheological medication Pentoxifylline. Other drugs such as aspirin are also used in combination with these drugs [25]. While these approaches are targeted specifically at management of PVD, patients are also likely to be treated with statins, anti-hypertensive and hypoglycaemic agents, to limit progression and secondary events. Antiplatelet therapies are widely used, and are effective in reducing systemic thrombotic events, however their efficacy in reducing acute limb ischemia, amputation or symptoms of claudication are limited, particularly in the case of aspirin [26]. Cilostazol has been shown to cause modest improvements in walking distance [27], however it does not improve all cause, or cardiovascular mortality [28]. Additionally, while Cilostazol is broadly considered safe, it is not suitable for use in patients with heart failure, or reduced ejection fraction (which constitute a large proportion of patients), and has a number of side-effects, making durability a potential issue [26]. Pentoxifylline has been shown to improve perfusion in patients with PVD, through reduced blood viscosity and increased cellular deformability [29]. However, the clinical benefits of Pentoxifylline are small, making it rarely prescribed, typically only used in those who cannot tolerate other agents [26]. The small benefits, limitations, and side-effects of these pharmacological agents, makes their widespread use and impact minimal, particularly in significant outcomes such as mortality. In fact, neither Cilostazol nor Pentoxifylline are approved for management of PVD in Australia, paving the way for novel agents to reduce the clinical burden of PVD.

While these medications are hampered by poor efficacy and adverse events, other commonly used cardiometabolic medications are also used in the management of PVD. The angiotensin converting enzyme (ACE) inhibitors and statins are widely used to manage the hypertension and dyslipidaemia, respectively, commonly seen alongside PVD. ACE inhibitors have been shown to have anti-atherogenic, antiproliferative and antimigratory effects on mononuclear cells and protect against rupture of atherosclerotic plaques [30]. ACE inhibitors also have the ability to improve vascularisation by boosting endogenous fibrinolysis [30]. ACE inhibitors primarily exert their activity by inhibiting production of Angiotensin II in the kidney, which results in vasodilation of renal and peripheral arteries. The statins improve plasma hypercholesterolemia via inhibiting the action of HMG-CoA reductase, a crucial enzyme in the production of plasma LDL [31]. Statins have been shown to improve endothelial dysfunction and also

provide anti-inflammatory, anti-proliferative and anti-thrombogenic effects [31], which are all relevant in the context of PVD. Similar to carnosine, statins can also stabilise atherosclerotic plaque formation and inactivate NFkB, a potent activator of inflammatory cascades [31], providing further potential for utility in PVD. However, unlike carnosine, ACE inhibitors and statins have side effects, which can prevent their use.

The last line of therapeutic intervention is revascularization, which can be performed via endovascular or open surgical approach, depending upon location and extent of atherosclerosis. Revascularization is generally effective in restoring effective perfusion to an area, and reducing symptoms of the disease [32]. It is shown to be more effective than exercise interventions, however the best outcomes are achieved through a combined approach of surgical and supervised walking [8]. Endovascular revascularization has been associated with improved long-term amputation free survival compared with open surgery [33]. A recent Cochrane review suggests combination therapy, such as endovascular revascularization with either supervised exercise or drug therapy offers greater clinical improvement than either supervised exercise therapy or drug therapy alone [34]. However, it is typically reserved for patients with chronic critical limb ischemia (CCLI), characterized by extreme circulation limitation and pain at rest, or with severe claudication, due to risks associated with the procedures due to risk of adverse limb, or cardiovascular events [35]. Additionally, it is commonly not recommended for those with key risk factors for critical events, such as in smokers, type 2 diabetics, limiting its applicability [36]. Importantly, given that even asymptomatic and undiagnosed PVD is associated with negative outcomes, revascularization is unable to be applied to many patients.

While therapeutic interventions are available for PVD, they are limited by compliance, limited efficacy, or inability for broad application to patients. To combat this, novel, safe and cost-effective approaches are urgently needed to reduce the burden of PVD more broadly.

3. Prevent the plaque: carnosine as an inhibitor of atherogenesis

Advanced glycation end products (AGEs) are well known to be associated with the development of atherosclerosis [37] and type 2 diabetes [38]. AGEs cause significant oxidative stress, leading to inflammatory endothelial dysfunction, a critical precursor to formation of atherosclerotic plaques [39]. Importantly, AGE formation is associated with adverse outcomes in patients with PVD, being strongly associated with disease progression and cardiovascular outcomes [40].

Carnosine strongly inhibits AGE formation [22], potentially reducing oxidative stress on the endothelium, and leading to decreased vascular injury and atherogenesis. Carnosine reduces the formation of pathogenic foam cells – cholesterol laden macrophages which contribute to the formation of plaques [41]. It was proposed that this also occurred through a decrease in the glycation of LDL by reactive aldehydes, a key mediator of AGE formation [41]. This effect on aldehyde reduction has also been indirectly inferred in humans [42]. Several animal models have identified that carnosine is able to protect against the development of atherosclerotic plaques in hypercholesteremic and diabetic mice, through its antiglycating actions [43–46]. In addition to the direct impact on plaque formation, carnosine has also been shown to protect against the harmful effects of obesity, ameliorating the associated dyslipidaemia in animal models [47].

In addition to these direct pathogenic actions, carnosine influences a significant number of risk factors strongly associated with PVD. Animal studies show that carnosine reduces obesity, improves glucose metabolism, blood pressure, markers of chronic low-grade inflammation and oxidative stress, AGE/ALEs, lipid levels and peroxidation in a dose-dependent fashion - each of which could individually significantly affect the progression of PVD [12,45,47–51].

Taken together, these findings paint a compelling picture of carnosine as a means of preventing the onset of PVD as well as limiting its progression. However, carnosine may also have a role to play in protecting patients against the ischemic injury to peripheral tissues caused by the ongoing obstruction and hypoxia caused by PVD.

4. Limiting the ischemia: carnosine as an anti-ischemic agent

Critical ischemia is the underlying cause of tissue loss in PVD patients [52], and is the central mechanism underpinning it's characteristic symptoms of exercise intolerance. The ischemia of PVD is caused by progressive occlusion in peripheral vessels, and a failure of the circulation to generate collateral supply. Current studies show that post-natal vessel growth is regulated by the transcription factor, HIF-1α [53,54]. Activation of HIF-1α leads to the expression of angiogenic genes such as VEGF [55], essential in promoting the mobilization of pro-angiogenic endothelial progenitor cells (EPCs) [56–58] as well as promoting an inflammatory response, which is essential for collateral growth [59,60]. Under normal conditions, HIF-1α is targeted for proteasomal degradation through the activity of prolyl hydroxylases (PHDs), which require iron for their activity. Recent studies show that metal chelators and pharmaceutical inhibitors of PHDs prevent HIF-1α proteasomal degradation and promote revascularization [61]. However, the use of these chelators or inhibitors can cause toxicity. Similarly, clinical trials using HIF-1α and VEGF gene therapy in patients with chronic limb ischemia were largely negative or inconclusive because the effect of HIF-1α was localized at the site of injection [62,63]. Carnosine is an efficient chelator of metals [64], through which it imparts some of it's antioxidant effect. However, it may also be a non-toxic means of limiting HIF-1α degradation through iron sequestration, increasing its angiogenesis. This effect has been shown in mouse models of hind limb ischemia, in which carnosine

administration led to increased expression of HIF-1α and VEGF, and enhanced perfusion [65].

While it is unclear whether atherosclerotic plaques can be reduced after onset, their impact can be mitigated through maintenance of circulation. Ongoing endothelial dysfunction commonly leads to altered function in endothelial nitric oxide synthase [66]. This dysfunction leads to excess production of superoxide free radicals, and a subsequent decrease in the powerful vasodilator nitric oxide (NO) [66]. This decrease in NO production leads to increased vasoconstriction, leading to progression or exacerbation of a number of cardiovascular conditions [67]. Interestingly, this process is reversed through application of antioxidants, increasing the bioavailability of NO [68]. Carnosine has been shown to improve vasodilation in animal models [69], however the mechanism for this effect has yet to be identified. This could lead to increased tissue perfusion, and limit hypoxia for those living with PVD. In addition to improving perfusion, carnosine may be able to limit the injury associated with hypoxia.

Carnosine has also been shown to protect against ischemia in a number of animal models of organ damage induced by ischemia-reperfusion [70–72]. The mechanisms underlying these effects are not fully understood but are potentially due in part to its proton buffering effect. As cells breakdown due to hypoxic injury, protons are released from both breakdown of tissue, mitochondrial dysfunction and the action of innate immune cells [73]. The decreased pH and resulting oxidative damage lead to ongoing tissue stress and injury. Carnosine has significant pH buffering activity [74], offering a protective benefit against this, as well as inhibiting the respiratory burst of innate immune cells [75]. This is likely to protect against some level of the hypoxia induced injury caused by PVD.

While increasing perfusion is likely to aid in ameliorating the symptoms associated with exercise induced hypoxia and claudication, carnosine has a number of physiological impacts at the skeletal muscle level, which are likely to improve exercise tolerance.

5. Improving exercise tolerance

The major symptom of PVD is a lack of exercise tolerance, with onset of claudication during physical exertion. As obstructive disease progresses the capacity for exertion decreases, and lack of physical activity subsequently worsens cardiovascular health. Perhaps carnosine's most well-known benefit is an increase in muscular performance, leading to its use as an athletic supplement [74]. However, its powerful action in skeletal muscle physiology could also aid in ameliorating the symptoms of patients living with PVD, improving quality of life, and increasing exercise capacity. Carnosine reduces lactic acid formation due to its buffering capacity, which may on its own promote walking endurance in patients with PVD by preventing muscle acidosis [76], however this is yet to be tested in those with PVD induced claudication. Administration of carnosine to individuals with heart failure was able to improve 6-min walk test, health related quality of life and key VO2 measures, with non-significant ($p = 0.07$) improvement in systolic cardiac function, but muscular effects were not evaluated [77]. There has also been some suggestion that carnosine can improve excitation coupling, and therefore muscular performance and cardiac function [78], however this has not been directly observed [79].

Carnosine has been widely used to increase athletic performance, and while it may seem logical that it would improve outcomes for those with PVD, there is little data available in humans. High-quality randomised controlled trials studying the impact of carnosine supplementation on exercise endurance and performance are required to guide its recommendation.

6. Why carnosine?

While carnosine has been more extensively studied in chronic diseases, there may be some utility in the study of cheaper β-alanine rather than carnosine for managing the disease. As the key rate-limiting component of *in vivo* carnosine synthesis, supplementation with β-alanine effectively increases concentrations of carnosine in muscle [15]. However, to our knowledge, there are no studies which investigated β-alanine, in any setting of cardiometabolic disease, or compared β-alanine and carnosine. Indeed, the other amino acid component of carnosine, histidine, has also been shown to have physiological effects, improving metabolic syndrome, giving a potential dual mechanism for carnosine supplementation [80]. There are also a number of other histidine containing dipeptides which have physiological functions similar to carnosine, the most important of these being anserine and acetylcarnosine. These two molecules are known to have a range of shared functions with carnosine, including its metal chelating and anti-oxidative properties, however, their therapeutic effect has not been evaluated in cardiometabolic diseases. While there may be no evidence on these compounds in cardiovascular disease, they may offer advantages over carnosine in some settings. Anserine and acetylcarnosine are more stable in the circulation, and resistant to carnosinase mediated degradation [81], potentially allowing for more long-lasting effects. While, these are worthy candidates for future investigation, carnosine remains the front-running therapeutic candidate for clinical use at present.

7. Carnosine prescription for PVD: what is its role?

As previously described, carnosine is particularly suited to managing the symptoms of PVD as part of a multimodal treatment. Its broad physiological action, targets a number of key mechanisms which may provide benefit to those with PVD. Importantly, carnosine is well tolerated, with few reports of serious side-effects, beyond occasional transient paraesthesia with high doses [82]. This, coupled with its widespread benefits on key cardiometabolic risk factors, makes it a strong candidate for use regardless of direct impact on PVD [12,51]. However, it is also likely to have a beneficial effect on lower limb tissue perfusion,

prevent formation and progression of atherosclerosis, and increase exercise capacity in those living with PVD. Given the strong associations with even asymptomatic PVD, decreased quality of life and mortality, novel treatments for the disease are vital. It is likely that carnosine would play an adjunctive role alongside standard care such as supervised exercise training. Carnosine also has a potential synergistic effect when combined with endovascular revascularization. Importantly, while PVD is on its own, a significant health challenge, the multimorbid nature of atherosclerotic disease cannot be forgotten. Patients with PVD are also likely to have coronary heart disease, hypertension, metabolic syndrome, and kidney disease, however given the shared pathology, it is likely that carnosine will have a beneficial impact across the cardiovascular risk factor and disease spectrum. Indeed, it is known to have benefits in type 2 diabetes [12,45,48], renal disease [83], and heart failure [77], strengthening the case for its use in this population.

While the preliminary evidence on the use of carnosine in PVD is supportive, however there is a critical lack of human studies in the field. High quality trials are needed evaluating supplementation of carnosine alongside standard care, such as exercise or pharmacological intervention. Additionally, dose finding studies, and long-term evaluation of its outcomes are required to guide recommendations for its use. Finally, thorough investigation into the mechanisms underpinning its effects is required to further inform its use, and identify any relevant contraindications or interactions.

8. Conclusion

As the burden of atherosclerotic disease such as PVD continues its steady increase, identification of novel therapeutic strategies for its prevention and management are of critical importance. Particularly, in the instance of PVD, where current interventions are of limited efficacy. Carnosine is a strong candidate for therapeutic evaluation in this setting, with a wide range of physiological effects on factors central to the pathology of PVD. Its metal chelating, antioxidant, anti-inflammatory, antiglycating and pH buffering effects prevent the development and progression of plaques, as well as potentially increase tissue perfusion, and improve exercise capacity. Future human studies will help evaluate the clinical outcomes of supplementation, and thus guide translation into this underserved cohort.

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Declaration of competing interest

The authors declare no conflicts of interest.

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