Pathophysiology of chronic peripheral ischemia: new perspectives

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Abstract: Peripheral arterial disease (PAD) affects individuals particularly over 65 years old in the more advanced countries. Hemodynamic, inflammatory, and oxidative mechanisms interact in the pathophysiological scenario of this chronic arterial disease. We discuss the hemodynamic, muscle tissue, and oxidative stress (OxS) conditions related to chronic ischemia of the peripheral arteries. This review summarizes the results of evaluating both metabolic and oxidative markers, and also therapy to counteract OxS. In conclusion, we believe different pathways should be highlighted to discover new drugs to treat patients suffering from PAD.

Keywords: biomarkers, inflammation, pathophysiology, peripheral arterial disease, therapy

Introduction

Peripheral arterial disease (PAD) is one aspect of atherosclerosis. A call to action against PAD is needed because it is often undiagnosed or underdiagnosed. In addition, PAD frequency is higher in older individuals (over 65 years old) in socially and economically advanced countries. PAD patients have a high risk of major cardiovascular events (MACE) in both the coronary and carotid arteries, although there are several contributory risk factors that initiate and progress PAD. Advances in research have led to a focus on the progressive pathophysiology pathways of PAD. This pathophysiology has shifted from a hemodynamic scenario to a lack of vasodilation, to the more recent inclusion of the role of oxidative and inflammatory processes. This review summarizes old and progressive pathophysiology, targeting the most helpful long-term therapies for PAD patients.

Methods

Data sources and search

A literature search strategy was developed by an experienced team to screen the medical scientific web platform (MEDLINE). The literature search included most published papers or reviews dated up to 2018. The search used a combination of keywords (e.g. peripheral arterial disease, inflammation, biomarkers, pathophysiology, and therapy). Search process results were limited to papers published in English.

Data extraction

Each participant in the literature search process extracted all relevant data and knowledge on the field of the present review, and other participants verified the extracted data for accuracy and completeness. Each author made a judgement regarding whether the results from the search process were different or confounding in order to release a complete overview of this field in the present review.

Background to the review

A number of studies have indicated that, globally, over 200 million adults have PAD.[1–7] PAD largely affects the over 65s,[8] yet PAD symptoms are largely overlooked.[9] PAD is an expression of systemic atherosclerosis and is well-established as heightening the risk of MACE.[10,11] It has been shown by a number of published studies (Table 1)

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Table 1. List of studies on prevalence of PAD.

First author	Journal, year	Prevalence (%)	Number of enrolments	Study population
Murabito[12]	*Am Heart J*, 2002	3.9%	5124	Population-based
Selvin[13]	*Circulation*, 2004	4.3%	2174	From National Survey (NHANES),
Sigvant[14]	*J Vasc Surg*, 2007		8000	Randomly selected population sample
Mostaza[15]	*Med Clin (Barc)*, 2008	(a) 33.8 (b) 32.4 (c) 53.9	1203	Outpatients forwarded to internal medicine unit. (a) previous coronary event, (b) cerebra-vascular disease disease in coronary and carotid
Ramos[16]	*Eur Soc Vasc Surg*, 2009	4.5	6262	Population-based cross-sectional survey
Alzamora[17]	*BMC Public Health*, 2010	7.6	3786	Population-based
Signorelli[18]	*Angiology*, 2010	2.3	9100	Population-based from general physicians files
Fowkes[19]	*Lancet*, 2013	PAD prevalence increases by 28.7% in countries with low income, LMIC and 13.1% in countries with high income.	Review on 34 published studies ranged between 2000 and 2010	Literature review
Sigvant[20]	*J Vasc Surg*, 2017	Primary PAD: 40,136 out 66,189	66,189 patients diagnosed as PAD (2006–2013)	Cohort study (retrospective analysis)

LMIC, low and middle income countries; NHANES, National Health and Nutrition Examination Survey; PAD, peripheral arterial disease.

that many individuals are affected by this chronic arterial disease but without knowing they have PAD,[12–20] as we have also demonstrated in a study focused on the prevalence of PAD in the general population, and on comorbidities in PAD patients.[21] In our study, we found an ankle/brachial index lower than 0.9 in 80 out 3332 individuals from the lists of general practitioners (GPs) (Table 2). PAD patients have been classified by using Fontaine's classification of the crucial symptoms of PAD progression. As pain in the lower limbs caused by muscular effort (intermittent claudication) progresses, walking distance and muscular performance is reduced, and may be considered a pivotal symptom in suspecting PAD. Furthermore, chronic ischemia in PAD causes progressive or severe damage to muscle cells and skin tissue. Skin lesions are expressions of PAD. What can staging PAD patients according to clinical classifications (Table 3: Fontaine's, Rutherford's) achieve: first, evaluation of the clinical situation by eliciting pain in the lower limbs, pain-free walking distance, skin color modifications (range: red, cyan, white), and loss of skin integrity from ischemic or necrotic evidence. Second, evaluation of the pathophysiology of PAD, and the different therapeutic challenges that may need to be considered as

Table 2. Demographic characteristic of general population of Catania city enrolled to estimate frequency of PAD. PAD was diagnosed by using the ABI (ABI $\leqslant$ 0.90).

	PAD	Controls
	80 (2.34%)	3332
Age	62.7 ± 10.5	54.4 ± 12.6
Male	52 (65%)	1312 (38.5%)
Female	28 (35%)	2020 (61.5%)
Smokers	48 (60)	680 (22.2%)
Past smoker	12 (15%)	508 (16.5%)
TDM2	24 (30%)	200 (6.5%)
Hypertension	40 (50%)	1016 (33.1%)
Dyslipidemia	40 (50%)	908 (29.6%)
BMI	27.3 ± 3.9	26.3 ± 5.3
Waist:hip ratio	97.2 ± 10.3	92.5 ± 5.3
Ankle brachial index $\leqslant$ 0.90	0.81 ± 0.11 80 out 3332	1.18 ± 0.10

ABI, ankle brachial index; BMI, body mass index; TDM2, type 2 diabetes mellitus; PAD, peripheral arterial disease.

Table 3. Clinical and functional classifications of PAD.

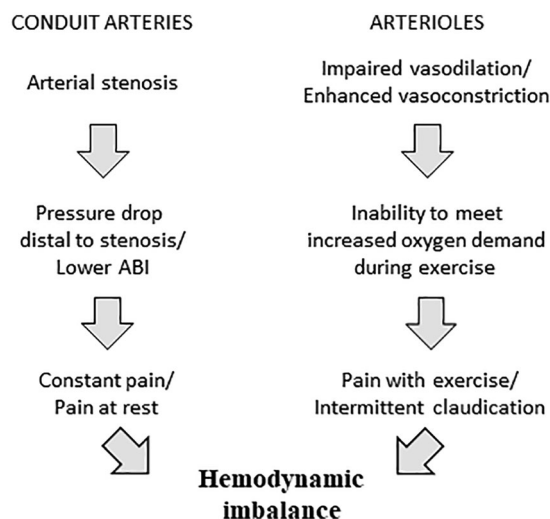| Fontaine[22]			Rutherford[23]		
Stage	Clinical	Symptoms	Pathophysiology	Clinical	Grade
1st	No symptoms	Occasional discovery of aortic and iliac calcification	Ats plaque risk plaque inflammation	Asymptomatic	0/0
2nd A	Claudication	ACD >200 m; recovery time <2 min	Discrepancy oxygen request arterial supply	Mild claudication moderate claudication	I/1 I/2
2nd B	Claudication	ACD < 200 m; recovery time >2 min	Discrepancy oxygen request arterial supply	Severe claudication	I/3
		ACD < 100 m; recovery time >2 min	Highest discrepancy and acidosis		3rd
Ischaemic rest pain	Ischaemic rest pain	Skin hypoxia acidosis	Ischaemic rest pain	II/4	4th
Ulceration or gangrene	Skin necrosis Gangrene	Severe skin hypoxia acidosis	Minor tissue loss major tissue loss	III/5 III/6	

ACD, absolute claudication distance; Ats, atherosclerotic; PAD, peripheral arterial disease.

helpful management strategies (i.e. medical or interventional or open surgery options) for successful PAD patient outcomes.

There is a need to focus on the growing and still debated issues surrounding PAD, as follows.

(1) Epidemiology: PAD is now listed as a chronic arterial disease affecting individuals over 60–65 years. PAD epidemiology and frequency are closely related to longer life expectancy, particularly in socially and economically advanced countries.

(2) Clinic- and patient-related: PAD is still underdiagnosed compared with other ischemic arterial diseases (i.e. coronary and carotid diseases), although atherosclerosis is a common pathogenic symptom for both.

(3) Diagnosis: ankle brachial index (ABI) is an easy, noninvasive, and repeatable diagnostic tool. It is a specific and sensitive method for diagnosing PAD. However, it is not widely applied, particularly by GPs. ABI is helpful in monitoring PAD patient outcomes.

(4) Outcome, social: PAD lowers physical capability and performance, thus it modifies quality of life.

(5) Clinic and prognosis: PAD patients have a risk of a cardiovascular event that is two to three times higher a than that of the non-PAD population.

(6) Treatment: Drugs applied in PAD do not really affect clinical symptoms or the potency of interventional procedures. Moreover, drugs seem not to be effective in reducing the burden of PAD patients, or their long-term outcomes.

There are effectively two players in PAD: the gradual narrowing of arteries, and the reduced vasodilative ability of peripheral arteries. More strategies, new drugs, and more research are needed to achieve effective goals for PAD outcomes and treatment. So, improved understanding of the pathophysiology of limb symptoms in PAD may be helpful in accelerating the development of novel medical, interventional, or surgical therapies for PAD patients. It has long been known that PAD may be considered as a model of prevalently chronic ischemia; however, it is less frequently expressed as a model of acute ischemia.

Figure 1. Hemodynamic disarrangement in peripheral chronic ischemia.
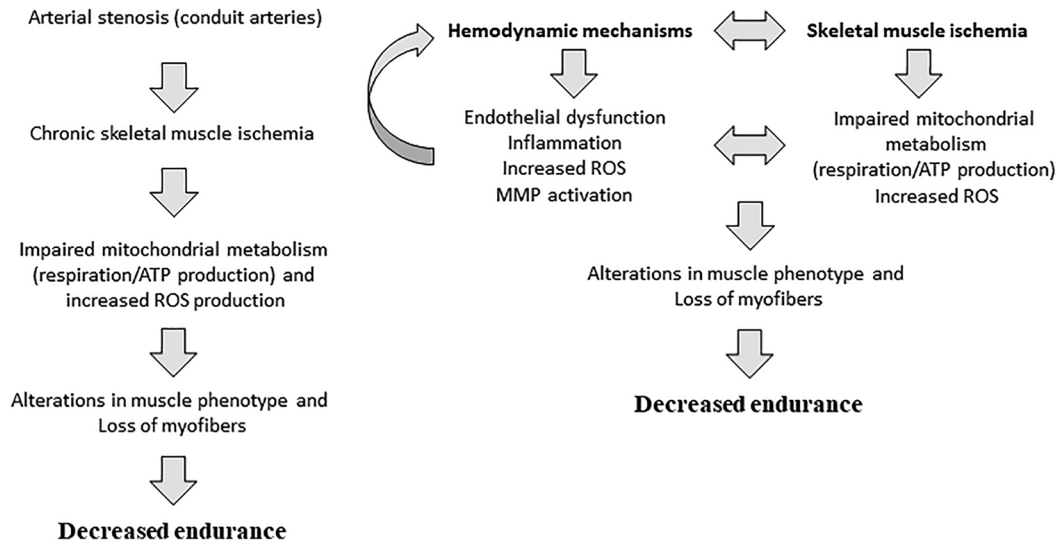ABI, ankle brachial index.

So, we would like to highlight any progressive research related to the pathophysiology of chronic ischemic arterial disease, as PAD is also known.

Pathophysiology of chronic limb ischemia: hemodynamic scenario

Historically, the hemodynamics of arterial stenosis dominated the pathophysiological PAD scenario. Single or multiple obstructive atherosclerotic lesions, and a drop in blood pressure and flow, are additional to reducing ankle pressure. With exercise, the flow to the lower extremity increases, magnifying the pressure drop across fixed lesions and thereby increasing the sensitivity of PAD detection.[24–26]

Furthermore, endothelium-dependent vasomotion and vasodilation play a role in hemodynamic balance, and endothelial membrane integrity plays a fundamental role in arterial vasomotion and vasodilation. Both these arterial capabilities are seriously compromised in peripheral chronic ischemia (Figure 1).

There is evidence of diminished release of nitric oxide (NO) in PAD patients; consequently, a number of active capabilities (e.g. vasoconstrictive effects of agents, flow mediated dilatation, exercise improving arterial flow, etc.) are limited or highly reduced. Moreover, reactive hyperemia, such as

Figure 2. Mitochondrial and muscle disorders originated by peripheral chronic ischemia.
ATP, adenosine triphosphate; MMP, matrix metalloproteinase; ROS, reactive oxygen species.

the inflow compensation mechanism in the post-stenotic regions or after extended muscle performance (i.e. walking test) is significantly lowered in PAD. Endothelial dysfunction is the origin of these deleterious effects on blood flow, as shown by results from studies on PAD populations.[27–32]

The diminished bioavailability of NO is crucial because this molecule is a pluripotent agent. NO is able to combat the deleterious effects on blood-stream cells such as platelets (promoting adhesion, aggregability, microvesculation etc.) and leukocytes (inducing adhesion, immunological activation, and reactive molecules release). NO also counteracts proliferation of smooth muscle cells, and promotes angiogenesis.[33–44]

Angiogenesis actively creates new capillaries from existing ones to supply oxygen to tissues and cells suffering from low flow rates due to arterial stenosis. However, angiogenesis is not sufficient to counteract the hemodynamic disturbances due to arterial stenosis. The resistance to blood flow in peripheral arteries cannot be compensated by angiogenesis, which is characterized by high resistance to flow.[45]

It is interesting to focus on the muscle cell pathways associated with tissue ischemia leading to reduced muscular capability (i.e. walking performance) in PAD patients. Where there is lowered

arterial perfusion, there is a reduction in the number of muscle myofibres, impaired mitochondrial function, muscle damage or degeneration, and finally peripheral nerve dysfunction.

Muscular fibers are related to established oxidative damage

Studies have focused on muscular damage in PAD patients when chronic or critical ischemia occurs.[46,47] In patients affected by atherosclerotic diseases, carbonyl and 4-hydroxynonenale damage of myofibers has been found. In PAD patients, high levels of oxidative agents cause greater myofiber damage (40%) than in coronary artery patients (Figure 2). There are two kinds of myofiber (I, II, fibers) with different characteristics: high mitochondrial content, high oxidative metabolism in type I, and a close relationship with glycolysis in type II. In PAD patients, the ratio of type I to type II fibers is lowered, which is crucial in explaining the selective damage in type II fibers. Oxidative stress (OxS) occurs in chronic ischemia (PAD) and is closely related to the frequency of type II fibers in PAD patients Notably, type II fibers are more prone to oxidative damage.[46,47]

Muscular tissue is dependent on ischemia

Tissue oxygen extraction by muscular tissue is modified, explaining the imbalance that occurs

under oxygen demand in PAD patients. Two metabolic phenomena that occur in PAD-altered cell respiratory capability can be demonstrated using O_2/CO_2 transcutaneous measurements. The $TcpO_2/CO_2$ technique is a simple, noninvasive method to evaluate respiratory tissue ability. Study results demonstrated a severe decrease in oxygen tissue extraction in PAD patients, both at rest, and, particularly, at the peak of muscular exercise.[48,49] Note the inverse relationship between the extraction of tissue oxygen and ABI values. Furthermore, after muscle work, prolonged oxygen desaturation is found in PAD patients. These findings are proof of the concept of a close relationship between arterial perfusion and mitochondrial cell damage in PAD patients. Respiratory cell parameters include the base respiratory rate (V0), the respiratory rate after the addition of substrates (VSUB), the respiratory rate after the further addition of adenosine diphosphate (ADP) (VADP), and the respiratory rate after the addition of atractyloside (VAT). The main mitochondrial respiratory phenomenon is adenosine triphosphate (ATP)–ADP translocation: the absence of ADP is highly deleterious for cell respiration/function.

In PAD patient mitochondria, there is a similar base respiratory rate compared with healthy patients. However, in PAD patients, VSUB is lowered, VADP is lowered significantly, and VAT does not differ compared with healthy individuals.[50] The mitochondrial dysfunction shown by PAD patients gives rise to two negative consequences. First, there is a difference in the amount of ATP produced in normal skeletal muscles compared with that produced in PAD patients. In PAD patients there are double-negative effects: a decreased supply of nutrients and oxygen, and a defect in mitochondrial respiration. Therefore, there is less ATP production, limited O_2 supply, and a reduction in nutrients. Second, mitochondria dysfunction produces high levels of reactive oxygen species (ROS), leading to very dangerous mechanisms that combat normal cellular structure and function. In PAD patients, ROS assists the destruction of skeletal myocytes.

OxS in pathophysiology of PAD

We would like to draw attention to the other scenario concerning the pathophysiology of chronic ischemia in PAD: the role played by inflammation,

the hypercoagulative condition, and the lack of fibrinolytic capabilities are closely connected. All these factors act directly, or intermediately, in emerging and maintaining or worsening PAD. OxS plays a key role in promoting a number of arterial diseases. Lipid peroxidation is the oxidative degradation of lipids, resulting in cell damage. Malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE) are the principal end products of lipid peroxidation, the accumulation of which plays a significant role in human tissues. MDA is the breakdown product of lipid peroxidation, and its assessment is a reliable indirect marker of oxidative damage. 4-HNE is abundant within the vasculature, and its concentration induces effects on vascular endothelial and smooth muscle cells: kinase activation, proliferation, and the induction of phase II enzymes. In high doses, 4-HNE reduces the activation of enzymatic processes, and, finally, induces apoptosis.[51] Among the effects of OxS in PAD, we measured such surrogate markers of OxS. Plasma levels of MDA were found higher in PAD than controls at rest. Concentrations of MDA rose after the strenuous walking test in both groups; interestingly, MDA increase was most significant in PAD patients. The baseline value of 4-HNE was also found to be higher in PADs than in controls, and differences rose at the end of the treadmill test. Moreover, oxidized lipoproteins (OxL) in PAD patients were different to controls at rest.[52] The inflammatory process stimulates vascular smooth muscle cell proliferation, and, in late neo-intimal growth, endothelial membrane damage increases coagulative capability.[53]

In PAD patients with risk factors for atherosclerosis, interleukin-6, tumor necrosis factor alfa, ICAM-1 and VCAM-1, selectines (leukocyte, endothelial, platelet selectines) were tested to understand the interplay between hemodynamic imbalance and cell dysfunction.[54,55] We found higher plasma concentrations of biomarkers at rest in PAD patients compared with healthy controls. Concentrations increased strongly after maximal walking test inducing pain of limbs, differences between PAD patients and controls were enhanced. These results are helpful in clarifying cell environment and metabolic tissue factors in PAD patients. As for inflammatory markers, we measured both fibrinogen and C reactive proteins as two markers of acute inflammation. In PAD patients, plasma concentrations of these markers were higher than in controls.[54] To estimate cell

activation (i.e. platelet aggregation) in chronic ischemia, the concentration of matrix metalloproteinases (MMPs) was measured in PAD patients and controls. It is known that MMPs are involved in many physiological processes, such as tissue remodeling and cell aggregation. MMPs also play other roles in pathological processes such as inflammation and tissue repair. MMP deregulation contributes to arterial lesions by facilitating monocyte invasion.[55] On this crucial issue, we searched for oxidized lipoproteins (OxL) in PAD patients and controls. We know that PAD patients suffer from modified acetyl-Co ester accumulation when the concentration of carnitine in muscle cells is lowered. In PAD patients, there is inadequate ATP generation, thus cell respiratory activity is worsened. PAD patients show an increased level of esterified derivatives of acetyl-CoA; this may be closely related to lowered blood perfusion. Metabolic imbalance occurs when muscle and plasma levels of carnitine are low, as in patients suffering from progressive PAD.[56] Results suggest that carnitine stimulates glucose disposal and oxidation, leading to the efficient utilization of glucose under ischemia, as occurs in PAD patients.[57,58]

The anti-oxidative drug propionyl l-carnitine has been shown to modify oxidative stress in PADs.[59] It is worth clarifying the role played by biochemical agents in cardiovascular tissue.[56–59] We measured heme oxygenase-1 (HO-1) in PAD, and showed conclusively that HO-1 plasma levels are low in these patients. This seems to agree with the differences found in lactic acid plasma levels in PAD patients and controls.[60] Concerning oxidative stress markers, we want to highlight glutathione (GSH) levels in PAD. We found lower GSH higher plasma level in progressed PAD patients (2nd B of Leriche's classification) than in PAD patients at the 2nd A stage. We postulate that the reduced HO-1 levels may reflect reduced intracellular content in PAD patients.[60] Plasma HO-1 reduction may also be part of the compensatory mechanisms that maintain cellular redox status.[61–64] Moreover, severe metabolic tissue disorders, such as oxidative stress originating from chronic repetitive (intermittent claudication, walking-related pain occurrence) ischemia is a characteristic of PAD patients.[65–69] Based on our knowledge of OxS in PAD, there is consecutive production of ROS, mitochondrial damage, endothelial dysfunction, and selective damage of myofibers of muscles. Thus, OxS plays a role as a crucial mechanism, both in determining PAD and in its progression. It is very intriguing to note that inflammatory markers are closely linked with predictors of arterial disease, such as arterial stiffness. Arterial stiffness may be considered an early signal of vessels changes, thus arterial stiffness is now a helpful predictor of cardiovascular disorders. These findings clearly show crosstalk between bloodstream cells and arterial wall properties.[70]

NADPH oxidases

OxS represents an imbalance between ROS production and removal by the endogenous antioxidant defense system, mediating damage to lipids, membranes, proteins, and DNA. OxS and ROS production have long been regarded as a key pathophysiological mediators that ultimately lead to cardiovascular diseases.[71] OxS in PAD is believed to contribute to consequences and disease progression.[72] Enhanced levels of ROS are involved in the disability associated with PAD, including decreased walking distance and quality of life.[73,74] Interestingly, ROS generated by myeloperoxidase, xanthine-oxidase, and nicotinamide-adenine dinucleotide phosphate (NADPH) oxidase (NOX) may be implicated in artery dysfunction.[75] NOX2 upregulation is associated with artery dysfunction in patients with PAD.[76,77] Indeed, besides mitochondria, the NADPH oxidase system is now widely recognized as a key player in intracellular ROS homeostasis, and as one of the major producers of ROS within the cell.[78] Seven distinct members of the NOX family have been characterized, of which four are named NOX1, 2, 4, and 5.[79] The catalytic core of NOX, gp91 (phox), is a membrane-bound subunit that is inactive until it binds to membrane-anchored p22phox, which stabilizes the catalytic subunit in the plasma or intracellular membrane. Activated NOX catalyzes the transfer of electrons from NADPH to molecular oxygen, generating superoxide anions (O_2^-) as the primary product.[80]

NOX has been described primarily in phagocytes, whose main task is to generate ROS to kill foreign pathogens.[81] Recently, it has become evident that NADPH oxidase is functionally expressed not only in phagocytes but also in several other cell types.[82,83] In the cardiovascular system, the nonphagocytic NADPH oxidases, NOX1, NOX2, and NOX4, have different

physiological functions. NOX2 is believed to have the greatest implication in vascular disease.[84,85] *In vitro* studies demonstrated that endothelial cells exposed to oxidized LDL showed increased NOX2 expression and ROS formation; NOX2 inhibition prevented the release of ROS.[86] Additionally, increased activation of NOX2 contributes to diminished bioavailability of NO, and thus, to endothelial dysfunction and vascular cell hypertrophy. NOX2 upregulation could explain OxS in PAD patients, and account for endothelial dysfunction.[87] Increasing ROS from NOX NOX2 contributes to arterial dysfunction, and to arterial hypertrophy through reduced bioavailability of NO and the formation of peroxynitrite (ONOO$^-$).[80] Interestingly, Shafique and colleagues demonstrated *in vivo* that above-physiological levels of endothelial cell-specific NADPH oxidase-derived ROS *in vivo* exerted distinct beneficial and adverse effects on vascular endothelium, depending on the duration of the ROS exposure and on subcellular ROS levels in mitochondria. An increase in peroxynitrite and mitochondrial dysfunction due to sustained elevation in endogenous ROS in the cytosol of endothelial cells may have resulted in decreased endothelium-dependent vasorelaxation and endothelial cells proliferation.[88] Cytokines have also been shown to regulate vascular NADPH oxidases, which links inflammation with OxS. In particular, tumor necrosis factor-α (TNF-α) stimulates NADPH oxidase NOX1, NOX2, and NOX4 expression and activation in a variety of vascular cells.[89] Increased NOX2-mediated superoxide production and NOX2 expression in T cells and monocytes in peripheral blood has been linked to the activation of these cells, and may be important in the pathogenesis of angiotensin II-mediated hypertension.[80] It has been found that natural antioxidant compounds (i.e. flavonoids) can affect NADPH oxidase activity and induce cellular cytoprotective systems. These phenolic compounds potentially improve endothelial dysfunction and decrease overall OxS.[90] Induction of HO-1, a critical cytoprotective system, is activated during cellular stress.[91,92] Epoxyeicosatrienoic intervention improves non-alcoholic *fatty liver* disease (NAFLD) in leptin receptor deficient mice by an increase in PGC1α-HO-1-PGC1α-mitochondrial signaling,[93] resulting in decreased cardiac levels of superoxide and NOX2 expression, which may be due to a decrease in the levels of NADPH oxidase, a heme-dependent protein, or an increase in the levels of superoxide dismutase EC-SOD.[94] Promising inhibition of NOX acts *via* apocynin, which inhibits the binding of p47phox to p22phox.[95,96] A number of studies have examined the effects on NADPH oxidase and NO bioavailability in a variety of mouse models. However, a large body of evidence in the literature supports apocynin as a nonspecific NOX inhibitor. NOX2 has been studied as a potential therapeutic target for cardiovascular diseases.[97] Particularly, selective platelet NOX2 inhibition might represent a promising strategy to prevent thrombosis, since NOX2 plays an important role in platelet activation in thrombosis.[98] Understanding the importance of vascular NADPH oxidases and their potential value as therapeutic targets triggered a search for specific and efficient NOX enzyme inhibitors.

Novel agents for PAD

The objectives for PAD patients are as follows: ameliorate intermittent claudication and quality of life, improve long-term prognosis for MACE, and prevent or treat critical limb ischemia. According to a consensus of studies, a number of drugs have been suggested and tested on PAD patients.[99–101] Although aggressive, they do not promote positive effects on arterial hemodynamics, which, in turn, is effective on the symptoms and prognosis of PAD. In contrast, surgical or endovascular options are now the first-line therapies used to relieve PAD symptoms. However, it is notable that drugs are not very effective in achieving positive objectives for PAD patients, whilst surgical or interventional strategies suffer from a lack of long-term potency.[71,98,99]

So, novel agents are needed to promote alternative approaches for PAD, although research does not show any conclusive results. However, there are intriguing findings on novel therapies, targeted mainly at promoting arterial angiogenesis.

Vascular endothelial growth factor
Data from studies on angiogenic factors, including vascular endothelial growth factor (VEGF) hepatocyte growth factor and fibroblast growth factor is insufficient to show efficacy in PAD treatment. Beneficial effects were found in

improving leg endothelial function and flow reserve by administration of VEGF165 and VEGF121.[101,102] The efficacy of clinical gene therapy for angiogenesis was initially recognized, with intramuscular injections having beneficial effects. Unfortunately, negative results were found (death, leg amputation) in long-term studies, including a number of PAD patients treated with AdVEGF121 or VEGF-A gene transfer.[103,104]

Fibroblast growth factor

Fibroblast growth factor is an angiogenic factor for PAD treatment administered using a plasmid-based delivery (NV1FGF) for local expression. NV1FGF proved effective for pain and skin ulceration, and it increased the ABI value. Conversely, controversial data resulted from the risk of leg amputation and death in PAD patients.

Hepatocyte growth factor

Hepatocyte growth factor (HGF) can induce angiogenesis but is ineffective on vascular inflammation and permeability. HGF used against ischemia in PAD patients has shown increased blood flow, and increased microcirculatory density.[105] Data from observational studies (phase II, III, and IV) have proved promising for PAD patients to avoid amputation.[106,107]

Cell-based therapy

Endothelial progenitor cell (EPCs) vasculogenesis was induced by bone marrow-derived EPCs in ischemic sites. In patients affected by critical limb ischemia, EPCs ameliorated the efficacy score.[108,109] Mononuclear cells (MNCs) are able to secrete angiogenic factors, and were injected into patients with critical limb ischemia. They improved ABI (macrocirculatory efficacy), transcutaneous oxygen pressure (microcirculation), rest pain, and pain-free walking time (clinical end points). Interestingly, these positive effects remained for some time after therapy.[110–112] Mesenchymal stem cells (MSCs) are also able to induce angiogenic activity. Bone marrow MSC results from a clinical trial showed positive effects on intermittent claudication (free walking distance), healing skin damage, and percutaneous tissue oxygen.[113,114] Currently, there are unequivocal results on new therapeutic strategies for PAD

patients. Angiogenic and cell-based therapies have been approved as advanced medical opportunities for PAD treatment; however, the regulatory agencies have not approved any of the new therapies as standard for PAD.

MicroRNAs

Some emerging biomarkers, including microRNAs (miRNAs), now seem to be additional tools that can be used to establish role of multiple risk factors in PAD. To date, there is a comprehensive understanding of the role of miRNA in regulating angiogenesis, and in maintaining vascular integrity. Furthermore, such miRNAs could act as a diagnostic tools to facilitate new therapeutic strategies such as gene therapy in patients threating to develop PAD. It is known that miR-130a, miR-27b, and miR-210 are activated under hypoxic conditions; thus, they could play a role in PAD, as we demonstrated by showing miR-130a, miR-27b, and miR-210 in PAD patients. In this regard, we know such miRNAs are upregulated in hypoxia (i.e. PAD) so they are interesting inhibitors of OxS. However, to date, any effective role of miRNAs as a target for PAD therapy remains to be clarified.[115]

It is interesting to highlight the role of leptin (L) in inducing vascular disorders. L plays a role in provoking OxS, and, interestingly, it promotes both angiogenesis and aggregation of platelets.[116] High values of L were found to be associated with PAD in patients with favorable conditions for developing PAD, such as arterial hypertension.[117]

Concluding remarks

To date, PAD patients suffer from several debated and unresolved concerns, such as the high risk of acute adverse cardiac events, modified or worsened quality of life induced by intermittent claudication or by progressive pain in the lower limbs. Several medical therapies (i.e. drugs to lower serum cholesterol, inhibitors of platelet aggregation, anticoagulants) have been suggested to treat PAD, but these have not provided any clarity in achieving long-term results on clinical outcomes of PAD patients, whereas supervised exercise programs may be considered as very effective in treating walking performance of PAD patients, improving quality of life also. The

pathophysiology of PAD is complex, including lowered hematic load, reduced tissue and cell perfusion, and respiratory capability. Both distributive arterial circulation (macrocirculation) and nutritional arterial circulation (microcirculation) are progressively involved and severely dysfunctional. The screening and monitoring of several oxidative and inflammatory biomarkers and continuously supervised exercise as therapeutic strategies have proved effective in ameliorating the clinic parameters as well as pain-free walking distance.[118–120] It is mandatory now to highlight more pathophysiological pathways aiming to discover new medical drugs to achieve crucial objectives for PAD patients.

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References

1. Ankle Brachial Index Collaboration, Fowkes FG, Murray GD, *et al*. Ankle brachial index combined with Framingham risk score to predict cardiovascular events and mortality: a meta-analysis. *JAMA* 2008; 300: 197–208.

2. Steg PG, Bhatt DL, Wilson PW, *et al*. One-year cardiovascular event rates in outpatients with atherothrombosis. *JAMA* 2007; 297: 1197–1206.

3. Bhatt DL, Eagle KA, Ohman EM, *et al*. Comparative determinants of 4-year cardiovascular event rates in stable outpatients at risk of or with atherothrombosis. *JAMA* 2010; 304: 1350–1357.

4. Sampson UK, Fowkes FG, McDermott MM, *et al*. Global and regional burden of death and disability from peripheral artery disease: 21 world regions, 1990 to 2010. *Global Heart* 2014; 9: 145–158.e21.

5. Fowkes FG, Housley E, Cawood EH, *et al*. Edinburgh artery study: prevalence of asymptomatic and symptomatic peripheral arterial disease in the general population. *Int J Epidemiol* 1991; 20: 384–392.

6. Allison MA, Ho E, Denenberg JO, *et al*. Ethnic-specific prevalence of peripheral arterial disease in the United States. *Am J Prev Med* 2007; 32: 328–333.

7. Hirsch AT, Criqui MH, Treat-Jacobson D, *et al*. Peripheral arterial disease detection, awareness, and treatment in primary care. *JAMA* 2001; 286: 1317–1324.

8. Malyar N, Fürstenberg T, Wellmann J, *et al*. Recent trends in morbidity and in-hospital outcomes of in-patients with peripheral arterial disease: a nationwide population-based analysis. *Eur Heart J* 2013; 34: 2706–2714.

9. Kröger K, Stang A, Kondratieva J, *et al*. Prevalence of peripheral arterial disease - results of the Heinz Nixdorf recall study. *Eur J Epidemiol* 2006; 21: 279–285.

10. Bowlin SJ, Medalie JH, Flocke SA, *et al*. Epidemiology of intermittent claudication in middle-aged men. *Am J Epidemiol* 1994; 140: 418–430.

11. Hiatt WR, Hoag S and Hamman RF. Effect of diagnostic criteria on the prevalence of peripheral arterial disease. The San Luis valley diabetes study. *Circulation* 1995; 91: 1472–1479.

12. Murabito JM, Evans JC, Nieto K, *et al*. Prevalence and clinical correlates of peripheral arterial disease in the Framingham offspring study. *Am Heart J* 2002; 143: 961–965.

13. Selvin E and Erlinger TP. Prevalence of and risk factors for peripheral arterial disease in the United States: results from the National health and nutrition examination survey, 1999–2000. *Circulation* 2004; 110: 738–743.

14. Sigvant B, Wiberg-Hedman K, Bergqvist D, *et al*. A population- based study of peripheral arterial disease prevalence with special focus on critical limb ischemia and sex differences. *J Vasc Surg* 2007; 45: 1185–1191.

15. Mostaza JM, Manzano L, Suárez C, *et al*. Prevalence of asymptomatic peripheral artery disease detected by the ankle-brachial index in patients with cardiovascular disease. MERITO II study. *Med Clin (Barc)* 2008; 131: 561–565.

16. Ramos R, Quesada M, Solanas P, *et al*. Prevalence of symptomatic and asymptomatic peripheral arterial disease and the value of the ankle-brachial index to stratify cardiovascular

risk. *Eur J Vasc Endovasc Surg* 2009; 38: 305–311.

17. Alzamora MT, Forés R, Baena-Díez JM, *et al.* The peripheral arterial disease study (PERART/ARTPER): prevalence and risk factors in the general population. *BMC Public Health* 2010; 10: 38.

18. Signorelli SS, Anzaldi M, Fiore V, *et al.* Study on unrecognized peripheral arterial disease (PAD) by ankle/brachial index and arterial comorbidity in Catania, Sicily, Italy. *Angiology* 2010; 61: 524–529.

19. Fowkes FG, Rudan D, Rudan I, *et al.* Comparison of global estimates of prevalence and risk factors for peripheral artery disease in 2000 and 2010: a systematic review and analysis. *Lancet* 2013; 382: 1329–1340.

20. Sigvant B, Hasvold P, Kragsterman B, *et al.* Cardiovascular outcomes in patients with peripheral arterial disease as an initial or subsequent manifestation of atherosclerotic disease: results from a Swedish nationwide study. *J Vasc Surg* 2017; 66: 507–514.

21. Signorelli S, Anzaldi M, Fiore V, *et al.* Patients with unrecognized peripheral arterial disease (PAD) assessed by ankle-brachial index (ABI) present a defined profile of proinflammatory markers compared to healthy subjects. *Cytokine* 2012; 59: 294–298.

22. Fontaine R, Kim M and Kieny R. Surgical treatment of peripheral circulation disorders [article in German]. *Helv Chir Acta* 1954; 21: 499–533.

23. Rutherford RB, Baker JD, Ernst C, *et al.* Recommended standards for reports dealing with lower extremity ischemia: revised version. *J Vasc Surg* 1997; 26: 517–538.

24. Campia U, Gerhard-Herman M, Piazza G, *et al.* Peripheral artery disease: past, present, and future. *Am J Med* 2019; 132: 1133–1141.

25. Habib A, Petrucci G and Rocca B. Pathophysiology of thrombosis in peripheral arterial disease. *Curr Vasc Pharmacol.* Epub ahead of print 6 February 2019. DOI: 10.2174/1570161117666190206234046.

26. Morley RL, Sharma A, Horsch AD, *et al.* Peripheral arterial disease. *BMJ* 2018; 360: j5842.

27. Kashyap VS, Lakin RO, Feiten LE, *et al.* In vivo assessment of endothelial function in human lower extremity arteries. *J Vasc Surg* 2013; 58: 1259–1266.

28. Igari K, Kudo T, Toyofuku T, *et al.* The relationship between endothelial dysfunction and endothelial cell markers in peripheral arterial disease. *PLoS One* 2016; 11: e0166840.

29. Maruhashi T, Nakashima A, Matsumoto T, *et al.* Relationship between nitroglycerine-induced vasodilation and clinical severity of peripheral artery disease. *Atherosclerosis* 2014; 235: 65–70.

30. Brevetti G, Schiano V and Chiariello M. Endothelial dysfunction: a key to the pathophysiology and natural history of peripheral arterial disease? *Atherosclerosis* 2008; 197: 1–11.

31. Sydow K, Hornig B, Arakawa N, *et al.* Endothelial dysfunction in patients with peripheral arterial disease and chronic hyperhomocysteinemia: potential role of ADMA. *Vasc Med* 2004; 9: 93–101.

32. Boger RH, Endres HG, Schwedhelm E, *et al.* Asymmetric dimethylarginine as an independent risk marker for mortality in ambulatory patients with peripheral arterial disease. *J Intern Med* 2011; 269: 349–361.

33. Hart CR, Layec G, Trinity JD, *et al.* Increased skeletal muscle mitochondrial free radical production in peripheral arterial disease despite preserved mitochondrial respiratory capacity. *Exp Physiol* 2018; 103: 838–850.

34. Pipinos II, Judge AR, Zhu Z, *et al.* Mitochondrial defects and oxidative damage in patients with peripheral arterial disease. *Free Radic Biol Med* 2006; 41: 262–269.

35. Pipinos II, Judge AR, Selsby JT, *et al.* The myopathy of peripheral arterial occlusive disease: part 1. Functional and histomorphological changes and evidence for mitochondrial dysfunction. *Vasc Endovascular Surg* 2007; 41: 481–489.

36. Brass EP and Hiatt WR. Acquired skeletal muscle metabolic myopathy in atherosclerotic peripheral arterial disease. *Vasc Med* 2000; 5: 55–59.

37. Koutakis P, Ismaeel A, Farmer P, *et al.* Oxidative stress and antioxidant treatment in patients with peripheral artery disease. *Physiol Rep* 2018; 6: e13650.

38. Anderson JD, Epstein FH, Meyer CH, *et al.* Multifactorial determinants of functional capacity in peripheral arterial disease: uncoupling of calf muscle perfusion and metabolism. *J Am Coll Cardiol* 2009; 54: 628–635.

39. Vita JA and Hamburg NM. Does endothelial dysfunction contribute to the clinical status of patients with peripheral arterial disease? *Can J Cardiol* 2010; 26(Suppl. A): 45A–50A.

40. Meredith IT, Currie KE, Anderson TJ, *et al.* Postischemic vasodilation in human forearm is dependent on endothelium-derived nitric oxide. *Am J Physiol* 1996; 270: H1435–H1440.

41. Liao JK, Bettmann MA, Sandor T, *et al.* Differential impairment of vasodilator responsiveness of peripheral resistance and conduit vessels in humans with atherosclerosis. *Circ Res* 1991; 68: 1027–1034.

42. Makris KI, Nella AA, Zhu Z, *et al.* Mitochondriopathy of peripheral arterial disease. *Vascular* 2007; 15: 336–343.

43. Tzagoloff A and Myers AM. Genetics of mitochondrial biogenesis. *Annu Rev Biochem* 1986; 55: 249–285.

44. Scholz D, Cai WJ and Schaper W. Arteriogenesis a new concept of vascular adaptation in occlusive diseases. *Angiogenesis* 2001; 4: 247–257.

45. Tuppen HA, Blakely EL, Turnbull DM, *et al.* Mitochondrial DNA mutations and human disease. *Biochim Biophys Acta* 2010; 1797: 113–128.

46. Pipinos II, Sharov VG, Shepard AD, *et al.* Abnormal mitochondrial respiration in skeletal muscle in patients with peripheral arterial disease. *J Vasc Surg* 2003; 38: 827–832.

47. Andreozzi GM, Riggio F, Butto G, *et al.* Transcutaneous PCO2 level as an index of tissue resistance to ischemia. *Angiology* 1995; 46: 1097–102.

48. Comerota AJ, Throm RC, Kelly P, *et al.* Tissue (muscle) oxygen saturation (StO2): a new measure of symptomatic lower-extremity arterial disease. *J Vasc Surg* 2003; 38: 724–729.

49. Bauer TA, Brass EP and Hiatt WR. Impaired muscle oxygen use at onset of exercise in peripheral arterial disease. *J Vasc Surg* 2004; 40: 488–493.

50. Chapple SJ, Cheng X and Mann GE. Effects of 4-hydroxynonenal on vascular endothelial and smooth muscle cell redox signaling and function in health and disease. *Redox Biol* 2013; 1: 319–331.

51. Signorelli SS, Malaponte G, Di Pino L, *et al.* Effects of ischaemic stress on leukocyte activation processes in patients with chronic peripheral occlusive arterial disease: role of L-propionyl carnitine administration. *Pharmacol Res* 2001; 44: 305–309.

52. Kornowski R, Hong MK, Tio FO, *et al.* In-stent restenosis: contributions of inflammatory responses and arterial injury to neointimal hyperplasia. *J Am Coll Cardiol* 1998; 31: 224–230.

53. Signorelli SS, Malaponte G, Libra M, *et al.* Plasma levels and zymographic activities of matrix metalloproteinases 2 and 9 in type II diabetics with peripheral arterial disease. *Vasc Med* 2005; 10: 1–6.

54. Libra M, Signorelli SS, Bevelacqua Y, *et al.* Analysis of G(-174)C IL-6 polymorphism and plasma concentrations of inflammatory markers in patients with type 2 diabetes and peripheral arterial disease. *J Clin Pathol* 2006; 59: 211–215.

55. Sawicki G, Sanders EJ, Salas E, *et al.* Localization and translocation of MMP-2 during aggregation of human platelets. *Thromb Haemost* 1998; 80: 836–839.

56. Dollery CM, McEwan JR and Henney AM. Matrix metalloproteinases and cardiovascular disease. *Circ Res* 1995; 77: 863–868.

57. Brass EP and Hiatt WR. The role of carnitine and carnitine supplementation during exercise in man and in individuals with special needs. *J Am Coll Nutr* 1998; 17: 207–215.

58. Brevetti G, Angelini C, Rosa M, *et al.* Muscle carnitine deficiency in patients with severe peripheral vascular disease. *Circulation* 1991; 84: 1490–1495.

59. Hiatt WR. Carnitine and peripheral arterial disease. *Ann N Y Acad Sci* 2004; 1033: 92–98.

60. Santo SS, Sergio N, Luigi DP, *et al.* Effect of PLC on functional parameters and oxidative profile in type 2 diabetes-associated PAD. *Diabetes Res Clin Pract* 2006; 72: 231–237.

61. Signorelli SS, Li Volsi G, Fiore V, *et al.* Plasma heme oxygenase-1 is decreased in peripheral artery disease patients. *Mol Med Rep* 2016; 14: 3459–3463.

62. Li Volti G, Seta F, Schwartzman ML, *et al.* Heme oxygenase attenuates angiotensin II-mediated increase in cyclooxygenase-2 activity in human femoral endothelial cells. *Hypertension* 2003; 41: 715–719.

63. Barbagallo I, Galvano F, Frigiola A, *et al.* Potential therapeutic effects of natural heme oxygenase-1 inducers in cardiovascular diseases. *Antioxid Redox Signal* 2013; 18: 507–521.

64. Abraham NG, Rezzani R, Rodella L, *et al.* Overexpression of human heme oxygenase-1 attenuates endothelial cell sloughing in experimental diabetes. *Am J Physiol Heart Circ Physiol* 2004; 287: H2468–H2477.

65. Rodella LF, Vanella L, Peterson SJ, *et al.* Heme oxygenase-derived carbon monoxide restores vascular function in type 1 diabetes. *Drug Metab Lett* 2008; 2: 290–300.

66. Signorelli SS, Mazzarino MC, Spandidos DA, *et al.* Proinflammatory circulating molecules in peripheral arterial disease. *Int J Mol Med* 2007; 20: 279–286.

67. Hamburg NM and Creager MA. Pathophysiology of intermittent claudication in peripheral artery disease. *Circ J* 2017; 81: 281–289.

68. Cassar K, Bachoo P, Ford I, *et al.* Platelet activation is increased in peripheral arterial disease. *J Vasc Surg* 2003; 38: 99–103.

69. Antithrombotic Trialists' (ATT) Collaboration, Baigent C, Blackwell L, *et al.* Aspirin in the primary and secondary prevention of vascular disease: collaborative meta-analysis of individual participant data from randomised trials. *Lancet* 2009; 373: 1849–1860.

70. Mozos I, Malainer C, Horbanczuk J, *et al.* Inflammatory markers for arterial stiffness in cardiovascular disease. *Front Immunol* 2017; 8: 1058.

71. Sugamura K and Keaney JF Jr. Reactive oxygen species in cardiovascular disease. *Free Radic Biol Med* 2011; 51: 978–992.

72. Drummond GR and Sobey CG. Endothelial NADPH oxidases: which NOX to target in vascular disease? *Trends Endocrinol Metab* 2014; 25: 452–463.

73. Ismaeel A, Brumberg RS, Kirk JS, *et al.* Oxidative stress and arterial dysfunction in peripheral artery disease. *Antioxidants (Basel)* 2018; 7: pii: E145.

74. Steven S, Daiber A, Dopheide JF, *et al.* Peripheral artery disease, redox signaling, oxidative stress - basic and clinical aspects. *Redox Biol* 2017; 12: 787–797.

75. Berger JS, Krantz MJ, Kittelson JM, *et al.* Aspirin for the prevention of cardiovascular events in patients with peripheral artery disease: a meta-analysis of randomized trials. *JAMA* 2009; 301: 1909–1919.

76. Fowkes FG, Price JF, Stewart MC, *et al.* Aspirin for prevention of cardiovascular events in a general population screened for a low ankle brachial index: a randomized controlled trial. *JAMA* 2010; 303: 841–848.

77. Chi YW, Lavie CJ, Milani RV, *et al.* Safety and efficacy of cilostazol in the management of intermittent claudication. *Vasc Health Risk Manag* 2008; 4: 1197–1203.

78. Mangiafico RA and Mangiafico M. Medical treatment of critical limb ischemia: current state and future directions. *Curr Vasc Pharmacol* 2011; 9: 658–676.

79. Anderson JL, Halperin JL, Albert NM, *et al.* Management of patients with peripheral artery disease (compilation of 2005 and 2011 ACCF/AHA guideline recommendations): a report of the American college of cardiology foundation/American heart association task force on practice guidelines. *Circulation* 2013; 127: 1425–1443.

80. Loffredo L, Carnevale R, Cangemi R, *et al.* NOX2 up-regulation is associated with artery dysfunction in patients with peripheral artery disease. *Int J Cardiol* 2013; 165: 184–192.

81. Sun QA, Runge MS and Madamanchi NR. Oxidative stress, NADPH oxidases, and arteries. *Hamostaseologie* 2016; 36: 77–88.

82. Lassègue B, San Martín A and Griendling KK. Biochemistry, physiology, and pathophysiology of NADPH oxidases in the cardiovascular system. *Circ Res* 2012; 110: 1364–1390.

83. Konior A, Schramm A, Czesnikiewicz-Guzik M, *et al.* NADPH oxidases in vascular pathology. *Antioxid Redox Signal* 2014; 20: 2794–2814.

84. Panday A, Sahoo MK, Osorio D, *et al.* NADPH oxidases: an overview from structure to innate immunity-associated pathologies. *Cell Mol Immunol* 2015; 12: 5–23.

85. Nguyen GT, Green ER and Mecsas J. Neutrophils to the ROScue: mechanisms of NADPH oxidase activation and bacterial resistance. *Front Cell Infect Microbiol* 2017; 7: 373.

86. Seno T, Inoue N, Gao D, *et al.* Involvement of NADH/NADPH oxidase in human platelet ROS production. *Thromb Res* 2001; 103: 399–409.

87. Fuentes E, Gibbins JM, Holbrook LM, *et al.* NADPH oxidase 2 (NOX2): a key target of oxidative stress-mediated platelet activation and thrombosis. *Trends Cardiovasc Med* 2018; 28: 429–434.

88. Shafique E, Anali Torina A, Reichert K, *et al*. Mitochondrial redox plays a critical role in the paradoxical effects of NAPDH oxidase-derived ROS on coronary endothelium. *Cardiovasc Res* 2017; 113: 234–246.

89. Zhao R, Ma X, Xie X, *et al*. Involvement of NADPH oxidase in oxidized LDL-induced upregulation of heat shock factor-1 and plasminogen activator inhibitor-1 in vascular endothelial cells. *Am J Physiol Endocrinol Metab* 2009; 297: E104–E111.

90. Förstermann U. Nitric oxide and oxidative stress in vascular disease. *Pflugers Arch* 2010; 459: 923–939.

91. Li Volti G, Sorrenti V, Murabito P, *et al*. Pharmacological induction of hemeoxygenase-1 inhibits iNOS and oxidative stress in renal ischemia-reperfusion inhibits iNOS and oxidative stress in renal ischemia-reperfusion injury. *Transplant Proc* 2007; 39: 2986–2991.

92. Brown DI and Griendling KK. Nox proteins in signal transduction. *Free Radic Biol Med* 2009; 47: 1239–1253.

93. Konior A, Schramm A, Czesnikiewicz-Guzik M, *et al*. NADPH oxidases in vascular pathology. *Antioxid Redox Signal* 2014; 20: 2794–2814.

94. Yousefian M, Shakour N, Hosseinzadeh H, *et al*. The natural phenolic compounds as modulators of NADPH oxidases in hypertension. *Phytomedicine* 2019; 55: 200–213.

95. Raffaele M, Li Volti G, Barbagallo IA, *et al*. Therapeutic efficacy of stem cells transplantation in diabetes: role of heme oxygenase. *Front Cell Dev Biol* 2016; 4: 80.

96. Raffaele M, Bellner L, Singh SP, *et al*. Epoxyeicosatrienoic intervention improves NAFLD in leptin receptor deficient mice by an increase in PGC1α-HO-1-PGC1α-mitochondrial signaling. *Exp Cell Res* 2019; 380: 180–187.

97. Sorrenti V, Raffaele M, Vanella L, *et al*. Protective effects of caffeic acid phenethyl ester (CAPE) and novel cape analogue as inducers of heme oxygenase-1 in streptozotocin-induced type 1 diabetic rats. *Int J Mol Sci* 2019; 20. pii: E2441.

98. Cao J, Sodhi K, Puri N, *et al*. High fat diet enhances cardiac abnormalities in SHR rats: protective role of heme oxygenase-adiponectin axis. *Diabetol Metab Syndr* 2011; 3: 37.

99. Tanriverdi LH, Parlakpinar H, Ozhan O, *et al*. Inhibition of NADPH oxidase by apocynin promotes myocardial antioxidant response and prevents isoproterenol-induced myocardial oxidative stress in rats. *Free Radic Res* 2017; 51: 772–786.

100. Costa CA, Amaral TA, Carvalho LC, *et al*. Antioxidant treatment with tempol and apocynin prevents endothelial dysfunction and development of renovascular hypertension. *Am J Hypertens* 2009; 22: 1242–1249.

101. Rey FE, Cifuentes ME, Kiarash A, *et al*. Novel competitive inhibitor of NAD(P)H oxidase assembly attenuates vascular O_2^- and systolic blood pressure in mice. *Circ Res* 2001; 89: 408–414.

102. Fuentes E, Gibbins JM, Holbrook LM, *et al*. NADPH oxidase 2 (NOX2): a key target of oxidative stress-mediated platelet activation and thrombosis. *Trends Cardiovasc Med* 2018; 28: 429–434.

103. Hirsch AT, Haskal ZJ, Hertzer NR, *et al*. ACC/AHA guidelines for the management of patients with peripheral arterial disease (lower extremity, renal, mesenteric, and abdominal aortic): a collaborative report from the American associations for vascular surgery/society for vascular surgery. society for cardiovascular angiography and interventions, society for vascular medicine and biology, society of interventional radiology, and the ACC/AHA task force on practice guidelines (writing committee to develop guidelines for the management of patients with peripheral arterial disease). Summary of recommendations. *J Vasc Interv Radiol* 2006; 17: 1383–1397; quiz 1398.

104. Isner JM, Pieczek A, Schainfeld R, *et al*. Clinical evidence of angiogenesis after arterial gene transfer ofphVEGF165 in patient with ischaemic limb. *Lancet* 1996; 348: 370–374.

105. Baumgartner I, Pieczek A, Manor O, *et al*. Constitutive expression of phVEGF165 after intramuscular gene transfer promotes collateral vessel development in patients with critical limb ischemia. *Circulation* 1998; 97: 1114–1123.

106. Comerota AJ, Throm RC, Miller KA, *et al*. Naked plasmid DNA encoding fibroblast growth factor type 1 for the treatment of end-stage unreconstructible lower extremity ischemia: preliminary results of a phase I trial. *J Vasc Surg* 2002; 35: 930–936.

107. Nikol S, Baumgartner I, van Belle E, *et al*. Therapeutic angiogenesis with intramuscular NV1FGF improves amputation-free survival in patients with critical limb ischemia. *Mol Ther* 2008; 16: 972–978.

108. Ogihara T and Morishita R. Randomized, double- blind, placebo-controlled clinical trial of hepatocyte growth factor plasmid for critical limb ischemia. *Gene Ther* 2010; 17: 1152–1161.

109. Shigematsu H, Yasuda K, Sasajima T, *et al.* Transfection of human HGF plasmid DNA improves limb salvage in Buerger's disease patient with critical limb ischemia. *Int Angiol* 2011; 30: 140–149.

110. Belch J, Hiatt WR, Baumgartner I, *et al.* Effect of fibroblast growth factor NV1FGF on amputation and death: a randomised placebo-controlled trial of gene therapy in critical limb ischaemia. *Lancet* 2011; 377: 1929–1937.

111. Kaga T, Kawano H, Sakaguchi M, *et al.* Hepatocyte growth factor stimulated angiogenesis without inflammation: differential actions between hepatocyte growth factor, vascular endothelial growth factor and basic fibroblast growth factor. *Vasc Pharmacol* 2012; 57: 3–9.

112. Tateishi-Yuyama E, Matsubara H, Murohara T, *et al.* Therapeutic angiogenesis for patients with limb ischaemia by autologous transplantation of bone marrow cells: a pilot study and a randomised controlled trial. *Lancet* 2002; 360: 427–435.

113. Matoba S, Tatsumi T, Murohara T, *et al.* Long-term clinical outcome after intramuscular implantation of bone marrow mononuclear cells (therapeutic angiogenesis by cell transplantation [TACT] trial) in patients with chronic limb ischemia. *Am Heart J* 2008; 156: 1010–1018.

114. Lu D, Chen B, Liang Z, *et al.* Comparison of bone marrow mesenchymal stem cells with bone marrow-derived mononuclear cells for treatment of diabetic critical limb ischemia and foot ulcer: a double-blind, randomized, controlled trial. *Diabetes Res Clin Pract* 2011; 92: 26–36.

115. Signorelli SS, Volsi GL, Pitruzzella A, *et al.* Circulating miR-130a, miR-27b, and miR-210 in patients with peripheral artery disease and their potential relationship with oxidative stress. *Angiology* 2016; 67: 945–950.

116. Opratilova R, Caprnda M, Kubakta P, *et al.* Adipokine in neurovascular disease. *Biomed Pharmaother* 2018; 98: 424–432.

117. Huang IC, Chang CC, Hsu BG, *et al.* Association of hyperleptinemia with peripheral arterial disease in hypertensive patients. *Ci Ji Yi Xue Za Zhi* 2017; 29: 148–153.

118. Writing Committee Members, Gerhard-Herman MD, Gornik HL, *et al.* 2016 AHA/ACC Guideline on management of patients with lower extremity peripheral arterial disease. *Vasc Med* 2017; 22: NP1–NP43.

119. Parvar SL, Fitridge R, Dawson J, *et al.* Medical and lifestyle management of peripheral arterial disease. *J Vasc Surg* 2018; 68: 1595–1606.

120. McDermott MM. Medical management of functional impairment in peripheral artery disease: a review. *Prog Cardiovasc Dis* 2018; 60: 586–592.

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