ELSEVIER

The therapeutic prospects of using L-carnitine to manage hypertension-related organ damage

Alfonso Mate, José L. Miguel-Carrasco and Carmen M. Vázquez

Departamento de Fisiología y Zoología, Facultad de Farmacia, Universidad de Sevilla, CL Profesor García González 2, E-41012 Sevilla, Spain

Subclinical organ damage is a very important aspect when assessing total cardiovascular risk in hypertensive subjects. Therapeutic strategies in those patients should consider treatment of hypertension-related cardiovascular and renal damage in addition to achieving the recommended blood pressure targets. L-carnitine (LC) is a naturally occurring compound that is administered exogenously for treatment of patients that are deficient in carnitine. The currently available data do not support a preferential role of LC as an antihypertensive agent compared to other available drugs. However, its ability to simultaneously modulate several targets and/or pathways provides antioxidant and anti-inflammatory properties. These additional properties might justify the therapeutic use of LC as a protective agent against cardiovascular and renal remodelling in arterial hypertension.

The term carnitine (after the Latin 'carnus', or flesh, as it was first isolated from meat) usually refers to several compounds, including the free form of L-carnitine (LC) and LC-derived small esters, such as acetyl-LC and propionyl-LC. Because it is a widely distributed naturally occurring compound and can be endogenously synthesized under physiologic conditions, LC is considered a 'conditionally essential' nutrient. That is, LC requirements might be higher than the individual's intake and/or capacity of synthesis under certain conditions.

Since the discovery of LC deficiency syndromes in the early 1970s, there has been a growing interest in exploring the potential therapeutic uses of LC as a medicinal agent and nutritional supplement [1]. In this regard, LC has become an increasingly popular ingredient in over-the-counter dietary supplements; these formulations have been advertised as an aid to weight loss, to improve exercise performance and to enhance the sense of well-being. Whereas there is a general consensus that LC treatment is beneficial in patients with carnitine deficiency, studies regarding the benefits of carnitine supplementation in individuals who are carnitine-sufficient have not been conclusive. However, recent experimental and clinical data support the notion that supraphysiological concentrations of LC in plasma and target organs might exert beneficial effects on several disorders of a common origin, including insulin resistance, type 2 diabetes, dyslipidemia, cardiovascular disease and hypertension [2].

Arterial hypertension causes serious structural and functional alterations in target organs such as the heart and kidneys. Presently, subclinical organ damage is considered to be an important component of total cardiovascular risk. Asymptomatic alterations in the cardiovascular system and the kidneys are crucial intermediate stages in the disease continuum that links hypertension to cardiovascular events and ultimately death [3]. Therefore, it is important to seek therapeutic strategies that, in addition to lowering blood pressure, are able to manage or treat hypertension-related organ damage. This article will discuss the potential role of LC in treatment of hypertension-related organ damage based upon current literature and relevant conclusions (Table 1).

The physiological function and metabolism of LC

Since its discovery in 1905 from muscle extract, research regarding carnitine aimed to initially establish its chemical structure and, subsequently, delineate its major physiological function, biosynthetic pathway and transport mechanisms [4]. Carnitine has two major functions: (i) transport of long-chain fatty acids in the form of acylcarnitine from the cytosol into the mitochondria for their subsequent use as a source of energy (via acetyl-CoA formation in the process known as β-oxidation) and (ii) the removal of short- and medium-chain fatty acids formed as a consequence of normal

Corresponding author:. Mate, A. (mate@us.es)

1359-6446/06/$ - see front matter doi:10.1016/j.drudis.2010.03.014

TABLE 1

Summary of studies on antioxidant, anti-inflammatory and antihypertensive properties of L-carnitine

Biological sample	AO	AI	AH	Refs.	Observations
Nondiabetic subjects with increased cardiovascular risk	ND	ND	+/−	[18]	2 g per day of acetyl-LC during 24 weeks; partial reduction of systolic BP, diastolic BP reduced only in patients with low insulin resistance
Serum of uraemic patients	ND	+	ND	[22]	20 mg/kg after dialysis sessions during six months (infusion)
Brain of aged rats	+/−	ND	ND	[48]	75 mg/kg per day during four weeks; effect achieved with acetyl-LC, not with LC
Brain of rats with induced neurotoxicity	+	ND	ND	[49]	100 mg/kg per day during 5–30 days
Lymphocytes of aged rats	+	+	ND	[51]	300 mg/kg per day during 21 days
Human endothelial cells	+	+	ND	[52]	0.05–2.00 mM of LC, acetyl-LC or propionyl-LC
Serum/liver of ethanol-treated rats	+	ND	ND	[54]	1.5 g/L *ad libitum* during five weeks
Serum of patients with peripheral arterial disease	+	ND	ND	[55]	6 g per day during seven days (propionyl-LC)
Liver/heart/aorta of young SHR	+	ND	–	[59,60]	200 mg/kg per day during eight weeks (LC or propionyl-LC)
Fructose-fed hypertensive rats	+	ND	+	[62]	300 mg/kg (intraperitoneal)
Serum/heart of L-NAME-induced hypertension	+	ND	+	[63]	300 mg/kg per day during 12 weeks; incomplete reduction of BP
Serum/heart of L-NAME-induced hypertension	ND	+	+	[65]	300 mg/kg per day during 12 weeks; incomplete reduction of BP
Rat cardiac fibroblasts	+	+	ND	[67]	1–30 mM
SHR	ND	ND	+/−	[70]	200 mg/kg per day during six weeks; partial reduction of systolic BP, no effect on diastolic BP
Blood/heart of adult SHR	+	ND	+	[71]	300 mg/kg per day during 12 weeks; incomplete reduction of BP

Abbreviations: AH, antihypertensive; AI, anti-inflammatory; AO, antioxidant; BP, blood pressure; L-NAME, N-ω-nitro-L-arginine methyl ester; LC, L-carnitine; ND, not determined; SHR, spontaneously hypertensive rats.

metabolism, preventing a toxic accumulation of these compounds in the mitochondria and leading to an increase of free CoA. An increase in free CoA results in activation of the pyruvate dehydrogenase complex and subsequently improves coupling between glycolysis and glucose oxidation (Figure 1). Therefore, it is not surprising that carnitine is highly concentrated in tissues that use fatty acids as their main source of energy, such as skeletal and cardiac muscle.

LC is a naturally occurring compound that is widely distributed in nature, especially in red meats and dairy products. It is absorbed in the intestine and distributed to various tissues, with skeletal and cardiac muscle stores accounting for more than 98% of the total carnitine pool. In addition, low levels of carnitine can be synthesized, primarily in the liver and, to a lesser extent, in the kidneys and brain [5]. The kinetics and pharmacokinetics of LC have been reviewed extensively elsewhere [6].

Carnitine deficiency and carnitine supplementation

Although LC is synthesized endogenously and supplemented exogenously by a normal diet, there is evidence that both primary and secondary deficiencies of LC can occur. Primary LC deficiency can have a variety of causes, including defects in LC metabolism, abnormal renal handling, impaired tissue uptake and malabsorption of dietary LC [6]. Secondary carnitine deficiencies are more common than primary deficiencies and can arise from excessive LC loss owing to dialysis [7], inborn metabolic dysfunction [8], the administration of certain drugs [9,10] or different underlying pathologic disorders, as reviewed below. Regardless of its aetiology, LC deficiency can result in severe symptoms such as muscle weakness, cardiomyopathy, congestive heart failure, encephalopathy, hepatic insufficiency, impaired growth and development in children and neuromuscular disorders [1,11].

Exogenous LC is typically administered for most pathologies caused by LC deficiency (hence the 'conditionally essential' term used above). Because of its low bioavailability [6] and high levels of excretion when renal tubular reabsorption is saturated, high doses of LC are required to obtain significant supraphysiological concentrations in plasma and target organs. The approved conditions for use are mainly myopathies and cardiovascular disease presenting with carnitine deficiency. LC is also used to restore carnitine levels in chronic haemodialysis patients and has historically been used in parenteral nutrition [12]. Many other potential applications of LC have been studied so far, some of which are discussed below.

Cardiovascular disease and diabetes

In the past two decades, many studies concerning the therapeutic effects of carnitine on cardiovascular disease have been conducted. Initial interest arose just after the recognition of carnitine deficiency syndrome, which was associated with cardiac and skeletal myopathies, among other symptoms [13]. The efficacy of carnitine treatment in ischaemic heart disease was initially attributed to an increase in lipid oxidation in cardiomyocytes and a subsequent extra energy supply. Further evidence, however, has suggested that LC had additional relevant roles in the protection of cardiac cells against ischaemia, hypoxia and oxidative stress. Thus, it is currently believed that carnitine is cardioprotective through its indirect effect on decreasing the levels of toxic acyl-CoA derivatives and regulating carbohydrate metabolism [8,14]. The administration of LC reduces the intramitochondrial ratio of acetyl-CoA to free CoA, which stimulates pyruvate dehydrogenase activity and glucose oxidation (Figure 1). In addition to its effects on ischaemic heart disease, beneficial functions of LC have been reported in arrhythmia, cardiopulmonary bypass surgery, circulatory failure, endothelial function and peripheral blood diseases [14–16].

Because of its ability to regulate both lipid and glucose metabolism, LC might also have a role in the pathogenesis of insulin resistance. Chronically elevated levels of glucose and free fatty acids, as occur in type 2 diabetes, contribute to cell dysfunction by

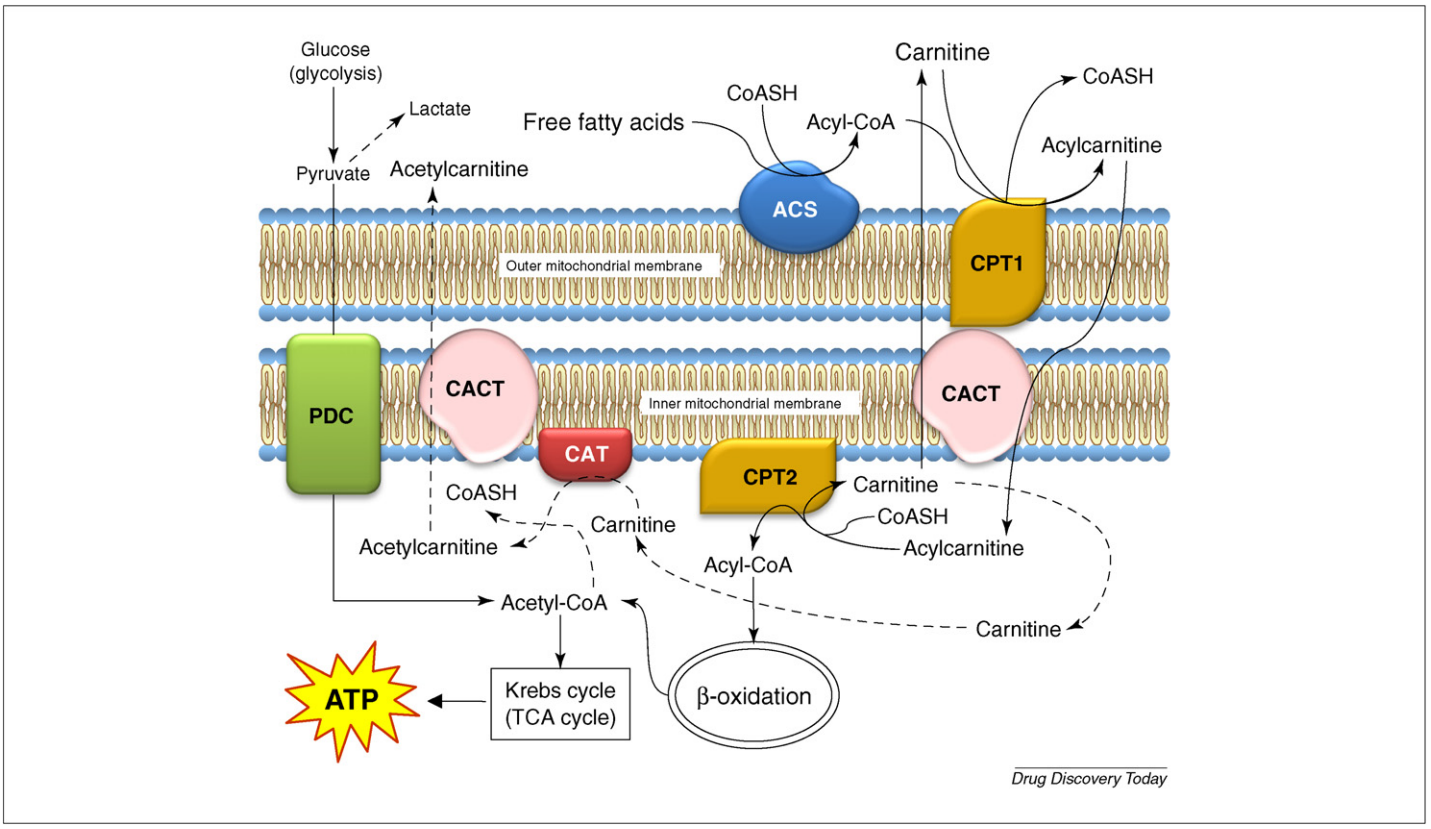

FIGURE 1

The carnitine system in mitochondria. The carnitine system is primarily involved in the incorporation of long-chain fatty acids inside the mitochondria for ATP production. Fatty acid transport across the mitochondrial membrane involves three enzymes. After the initial activation of fatty acids by acyl-CoA synthetase (ACS), the enzyme carnitine palmitoyltransferase I (CPT1) – located in the outer mitochondrial membrane – catalyzes the transfer of acyl groups from acyl-CoA to carnitine to synthesize acylcarnitine and release free CoA (CoASH). The enzyme carnitine–acylcarnitine translocase (CACT), located within the inner mitochondrial membrane, exchanges cytoplasmic acylcarnitine for free mitochondrial carnitine. Finally, the enzyme carnitine palmitoyltransferase II (CPT2), located on the matrix side of the inner mitochondrial membrane, catalyzes the reverse reaction of CPT1, reconverting acylcarnitine to acyl-CoA (conveyed to β-oxidation) and releasing free carnitine. CACT is also able to act as a uniport carrier. This function, together with the presence of the enzyme carnitine acetyltransferase (CAT) on the matrix side of the inner mitochondrial membrane, is important for the export of potentially toxic short-chain acylcarnitine compounds (such as acetylcarnitine) out of mitochondria and for the production of free CoA. Through this mechanism, LC ensures that a viable pool of free CoA is maintained for the continuation of pyruvate dehydrogenase complex (PDC) and TCA cycle reactions, allowing for improved coupling between glycolysis and glucose oxidation [20,29].

increasing the basal rate of insulin secretion, which can lead to insulin resistance. The exogenous administration of LC improves insulin-stimulated glucose disposal [17,18]. In addition, its antioxidant properties (discussed below) might have an important protective role against diabetic vascular complications [19]. Because muscular carnitine content can be increased by insulin [20], it seems plausible that patients suffering from diabetes with hyperinsulinaemia would benefit from exogenous LC adsorption, which would improve glycaemic control and reduce the impact of chronic complications.

Renal failure and dialysis

It has been well documented that haemodialysis substantially reduces plasma and tissue carnitine levels. In fact, LC has been used for ten years in haemodialysis-associated carnitine deficiency treatment [21]. In addition to restoring carnitine levels, it has been suggested that the antioxidant and anti-inflammatory properties of LC might be beneficial in uraemic patients [22,23]. Many nephrologists are reluctant to prescribe LC, however, because of the lack of large-scale randomized and registered trials [7,24].

Nutrition disorders, chronic fatigue and sports medicine

There is no substantial evidence regarding the efficacy of LC in weight loss in healthy, non-obese subjects [25,26], despite current advertisement claims. However, LC shows the most promise among supplement therapies for the management of fatigue in cancer patients [27]. A large amount of research has also been directed towards investigating the effects of LC as an ergogenic agent; the main hypothesis is that increased LC availability would increase fat burning during prolonged exercise, spare muscular glycogen stores and, therefore, delay the onset of fatigue. Limitations in the study design of some reports, although, have elicited controversial conclusions in the literature [26,28].

Other uses

Supplementation with LC has been used to treat adriamycin-induced cardiotoxicity [29] and valproic acid-induced hepatotoxicity [10], among others. Carnitine has also been proposed for treatment of neurodegenerative disorders [30], male infertility [31] and AIDS patients [1] and has even been suggested as an additive in extended platelet storages [32].

The role of LC in treatment of arterial hypertension

Hypertension is a current major global public health problem and represents a major risk factor for cardiovascular and related diseases in a direct or indirect manner. Considerable progress has been achieved in recent years concerning the pathophysiology of hypertension and therapeutic approaches to treatment. However, in spite of a wide range of prescription possibilities and globally available guidelines [33–36], less than half of patients remain controlled. Additional basic research in animals and clinical trials are necessary to improve the control of elevated blood pressure and organ protection in hypertensive patients [37]. Hypertension-associated organ damage is an important component that contributes to total cardiovascular risk. In particular, structural and functional alterations in the cardiovascular system and the kidneys are crucial steps in the appearance of cardiovascular events after a maintained rise in blood pressure [3]; therefore, therapeutic approaches should not only reduce blood pressure but also protect the heart, the vasculature and the kidneys against hypertension-induced damage.

Oxidative stress and inflammation in the pathophysiology of hypertension

Molecular mechanisms contributing to the pathoaetiology of hypertension are complex and involve many interacting systems. In the past few years, there has been increasing evidence that the interplay between oxidative stress and inflammation is involved in the initiation, progression and long-term complications of cardiovascular disease, including various forms of hypertension [38–41]. The term 'oxidative stress' refers to increased levels of reactive oxygen species (ROS) that cannot be adequately controlled by intrinsic antioxidant systems. ROS are reactive chemical entities that consist of either free oxygen radicals (e.g. superoxide anion, $^{\bullet}O_2^-$) or nonradical O_2 derivatives (e.g. hydrogen peroxide, H_2O_2). The organism constantly buffers ROS generation via an antioxidant system comprising both nonenzymatic elements (mainly glutathione, or GSH) and enzymatic elements (glutathione peroxidase, or GSH-Px; glutathione reductase, or GSH-Red; catalase, or Cat; and superoxide dismutase, or SOD). However, excessive ROS generation triggers tissue injury and dysfunction through the activation of pro-inflammatory, profibrotic and mitogenic signalling pathways. This might lead to endothelial dysfunction, cardiovascular and renal remodelling and increased peripheral resistance with subsequent elevated blood pressure [39,42,43].

Because oxidative stress can cause hypertension and vice versa, it seems that both elements could be involved in a self-perpetuating cycle in regard to the generation and maintenance of hypertension [41]. The mechanisms by which oxidative stress can raise blood pressure include structural and functional alterations within the vasculature. These are mediated by increases in the intracellular calcium concentration of vascular smooth muscle cells, stimulation of inflammatory and growth-signalling events, generation of vasoconstrictor pro-inflammatory lipid peroxidation products (e.g. isoprostanes) and reduction in the bioavailability of the vasodilator nitric oxide (NO) [41,44]. NO depletion can be secondary to either quenching by ROS, such as superoxide anions, or NO synthase (NOS) uncoupling. The latter might occur in the absence of NOS substrate (L-arginine) or its cofactor (tetrahydrobiopterin, BH_4). Under these conditions, NOS oxidation of NADPH does not generate NO but produces superoxide and hydrogen peroxide; thus, the enzyme NOS is said to be 'uncoupled'. Endothelial NOS uncoupling is an important mechanism underlying the pathogenesis of endothelial dysfunction, especially when added to cardiovascular risk factors such as hypercholesterolemia, diabetes and hypertension. The exogenous administration of BH_4 and/or strategies that augment endogenous levels of BH_4 are gaining popularity for the prevention and treatment of vascular disease [45].

In addition to the above-mentioned uncoupled NOS mechanism, the most relevant enzymatic sources of ROS in vascular disease and hypertension are xanthine oxidase and NAD(P)H oxidase. Evidence from animal studies supports a role for xanthine oxidase in hypertensive end-organ damage rather than in the development of hypertension *per se* [39]. However, recent reviews have highlighted that NAD(P)H oxidases (the Nox family) are the major source of free radicals in the cardiovascular and renal systems [39,42,46]. The prototypical NAD(P)H oxidase contains a membrane-bound heterodimeric flavoprotein termed Cytochrome *b*558 that comprises the p22phox subunit and the catalytic subunit Nox2 (formerly known as gp91phox), and several cytosolic regulatory subunits. This configuration was originally described in neutrophils (hence the term 'phox', standing for phagocyte oxidase). Upon stimulation, the cytosolic subunits translocate to the flavocytochrome complex in the membrane and form the active oxidase, which transfers electrons from NAD(P)H to O_2 and ultimately releases $^{\bullet}O_2^-$ (Figure 2).

Seven members of the Nox family have been described to date, based on the discovery of Nox2 homology. Although the regulation and function of each Nox still remains unknown, there is evidence that renal and cardiovascular NAD(P)H oxidases are strongly regulated by the renin–angiotensin system (RAS) [39,42,46,47]. At the molecular level, angiotensin II (Ang II) activates NAD(P)H oxidase via AT1 receptors through stimulation of diverse signal transduction pathways (Figure 2), resulting in extracellular matrix protein deposition, activation of matrix metalloproteinases, inflammation, endothelial dysfunction and increased vascular tone. All of these symptoms are characteristics of the hypertension vascular phenotype. It should be noted, though, that studies of hypertension in humans have not been as conclusive as those in animal models of hypertension and that further research on oxidative stress and hypertension from a clinical point of view is needed.

Antioxidant and anti-inflammatory properties of LC

The antioxidant properties of LC and its short acyl congeners (acetyl-LC and propionyl-LC) have been demonstrated in many adverse conditions under high oxidative stress. For example, decreased functional capacity in aged organisms is believed to result from the increased generation of reactive oxygen and nitrogen species. Supplementation with 75 mg/kg per day of acetyl-LC during four weeks was able to decrease several markers of oxidative stress (namely lipid peroxidation, estimated as malondialdehyde [MDA] production, and extent of oxidized nucleotides and nitrotyrosine formation) in the brain of aged rats [48]. Interestingly, these effects could not be reproduced by an equivalent treatment with LC. A decreased LC-mediated effect could be attributed to a higher efficiency of acetyl-LC in crossing the blood–brain barrier

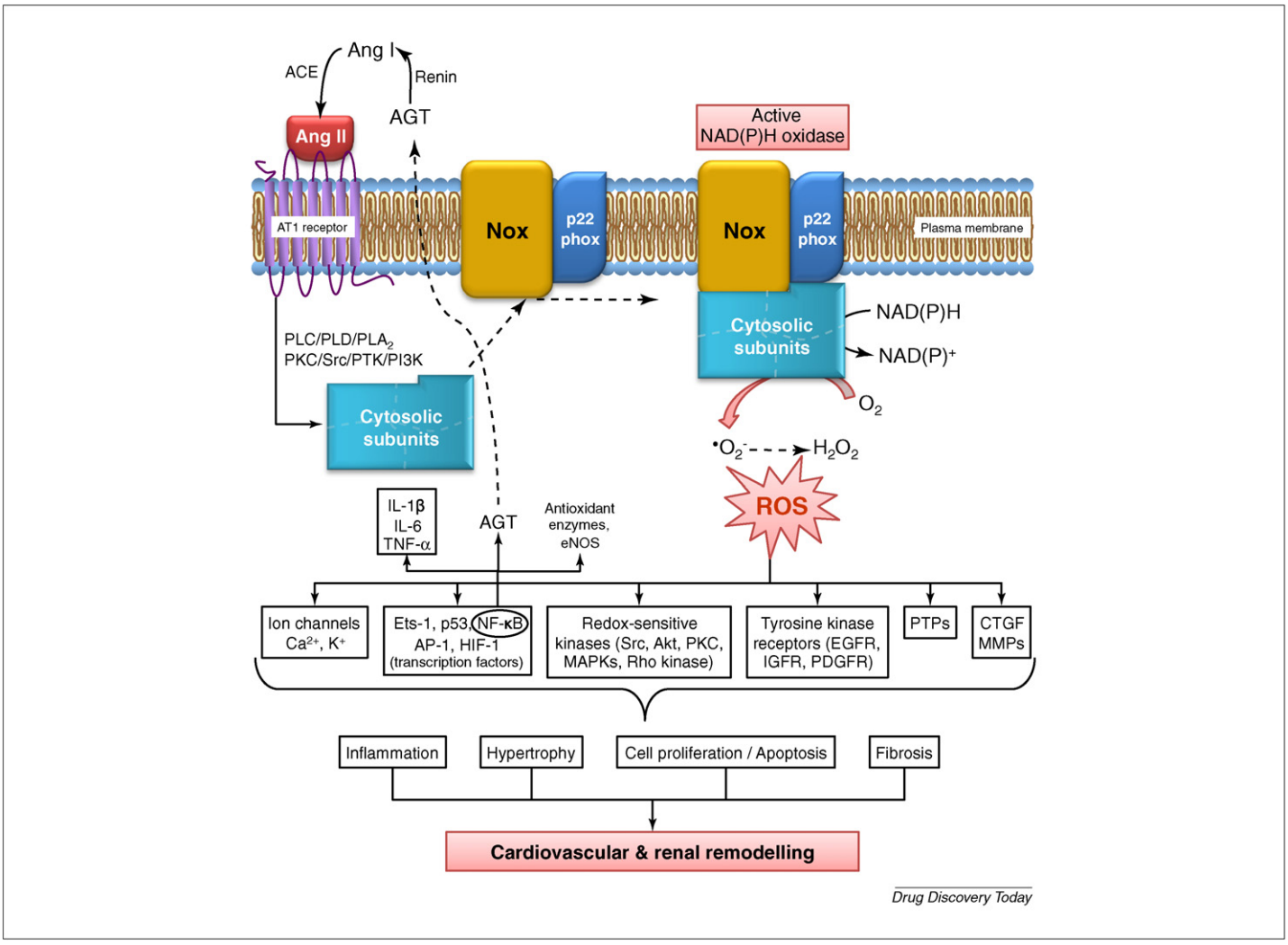

FIGURE 2

NAD(P)H oxidase and angiotensin II (Ang II) receptor signalling pathways. Many of the pathophysiological effects of Ang II are due to its ROS-dependent pro-growth properties. When Ang II binds to its receptor (AT1 receptor), a series of kinase proteins are activated and the cytosolic subunits of NAD(P)H oxidase form a complex that translocates to the membrane, where it associates with additional subunits to form the active oxidase. ROS produced by NAD(P)H oxidase (especially the superoxide anion, $^{\bullet}O_2^-$, and hydrogen peroxide, H_2O_2) activate multiple and complex signalling pathways depending on the cell types proximal to where these compounds are released. In the cardiovascular and renal systems, this process activates transcription factors such as nuclear factor-kappa B (NF-κB). This, in turn, activates the expression of pro-inflammatory cytokines, such as interleukin (IL)-1β, IL-6 and tumour necrosis factor-α (TNF-α). The inflammatory response is amplified through stimulation of expression of the Ang II precursor, angiotensinogen (AGT). However, NF-κB also regulates the expression of antioxidant enzymes and endothelial nitric oxide synthase (eNOS). The process culminates with remodelling of the cardiovascular and renal tissue, a characteristic feature of hypertension-related organ disease. Abbreviations: ACE, angiotensin-converting enzyme; AP-1, activator protein 1; CTGF, connective tissue growth factor; EGFR, epidermal growth factor receptor; Ets-1, E26 transformation-specific sequence; HIF-1, hypoxia inducible factor 1; IGFR, insulin-like growth factor receptor; MAPKs, mitogen-activated protein kinases; MMPs, matrix metalloproteinases; PDGFR, platelet-derived growth factor receptor; PI3K, phosphatidylinositol 3-kinase; PKC, protein kinase C; PLA_2, phospholipase A_2; PLC, phospholipase C; PLD, phospholipase D; PTK, protein tyrosine kinase; PTPs, protein tyrosine phosphatases.

[30]. However, a more recent study showed that the administration of 100 mg/kg per day of LC during 5–30 days reduced ROS formation, reduced lipid peroxidation and improved mitochondrial dysfunction in the brain of rats with induced neurotoxicity [49]. In addition, the observation that lymphocytes are enriched in carnitine has suggested that the carnitine system might have a considerable role in counteracting the impaired immune responses associated with ageing [50]. It has been reported recently that LC (300 mg/kg per day for 21 days), in addition to reducing lipid peroxidation and increasing the levels of antioxidant enzymes (SOD, Cat, GSH-Px and GSH-Red), was able to reduce DNA damage, reduce apoptosis and decrease tumour necrosis factor-α (TNF-α) levels in lymphocytes of aged rats. Thus, the authors concluded that LC might improve immune system function by its antioxidant action [51]. Evidence also suggests a role for carnitine in the upregulation of cytoprotective heat-shock proteins, which form a highly conserved system responsible for the preservation and repair of proteins in the so-called 'longevity assurance processes' [52,53].

We have previously mentioned in this article the protective effect of carnitine against the toxic actions of different drugs, some of which induce oxidative stress and/or carnitine deficiency. Recently, ethanol-induced oxidative stress has also been reversed in Wistar rats after LC treatment [54]. In cardiovascular diseases,

the effects of carnitine therapy have been extensively investigated beyond its regulatory effects on lipid and carbohydrate metabolism. Loffredo *et al*. [55] performed a cross-sectional study that compared flow-mediated dilatation of the brachial artery, oxidative stress and NO generation (assessed by serum levels of nitrite and nitrate, NOx) in a population of control and peripheral arterial disease patients. The authors found that a seven-day administration of propionyl-LC (6 g per day) resulted in an increase in flow-mediated dilatation and NOx and a decrease in the oxidative stress markers, improving the alterations of these three parameters observed in peripheral arterial disease patients with respect to the control group. In streptozotocin-induced diabetic rats, the administration of 1 g/kg per day of LC for three weeks after diabetes induction reduced the activity of angiotensin-converting enzyme (ACE) in aorta, heart and kidney homogenates [56]. ACE inhibition might result in lower Ang II and NAD(P)H oxidase-dependent ROS production (Figure 2), thus improving NO bioavailability owing to a lower depletion by ROS.

The spontaneously hypertensive rat (SHR) is a model of hypertension characterized by an impaired antioxidant defence capacity [57,58]. The antioxidant properties of carnitine have also been observed in the SHR model. Gómez-Amores *et al*. [59] administered propionyl-LC (200 mg/kg per day) to four-week-old SHRs (these animals still have mild hypertension at that age) for a total of eight weeks; Wistar-Kyoto (WKY) rats were used as a control normotensive group. They saw a reduced carnitine content, a decreased ratio of reduced to oxidized glutathione (GSH/GSSG) and higher lipid peroxidation levels (estimated as MDA formation) in the liver and heart of SHR compared with WKY rats. Propionyl-LC treatment restored carnitine and GSH levels to normal in hypertensive animals and reduced the MDA to normal levels measured in the normotensive group. In general, no effect was observed in WKY rats treated with propionyl-LC, except for increased carnitine levels in the serum, liver and heart. Similar results were obtained after the administration of LC in young SHRs following the same experimental design [60]. Additional measurements confirmed that LC was also able to reverse the reduced plasma NOx levels and the total antioxidant status observed in the SHR model. The increase in NO bioavailability in SHRs after treatment might have been due to a reduction in ROS production because LC down-regulated the p22phox subunit of NAD(P)H oxidase. As a consequence, an increased NO effect on endothelium-dependent relaxation was observed in the aortic rings from LC-treated SHRs, suggesting that LC supplementation resulted in an improvement of endothelial function [61]. Evidence suggests that LC also reduces oxidative stress in other experimental models of hypertension, such as the fructose-fed hypertensive rat [62], or rats with hypertension caused by NO depletion subsequent to N-ω-nitro-L-arginine methyl ester (L-NAME) treatment [63].

A new era for carnitine applications was announced in the early 1990s, after experiments that demonstrated the immunomodulating and anti-apoptotic properties of LC. These effects were related to a reduction in the serum levels of pro-inflammatory cytokines, specifically interleukin (IL)-1β, IL-6 and TNF-α [64]. In uraemic patients, infusion of 20 mg/kg LC at the end of each dialysis session for a total period of six months resulted in a notable decrease in levels of serum C-reactive protein, which is a pro-inflammatory cytokine known to inhibit erythropoiesis [22]. The authors concluded that the clinical benefits of LC in patients with renal disease were supported by its anti-inflammatory effects. More recently, the anti-inflammatory properties of LC (estimated as a reduction in plasma levels and cardiac expression of IL-1β, IL-6 and TNF-α) have been reported in L-NAME-induced hypertension [65]. These effects were accompanied by a reduction in the protein and mRNA expression of RAS components, namely ACE and the Ang II AT1 receptor, in the heart. It seems probable that the immunomodulatory action of LC is mediated by transcription factor NF-κB regulation (Figure 2). In fact, it has been suggested previously that Ang II is involved in the stimulation of inflammatory mediators through NF-κB system activation in the SHR model of hypertension [66].

As we have already mentioned, an excessive activation of NAD(P)H oxidase by Ang II can induce hypertrophy, cell proliferation and fibrosis (Figure 2); these cardiovascular and renal remodelling processes might be involved in hypertension progression towards ischaemic injury and heart failure. It has been reported recently that LC attenuates Ang II-induced proliferation of cardiac fibroblasts via NAD(P)H oxidase inhibition in a process mediated by prostacyclin activation of the peroxisome proliferator-activated receptor alpha (PPARα) [67]. The important role of PPARs in cardiovascular disease has been increasingly recognized through modification of the immune system and inflammation. PPAR agonists might be beneficial, alone or in combination with other drugs that modify the inflammatory response, for hypertension treatment and associated inflammatory disease complications [68]. Interestingly, LC supplementation has been reported recently to confer partial metabolic recovery in mice that do not express the PPARα receptor (PPARα−/−) [69]. PPARα knockout mice have a marked depletion of whole-body carnitine reserves owing to the low expression of genes involved in carnitine synthesis and transport. These novel findings could open new possibilities for exploration between the interaction of carnitine and PPAR systems and, specifically, their role in the pathophysiology of arterial hypertension through modulation of oxidative stress and inflammation.

LC can lower blood pressure

Despite the involvement of RAS, oxidative stress and inflammation in the development of hypertension and the ability of LC to counteract all of these components, very few studies have been designed to specifically address the effect of carnitine supplementation on arterial blood pressure in hypertensive patients or animal models of hypertension. Rauchová *et al*. [70] demonstrated for the first time an antihypertensive effect of carnitine in eight-week-old SHR rodents treated for six weeks with an LC oral dose of 200 mg/kg per day. According to the authors, LC supplementation maintained a plasma level of carnitine approximately three times higher than that of the control animals. LC treatment significantly lowered the systolic and mean arterial pressure, but not the diastolic blood pressure, of the SHR. However, neither LC nor propionyl-LC chronic treatment (200 mg/kg per day for eight weeks) was able to prevent the development of hypertension in young (four-week-old) SHRs [59,60], although the same authors found significant reductions in both the systolic and the diastolic blood pressures of 20- to 22-week-old SHRs after LC chronic administration of 300 mg/kg per day for 12 weeks [71]. The

different results could be attributed to higher dosing and longer treatment in the latter study or to the use of SHRs with fully established hypertension instead of young pre-hypertensive animals. Using a similar experimental design, these authors have recently reported a significant antihypertensive effect of LC in another experimental model of hypertension, specifically L-NAME-treated rats [65]. Finally, Rajasekar *et al.* [62] have also shown that carnitine lowers blood pressure in fructose-fed hypertensive rats treated with 300 mg/kg per day of LC. On the whole, it should be noted that the reduction in blood pressure of hypertensive animals subjected to LC treatment, although significant, was incomplete because the values at the end of the treatment were still higher than those of the corresponding control groups. Moreover, LC did not modify blood pressure values in normotensive animals, indicating that carnitine exerts a specific action only when blood pressure rises beyond a threshold.

Ruggenenti *et al.* [18] recently published the only paper that describes the clinically relevant antihypertensive effects of carnitine in humans. In this study, the authors included individuals expected to have decreased insulin sensitivity because of the presence of several risk factors, including arterial hypertension. The subjects entered a 24-week treatment period with 2 g per day of oral acetyl-LC, followed by a 16-week recovery period after active treatment withdrawal. Systolic blood pressure decreased significantly after the treatment and progressively recovered towards baseline over eight weeks post-treatment. Diastolic blood pressure was also diminished by LC administration, but only in those subjects presenting with less severe insulin resistance before the treatment. As was stated by the authors, the lack of a placebo control group is a limitation of their study. However, this was a sequential off–on–off study in which each patient served as his or her own control.

Future perspectives

Experimental evidence regarding the importance of oxidative stress in cardiovascular and renal damage has enormously encouraged the development of various strategies to target ROS generation in the treatment of hypertension and other related cardiovascular diseases. However, the overall results of clinical studies with antioxidant supplementation (e.g. vitamins A, C and E, selenium and other agents) have been disappointing, considering the consistent and promising findings in animal models. One possible explanation is that antioxidant therapies are limited to scavenging already-formed oxidants but cannot target the sources of ROS, such as NAD(P)H oxidases [19,39,42]. However, treatment with carnitine represents a 'causal' rather than a 'symptomatic' antioxidant therapy. Carnitine is able to act as an intracellular ROS scavenger [19], can reduce the abnormally high expression of NAD(P)H oxidase, can decrease pro-inflammatory cytokines and can decrease RAS components in several animal models of hypertension [60,65] (Figure 3). To date, the therapeutic indications of LC are mostly limited to pathologies in which a clear deficiency of this compound exists. Although there is no clear relationship between carnitine content and blood pressure levels [59], recent evidence indicates new multifactorial roles for LC and its derivatives and supports the idea that carnitine is not merely a cofactor in β-oxidation but rather has many known and undiscovered functions in physiology and in the pathophysiology of different diseases, including arterial hypertension. Despite the available antihypertensive medications, achieving the blood pressure targets recommended by the different guidelines is often difficult. The currently available data do not support a preferential role for LC as an antihypertensive agent compared to other available drugs. However, the multiple mechanisms of LC action enable this compound to modulate several targets and/or pathways simultaneously. It has been proposed recently that drug discovery strategies should consider the design of molecules that act against multiple targets rather than the currently preferred single-target drugs [72]. This novel drug design paradigm might justify a therapeutic use of LC as a protective agent against cardiovascular and renal remodelling in hypertensive subjects. The possible antifibrotic properties and additional signalling pathways involved in the beneficial effects of LC in these tissues are yet to be discovered.

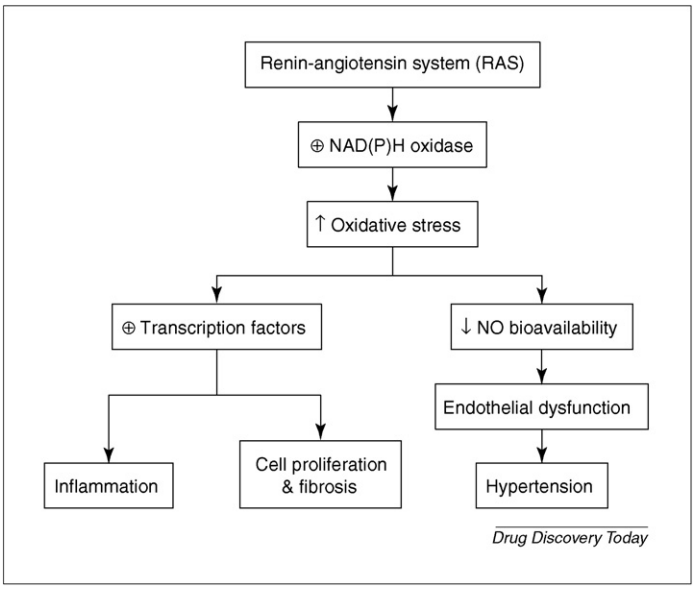

FIGURE 3

Multiple mechanisms of carnitine action. L-carnitine (LC) has been reported to have beneficial effects at the different stages depicted in the figure, which are all of relevance in the pathophysiology of hypertension and related organ damage. The ability of LC to modulate several targets/pathways simultaneously might justify a therapeutic use of this compound to reduce total cardiovascular risk in hypertensive subjects.

References

1 (2005) Monograph. L-carnitine. *Altern. Med. Rev.* 10, 42–50
2 Arduini, A. *et al.* (2008) Carnitine in metabolic disease: potential for pharmacological intervention. *Pharmacol. Ther.* 120, 149–156
3 Mancia, G. *et al.* (2009) Reappraisal of European guidelines on hypertension management: a European Society of Hypertension Task Force document. *J. Hypertens.* 27, 2121–2158
4 Wolf, G. (2006) The discovery of a vitamin role for carnitine: the first 50 years. *J. Nutr.* 136, 2131–2134
5 Vaz, F.M. and Wanders, R.J. (2002) Carnitine biosynthesis in mammals. *Biochem. J.* 361, 417–429
6 Evans, A.M. and Fornasini, G. (2003) Pharmacokinetics of L-carnitine. *Clin. Pharmacokinet.* 42, 941–967

7 Hedayati, S.S. (2006) Dialysis-related carnitine disorder. *Semin. Dial.* 19, 323–328
8 Ferrari, R. *et al.* (2004) Therapeutic effects of L-carnitine and propionyl-L-carnitine on cardiovascular diseases: a review. *Ann. N. Y. Acad. Sci.* 1033, 79–91
9 Broderick, T.L. (2006) Hypocarnitinaemia induced by sodium pivalate in the rat is associated with left ventricular dysfunction and impaired energy metabolism. *Drugs R. D.* 7, 153–161
10 Lheureux, P.E. and Hantson, P. (2009) Carnitine in the treatment of valproic acid-induced toxicity. *Clin. Toxicol. (Phila.)* 47, 101–111
11 Crill, C.M. and Helms, R.A. (2007) The use of carnitine in pediatric nutrition. *Nutr. Clin. Pract.* 22, 204–213
12 Borum, P.R. (2009) Carnitine in parenteral nutrition. *Gastroenterology* 137, S129–S134
13 Atar, D. *et al.* (1997) Carnitine – from cellular mechanisms to potential clinical applications in heart disease. *Eur. J. Clin. Invest.* 27, 973–976
14 Lango, R. *et al.* (2001) Influence of L-carnitine and its derivatives on myocardial metabolism and function in ischemic heart disease and during cardiopulmonary bypass. *Cardiovasc. Res.* 51, 21–29
15 Shankar, S.S. *et al.* (2004) L-carnitine may attenuate free fatty acid-induced endothelial dysfunction. *Ann. N. Y. Acad. Sci.* 1033, 189–197
16 Carrero, J.J. and Grimble, R.F. (2006) Does nutrition have a role in peripheral vascular disease? *Br. J. Nutr.* 95, 217–229
17 Mynatt, R.L. (2009) Carnitine and type 2 diabetes. *Diabetes Metab. Res. Rev.* 25 (Suppl. 1), S45–S49
18 Ruggenenti, P. *et al.* (2009) Ameliorating hypertension and insulin resistance in subjects at increased cardiovascular risk: effects of acetyl-L-carnitine therapy. *Hypertension* 54, 567–574
19 Ceriello, A. (2003) New insights on oxidative stress and diabetic complications may lead to a ''causal'' antioxidant therapy. *Diabetes Care* 26, 1589–1596
20 Stephens, F.B. *et al.* (2007) New insights concerning the role of carnitine in the regulation of fuel metabolism in skeletal muscle. *J. Physiol.* 581, 431–444
21 Schreiber, B.D. (2006) Debate forum: levocarnitine therapy is rational and justified in selected dialysis patients. *Blood Purif.* 24, 128–139
22 Savica, V. *et al.* (2004) Carnitine system in uremic patients: molecular and clinical aspects. *Semin. Nephrol.* 24, 464–468
23 Schreiber, B.D. (2003) Congestive heart failure in patients with chronic kidney disease and on dialysis. *Am. J. Med. Sci.* 325, 179–193
24 Handelman, G.J. (2006) Debate forum: carnitine supplements have not been demonstrated as effective in patients on long-term dialysis therapy. *Blood Purif.* 24, 140–142
25 Saper, R.B. *et al.* (2004) Common dietary supplements for weight loss. *Am. Fam. Physician* 70, 1731–1738
26 Karlic, H. and Lohninger, A. (2004) Supplementation of L-carnitine in athletes: does it make sense? *Nutrition* 20, 709–715
27 Harris, J.D. (2008) Fatigue in chronically ill patients. *Curr. Opin. Support. Palliat. Care* 2, 180–186
28 Brass, E.P. (2004) Carnitine and sports medicine: use or abuse? *Ann. N. Y. Acad. Sci.* 1033, 67–78
29 Peluso, G. *et al.* (2000) Cancer and anticancer therapy-induced modifications on metabolism mediated by carnitine system. *J. Cell. Physiol.* 182, 339–350
30 Jones, L.L. *et al.* (2010) Acylcarnitines: role in brain. *Prog. Lipid Res.* 49, 61–75
31 Ng, C.M. *et al.* (2004) The role of carnitine in the male reproductive system. *Ann. N. Y. Acad. Sci.* 1033, 177–188
32 Sweeney, J.D. and Arduini, A. (2004) L-carnitine and its possible role in red cell and platelet storage. *Transfus. Med. Rev.* 18, 58–65
33 Mancia, G. *et al.* (2007) 2007 Guidelines for the Management of Arterial Hypertension: The Task Force for the Management of Arterial Hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). *J. Hypertens.* 25, 1105–1187
34 Rosendorff, C. *et al.* (2007) Treatment of hypertension in the prevention and management of ischemic heart disease: a scientific statement from the American Heart Association Council for High Blood Pressure Research and the Councils on Clinical Cardiology and Epidemiology and Prevention. *Circulation* 115, 2761–2788
35 Sanchez, R.A. *et al.* (2009) Latin American guidelines on hypertension. Latin American Expert Group. *J. Hypertens.* 27, 905–922
36 Ogihara, T. *et al.* (2009) The Japanese Society of Hypertension Guidelines for the Management of Hypertension (JSH 2009). *Hypertens. Res.* 32, 3–107
37 Simko, F. and Pechanova, O. (2009) Recent trends in hypertension treatment: perspectives from animal studies. *J. Hypertens.* 27 (Suppl. 6), S1–S4
38 Tousoulis, D. *et al.* (2008) Role of inflammation and oxidative stress in endothelial progenitor cell function and mobilization: therapeutic implications for cardiovascular diseases. *Atherosclerosis* 201, 236–247
39 Paravicini, T.M. and Touyz, R.M. (2008) NADPH oxidases, reactive oxygen species, and hypertension: clinical implications and therapeutic possibilities. *Diabetes Care* 31 (Suppl. 2), S170–S180
40 Mathieu, P. *et al.* (2009) Visceral obesity: the link among inflammation, hypertension, and cardiovascular disease. *Hypertension* 53, 577–584
41 Vaziri, N.D. (2008) Causal link between oxidative stress, inflammation, and hypertension. *Iran J. Kidney Dis.* 2, 1–10
42 Sedeek, M. *et al.* (2009) Molecular mechanisms of hypertension: role of Nox family NADPH oxidases. *Curr. Opin. Nephrol. Hypertens.* 18, 122–127
43 Cachofeiro, V. *et al.* (2008) Oxidative stress and inflammation, a link between chronic kidney disease and cardiovascular disease. *Kidney Int.* 74 (Suppl. 111), S4–S9
44 Ward, N.C. and Croft, K.D. (2006) Hypertension and oxidative stress. *Clin. Exp. Pharmacol. Physiol.* 33, 872–876
45 Katusic, Z.S. *et al.* (2009) Vascular protection by tetrahydrobiopterin: progress and therapeutic prospects. *Trends Pharmacol. Sci.* 30, 48–54
46 Garrido, A.M. and Griendling, K.K. (2009) NADPH oxidases and angiotensin II receptor signaling. *Mol. Cell. Endocrinol.* 302, 148–158
47 San Jose, G. *et al.* (2009) The angiotensin-converting enzyme insertion/deletion polymorphism is associated with phagocytic NADPH oxidase-dependent superoxide generation: potential implication in hypertension. *Clin. Sci. (Lond.)* 116, 233–240
48 Liu, J. *et al.* (2004) Comparison of the effects of L-carnitine and acetyl-L-carnitine on carnitine levels, ambulatory activity, and oxidative stress biomarkers in the brain of old rats. *Ann. N. Y. Acad. Sci.* 1033, 117–131
49 Silva-Adaya, D. *et al.* (2008) Excitotoxic damage, disrupted energy metabolism, and oxidative stress in the rat brain: antioxidant and neuroprotective effects of L-carnitine. *J. Neurochem.* 105, 677–689
50 Famularo, G. *et al.* (2004) Carnitines and its congeners: a metabolic pathway to the regulation of immune response and inflammation. *Ann. N. Y. Acad. Sci.* 1033, 132–138
51 Thangasamy, T. *et al.* (2009) L-carnitine mediates protection against DNA damage in lymphocytes of aged rats. *Biogerontology* 10, 163–172
52 Calo, L.A. *et al.* (2006) Antioxidant effect of L-carnitine and its short chain esters: relevance for the protection from oxidative stress related cardiovascular damage. *Int. J. Cardiol.* 107, 54–60
53 Calabrese, V. *et al.* (2006) Acetylcarnitine and cellular stress response: roles in nutritional redox homeostasis and regulation of longevity genes. *J. Nutr. Biochem.* 17, 73–88
54 Augustyniak, A. and Skrzydlewska, E. (2009) L-carnitine in the lipid and protein protection against ethanol-induced oxidative stress. *Alcohol* 43, 217–223
55 Loffredo, L. *et al.* (2007) Oxidative-stress-mediated arterial dysfunction in patients with peripheral arterial disease. *Eur. Heart J.* 28, 608–612
56 Sharifi, A.M. *et al.* (2009) Effect of short term treatment of L-carnitine on tissue ACE activity in streptozotocin-induced diabetic rats. *Pathophysiology* 16, 53–56
57 Cediel, E. *et al.* (2003) Effect of AT1 receptor blockade on hepatic redox status in SHR: possible relevance for endothelial function? *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 285, R674–R681
58 Ulker, S. *et al.* (2003) Impaired activities of antioxidant enzymes elicit endothelial dysfunction in spontaneous hypertensive rats despite enhanced vascular nitric oxide generation. *Cardiovasc. Res.* 59, 488–500
59 Gomez-Amores, L. *et al.* (2006) Antioxidant activity of propionyl-L-carnitine in liver and heart of spontaneously hypertensive rats. *Life Sci.* 78, 1945–1952
60 Gomez-Amores, L. *et al.* (2007) L-carnitine attenuates oxidative stress in hypertensive rats. *J. Nutr. Biochem.* 18, 533–540
61 Bueno, R. *et al.* (2005) L-carnitine and propionyl-L-carnitine improve endothelial dysfunction in spontaneously hypertensive rats: different participation of NO and COX-products. *Life Sci.* 77, 2082–2097
62 Rajasekar, P. *et al.* (2007) Increase in nitric oxide and reductions in blood pressure, protein kinase C beta II and oxidative stress by L-carnitine: a study in the fructose-fed hypertensive rat. *Clin. Exp. Hypertens.* 29, 517–530
63 Miguel-Carrasco, J.L. *et al.* (2009) L-carnitine in hypertension-related heart damage. *Res. Adv. Hypertens.* 1, 21–38
64 Famularo, G. and De Simone, C. (1995) A new era for carnitine? *Immunol. Today* 16, 211–213
65 Miguel-Carrasco, J.L. *et al.* (2008) The role of inflammatory markers in the cardioprotective effect of L-carnitine in L-NAME-induced hypertension. *Am. J. Hypertens.* 21, 1231–1237
66 Sanz-Rosa, D. *et al.* (2005) Effect of AT1 receptor antagonism on vascular and circulating inflammatory mediators in SHR: role of NF-kappaB/IkappaB system. *Am. J. Physiol. Heart Circ. Physiol.* 288, H111–H115
67 Chao, H.H. *et al.* (in press) L-carnitine attenuates angiotensin II-induced proliferation of cardiac fibroblasts: role of NADPH oxidase inhibition and decreased sphingosine-1-phosphate generation. *J. Nutr. Biochem*
68 Duan, S.Z. *et al.* (2009) PPARs: the vasculature, inflammation and hypertension. *Curr. Opin. Nephrol. Hypertens.* 18, 128–133

69 Makowski, L. *et al.* (2009) Metabolic profiling of PPARalpha−/− mice reveals defects in carnitine and amino acid homeostasis that are partially reversed by oral carnitine supplementation. *FASEB J.* 23, 586–604
70 Rauchova, H. *et al.* (1998) The effect of chronic L-carnitine treatment on blood pressure and plasma lipids in spontaneously hypertensive rats. *Eur. J. Pharmacol.* 342, 235–239
71 Miguel-Carrasco, J.L. *et al.* (2010) Comparative effects of captopril and L-carnitine on blood pressure and antioxidant enzyme gene expression in the heart of spontaneously hypertensive rats. *Eur. J. Pharmacol.* 632, 65–72
72 Zimmermann, G.R. *et al.* (2007) Multi-target therapeutics: when the whole is greater than the sum of the parts. *Drug Discov. Today* 12, 34–42