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Review

Arginine and Endothelial Function

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Abstract: Arginine (L-arginine), is an amino acid involved in a number of biological processes, including the biosynthesis of proteins, host immune response, urea cycle, and nitric oxide production. In this systematic review, we focus on the functional role of arginine in the regulation of endothelial function and vascular tone. Both clinical and preclinical studies are examined, analyzing the effects of arginine supplementation in hypertension, ischemic heart disease, aging, peripheral artery disease, and diabetes mellitus.

Keywords: ADMA; arginine; arginine paradox; BH4; blood pressure; COVID-19; dietary supplements; endothelial dysfunction; endothelium; eNOS uncoupling; heart failure; hypertension; L-arginine; myocardial infarction; NADPH; nitric oxide; oxidative stress; peripheral artery disease.

1. Pleiotropic Effects of Arginine

L-arginine, hereinafter referred to as arginine, is a semi-essential or conditionally essential amino acid, since it can be synthetized by healthy individuals but not by preterm infants [1]. From a chemical point of view, arginine is a 2-amino-5-guanidinopentanoic acid (Figure 1). Its name derives from the Greek word ἄργυρος (silver), indicating the color of arginine nitrate crystals.

Arginine is involved in a number of biological processes, it is the substrate for a series of reactions leading to the synthesis of other amino acids, and it is a substrate for two enzymes, namely nitric oxide (NO) synthase (NOS) and arginase, which are fundamental for the generation of NO and urea, respectively. Arginine is known to act as a substrate for NO production by endothelial cells, thus regulating vascular tone and, overall, cardiovascular homeostasis [2]. NO is synthesized from arginine by the enzyme NOS in a reaction that involves the transfer of electrons from nicotinamide adenine dinucleotide phosphate (NADPH)—via the flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN) in the C-terminal reductase domain [3,4]—to the heme in the N-terminal oxygenase domain, where the substrate arginine is oxidized to citrulline and NO [5,6], as shown in Figure 1. Arginine is also implicated in T-cell proliferation and host immune responses, as well as in creatine and collagen synthesis [7–11].

There are three isoforms of NOS, two of which—endothelial (eNOS) [12,13] and neuronal (nNOS) [14–16]—are constitutively expressed, while the third one, inducible NOS (iNOS) [17–19], is expressed in response to cytokines and is related to the inflammatory response [6,20]. NO generation

occurs in two steps: first, NOS hydroxylates arginine to N^{ω}-hydroxy-arginine (which remains largely bound to the enzyme); in a second step, NOS oxidizes N^{ω}-hydroxy-arginine to citrulline and NO [21–29].

In normal conditions, NOS catalyzes the transformation of arginine, O_2, and NADPH-derived electrons to NO and citrulline (Figure 1). However, in the presence of pathologic conditions like atherosclerosis and diabetes, the NOS function is altered, and the enzyme catalyzes the reduction of O_2 to superoxide (O_2^-), a phenomenon that is generally referred to as "NOS uncoupling" [30–41], and has been linked to a limited bioavailability of tetrahydrobiopterin (BH4, also known as sapropterin) [42–47]. Indeed, the donation of an electron by BH4 to produce a transient $BH4^{\bullet+}$ radical is required for the oxidation of arginine to citrulline and the associated formation of a ferrous iron–NO complex at the NOS heme catalytic center [48–51]. BH4 is synthesized from guanosine triphosphate (GTP) by GTP cyclohydrolase I (GTPCH) and recycled from 7,8-dihydrobiopterin (BH2) by dihydrofolate reductase (Figure 2). Of note, NOS is inhibited by arginine analogs that are substituted at the guanidino nitrogen atom, like NG-monomethyl-arginine or NG-nitro-arginine [52–58].

As mentioned above, in the urea cycle arginine is converted by arginase, a manganese metalloenzyme, in ornithine and urea; this cycle is crucial not only for allowing urea excretion, but also for producing bicarbonate, which is critical for maintaining acid/base homeostasis [59–63]. Arginase exists in two distinct isoforms, arginase I and II, that share ~60% sequence homology; arginase I is a cytosolic enzyme mainly localized in the liver, whereas arginase II is a mitochondrial enzyme with a wide distribution and is expressed in the kidney, prostate, gastrointestinal tract, and the vasculature [64–67].

The enzyme arginase is a key modulator of NO production by competing for arginine: in other words, NO generation is dependent on the relative expression and activities of arginase and NOS. More specifically, increased arginase activity may lead to a decreased bioavailability of arginine for NOS, thereby diminishing NO production. This mechanism has emerged as an essential factor underlying impaired endothelial functions [68,69]. Specifically, an increased arginase activity has been associated with endothelial dysfunction in a number of experimental models of hypertension, atherosclerosis, diabetes, and aging [70–92].

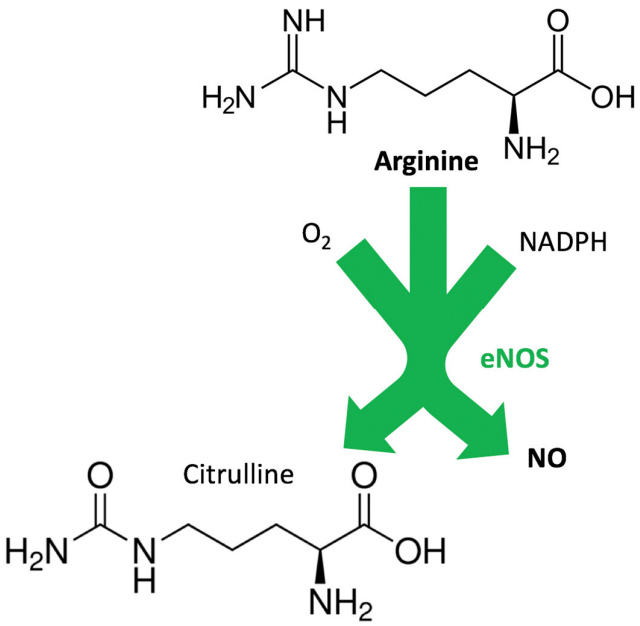

Figure 1. Functional role of arginine in the synthesis of nitric oxide (NO). NADPH: nicotinamide adenine dinucleotide phosphate; eNOS: endothelial NO synthase.

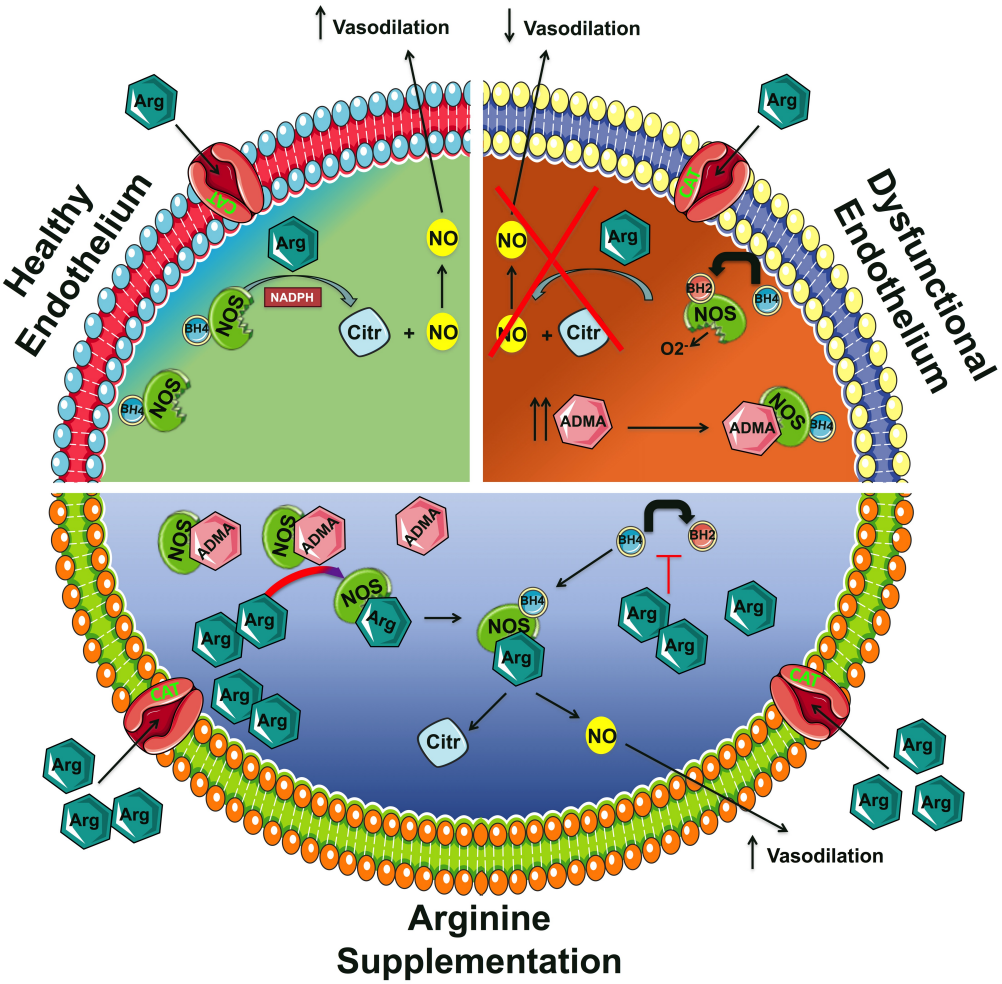

Figure 2. Functional role of arginine in endothelial (dys)function. ADMA: asymmetric dimethylarginine; Arg: arginine; BH2: 7,8-dihydrobiopterin; BH4: tetrahydrobiopterin; CAT: cationic amino acid transporter; Citr: citrulline; NADPH: nicotinamide adenine dinucleotide phosphate; NO: nitric oxide; NOS: NO synthase.

2. Arginine and NO Production in Physiological Conditions: The Arginine Paradox

Indeed, endothelial dysfunction is a leading cause of several pathological conditions affecting the cardiovascular system, including hypertension, atherosclerosis, diabetes, and atherothrombosis [46,93–119]. Moreover, in April 2020, we were the first group to show that the systemic manifestations observed in coronavirus disease (COVID-19), caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), could be explained by endothelial dysfunction [120]. Indeed, alterations in endothelial function have been linked to hypertension, diabetes, thromboembolism, and kidney failure, all featured, to different extents, in COVID-19 patients [121–123]. Other investigators have later confirmed our view [124–133]. On these grounds, based on the positive effects of arginine on endothelial function, we can also speculate that arginine supplementation could be helpful, while not being harmful, for contrasting endothelial dysfunction in COVID-19 patients.

An increasing interest in the potential therapeutic effects of arginine supplementation, especially in cardiovascular disorders, has recently emerged. An impaired NO synthesis is considered a main feature of a dysfunctional endothelium [107,134–136]; however, several studies suggest that arginine supplementation in healthy subjects does not lead to a significant increase in NO production [11,137–140]. For instance, the daily administration of arginine for 1 week did not affect the serum concentration of two established indicators of NO production, namely NO_2^- and NO_3^-, in twelve

healthy subjects [138]. In another study, 20 healthy subjects received daily arginine supplementation in both sustained-release or immediate-release form; despite the significant increase in the plasma arginine concentration, which proved the effectiveness of the administration protocol, the authors did not observe significant differences in urinary extraction of nitrate [141].

One reason for the absence of significant results in normal conditions could be that the NO synthesis machinery seems to be saturated by the endogenous arginine. Indeed, the Michaelis–Menten constant (K_m) of NO synthase is in the micromolar range, specifically 2.9 μmol/L, as demonstrated by Bredt and colleagues [142]. Arginine plasma levels measured in healthy humans are 15–30-fold higher than this K_m, thereby making the levels of the substrate a non-limiting factor in the enzymatic reaction leading to NO production. Despite such a biochemical ratio, which in fact makes the enzyme physiologically saturated, various studies are also showing beneficial effects of arginine supplementation in healthy subjects. For instance, arginine supplementation has been tested in athletes, as vasodilation favors muscle perfusion and nutrient/oxygen delivery during exercise, enhancing muscle strength and recovery [143]. Controversial results come from these studies, sometimes yielding no effects of arginine supplementation on muscle performance, and sometimes demonstrating a significant improvement in exercise capability [137,144–148].

The phenomenon known as "arginine paradox" is born from this scenario, and indicates that we were losing part of the story concerning the alternative ways by which arginine can act on endothelial NO production. The arginine paradox refers to the fact that despite intracellular physiological concentrations of arginine being several hundred micromoles per liter, thereby exceeding the K_m of eNOS, the acute provision of exogenous arginine still increases NO production [149–151].

One of the mechanisms that may help explain the arginine paradox comes from the discovery of asymmetric dimethylarginine (ADMA), an endogenous inhibitor of NOS [152–155]. Given its own structure similar to arginine, ADMA is a direct competitor for NOS binding. Moreover, both ADMA and arginine are both transported into the cell via the cationic amino acid transporter (CAT, also known as "y^+ system"), a high-affinity, Na^+-independent transporter of the basic amino acids [156,157], and therefore also compete with each other on this level (Figure 2). Since ADMA competes with arginine for NOS and for cell transport, the bioavailability of NO depends on the balance between the two [158]. Plasma levels of ADMA increase during hypertension, hypercholesterolemia, diabetes, and atherosclerosis [95,159–170]. Hence, despite the range of endogenous arginine levels, they could still be sufficient to guarantee eNOS saturation, and so the arginine/ADMA ratio would be reduced, resulting in a net inhibition of NO production [171–173].

The arginine/ADMA ratio is widely considered to be an important indicator of NO bioavailability as well as of the risk of formation of atherosclerotic plaques [174]. The ratio has been shown to be a better predictor for all-cause mortality compared to ADMA alone [174,175]. Similarly, although plasma ADMA levels were a significant predictor of all-cause mortality in an elderly population, the effect disappeared in subjects with higher arginine levels [176], and the arginine/ADMA ratio (but not ADMA alone) was a significant risk factor for microangiopathy-related cerebral damage in an elderly population [177].

Arginine supplementation can equilibrate the arginine/ADMA ratio, recovering the production of NO. In other terms, the increased arginine availability, resulting from supplementation, competes with ADMA in binding eNOS (Figure 2). This interesting mechanism sheds light on the effectiveness of the increased arginine availability, implicating further therapeutic options for arginine supplementation. Furthermore, this phenomenon can explain some conflicting results about arginine supplementation studies, as ADMA levels should be considered in the study populations. Specifically, cardiovascular patients with increased ADMA plasma levels could be the best target of arginine supplementation.

Another potential explanation of the arginine paradox may be that arginine could be compartmentalized in the cytoplasm, and local concentrations in the vicinity of NOS may be lower than expected from arginine levels in whole-cell homogenates [178].

3. Impaired NO Production as a Mechanism of Endothelial Dysfunction and Arginine Intervention

The major determinants of cardiovascular risk, including dyslipidemia, glucose intolerance, smoking, hypercholesterolemia, and aging, have a direct impact on the endothelium [179–181]. Exposing the vasculature to these conditions induces endothelial dysfunction and alterations as an early phenomenon, able to evolve and contribute to the progression towards clinically relevant disorders like hypertension, atherosclerosis, and diabetes mellitus. Hence, the endothelium plays a key role in cardiovascular physiology and pathophysiology [182–194]. Fervent research has been conducted in recent years in order to understand the underlying mechanisms and identify therapeutic strategies to prevent or counteract endothelial dysfunction.

The ability of the endothelium to regulate vascular homeostasis is largely dependent on NO production, making endothelial vasodilator failure the main sign of endothelial dysfunction and a hot point to be targeted. The impaired endothelial NO availability in perturbed vasculature can be attributable to a diminished synthesis of NO or, indirectly, to an increased ROS production, which inactivates the NO source [195,196]. In addition to counteracting oxidative stress, the stimulation of NO synthesis represents an alternative and a potentially effective approach [197,198], for instance, by providing further substrates to NO synthase. Theoretically, arginine supplementation meets these needs, and thus, it has been tested in many cardiovascular disorders as a potential therapeutic strategy [199]. However, human studies on arginine supplementation have often been a source of debate. Indeed, in healthy subjects as well as in patients suffering from cardiovascular disorders, levels of plasma arginine range from ~45 to ~100 μmol/L [137,200–202], significantly higher than the eNOS K_m of 2.9 μmol/L [203]. Endocrine mechanisms may also contribute to vasodilation induced by arginine. Indeed, arginine stimulates the release of both insulin [204–206] and glucagon [207] from pancreatic islets of Langerhans. Interestingly, an intravenous infusion of arginine has been shown to induce vasodilation and insulin release in healthy humans, but when insulin secretion was blocked by octreotide co-infusion, no vasodilation occurred, whereas vasodilation was restored by insulin co-administration [208]. Since high intravenous doses of arginine (30 g) have also been shown to induce growth hormones (GHs), and secretion [209], the vasodilation induced by arginine could also be mediated by GHs via a signaling pathway that includes insulin-like growth factor-1 [210,211].

Substantial data indicate that endothelial dysfunction is highly prevalent in elderly individuals [212,213]. Endothelial dysfunction has also been implicated in age-associated declines in cognitive function, physical function, as well as in the pathogenesis of stroke, erectile dysfunction, and renal dysfunction. Clinical trials testing the effects of arginine in aging-induced endothelial dysfunction have yielded controversial results. An acute intravenous infusion of arginine (1 g/min for 30 min) had no effect on endothelial-dependent vasodilation in healthy older individuals [214]. Similarly, the intravenous infusion of arginine induced a significant increase in the renal plasma flow, glomerular filtration rate, natriuresis, and kaliuresis, in young but not in aged hypertensives [215]. Another study conducted in healthy postmenopausal women taking 9 g of arginine per day for 1 month confirmed that plasma arginine increased without a concomitant significant change in flow-mediated dilation [216]. On the contrary, in a prospective, double-blind, randomized crossover trial in 12 healthy, old participants (age 73.8 ± 2.7 years), chronic arginine supplementation (16 g/day for 2 weeks) markedly increased their plasma levels of arginine (114.9 ± 11.6 vs. 57.4 ± 5.0 mM) and significantly improved endothelial-dependent vasodilation [217].

4. Arginine Supplementation in Hypertension

The majority of studies in animal models supports a beneficial effect of arginine supplementation in hypertension, especially in the presence of salt-sensitive hypertension. For instance, both oral [218–220] and intraperitoneal [221,222] arginine administration in Dahl salt-sensitive (DSS) rats was shown to prevent the increase in blood pressure induced by a high salt diet. However, arginine was not effective in DSS pretreated with high salt for three weeks [218], suggesting that arginine is able to prevent and counteract hypertension when it is in the early stages, but probably not when some changes and pathological remodeling have already occurred.

The outcome of arginine supplementation could also depend on the method of administration. For instance, renal medullary interstitial infusion of arginine prevents the increase in blood pressure

in high salt-treated rats, while the intravenous dose necessary to obtain a similar increase in plasma arginine does not affect blood pressure [223]. A rat model of type 1 diabetes mellitus shows an important reduction in blood pressure after 4 weeks of oral arginine treatment [224]; oral arginine administration prevents fructose-induced hypertension [225]. Oral arginine administration does not correct hypertension in spontaneously hypertensive rats, although markedly reduces renal damage [226].

Although the beneficial effect of arginine supplementation in hypertension appears to be largely attributable to its impact on NO synthesis, arginine has also been shown to have antioxidant properties, thus affecting the activity of redox-sensitive proteins and lowering blood pressure [227–234]. Indeed, supplementation with 3 g/day arginine for two months increases the serum total antioxidant capacity in obese patients with prediabetes [235]; of note, in vitro experiments performed in endothelial cells have revealed that arginine reduces superoxide release and the cell-mediated breakdown of NO [236].

In the clinical scenario, the oral administration of arginine acutely improves endothelium-dependent, flow-mediated dilatation of the brachial artery in patients with essential hypertension [237]; however, the long-term effects of arginine were not investigated in this study [237]. In a Japanese population, the acute intravenous infusion of arginine (500 mg/kg for 30 min) is able to decrease arterial pressure of both salt-sensitive and salt-insensitive patients [238]. In a similar study, conducted on African-Americans, the same amount of arginine administration reduces blood pressure with a greater effect in the salt-sensitive population [239]. Interestingly, in hypertensive patients in which the control of blood pressure with angiotensin converting enzyme (ACE)-inhibitors and diuretics for three months was unsuccessful, the addition of oral arginine (6 g/day) was effective in reducing both systolic and diastolic blood pressure levels [240]. Unfortunately, many of the findings on the effects of arginine supplementation in hypertension derive from small clinical studies and, despite the promising efficacy, further investigations are needed, especially large, randomized, and controlled trials. The ability to modulate the renin-angiotensin-aldosterone system (RAAS) is another mechanism by which arginine can regulate blood pressure: specifically, arginine inhibits ACE activity, reducing angiotensin II production and its effects on vascular tone [241].

5. Arginine Supplementation in Ischemic Heart Disease and Peripheral Artery Disease

Alongside the preservation of endothelial-dependent vasodilation, the enhanced bioavailability of NO reduces the activation of pro-inflammatory genes and the expression of endothelial adhesion molecules [242]. These events strongly regulate the development and the fate of atherosclerosis [243–245]. For these reasons, it is not surprising that arginine has a powerful effect on atherogenesis and its evolution. In particular, preclinical investigations have shown that chronic arginine administration in LDL-receptor KO mice significantly reduces the extension of atherosclerotic plaques [246]. Similarly, arginine supplementation in humans reverses the increased monocyte–endothelial adhesion, mirrored by a normalization of platelet aggregation [247]. These effects make arginine a promising drug for disorders like coronary artery disease (CAD), heart failure, and peripheral artery disease (PAD).

In 1997, two important studies investigating the effects of arginine in CAD were published [248,249]. In a placebo-controlled study, Adams and collaborators showed that oral administration of arginine (21 g/day for 3 days) significantly improved the vasodilatory response of the brachial artery in premature CAD [248]. A double-blind placebo-controlled study conducted on 22 patients with stable angina pectoris revealed that the administration of arginine was able to improve their exercise capacity in just 3 days [249]. The following year, a clinical study confirmed the beneficial effects of long-term arginine supplementation (9 g for 6 months), showing significantly enhanced vascular responses to acetylcholine in patients with coronary atherosclerosis [250]. Preclinical studies were consistent with these findings. For instance, oral administration of arginine reduced the intimal hyperplasia in balloon-injured carotid arteries in spontaneously hypertensive rats [251]. This first encouraging evidence prompted further investigations about arginine's effects on CAD. Again, arginine treatment for 4 weeks preserved endothelial function in CAD patients, markedly reducing

LDL oxidation [252]. Another study highlighted the method of administration as a major determinant of the efficacy of high dose arginine supplementation: intra-arterial infusion, but not oral administration, was able to improve endothelial-dependent vasodilation in patients with stable angina pectoris [253].

The therapeutic potential of arginine has been also investigated in heart failure [254–258] and ischemia-reperfusion injury [259–261], often yielding controversial results. Endothelium-dependent vasodilation in response to acetylcholine and ischemic vasodilation during reactive hyperemia is attenuated in the forearm of patients with heart failure [262]. In a seminal paper, Hirooka and collaborators demonstrated that the intra-arterial infusion of arginine was effective in reversing the blunted endothelium-dependent vasodilation observed in heart failure [263]. Moreover, oral arginine supplementation (6 g twice a day for 6 weeks) enhanced endurance exercise tolerance in heart failure patients, an important determinant of daily-life activity in patients with chronic stable heart failure [264]. In line with these results, a clinical study carried out in 21 patients with class II/III heart failure (New York Heart Association, NYHA) established that improved endothelial function following exercise training is associated with increased arginine transport [265]. However, another investigation in 20 patients with NYHA class III/IV heart failure demonstrated that responses to acetylcholine and sodium nitroprusside determined using forearm plethysmography were not affected by arginine (20 g/day every day for 28 days), although the actual levels of arginine in the blood were not measured [266]. Exogenous arginine (3 g three times a day for 6 months) administered to patients after an acute myocardial infarction did not improve vascular stiffness measurements or ejection fractions; this clinical trial had to be interrupted due to excess mortality in the treated patients [267].

The improvement in peripheral circulation is critical in patients with PAD, as in severe cases the extensive damage of leg tissues can result in gangrene and amputation [268–270]. Intravenous arginine administration to PAD patients is able to increase the calf blood flow and walking distance [271]. Similarly, an acute intravenous arginine infusion (30 g in 60 min) improves NO production and blood flow of the femoral artery in PAD patients [272]. The oral consumption of arginine for 2 weeks is able to increase the pain-free walking distance, improving the quality of life of patients with hypercholesterolemia [273]. Nevertheless, if the short-term arginine administration seems to be effective in treating PAD, the results on long-term administration are less consistent. A randomized clinical trial testing the long-term (6 months) effects of arginine supplementation was conducted on 133 subjects. Despite an increase in plasma levels of arginine, the study revealed no significant effect of arginine treatment on NO-dependent vasodilation, as well as on the relative functional phenotype of PAD patients [274].

6. Arginine Supplementation in Diabetes Mellitus

Given the fundamental pathogenic role of endothelial dysfunction in diabetes and its complications [275,276], the therapeutic use of arginine supplementation has been tested. In addition to the direct impact of arginine on endothelial vasodilator capacity, a crosstalk with the insulin pathway has been suggested [150,277]. In particular, as mentioned above, arginine can induce the release of insulin from pancreatic beta cells [204–206]. On the other hand, insulin is able to reduce ADMA concentrations [278] and to stimulate the secretion of arginine [279,280]. The stimulation of insulin receptors induces NO release, producing an insulin-dependent vasodilation [281–285]. Of note, such a protective effect of insulin on arginine mobility and endothelial NO production is compromised in diabetes [286]. Henceforth, diabetic patients could be an optimal target population for arginine supplementation.

Preclinical studies corroborate this theory: in diabetic rats, the oral administration of arginine reverses endothelial dysfunction [287], restoring endothelium-dependent relaxation and decreasing oxidative stress [224]. Arginine administration in tap water (free base, 50 mg/kg/day) for 4 months has been shown to reduce both cardiac [288] and renal [289] fibrosis in *db*/*db* mice, by the interaction of arginine with reactive carbonyl residues of glycosylation adducts of collagen, thereby inhibiting glucose-mediated abnormal cross-linking of collagenous structures. These results were later

confirmed in a clinical setting, showing that 2 g of arginine free base administered orally as two daily doses of 1 g each reduced the lipid peroxidation product malondialdehyde in diabetic patients [290].

Clinical studies confirmed the reduction in blood pressure, platelet aggregation, and hemodynamic function in diabetic patients treated with intravenous arginine [291]. While in healthy subjects arginine treatment does not seem to affect insulin receptor sensitivity or density [292], in conditions of insulin resistance, arginine improves insulin sensitivity; indeed, the intravenous injection of arginine in obese or type 2 diabetic patients stimulates insulin responsiveness, restoring insulin-dependent vasodilation [151,293]. Similarly, the oral administration of arginine improves hepatic and peripheral insulin sensitivity in a cGMP dependent fashion [294]. A prospective, crossover clinical trial conducted in mildly hypertensive type 2 diabetic patients revealed a significant decrease in blood pressure in response to arginine, occurring two hours after the oral administration; the effect of lowering blood pressure was associated with increased plasma levels of citrulline, whereas no significant changes in insulin levels were detected, suggesting that the observed phenotype was dependent on arginine-induced NO synthesis [295].

Overall, the mentioned studies substantiate the use of arginine in the diabetic population, at least as a prophylactic treatment able to prevent cardiovascular complications of diabetes. One potential limitation for the use of arginine is the risk of reaction with precursors of advanced glycosylated products [296], which are particularly abundant in diabetes. Since the addition of methylglyoxal (abundant in diabetic patients [297]) to arginine has been shown in vitro to produce potent superoxide radicals in a dose-dependent manner [298], arginine supplementation has been suggested to be combined with antioxidants. A double-blind study on 24 diabetic patients verified this assumption evaluating the combination of *N*-acetylcysteine and arginine oral treatments: the combined treatment was able to reduce systolic and diastolic blood pressure, total cholesterol, C-reactive proteins, vascular adhesion molecules, and improved the intima-media thickness during endothelial post-ischemic vasodilation [299]. This last evidence indicates that the combination of arginine with an antioxidant agent should be potentially effective and well-tolerated.

7. Conclusions and Perspective: Arginine as a Therapeutic Tool

Overall, data available in the literature support and encourage the use of arginine supplementation in cardiovascular disorders, especially in preventing the evolution of hypertension and atherosclerosis. One limitation of using arginine supplementation remains the selection of the optimal target population. In this sense, we believe that ADMA levels could be very useful in selecting the target population, and patients with increased ADMA/arginine ratios are probably the most suitable population, in which arginine supplementation can actually be effective. Another limitation about arginine use concerns its dose. Indeed, available studies suggest a number of different doses, sometimes effective, sometimes not. For instance, the acute oral administration of arginine (9 g/day) has been shown to be not successful in inducing an effective NO production [216]. Instead, chronic administration of oral arginine (e.g., vials containing arginine salts-free 1.66 g/20 mL), has been shown to favor the utilization of arginine for NO synthesis [300], and we have data showing that oral arginine (3 g/day of Bioarginina®, Farmaceutici Damor, 2 vials/day) improves endothelial function in hypertensive patients via the regulation of non-coding RNAs (Gambardella et al., personal communication). Large, prospective randomized clinical trials are needed to better define the target population for arginine supplementation, alongside with correct dosage definitions. To date, a dose of ~3 g/day of arginine (e.g., Bioarginina®, 2 vials/day) seems to be effective in favoring the utilization of arginine for NO synthesis, without toxic effects.

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References

1. Lopez, M.J.; Mohiuddin, S.S. Biochemistry, Essential Amino Acids. In *StatPearls*; StatPearls Publishing: Treasure Island, FL, USA, 2020.
2. Luiking, Y.C.; Ten Have, G.A.; Wolfe, R.R.; Deutz, N.E. Arginine de novo and nitric oxide production in disease states. *Am. J. Physiol. Endocrinol. Metab.* **2012,** *303*, E1177–E1189.
3. Agapie, T.; Suseno, S.; Woodward, J.J.; Stoll, S.; Britt, R.D.; Marletta, M.A. NO formation by a catalytically self-sufficient bacterial nitric oxide synthase from Sorangium cellulosum. *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 16221–16226.
4. Jachymova, M.; Martasek, P.; Panda, S.; Roman, L.J.; Panda, M.; Shea, T.M.; Ishimura, Y.; Kim, J.J. Masters, B.S. Recruitment of governing elements for electron transfer in the nitric oxide synthase family. *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 15833–15838.
5. Serpe, M.J.; Zhang, X. The Principles, Development and Application of Microelectrodes for the In Vivo Determination of Nitric Oxide. In *Electrochemical Methods for Neuroscience*; Michael, A.C., Borland, L.M., Eds.; CRC Press/Taylor & Francis: Boca Raton, FL, USA, 2007.
6. Andrew, P.J.; Mayer, B. Enzymatic function of nitric oxide synthases. *Cardiovasc. Res.* **1999**, *43*, 521–531.
7. Sax, H.C. Arginine stimulates wound healing and immune function in elderly human beings. *JPEN J. Parenter. Enteral. Nutr.* **1994**, *18*, 559–560.
8. Barbul, A.; Lazarou, S.A.; Efron, D.T.; Wasserkrug, H.L.; Efron, G. Arginine enhances wound healing and lymphocyte immune responses in humans. *Surgery* **1990**, *108*, 331–336.
9. Durante, W.; Liao, L.; Reyna, S.V.; Peyton, K.J.; Schafer, A.I. Physiological cyclic stretch directs L-arginine transport and metabolism to collagen synthesis in vascular smooth muscle. *FASEB J.* **2000**, *14*, 1775–1783.
10. da Silva, R.P.; Nissim, I.; Brosnan, M.E.; Brosnan, J.T. Creatine synthesis: Hepatic metabolism of guanidinoacetate and creatine in the rat in vitro and in vivo. *Am. J. Physiol. Endocrinol. Metab.* **2009**, *296*, E256–E261.
11. Bai, Y.; Sun, L.; Yang, T.; Sun, K.; Chen, J.; Hui, R. Increase in fasting vascular endothelial function after short-term oral L-arginine is effective when baseline flow-mediated dilation is low: A meta-analysis of randomized controlled trials. *Am. J. Clin. Nutr.* **2009**, *89*, 77–84.
12. Raman, C.S.; Li, H.; Martasek, P.; Kral, V.; Masters, B.S.; Poulos, T.L. Crystal structure of constitutive endothelial nitric oxide synthase: A paradigm for pterin function involving a novel metal center. *Cell* **1998**, *95*, 939–950.
13. Garthwaite, J.; Charles, S.L.; Chess-Williams, R. Endothelium-derived relaxing factor release on activation of NMDA receptors suggests role as intercellular messenger in the brain. *Nature* **1988**, *336*, 385–388.
14. Bec, N.; Gorren, A.C.; Voelker, C.; Mayer, B.; Lange, R. Reaction of neuronal nitric-oxide synthase with oxygen at low temperature. Evidence for reductive activation of the oxy-ferrous complex by tetrahydrobiopterin. *J. Biol. Chem.* **1998**, *273*, 13502–13508.
15. Iwai, N.; Hanai, K.; Tooyama, I.; Kitamura, Y.; Kinoshita, M. Regulation of neuronal nitric oxide synthase in rat adrenal medulla. *Hypertension* **1995**, *25*, 431–436.
16. O'Dell, T.J.; Huang, P.L.; Dawson, T.M.; Dinerman, J.L.; Snyder, S.H.; Kandel, E.R.; Fishman, M.C. Endothelial NOS and the blockade of LTP by NOS inhibitors in mice lacking neuronal NOS. *Science* **1994**, *265*, 542–546.
17. Geller, D.A.; Lowenstein, C.J.; Shapiro, R.A.; Nussler, A.K.; Di Silvio, M.; Wang, S.C.; Nakayama, D.K.; Simmons, R.L.; Snyder, S.H.; Billiar, T.R. Molecular cloning and expression of inducible nitric oxide synthase from human hepatocytes. *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 3491–3495.
18. Geller, D.A.; Nussler, A.K.; Di Silvio, M.; Lowenstein, C.J.; Shapiro, R.A.; Wang, S.C.; Simmons, R.L.; Billiar, T.R. Cytokines, endotoxin, and glucocorticoids regulate the expression of inducible nitric oxide synthase in hepatocytes. *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 522–526.
19. Hokari, A.; Zeniya, M.; Esumi, H. Cloning and functional expression of human inducible nitric oxide synthase (NOS) cDNA from a glioblastoma cell line A-172. *J. Biochem.* **1994**, *116*, 575–581.

20. Forstermann, U.; Sessa, W.C. Nitric oxide synthases: Regulation and function. *Eur. Heart J.* **2012**, *33*, 829–837, 837a–837d.
21. Rafikov, R.; Fonseca, F.V.; Kumar, S.; Pardo, D.; Darragh, C.; Elms, S.; Fulton, D.; Black, S.M. eNOS activation and NO function: Structural motifs responsible for the posttranslational control of endothelial nitric oxide synthase activity. *J. Endocrinol.* **2011**, *210*, 271–284.
22. Alderton, W.K.; Cooper, C.E.; Knowles, R.G. Nitric oxide synthases: Structure, function and inhibition. *Biochem. J.* **2001**, *357 Pt 3*, 593–615.
23. Stuehr, D.J. Enzymes of the L-arginine to nitric oxide pathway. *J. Nutr.* **2004**, *134* (Suppl. 10), 2748S–2751S.
24. Meulemans, A. Electrochemical detection of nitroso-arginine as an intermediate between N-hydroxy-arginine and citrulline. An in vitro versus in vivo study using microcarbon electrodes in neuronal nitric oxide synthase and mice brain. *Neurosci. Lett.* **2000**, *294*, 125–129.
25. Tsuboi, T.; Maeda, M.; Hayashi, T. Administration of L-arginine plus L-citrulline or L-citrulline alone successfully retarded endothelial senescence. *PLoS ONE* **2018**, *13*, e0192252.
26. de Betue, C.T.I.; Garcia Casal, X.C.; van Waardenburg, D.A.; Schexnayder, S.M.; Joosten, K.F.M.; Deutz, N.E.P.; Engelen, M. 24-Hour protein, arginine and citrulline metabolism in fed critically ill children—A stable isotope tracer study. *Clin. Nutr.* **2017**, *36*, 876–887.
27. Arnett, D.C.; Persechini, A.; Tran, Q.K.; Black, D.J.; Johnson, C.K. Fluorescence quenching studies of structure and dynamics in calmodulin-eNOS complexes. *FEBS Lett.* **2015**, *589*, 1173–1178.
28. Li, H.; Raman, C.S.; Glaser, C.B.; Blasko, E.; Young, T.A.; Parkinson, J.F.; Whitlow, M.; Poulos, T.L. Crystal structures of zinc-free and -bound heme domain of human inducible nitric-oxide synthase. Implications for dimer stability and comparison with endothelial nitric-oxide synthase. *J. Biol. Chem.* **1999**, *274*, 21276–21284.
29. Crane, B.R.; Rosenfeld, R.J.; Arvai, A.S.; Ghosh, D.K.; Ghosh, S.; Tainer, J.A.; Stuehr, D.J.; Getzoff, E.D. N-terminal domain swapping and metal ion binding in nitric oxide synthase dimerization. *EMBO J.* **1999**, *18*, 6271–6281.
30. Daiber, A.; Kroller-Schon, S.; Oelze, M.; Hahad, O.; Li, H.; Schulz, R.; Steven, S.; Munzel, T. Oxidative stress and inflammation contribute to traffic noise-induced vascular and cerebral dysfunction via uncoupling of nitric oxide synthases. *Redox Biol.* **2020**, *34*, 101506.
31. Jayaram, R.; Goodfellow, N.; Zhang, M.H.; Reilly, S.; Crabtree, M.; De Silva, R.; Sayeed, R.; Casadei, B. Molecular mechanisms of myocardial nitroso-redox imbalance during on-pump cardiac surgery. *Lancet* **2015**, *385* (Suppl. 1), S49.
32. Siu, K.L.; Lotz, C.; Ping, P.; Cai, H. Netrin-1 abrogates ischemia/reperfusion-induced cardiac mitochondrial dysfunction via nitric oxide-dependent attenuation of NOX4 activation and recoupling of NOS. *J. Mol. Cell Cardiol.* **2015**, *78*, 174–85.
33. Gebhart, V.; Reiss, K.; Kollau, A.; Mayer, B.; Gorren, A.C.F. Site and mechanism of uncoupling of nitric-oxide synthase: Uncoupling by monomerization and other misconceptions. *Nitric Oxide* **2019**, *89*, 14–21.
34. Yang, Y.M.; Huang, A.; Kaley, G.; Sun, D. eNOS uncoupling and endothelial dysfunction in aged vessels. *Am. J. Physiol. Heart Circ. Physiol.* **2009**, *297*, H1829–H1836.
35. Musicki, B.; Burnett, A.L. Constitutive NOS uncoupling and NADPH oxidase upregulation in the penis of type 2 diabetic men with erectile dysfunction. *Andrology* **2017**, *5*, 294–298.
36. Bohmer, A.; Gambaryan, S.; Flentje, M.; Jordan, J.; Tsikas, D. [Ureido-(1)(5)N]citrulline UPLC-MS/MS nitric oxide synthase (NOS) activity assay: Development, validation, and applications to assess NOS uncoupling and human platelets NOS activity. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* **2014**, *965*, 173–182.
37. Kietadisorn, R.; Juni, R.P.; Moens, A.L. Tackling endothelial dysfunction by modulating NOS uncoupling: New insights into its pathogenesis and therapeutic possibilities. *Am. J. Physiol. Endocrinol. Metab.* **2012**, *302*, E481–E495.
38. Xie, L.; Liu, Z.; Lu, H.; Zhang, W.; Mi, Q.; Li, X.; Tang, Y.; Chen, Q.; Ferro, A.; Ji, Y. Pyridoxine inhibits endothelial NOS uncoupling induced by oxidized low-density lipoprotein via the PKCalpha signalling pathway in human umbilical vein endothelial cells. *Br. J. Pharmacol.* **2012**, *165*, 754–764.
39. Crijns, H.J.; Schotten, U.; Moens, A.L., Is NOS uncoupling the missing link between atrial fibrillation and chronic non-ischaemic cardiomyopathy? *Cardiovasc. Res.* **2011**, *91*, 556.
40. Moens, A.L.; Leyton-Mange, J.S.; Niu, X.; Yang, R.; Cingolani, O.; Arkenbout, E.K.; Champion, H.C.; Bedja, D.; Gabrielson, K.L.; Chen, J.; et al. Adverse ventricular remodeling and exacerbated NOS uncoupling from pressure-overload in mice lacking the beta3-adrenoreceptor. *J. Mol. Cell Cardiol.* **2009**, *47*, 576–585.

41. Mollnau, H.; Schulz, E.; Daiber, A.; Baldus, S.; Oelze, M.; August, M.; Wendt, M.; Walter, U.; Geiger, C.; Agrawal, R.; et al. Nebivolol prevents vascular NOS III uncoupling in experimental hyperlipidemia and inhibits NADPH oxidase activity in inflammatory cells. *Arterioscler. Thromb. Vasc. Biol.* **2003**, *23*, 615–621.
42. Dikalova, A.; Aschner, J.L.; Kaplowitz, M.R.; Cunningham, G.; Summar, M.; Fike, C.D. Combined l-citrulline and tetrahydrobiopterin therapy improves NO signaling and ameliorates chronic hypoxia-induced pulmonary hypertension in newborn pigs. *Am. J. Physiol. Lung Cell Mol. Physiol.* **2020**, *318*, L762–L772.
43. Picciano, A.L.; Crane, B.R. A nitric oxide synthase-like protein from Synechococcus produces NO/NO3 (-) from l-arginine and NADPH in a tetrahydrobiopterin- and Ca(2+)-dependent manner. *J. Biol. Chem.* **2019**, *294*, 10708–10719.
44. Nagarkoti, S.; Sadaf, S.; Awasthi, D.; Chandra, T.; Jagavelu, K.; Kumar, S.; Dikshit, M. L-Arginine and tetrahydrobiopterin supported nitric oxide production is crucial for the microbicidal activity of neutrophils. *Free Radic. Res.* **2019**, *53*, 281–292.
45. Latini, A.; de Bortoli da Silva, L.; da Luz Scheffer, D.; Pires, A.C.S.; de Matos, F.J.; Nesi, R.T.; Ghisoni, K.; de Paula Martins, R.; de Oliveira, P.A.; Prediger, R.D.; et al. Tetrahydrobiopterin improves hippocampal nitric oxide-linked long-term memory. *Mol. Genet. Metab.* **2018**, *125*, 104–111.
46. Chuaiphichai, S.; Rashbrook, V.S.; Hale, A.B.; Trelfa, L.; Patel, J.; McNeill, E.; Lygate, C.A.; Channon, K.M.; Douglas, G. Endothelial Cell Tetrahydrobiopterin Modulates Sensitivity to Ang (Angiotensin) II-Induced Vascular Remodeling, Blood Pressure, and Abdominal Aortic Aneurysm. *Hypertension* **2018**, *72*, 128–138.
47. Ramasamy, S.; Haque, M.M.; Gangoda, M.; Stuehr, D.J., Tetrahydrobiopterin redox cycling in nitric oxide synthase: Evidence supports a through-heme electron delivery. *FEBS J.* **2016**, *283*, 4491–4501.
48. Stuehr, D.J.; Kwon, N.S.; Nathan, C.F.; Griffith, O.W.; Feldman, P.L.; Wiseman, J. N omega-hydroxy-L-arginine is an intermediate in the biosynthesis of nitric oxide from L-arginine. *J. Biol. Chem.* **1991**, *266*, 6259–6263.
49. Vasquez-Vivar, J.; Kalyanaraman, B.; Martasek, P.; Hogg, N.; Masters, B.S.; Karoui, H.; Tordo, P.; Pritchard, K.A., Jr. Superoxide generation by endothelial nitric oxide synthase: The influence of cofactors. *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 9220–9225.
50. Xia, Y.; Tsai, A.L.; Berka, V.; Zweier, J.L. Superoxide generation from endothelial nitric-oxide synthase. A Ca2+/calmodulin-dependent and tetrahydrobiopterin regulatory process. *J. Biol. Chem.* **1998**, *273*, 25804–25808.
51. Landmesser, U.; Dikalov, S.; Price, S.R.; McCann, L.; Fukai, T.; Holland, S.M.; Mitch, W.E.; Harrison, D.G. Oxidation of tetrahydrobiopterin leads to uncoupling of endothelial cell nitric oxide synthase in hypertension. *J. Clin. Invest.* **2003**, *111*, 1201–1209.
52. Moore, P.K.; al-Swayeh, O.A.; Chong, N.W.; Evans, R.A.; Gibson, A. L-NG-nitro arginine (L-NOARG), a novel, L-arginine-reversible inhibitor of endothelium-dependent vasodilatation in vitro. *Br. J. Pharmacol.* **1990**, *99*, 408–412.
53. Gibson, A.; Mirzazadeh, S.; Hobbs, A.J.; Moore, P.K. L-NG-monomethyl arginine and L-NG-nitro arginine inhibit non-adrenergic, non-cholinergic relaxation of the mouse anococcygeus muscle. *Br. J. Pharmacol.* **1990**, *99*, 602–606.
54. O'Kane, K.P.; Webb, D.J.; Collier, J.G.; Vallance, P.J. Local L-NG-monomethyl-arginine attenuates the vasodilator action of bradykinin in the human forearm. *Br. J. Clin. Pharmacol.* **1994**, *38*, 311–315.
55. Fukuda, N.; Izumi, Y.; Soma, M.; Watanabe, Y.; Watanabe, M.; Hatano, M.; Sakuma, I.; Yasuda, H. L-NG-monomethyl arginine inhibits the vasodilating effects of low dose of endothelin-3 on rat mesenteric arteries. *Biochem. Biophys. Res. Commun.* **1990**, *167*, 739–745.
56. Gaw, A.J.; Aberdeen, J.; Humphrey, P.P.; Wadsworth, R.M.; Burnstock, G. Relaxation of sheep cerebral arteries by vasoactive intestinal polypeptide and neurogenic stimulation: Inhibition by L-NG-monomethyl arginine in endothelium-denuded vessels. *Br. J. Pharmacol.* **1991**, *102*, 567–572.
57. Toda, N.; Minami, Y.; Okamura, T. Inhibitory effects of L-NG-nitro-arginine on the synthesis of EDRF and the cerebroarterial response to vasodilator nerve stimulation. *Life Sci.* **1990**, *47*, 345–351.
58. Cozzi, M.R.; Guglielmini, G.; Battiston, M.; Momi, S.; Lombardi, E.; Miller, E.C.; De Zanet, D.; Mazzucato, M.; Gresele, P.; De Marco, L. Visualization of nitric oxide production by individual platelets during adhesion in flowing blood. *Blood* **2015**, *125*, 697–705.
59. Haussinger, D.; Gerok, W.; Sies, H. The effect of urea synthesis on extracellular pH in isolated perfused rat liver. *Biochem. J.* **1986**, *236*, 261–265.

60. Mavri-Damelin, D.; Eaton, S.; Damelin, L.H.; Rees, M.; Hodgson, H.J.; Selden, C. Ornithine transcarbamylase and arginase I deficiency are responsible for diminished urea cycle function in the human hepatoblastoma cell line HepG2. *Int. J. Biochem. Cell Biol.* **2007**, *39*, 555–564.
61. Callery, E.M.; Elinson, R.P. Developmental regulation of the urea-cycle enzyme arginase in the direct developing frog Eleutherodactylus coqui. *J. Exp. Zool.* **1996**, *275*, 61–66.
62. Snellman, K.; Aperia, A.; Broberger, O. Studies of renal urea cycle enzymes. II. Human renal arginase activity and location of the adaptive changes of renal arginase in the protein deprived rat. *Scand. J. Clin. Lab. Invest.* **1979**, *39*, 337–342.
63. Chan, P.Y.; Cossins, E.A. Regulation of arginase levels by urea and intermediates of the Krebs-Henseleit cycle in Saccharomyces cerevisiae. *FEBS Lett.* **1972**, *19*, 335–339.
64. Pernow, J.; Jung, C. Arginase as a potential target in the treatment of cardiovascular disease: Reversal of arginine steal? *Cardiovasc. Res.* **2013**, *98*, 334–343.
65. Pandey, D.; Romer, L.; Berkowitz, D.E., Arginase II: Atherogenesis beyond enzyme activity. *J. Am. Heart Assoc.* **2013**, *2*, e000392.
66. Kuhn, N.J.; Ward, S.; Piponski, M.; Young, T.W., Purification of human hepatic arginase and its manganese (II)-dependent and pH-dependent interconversion between active and inactive forms: A possible pH-sensing function of the enzyme on the ornithine cycle. *Arch. Biochem. Biophys.* **1995**, *320*, 24–34.
67. Sumitani, A. Immunological studies of liver arginase in man and various kinds of vertebrates. Part I: Microquantification of arginase enzyme in liver tissue by quantitative immunoelectrophoresis. Part II: Immunological studies of human liver arginase. *Hiroshima J. Med. Sci.* **1977**, *26*, 59.
68. Kim, J.H.; Bugaj, L.J.; Oh, Y.J.; Bivalacqua, T.J.; Ryoo, S.; Soucy, K.G.; Santhanam, L.; Webb, A.; Camara, A.; Sikka, G.; et al. Arginase inhibition restores NOS coupling and reverses endothelial dysfunction and vascular stiffness in old rats. *J. Appl. Physiol. (1985)* **2009**, *107*, 1249–1257.
69. Romero, M.J.; Platt, D.H.; Tawfik, H.E.; Labazi, M.; El-Remessy, A.B.; Bartoli, M.; Caldwell, R.B.; Caldwell, R.W. Diabetes-induced coronary vascular dysfunction involves increased arginase activity. *Circ. Res.* **2008**, *102*, 95–102.
70. Mahdi, A.; Kovamees, O.; Pernow, J., Improvement in endothelial function in cardiovascular disease—Is arginase the target? *Int. J. Cardiol.* **2020**, *301*, 207–214.
71. Wernly, B.; Pernow, J.; Kelm, M.; Jung, C. The role of arginase in the microcirculation in cardiovascular disease. *Clin. Hemorheol. Microcirc.* **2020**, *74*, 79–92.
72. Chandrasekharan, U.M.; Wang, Z.; Wu, Y.; Wilson Tang, W.H.; Hazen, S.L.; Wang, S.; Elaine Husni, M. Elevated levels of plasma symmetric dimethylarginine and increased arginase activity as potential indicators of cardiovascular comorbidity in rheumatoid arthritis. *Arthritis Res. Ther.* **2018**, *20*, 123.
73. Yang, Z.; Ming, X.F. Functions of arginase isoforms in macrophage inflammatory responses: Impact on cardiovascular diseases and metabolic disorders. *Front. Immunol.* **2014**, *5*, 533.
74. Bagnost, T.; Ma, L.; da Silva, R.F.; Rezakhaniha, R.; Houdayer, C.; Stergiopulos, N.; Andre, C.; Guillaume, Y.; Berthelot, A.; Demougeot, C. Cardiovascular effects of arginase inhibition in spontaneously hypertensive rats with fully developed hypertension. *Cardiovasc. Res.* **2010**, *87*, 569–577.
75. Huang, J.; Liu, C.; Ming, X.F.; Yang, Z. Inhibition of p38mapk Reduces Adipose Tissue Inflammation in Aging Mediated by Arginase-II. *Pharmacology* **2020**, 1–14, doi:10.1159/000507635.
76. Masi, S.; Colucci, R.; Duranti, E.; Nannipieri, M.; Anselmino, M.; Ippolito, C.; Tirotta, E.; Georgiopoulos, G.; Garelli, F.; Nericcio, A.; et al. Aging Modulates the Influence of Arginase on Endothelial Dysfunction in Obesity. *Arterioscler. Thromb. Vasc. Biol.* **2018**, *38*, 2474–2483.
77. Cecilio, C.A.; Costa, E.H.; Simioni, P.U.; Gabriel, D.L.; Tamashiro, W.M. Aging alters the production of iNOS, arginase and cytokines in murine macrophages. *Braz. J. Med. Biol. Res.* **2011**, *44*, 671–681.
78. Katusic, Z.S. Mechanisms of endothelial dysfunction induced by aging: Role of arginase I. *Circ. Res.* **2007**, *101*, 640–641.
79. Sakai, Y.; Masuda, H.; Kihara, K.; Kurosaki, E.; Yamauchi, Y.; Azuma, H. Involvement of increased arginase activity in impaired cavernous relaxation with aging in the rabbit. *J. Urol.* **2004**, *172*, 369–373.
80. Berkowitz, D.E.; White, R.; Li, D.; Minhas, K.M.; Cernetich, A.; Kim, S.; Burke, S.; Shoukas, A.A.; Nyhan, D.; Champion, H.C.; et al. Arginase reciprocally regulates nitric oxide synthase activity and contributes to endothelial dysfunction in aging blood vessels. *Circulation* **2003**, *108*, 2000–2006.

81. Cheng, H.; Lu, T.; Wang, J.; Xia, Y.; Chai, X.; Zhang, M.; Yao, Y.; Zhou, N.; Zhou, S.; Chen, X.; et al. *HuangqiGuizhiWuwu* Decoction Prevents Vascular Dysfunction in Diabetes via Inhibition of Endothelial Arginase 1. *Front. Physiol* **2020**, *11*, 201.
82. Folley, S.J.; Greenbaum, A.L. Effect of experimental diabetes on tissue arginase levels. *J. Endocrinol.* **1949**, *6*, (2).
83. Yang, J.; Zheng, X.; Mahdi, A.; Zhou, Z.; Tratsiakovich, Y.; Jiao, T.; Kiss, A.; Kovamees, O.; Alvarsson, M.; Catrina, S.B.; et al. Red Blood Cells in Type 2 Diabetes Impair Cardiac Post-Ischemic Recovery Through an Arginase-Dependent Modulation of Nitric Oxide Synthase and Reactive Oxygen Species. *JACC Basic Transl. Sci.* **2018**, *3*, 450–463.
84. Zhou, Z.; Mahdi, A.; Tratsiakovich, Y.; Zahoran, S.; Kovamees, O.; Nordin, F.; Uribe Gonzalez, A.E.; Alvarsson, M.; Ostenson, C.G.; Andersson, D.C.; et al. Erythrocytes From Patients With Type 2 Diabetes Induce Endothelial Dysfunction Via Arginase, I. *J. Am. Coll. Cardiol.* **2018**, *72*, 769–780.
85. Zhang, H.; Liu, J.; Qu, D.; Wang, L.; Wong, C.M.; Lau, C.W.; Huang, Y.; Wang, Y.F.; Huang, H.; Xia, Y.; et al. Serum exosomes mediate delivery of arginase 1 as a novel mechanism for endothelial dysfunction in diabetes. *Proc. Natl. Acad. Sci. USA* **2018**, *115*, E6927–E6936.
86. Shosha, E.; Xu, Z.; Narayanan, S.P.; Lemtalsi, T.; Fouda, A.Y.; Rojas, M.; Xing, J.; Fulton, D.; Caldwell, R.W.; Caldwell, R.B. Mechanisms of Diabetes-Induced Endothelial Cell Senescence: Role of Arginase 1. *Int. J. Mol. Sci.* **2018**, *19*, 1215.
87. Kovamees, O.; Shemyakin, A.; Checa, A.; Wheelock, C.E.; Lundberg, J.O.; Ostenson, C.G.; Pernow, J. Arginase Inhibition Improves Microvascular Endothelial Function in Patients with Type 2 Diabetes Mellitus. *J. Clin. Endocrinol. Metab.* **2016**, *101*, 3952–3958.
88. Yao, L.; Chandra, S.; Toque, H.A.; Bhatta, A.; Rojas, M.; Caldwell, R.B.; Caldwell, R.W. Prevention of diabetes-induced arginase activation and vascular dysfunction by Rho kinase (ROCK) knockout. *Cardiovasc. Res.* **2013**, *97*, 509–519.
89. Elms, S.C.; Toque, H.A.; Rojas, M.; Xu, Z.; Caldwell, R.W.; Caldwell, R.B. The role of arginase I in diabetes-induced retinal vascular dysfunction in mouse and rat models of diabetes. *Diabetologia* **2013**, *56*, 654–662.
90. Shemyakin, A.; Kovamees, O.; Rafnsson, A.; Bohm, F.; Svenarud, P.; Settergren, M.; Jung, C.; Pernow, J. Arginase inhibition improves endothelial function in patients with coronary artery disease and type 2 diabetes mellitus. *Circulation* **2012**, *126*, 2943–2950.
91. Ren, B.; Van Kampen, E.; Van Berkel, T.J.; Cruickshank, S.M.; Van Eck, M. Hematopoietic arginase 1 deficiency results in decreased leukocytosis and increased foam cell formation but does not affect atherosclerosis. *Atherosclerosis* **2017**, *256*, 35–46.
92. Teupser, D.; Burkhardt, R.; Wilfert, W.; Haffner, I.; Nebendahl, K.; Thiery, J. Identification of macrophage arginase I as a new candidate gene of atherosclerosis resistance. *Arterioscler. Thromb. Vasc. Biol.* **2006**, *26*, 365–371.
93. Gimbrone, M.A., Jr.; Garcia-Cardena, G. Endothelial Cell Dysfunction and the Pathobiology of Atherosclerosis. *Circ. Res.* **2016**, *118*, 620–636.
94. Rajendran, P.; Rengarajan, T.; Thangavel, J.; Nishigaki, Y.; Sakthisekaran, D.; Sethi, G.; Nishigaki, I. The vascular endothelium and human diseases. *Int. J. Biol. Sci.* **2013**, *9*, 1057–1069.
95. Chirinos, J.A.; David, R.; Bralley, J.A.; Zea-Diaz, H.; Munoz-Atahualpa, E.; Corrales-Medina, F.; Cuba-Bustinza, C.; Chirinos-Pacheco, J.; Medina-Lezama, J. Endogenous nitric oxide synthase inhibitors, arterial hemodynamics, and subclinical vascular disease: The Prevencion Study. *Hypertension* **2008**, *52*, 1051–1059.
96. Bermejo-Martin, J.F.; Almansa, R.; Torres, A.; Gonzalez-Rivera, M.; Kelvin, D.J. COVID-19 as a cardiovascular disease: The potential role of chronic endothelial dysfunction. *Cardiovasc. Res.* **2020**, *116*, e132–e133.
97. Daiber, A.; Chlopicki, S. Revisiting pharmacology of oxidative stress and endothelial dysfunction in cardiovascular disease: Evidence for redox-based therapies. *Free Radic. Biol. Med.* **2020**, doi:10.1016/j.freeradbiomed.2020.02.026.
98. Lima, B.B.; Hammadah, M.; Kim, J.H.; Uphoff, I.; Shah, A.; Levantsevych, O.; Almuwaqqat, Z.; Moazzami, K.; Sullivan, S.; Ward, L.; et al. Association of Transient Endothelial Dysfunction Induced by Mental Stress With Major Adverse Cardiovascular Events in Men and Women With Coronary Artery Disease. *JAMA Cardiol.* **2019**, *4*, 988–996.
99. Yepuri, G.; Ramasamy, R. Significance and Mechanistic Relevance of SIRT6-Mediated Endothelial Dysfunction in Cardiovascular Disease Progression. *Circ. Res.* **2019**, *124*, 1408–1410.

100. Daiber, A.; Xia, N.; Steven, S.; Oelze, M.; Hanf, A.; Kroller-Schon, S.; Munzel, T.; Li, H. New Therapeutic Implications of Endothelial Nitric Oxide Synthase (eNOS) Function/Dysfunction in Cardiovascular Disease. *Int. J. Mol. Sci.* **2019**, *20*, 187.
101. Maruhashi, T.; Soga, J.; Fujimura, N.; Idei, N.; Mikami, S.; Iwamoto, Y.; Iwamoto, A.; Kajikawa, M.; Matsumoto, T.; Oda, N.; et al. Endothelial Dysfunction, Increased Arterial Stiffness, and Cardiovascular Risk Prediction in Patients with Coronary Artery Disease: FMD-J (Flow-Mediated Dilation Japan) Study A. *J. Am. Heart Assoc.* **2018**, *7*, e008588.
102. Akasaka, T.; Sueta, D.; Tabata, N.; Takashio, S.; Yamamoto, E.; Izumiya, Y.; Tsujita, K.; Kojima, S.; Kaikita, K.; Matsui, K.; et al. Effects of the Mean Amplitude of Glycemic Excursions and Vascular Endothelial Dysfunction on Cardiovascular Events in Nondiabetic Patients With Coronary Artery Disease. *J. Am. Heart Assoc.* **2017**, *6*, e004841.
103. Chello, M.; Nenna, A. Ethnicity, ABO group, endothelial dysfunction and cardiovascular disease: Multiple connections, multiple implications. *Atherosclerosis* **2016**, *251*, 514–515.
104. Erqou, S.; Kip, K.E.; Mulukutla, S.R.; Aiyer, A.N.; Reis, S.E. Endothelial Dysfunction and Racial Disparities in Mortality and Adverse Cardiovascular Disease Outcomes. *Clin. Cardiol.* **2016**, *39*, 338–344.
105. Bodolay, E.; Prohaszka, Z.; Paragh, G.; Csipo, I.; Nagy, G.; Laczik, R.; Demeter, N.; Zold, E.; Nakken, B.; Szegedi, G.; et al. Increased levels of anti-heat-shock protein 60 (anti-Hsp60) indicate endothelial dysfunction, atherosclerosis and cardiovascular diseases in patients with mixed connective tissue disease. *Immunol. Res.* **2014**, *60*, 50–59.
106. Moody, W.E.; Edwards, N.C.; Madhani, M.; Chue, C.D.; Steeds, R.P.; Ferro, C.J.; Townend, J.N. Endothelial dysfunction and cardiovascular disease in early-stage chronic kidney disease: Cause or association? *Atherosclerosis* **2012**, *223*, 86–94.
107. Versari, D.; Daghini, E.; Virdis, A.; Ghiadoni, L.; Taddei, S. Endothelial dysfunction as a target for prevention of cardiovascular disease. *Diabetes Care* **2009**, *32* (Suppl. 2), S314–S321.
108. Nozaki, T.; Sugiyama, S.; Koga, H.; Sugamura, K.; Ohba, K.; Matsuzawa, Y.; Sumida, H.; Matsui, K.; Jinnouchi, H.; Ogawa, H. Significance of a multiple biomarkers strategy including endothelial dysfunction to improve risk stratification for cardiovascular events in patients at high risk for coronary heart disease. *J. Am. Coll. Cardiol.* **2009**, *54*, 601–608.
109. Martin, B.J.; Anderson, T.J. Risk prediction in cardiovascular disease: The prognostic significance of endothelial dysfunction. *Can. J. Cardiol.* **2009**, *25* (Suppl. A), 15A–20A.
110. Nin, J.W.; Ferreira, I.; Schalkwijk, C.G.; Prins, M.H.; Chaturvedi, N.; Fuller, J.H.; Stehouwer, C.D.; Group E.P.C.S. Levels of soluble receptor for AGE are cross-sectionally associated with cardiovascular disease in type 1 diabetes, and this association is partially mediated by endothelial and renal dysfunction and by low-grade inflammation: The Eurodiab Prospective Complications Study. *Diabetologia* **2009**, *52*, 705–714.
111. Friedewald, V.E.; Giles, T.D.; Pool, J.L.; Yancy, C.W.; Roberts, W.C. The Editor's Roundtable: Endothelial dysfunction in cardiovascular disease. *Am. J. Cardiol.* **2008**, *102*, 418–423.
112. Rodford, J.L.; Torrens, C.; Siow, R.C.; Mann, G.E.; Hanson, M.A.; Clough, G.F. Endothelial dysfunction and reduced antioxidant protection in an animal model of the developmental origins of cardiovascular disease. *J. Physiol.* **2008**, *586*, 4709–4720.
113. Xu, Y.; Buikema, H.; van Gilst, W.H.; Henning, R.H. Caveolae and endothelial dysfunction: Filling the caves in cardiovascular disease. *Eur. J. Pharmacol.* **2008**, *585*, 256–260.
114. Subah Packer, C. Estrogen protection, oxidized LDL, endothelial dysfunction and vasorelaxation in cardiovascular disease: New insights into a complex issue. *Cardiovasc. Res.* **2007**, *73*, 6–7.
115. Ruef, J.; Marz, W.; Winkelmann, B.R. Markers for endothelial dysfunction, but not markers for oxidative stress correlate with classical risk factors and the severity of coronary artery disease. (A subgroup analysis from the Ludwigshafen Risk and Cardiovascular Health Study). *Scand. Cardiovasc. J.* **2006**, *40*, 274–279.
116. Hanson, M.; Gluckman, P., Endothelial dysfunction and cardiovascular disease: The role of predictive adaptive responses. *Heart* **2005**, *91*, 864–866.
117. Brevetti, G.; Silvestro, A.; Schiano, V.; Chiariello, M. Endothelial dysfunction and cardiovascular risk prediction in peripheral arterial disease: Additive value of flow-mediated dilation to ankle-brachial pressure index. *Circulation* **2003**, *108*, 2093–2098.
118. Gokce, N.; Keaney, J.F., Jr.; Hunter, L.M.; Watkins, M.T.; Nedeljkovic, Z.S.; Menzoian, J.O.; Vita, J.A. Predictive value of noninvasively determined endothelial dysfunction for long-term cardiovascular events in patients with peripheral vascular disease. *J. Am. Coll. Cardiol.* **2003**, *41*, 1769–1775.

119. Erhardt, L.R. Endothelial dysfunction and cardiovascular disease: The promise of blocking the renin-angiotensin system. *Int. J. Clin. Pract.* **2003**, *57*, 211–218.
120. Sardu, C.; Gambardella, J.; Morelli, M.B.; Wang, X.; Marfella, R.; Santulli, G. Is COVID-19 an Endothelial Disease? Clinical and Basic Evidence. *Preprints* **2020**, doi:10.20944/preprints202004.0204.v1.
121. Sardu, C.; Gambardella, J.; Morelli, M.B.; Wang, X.; Marfella, R.; Santulli, G. Hypertension, Thrombosis, Kidney Failure, and Diabetes: Is COVID-19 an Endothelial Disease? A Comprehensive Evaluation of Clinical and Basic Evidence. *J. Clin. Med.* **2020**, *9*, 1417.
122. Paules, C.I.; Marston, H.D.; Fauci, A.S., Coronavirus Infections-More Than Just the Common Cold. *JAMA* **2020**, *323*, 707–708.
123. Hui, D.S.; E., I.A.; Madani, T.A.; Ntoumi, F.; Kock, R.; Dar, O.; Ippolito, G.; McHugh, T.D.; Memish, Z.A.; Drosten, C.; et al. The continuing 2019-nCoV epidemic threat of novel coronaviruses to global health—The latest 2019 novel coronavirus outbreak in Wuhan, China. *Int. J. Infect. Dis.* **2020**, *91*, 264–266.
124. Colmenero, I.; Santonja, C.; Alonso-Riano, M.; Noguera-Morel, L.; Hernandez-Martin, A.; Andina, D.; Wiesner, T.; Rodriguez-Peralto, J.L.; Requena, L.; Torrelo, A. SARS-CoV-2 endothelial infection causes COVID-19 chilblains: Histopathological, immunohistochemical and ultraestructural study of 7 paediatric cases. *Br. J. Dermatol.* **2020**, doi:10.1111/bjd.19327.
125. Hanafi, R.; Roger, P.A.; Perin, B.; Kuchcinski, G.; Deleval, N.; Dallery, F.; Michel, D.; Hacein-Bey, L.; Pruvo, J.P.; Outteryck, O.; et al. COVID-19 Neurologic Complication with CNS Vasculitis-Like Pattern. *AJNR Am. J. Neuroradiol.* **2020**, doi:10.3174/ajnr.A6651.
126. Mosleh, W.; Chen, K.; Pfau, S.E.; Vashist, A. Endotheliitis and Endothelial Dysfunction in Patients with COVID-19: Its Role in Thrombosis and Adverse Outcomes. *J. Clin. Med.* **2020**, *9*, 1862.
127. Pons, S.; Fodil, S.; Azoulay, E.; Zafrani, L. The vascular endothelium: The cornerstone of organ dysfunction in severe SARS-CoV-2 infection. *Crit. Care* **2020,** *24*, 353.
128. Konopka, K.E.; Nguyen, T.; Jentzen, J.M.; Rayes, O.; Schmidt, C.J.; Wilson, A.M.; Farver, C.F.; Myers, J.L. Diffuse Alveolar Damage (DAD) from Coronavirus Disease 2019 Infection is Morphologically Indistinguishable from Other Causes of DAD. *Histopathology* **2020**, doi:10.1111/his.14180.
129. Benger, M.; Williams, O.; Siddiqui, J.; Sztriha, L. Intracerebral haemorrhage and COVID-19: Clinical characteristics from a case series. *Brain Behav. Immun.* **2020**, *88*, 940–944.
130. Wang, J.; Saguner, A.M.; An, J.; Ning, Y.; Yan, Y.; Li, G. Dysfunctional Coagulation in COVID-19: From Cell to Bedside. *Adv. Ther.* **2020**, *37*, 3033–3039.
131. Ackermann, M.; Verleden, S.E.; Kuehnel, M.; Haverich, A.; Welte, T.; Laenger, F.; Vanstapel, A.; Werlein, C.; Stark, H.; Tzankov, A.; et al. Pulmonary Vascular Endothelialitis, Thrombosis, and Angiogenesis in Covid-19. *N. Engl. J. Med.* **2020**, doi:10.1056/NEJMoa2015432.
132. Varga, Z.; Flammer, A.J.; Steiger, P.; Haberecker, M.; Andermatt, R.; Zinkernagel, A.S.; Mehra, M.R.; Schuepbach, R.A.; Ruschitzka, F.; Moch, H. Endothelial cell infection and endotheliitis in COVID-19. *Lancet* **2020**, *395*, 1417–1418.
133. Jones, V.G.; Mills, M.; Suarez, D.; Hogan, C.A.; Yeh, D.; Bradley Segal, J.; Nguyen, E.L.; Barsh, G.R.; Maskatia, S.; Mathew, R. COVID-19 and Kawasaki Disease: Novel Virus and Novel Case. *Hosp. Pediatr.* **2020**, *10*, 537-540.
134. Cyr, A.R.; Huckaby, L.V.; Shiva, S.S.; Zuckerbraun, B.S. Nitric Oxide and Endothelial Dysfunction. *Crit. Care Clin.* **2020,** *36*, 307–321.
135. Chen, J.; Zhang, J.; Shaik, N.F.; Yi, B.; Wei, X.; Yang, X.F.; Naik, U.P.; Summer, R.; Yan, G.; Xu, X.; et al. The histone deacetylase inhibitor tubacin mitigates endothelial dysfunction by up-regulating the expression of endothelial nitric oxide synthase. *J. Biol. Chem.* **2019,** *294*, 19565–19576.
136. Lomeli, O.; Perez-Torres, I.; Marquez, R.; Criales, S.; Mejia, A.M.; Chiney, C.; Hernandez-Lemus, E.; Soto, M.E. The Evaluation of Flow-Mediated Vasodilation in the Brachial Artery Correlates With Endothelial Dysfunction Evaluated by Nitric Oxide Synthase Metabolites in Marfan Syndrome Patients. *Front. Physiol.* **2018**, *9*, 965.
137. Evans, R.W.; Fernstrom, J.D.; Thompson, J.; Morris, S.M., Jr.; Kuller, L.H. Biochemical responses of healthy subjects during dietary supplementation with L-arginine. *J. Nutr. Biochem.* **2004**, *15*, 534–539.
138. Alvares, T.S.; Conte-Junior, C.A.; Silva, J.T.; Paschoalin, V.M. Acute L-Arginine supplementation does not increase nitric oxide production in healthy subjects. *Nutr. Metab.* **2012**, *9*, 54.

139. Meirelles, C.M.; Matsuura, C.; Silva, R.S., Jr.; Guimaraes, F.F.; Gomes, P.S.C. Acute Effects of L-Arginine Supplementation on Oxygen Consumption Kinetics and Muscle Oxyhemoglobin and Deoxyhemoglobin during Treadmill Running in Male Adults. *Int. J. Exerc. Sci.* **2019**, *12*, 444–455.
140. Liu, T.H.; Wu, C.L.; Chiang, C.W.; Lo, Y.W.; Tseng, H.F.; Chang, C.K. No effect of short-term arginine supplementation on nitric oxide production, metabolism and performance in intermittent exercise in athletes. *J. Nutr. Biochem.* **2009**, *20*, 462–468.
141. Schwedhelm, E.; Maas, R.; Freese, R.; Jung, D.; Lukacs, Z.; Jambrecina, A.; Spickler, W.; Schulze, F.; Boger, R.H. Pharmacokinetic and pharmacodynamic properties of oral L-citrulline and L-arginine: Impact on nitric oxide metabolism. *Br. J. Clin. Pharmacol.* **2008**, *65*, 51–59.
142. Bredt, D.S.; Snyder, S.H. Isolation of nitric oxide synthetase, a calmodulin-requiring enzyme. *Proc. Natl. Acad. Sci. USA* **1990**, *87*, 682–685.
143. Joyner, M.J.; Casey, D.P. Regulation of increased blood flow (hyperemia) to muscles during exercise: A hierarchy of competing physiological needs. *Physiol. Rev.* **2015**, *95*, 549–601.
144. Alvares, T.S.; Meirelles, C.M.; Bhambhani, Y.N.; Paschoalin, V.M.; Gomes, P.S. L-Arginine as a potential ergogenic aid in healthy subjects. *Sports Med.* **2011**, *41*, 233–248.
145. Doutreleau, S.; Rouyer, O.; Di Marco, P.; Lonsdorfer, E.; Richard, R.; Piquard, F.; Geny, B. L-arginine supplementation improves exercise capacity after a heart transplant. *Am. J. Clin. Nutr.* **2010**, *91*, 1261–1267.
146. Maughan, R.J. Nutritional ergogenic aids and exercise performance. *Nutr. Res. Rev.* **1999**, *12*, 255–280.
147. Suzuki, I.; Sakuraba, K.; Horiike, T.; Kishi, T.; Yabe, J.; Suzuki, T.; Morita, M.; Nishimura, A.; Suzuki, Y. A combination of oral L-citrulline and L-arginine improved 10-min full-power cycling test performance in male collegiate soccer players: A randomized crossover trial. *Eur. J. Appl. Physiol.* **2019**, *119*, 1075–1084.
148. Andrade, W.B.; Jacinto, J.L.; da Silva, D.K.; Roveratti, M.C.; Estoche, J.M.; Oliveira, D.B.; Balvedi, M.C.W.; da Silva, R.A.; Aguiar, A.F. l-Arginine supplementation does not improve muscle function during recovery from resistance exercise. *Appl. Physiol. Nutr. Metab.* **2018**, *43*, 928–936.
149. Dioguardi, F.S. To give or not to give? Lessons from the arginine paradox. *J. Nutrigenet. Nutrige.* **2011**, *4*, 90–98.
150. Ueda, S.; Petrie, J.R.; Cleland, S.J.; Elliott, H.L.; Connell, J.M. Insulin vasodilatation and the "arginine paradox". *Lancet* **1998**, *351*, 959–960.
151. Kurz, S.; Harrison, D.G. Insulin and the arginine paradox. *J. Clin. Investig.* **1997**, *99*, 369–370.
152. Vallance, P.; Leone, A.; Calver, A.; Collier, J.; Moncada, S. Endogenous dimethylarginine as an inhibitor of nitric oxide synthesis. *J. Cardiovasc. Pharmacol.* **1992**, *20* (Suppl. 12), S60–S62.
153. Bartnicki, P.; Kowalczyk, M.; Franczyk-Skora, B.; Baj, Z.; Rysz, J. Evaluation of Endothelial (dys)Function, Left Ventricular Structure and Function in Patients with Chronic Kidney Disease. *Curr. Vasc. Pharmacol.* **2016**, *14*, 360–367.
154. Bouras, G.; Deftereos, S.; Tousoulis, D.; Giannopoulos, G.; Chatzis, G.; Tsounis, D.; Cleman, M.W.; Stefanadis, C.,. Asymmetric Dimethylarginine (ADMA): A promising biomarker for cardiovascular disease? *Curr. Top. Med. Chem.* **2013**, *13*, 180–200.
155. Janes, F.; Cifu, A.; Pessa, M.E.; Domenis, R.; Gigli, G.L.; Sanvilli, N.; Nilo, A.; Garbo, R.; Curcio, F.; Giacomello, R.; et al. ADMA as a possible marker of endothelial damage. A study in young asymptomatic patients with cerebral small vessel disease. *Sci. Rep.* **2019**, *9*, 14207.
156. Strobel, J.; Muller, F.; Zolk, O.; Endress, B.; Konig, J.; Fromm, M.F.; Maas, R. Transport of asymmetric dimethylarginine (ADMA) by cationic amino acid transporter 2 (CAT2), organic cation transporter 2 (OCT2) and multidrug and toxin extrusion protein 1 (MATE1). *Amino Acids* **2013**, *45*, 989–1002.
157. Closs, E.I.; Basha, F.Z.; Habermeier, A.; Forstermann, U. Interference of L-arginine analogues with L-arginine transport mediated by the y+ carrier hCAT-2B. *Nitric Oxide* **1997**, *1*, 65–73.
158. Wijnands, K.A.; Hoeksema, M.A.; Meesters, D.M.; van den Akker, N.M.; Molin, D.G.; Briede, J.J.; Ghosh, M.; Kohler, S.E.; van Zandvoort, M.A.; de Winther, M.P.; et al. Arginase-1 deficiency regulates arginine concentrations and NOS2-mediated NO production during endotoxemia. *PLoS ONE* **2014**, *9*, e86135.
159. Boger, R.H.; Bode-Boger, S.M.; Szuba, A.; Tsao, P.S.; Chan, J.R.; Tangphao, O.; Blaschke, T.F.; Cooke, J.P. Asymmetric dimethylarginine (ADMA): A novel risk factor for endothelial dysfunction: Its role in hypercholesterolemia. *Circulation* **1998**, *98*, 1842–1847.
160. Nijveldt, R.J.; Teerlink, T.; Van Der Hoven, B.; Siroen, M.P.; Kuik, D.J.; Rauwerda, J.A.; van Leeuwen, P.A. Asymmetrical dimethylarginine (ADMA) in critically ill patients: High plasma ADMA concentration is an independent risk factor of ICU mortality. *Clin. Nutr.* **2003**, *22*, 23–30.

161. Abedini, S.; Meinitzer, A.; Holme, I.; Marz, W.; Weihrauch, G.; Fellstrom, B.; Jardine, A.; Holdaas, H. Asymmetrical dimethylarginine is associated with renal and cardiovascular outcomes and all-cause mortality in renal transplant recipients. *Kidney Int.* **2010**, *77*, 44–50.
162. Krzyzanowska, K.; Mittermayer, F.; Wolzt, M.; Schernthaner, G. Asymmetric dimethylarginine predicts cardiovascular events in patients with type 2 diabetes. *Diabetes Care* **2007**, *30*, 1834–1839.
163. Nanayakkara, P.W.; Teerlink, T.; Stehouwer, C.D.; Allajar, D.; Spijkerman, A.; Schalkwijk, C.; ter Wee, P.M.; van Guldener, C. Plasma asymmetric dimethylarginine (ADMA) concentration is independently associated with carotid intima-media thickness and plasma soluble vascular cell adhesion molecule-1 (sVCAM-1) concentration in patients with mild-to-moderate renal failure. *Kidney Int.* **2005**, *68*, 2230–2236.
164. Zoccali, C.; Bode-Boger, S.; Mallamaci, F.; Benedetto, F.; Tripepi, G.; Malatino, L.; Cataliotti, A.; Bellanuova, I.; Fermo, I.; Frolich, J.; et al. Plasma concentration of asymmetrical dimethylarginine and mortality in patients with end-stage renal disease: A prospective study. *Lancet* **2001**, *358*, 2113–2117.
165. Abbasi, F.; Asagmi, T.; Cooke, J.P.; Lamendola, C.; McLaughlin, T.; Reaven, G.M.; Stuehlinger, M.; Tsao, P.S. Plasma concentrations of asymmetric dimethylarginine are increased in patients with type 2 diabetes mellitus. *Am. J. Cardiol.* **2001**, *88*, 1201–1203.
166. Furuki, K.; Adachi, H.; Matsuoka, H.; Enomoto, M.; Satoh, A.; Hino, A.; Hirai, Y.; Imaizumi, T. Plasma levels of asymmetric dimethylarginine (ADMA) are related to intima-media thickness of the carotid artery: An epidemiological study. *Atherosclerosis* **2007**, *191*, 206–210.
167. Valkonen, V.P.; Paiva, H.; Salonen, J.T.; Lakka, T.A.; Lehtimaki, T.; Laakso, J.; Laaksonen, R. Risk of acute coronary events and serum concentration of asymmetrical dimethylarginine. *Lancet* **2001**, *358*, 2127–2128.
168. Surdacki, A.; Nowicki, M.; Sandmann, J.; Tsikas, D.; Boeger, R.H.; Bode-Boeger, S.M.; Kruszelnicka-Kwiatkowska, O.; Kokot, F.; Dubiel, J.S.; Froelich, J.C. Reduced urinary excretion of nitric oxide metabolites and increased plasma levels of asymmetric dimethylarginine in men with essential hypertension. *J. Cardiovasc. Pharmacol.* **1999**, *33*, 652–658.
169. Maas, R.; Xanthakis, V.; Polak, J.F.; Schwedhelm, E.; Sullivan, L.M.; Benndorf, R.; Schulze, F.; Vasan, R.S.; Wolf, P.A.; Boger, R.H.; et al. Association of the endogenous nitric oxide synthase inhibitor ADMA with carotid artery intimal media thickness in the Framingham Heart Study offspring cohort. *Stroke* **2009**, *40*, 2715–2719.
170. Bai, Y.; Sun, L.; Du, L.; Zhang, T.; Xin, W.; Lan, X.; Du, G. Association of circulating levels of asymmetric dimethylarginine (ADMA) with carotid intima-media thickness: Evidence from 6168 participants. *Ageing Res. Rev.* **2013**, *12*, 699–707.
171. Sundar, U.M.; Ugusman, A.; Chua, H.K.; Latip, J.; Aminuddin, A. Piper sarmentosum Promotes Endothelial Nitric Oxide Production by Reducing Asymmetric Dimethylarginine in Tumor Necrosis Factor-alpha-Induced Human Umbilical Vein Endothelial Cells. *Front. Pharmacol* **2019**, *10*, 1033.
172. Arlouskaya, Y.; Sawicka, A.; Glowala, M.; Giebultowicz, J.; Korytowska, N.; Talalaj, M.; Nowicka, G.; Wrzosek, M. Asymmetric Dimethylarginine (ADMA) and Symmetric Dimethylarginine (SDMA) Concentrations in Patients with Obesity and the Risk of Obstructive Sleep Apnea (OSA). *J. Clin. Med.* **2019**, *8*, 897.
173. Hsu, C.P.; Zhao, J.F.; Lin, S.J.; Shyue, S.K.; Guo, B.C.; Lu, T.M.; Lee, T.S. Asymmetric Dimethylarginine Limits the Efficacy of Simvastatin Activating Endothelial Nitric Oxide Synthase. *J. Am. Heart Assoc.* **2016**, *5*, e003327.
174. Notsu, Y.; Yano, S.; Shibata, H.; Nagai, A.; Nabika, T. Plasma arginine/ADMA ratio as a sensitive risk marker for atherosclerosis: Shimane CoHRE study. *Atherosclerosis* **2015**, *239*, 61–66.
175. Boger, R.H.; Endres, H.G.; Schwedhelm, E.; Darius, H.; Atzler, D.; Luneburg, N.; von Stritzky, B.; Maas, R.; Thiem, U.; Benndorf, R.A.; 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.
176. Pizzarelli, F.; Maas, R.; Dattolo, P.; Tripepi, G.; Michelassi, S.; D'Arrigo, G.; Mieth, M.; Bandinelli, S.; Ferrucci, L.; Zoccali, C. Asymmetric dimethylarginine predicts survival in the elderly. *Age* **2013**, *35*, 2465–2475.
177. Notsu, Y.; Nabika, T.; Bokura, H.; Suyama, Y.; Kobayashi, S.; Yamaguchi, S.; Masuda, J. Evaluation of asymmetric dimethylarginine and homocysteine in microangiopathy-related cerebral damage. *Am. J. Hypertens.* **2009**, 22, 257–262.

178. McDonald, K.K.; Zharikov, S.; Block, E.R.; Kilberg, M.S. A caveolar complex between the cationic amino acid transporter 1 and endothelial nitric-oxide synthase may explain the "arginine paradox". *J. Biol. Chem.* **1997**, *272*, 31213–31216.
179. Kim, J.A.; Montagnani, M.; Chandrasekran, S.; Quon, M.J. Role of lipotoxicity in endothelial dysfunction. *Heart Fail. Clin.* **2012**, *8*, 589–607.
180. Benowitz, N.L. Cigarette smoking and cardiovascular disease: Pathophysiology and implications for treatment. *Prog. Cardiovasc. Dis.* **2003**, *46*, 91–111.
181. Januzzi, J. *Cardiac Biomarkers in Clinical Practice*; Jones & Bartlett Learning: Burlington, MA, USA, 2009.
182. Rohde, D.; Busch, M.; Volkert, A.; Ritterhoff, J.; Katus, H.A.; Peppel, K.; Most, P. Cardiomyocytes, endothelial cells and cardiac fibroblasts: S100A1's triple action in cardiovascular pathophysiology. *Future Cardiol.* **2015**, *11*, 309–321.
183. Davies, P.F. Hemodynamic shear stress and the endothelium in cardiovascular pathophysiology. *Nat. Clin. Pract. Cardiovasc. Med.* **2009**, *6*, 16–26.
184. Santulli, G. microRNAs Distinctively Regulate Vascular Smooth Muscle and Endothelial Cells: Functional Implications in Angiogenesis, Atherosclerosis, and In-Stent Restenosis. *Adv. Exp. Med. Biol.* **2015**, *887*, 53–77.
185. Foster, W.; Carruthers, D.; Lip, G.Y.; Blann, A.D. Relationships between endothelial, inflammatory and angiogenesis markers in rheumatoid arthritis: Implications for cardiovascular pathophysiology. *Thromb. Res.* **2009**, *123*, 659–664.
186. Ciccarelli, M.; Santulli, G.; Campanile, A.; Galasso, G.; Cervero, P.; Altobelli, G.G.; Cimini, V.; Pastore, L.; Piscione, F.; Trimarco, B.; et al. Endothelial alpha1-adrenoceptors regulate neo-angiogenesis. *Br. J. Pharmacol.* **2008**, *153*, 936–946.
187. Luksha, L.; Agewall, S.; Kublickiene, K. Endothelium-derived hyperpolarizing factor in vascular physiology and cardiovascular disease. *Atherosclerosis* **2009**, *202*, 330–344.
188. Santulli, G.; Cipolletta, E.; Sorriento, D.; Del Giudice, C.; Anastasio, A.; Monaco, S.; Maione, A.S.; Condorelli, G.; Puca, A.; Trimarco, B.; et al. CaMK4 Gene Deletion Induces Hypertension. *J. Am. Heart Assoc.* **2012**, *1*, e001081.
189. Deedwania, P.C. Diabetes is a vascular disease: The role of endothelial dysfunction in pathophysiology of cardiovascular disease in diabetes. *Cardiol. Clin.* **2004**, *22*, 505–509.
190. Sorriento, D.; Santulli, G.; Del Giudice, C.; Anastasio, A.; Trimarco, B.; Iaccarino, G. Endothelial cells are able to synthesize and release catecholamines both in vitro and in vivo. *Hypertension* **2012**, *60*, 129–136.
191. Li, J.M.; Shah, A.M. Endothelial cell superoxide generation: Regulation and relevance for cardiovascular pathophysiology. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **2004**, *287*, R1014–R1030.
192. Ciccarelli, M.; Cipolletta, E.; Santulli, G.; Campanile, A.; Pumiglia, K.; Cervero, P.; Pastore, L.; Astone, D.; Trimarco, B.; Iaccarino, G. Endothelial beta2 adrenergic signaling to AKT: Role of Gi and SRC. *Cell Signal.* **2007**, *19*, 1949–1955.
193. Sellke, F.W.; Boyle, E.M., Jr.; Verrier, E.D. Endothelial cell injury in cardiovascular surgery: The pathophysiology of vasomotor dysfunction. *Ann. Thorac. Surg.* **1996**, *62*, 1222–1228.
194. Iaccarino, G.; Ciccarelli, M.; Sorriento, D.; Galasso, G.; Campanile, A.; Santulli, G.; Cipolletta, E.; Cerullo, V.; Cimini, V.; Altobelli, G.G.; et al. Ischemic neoangiogenesis enhanced by beta2-adrenergic receptor overexpression: A novel role for the endothelial adrenergic system. *Circ. Res.* **2005**, *97*, 1182–1189.
195. Ogita, H.; Liao, J. Endothelial function and oxidative stress. *Endothelium* **2004**, *11*, 123–132.
196. Skrypnyk, I.; Maslova, G.; Lymanets, T.; Gusachenko, I. L-arginine is an effective medication for prevention of endothelial dysfunction, a predictor of anthracycline cardiotoxicity in patients with acute leukemia. *Exp. Oncol.* **2017**, *39*, 308–311.
197. Ignarro, L.J.; Napoli, C. Novel features of nitric oxide, endothelial nitric oxide synthase, and atherosclerosis. *Curr. Diab. Rep.* **2005**, *5*, 17–23.
198. Nguyen, M.C.; Park, J.T.; Jeon, Y.G.; Jeon, B.H.; Hoe, K.L.; Kim, Y.M.; Lim, H.K.; Ryoo, S. Arginase Inhibition Restores Peroxynitrite-Induced Endothelial Dysfunction via L-Arginine-Dependent Endothelial Nitric Oxide Synthase Phosphorylation. *Yonsei Med. J.* **2016**, *57*, 1329–1338.
199. Wu, G.; Meininger, C.J. Arginine nutrition and cardiovascular function. *J. Nutr.* **2000**, *130*, 2626–2629.
200. Scaglia, F.; Brunetti-Pierri, N.; Kleppe, S.; Marini, J.; Carter, S.; Garlick, P.; Jahoor, F.; O'Brien, W.; Lee, B. Clinical consequences of urea cycle enzyme deficiencies and potential links to arginine and nitric oxide metabolism. *J. Nutr.* **2004,** *134* (Suppl. 10), 2775S-2782S.

201. Luneburg, N.; Xanthakis, V.; Schwedhelm, E.; Sullivan, L.M.; Maas, R.; Anderssohn, M.; Riederer, U.; Glazer, N.L.; Vasan, R.S.; Boger, R.H. Reference intervals for plasma L-arginine and the L-arginine:asymmetric dimethylarginine ratio in the Framingham Offspring Cohort. *J. Nutr.* **2011**, *141*, 2186–2190.
202. Contreras, M.T.; Gallardo, M.J.; Betancourt, L.R.; Rada, P.V.; Ceballos, G.A.; Hernandez, L.E.; Hernandez, L.F. Correlation between plasma levels of arginine and citrulline in preterm and full-term neonates: Therapeutical implications. *J. Clin. Lab. Anal.* **2017**, *31*, e22134.
203. Fernandez Diaz-Rullo, F.; Zamberlan, F.; Mewis, R.E.; Fekete, M.; Broche, L.; Cheyne, L.A.; Dall'Angelo, S.; Duckett, S.B.; Dawson, D.; Zanda, M. Synthesis and hyperpolarisation of eNOS substrates for quantification of NO production by (1)H NMR spectroscopy. *Bioorg. Med. Chem.* **2017**, *25*, 2730–2742.
204. Schmidt, H.H.; Warner, T.D.; Ishii, K.; Sheng, H.; Murad, F. Insulin secretion from pancreatic B cells caused by L-arginine-derived nitrogen oxides. *Science* **1992**, *255*, 721–723.
205. Fajans, S.S.; Floyd, J.C., Jr.; Knopf, R.F.; Conn, F.W. Effect of amino acids and proteins on insulin secretion in man. *Recent Prog. Horm. Res.* **1967**, *23*, 617–662.
206. Sener, A.; Best, L.C.; Yates, A.P.; Kadiata, M.M.; Olivares, E.; Louchami, K.; Jijakli, H.; Ladriere, L.; Malaisse, W.J. Stimulus-secretion coupling of arginine-induced insulin release: Comparison between the cationic amino acid and its methyl ester. *Endocrine* **2000**, *13*, 329–340.
207. Gerich, J.E.; Lorenzi, M.; Schneider, V.; Kwan, C.W.; Karam, J.H.; Guillemin, R.; Forsham, P.H. Inhibition of pancreatic glucagon responses to arginine by somatostatin in normal man and in insulin-dependent diabetics. *Diabetes* **1974**, *23*, 876–880.
208. Giugliano, D.; Marfella, R.; Verrazzo, G.; Acampora, R.; Coppola, L.; Cozzolino, D.; D'Onofrio, F. The vascular effects of L-Arginine in humans. The role of endogenous insulin. *J. Clin. Investig.* **1997**, *99*, 433–438.
209. Merimee, T.J.; Rabinowitz, D.; Riggs, L.; Burgess, J.A.; Rimoin, D.L.; McKusick, V.A. Plasma growth hormone after arginine infusion. Clinical experiences. *N. Engl. J. Med.* **1967**, *276*, 434–439.
210. Fryburg, D.A. NG-monomethyl-L-arginine inhibits the blood flow but not the insulin-like response of forearm muscle to IGF- I: Possible role of nitric oxide in muscle protein synthesis. *J. Clin. Investig.* **1996**, *97*, 1319–1328.
211. Bode-Boger, S.M.; Boger, R.H.; Loffler, M.; Tsikas, D.; Brabant, G.; Frolich, J.C. L-arginine stimulates NO-dependent vasodilation in healthy humans--effect of somatostatin pretreatment. *J. Investig. Med.* **1999**, *47*, 43–50.
212. Taddei, S.; Virdis, A.; Mattei, P.; Ghiadoni, L.; Gennari, A.; Fasolo, C.B.; Sudano, I.; Salvetti, A. Aging and endothelial function in normotensive subjects and patients with essential hypertension. *Circulation* **1995**, *91*, 1981–1987.
213. Celermajer, D.S.; Sorensen, K.E.; Gooch, V.M.; Spiegelhalter, D.J.; Miller, O.I.; Sullivan, I.D.; Lloyd, J.K.; Deanfield, J.E. Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. *Lancet* **1992**, *340*, 1111–1115.
214. Gates, P.E.; Boucher, M.L.; Silver, A.E.; Monahan, K.D.; Seals, D.R. Impaired flow-mediated dilation with age is not explained by L-arginine bioavailability or endothelial asymmetric dimethylarginine protein expression. *J. Appl. Physiol.* **2007**, *102*, 63–71.
215. Campo, C.; Lahera, V.; Garcia-Robles, R.; Cachofeiro, V.; Alcazar, J.M.; Andres, A.; Rodicio, J.L.; Ruilope, L.M. Aging abolishes the renal response to L-arginine infusion in essential hypertension. *Kidney Int. Suppl.* **1996**, *55*, S126–S128.
216. Blum, A.; Hathaway, L.; Mincemoyer, R.; Schenke, W.H.; Kirby, M.; Csako, G.; Waclawiw, M.A.; Panza, J.A.; Cannon, R.O.III. Effects of oral L-arginine on endothelium-dependent vasodilation and markers of inflammation in healthy postmenopausal women. *J. Am. Coll. Cardiol.* **2000**, *35*, 271–276.
217. Bode-Boger, S.M.; Muke, J.; Surdacki, A.; Brabant, G.; Boger, R.H.; Frolich, J.C. Oral L-arginine improves endothelial function in healthy individuals older than 70 years. *Vasc. Med.* **2003**, *8*, 77–81.
218. Chen, P.Y.; St John, P.L.; Kirk, K.A.; Abrahamson, D.R.; Sanders, P.W. Hypertensive nephrosclerosis in the Dahl/Rapp rat. Initial sites of injury and effect of dietary L-arginine supplementation. *Lab. Investig.* **1993**, *68*, 174–184.
219. Zhou, M.S.; Kosaka, H.; Tian, R.X.; Abe, Y.; Chen, Q.H.; Yoneyama, H.; Yamamoto, A.; Zhang, L. L-Arginine improves endothelial function in renal artery of hypertensive Dahl rats. *J. Hypertens.* **2001**, *19*, 421–429.

220. Fujii, S.; Zhang, L.; Igarashi, J.; Kosaka, H. L-arginine reverses p47phox and gp91phox expression induced by high salt in Dahl rats. *Hypertension* **2003**, *42*, 1014–1020.
221. Patel, A.; Layne, S.; Watts, D.; Kirchner, K.A. L-arginine administration normalizes pressure natriuresis in hypertensive Dahl rats. *Hypertension* **1993**, *22*, 863–869.
222. Chen, P.Y.; Sanders, P.W. L-arginine abrogates salt-sensitive hypertension in Dahl/Rapp rats. *J. Clin. Investig.* **1991**, *88*, 1559–1567.
223. Miyata, N.; Zou, A.P.; Mattson, D.L.; Cowley, A.W., Jr. Renal medullary interstitial infusion of L-arginine prevents hypertension in Dahl salt-sensitive rats. *Am. J. Physiol.* **1998**, *275*, R1667–73.
224. Ozcelikay, A.T.; Tay, A.; Guner, S.; Tasyaran, V.; Yildizoglu-Ari, N.; Dincer, U.D.; Altan, V.M. Reversal effects of L-arginine treatment on blood pressure and vascular responsiveness of streptozotocin-diabetic rats. *Pharmacol. Res.* **2000**, *41*, 201–209.
225. Tay, A.; Ozcelikay, A.T.; Altan, V.M. Effects of L-arginine on blood pressure and metabolic changes in fructose-hypertensive rats. *Am. J. Hypertens.* **2002**, *15 Pt 1*, 72–77.
226. Ono, H.; Ono, Y.; Frohlich, E.D. L-arginine reverses severe nephrosclerosis in aged spontaneously hypertensive rats. *J. Hypertens.* **1999**, *17*, 121–128.
227. Shan, L.; Wang, B.; Gao, G.; Cao, W.; Zhang, Y. L-Arginine supplementation improves antioxidant defenses through L-arginine/nitric oxide pathways in exercised rats. *J. Appl. Physiol.* **2013**, *115*, 1146–1155.
228. Lin, W.T.; Yang, S.C.; Chen, K.T.; Huang, C.C.; Lee, N.Y. Protective effects of L-arginine on pulmonary oxidative stress and antioxidant defenses during exhaustive exercise in rats. *Acta Pharmacol. Sin.* **2005**, *26*, 992–999.
229. Huang, C.C.; Tsai, S.C.; Lin, W.T. Potential ergogenic effects of L-arginine against oxidative and inflammatory stress induced by acute exercise in aging rats. *Exp. Gerontol.* **2008**, *43*, 571–577.
230. de Nigris, F.; Lerman, L.O.; Ignarro, S.W.; Sica, G.; Lerman, A.; Palinski, W.; Ignarro, L.J.; Napoli, C. Beneficial effects of antioxidants and L-arginine on oxidation-sensitive gene expression and endothelial NO synthase activity at sites of disturbed shear stress. *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 1420–1425.
231. Ahmad, A.; Sattar, M.Z.; Rathore, H.A.; Hussain, A.I.; Khan, S.A.; Fatima, T.; Afzal, S.; Abdullah, N.A.; Johns, E.J. Antioxidant Activity and Free Radical Scavenging Capacity of L-Arginine and Nahs: A Comparative in Vitro Study. *Acta Pol. Pharm.* **2015**, *72*, 245–252.
232. Wallner, S.; Hermetter, A.; Mayer, B.; Wascher, T.C. The alpha-amino group of L-arginine mediates its antioxidant effect. *Eur. J. Clin. Investig.* **2001**, *31*, 98–102.
233. Sulibursk a, J.; Bogdanski, P.; Krejpcio, Z.; Pupek-Musialik, D.; Jablecka, A. The effects of L-arginine, alone and combined with vitamin C, on mineral status in relation to its antidiabetic, anti-inflammatory, and antioxidant properties in male rats on a high-fat diet. *Biol. Trace Elem. Res.* **2014**, *157*, 67–74.
234. Zheng, P.; Yu, B.; He, J.; Tian, G.; Luo, Y.; Mao, X.; Zhang, K.; Che, L.; Chen, D. Protective effects of dietary arginine supplementation against oxidative stress in weaned piglets. *Br. J. Nutr.* **2013**, *109*, 2253–2260.
235. Fazelian, S.; Hoseini, M.; Namazi, N.; Heshmati, J.; Sepidar Kish, M.; Mirfatahi, M.; Some Olia, A.S. Effects of L-Arginine Supplementation on Antioxidant Status and Body Composition in Obese Patients with Pre-diabetes: A Randomized Controlled Clinical Trial. *Adv. Pharm. Bull.* **2014**, *4* (Suppl. 1), 449–454.
236. Wascher, T.C.; Posch, K.; Wallner, S.; Hermetter, A.; Kostner, G.M.; Graier, W.F. Vascular effects of L-arginine: Anything beyond a substrate for the NO-synthase? *Biochem Biophys Res. Commun* **1997**, *234*, 35–38.
237. Lekakis, J.P.; Papathanassiou, S.; Papaioannou, T.G.; Papamichael, C.M.; Zakopoulos, N.; Kotsis, V.; Dagre, A.G.; Stamatelopoulos, K.; Protogerou, A.; Stamatelopoulos, S.F. Oral L-arginine improves endothelial dysfunction in patients with essential hypertension. *Int. J. Cardiol.* **2002**, *86*, 317–323.
238. Higashi, Y.; Oshima, T.; Watanabe, M.; Matsuura, H.; Kajiyama, G. Renal response to L-arginine in salt-sensitive patients with essential hypertension. *Hypertension* **1996**, *27 Pt 2*, 643–648.
239. Campese, V.M.; Amar, M.; Anjali, C.; Medhat, T.; Wurgaft, A. Effect of L-arginine on systemic and renal haemodynamics in salt-sensitive patients with essential hypertension. *J. Hum. Hypertens* **1997**, *11*, 527–532.
240. Pezza, V.; Bernardini, F.; Pezza, E.; Pezza, B.; Curione, M. Study of supplemental oral l-arginine in hypertensives treated with enalapril + hydrochlorothiazide. *Am. J. Hypertens* **1998**, *11*, 1267–1270.
241. Higashi, Y.; Oshima, T.; Ono, N.; Hiraga, H.; Yoshimura, M.; Watanabe, M.; Matsuura, H.; Kambe, M.; Kajiyama, G. Intravenous administration of L-arginine inhibits angiotensin-converting enzyme in humans. *J. Clin. Endocrinol. Metab.* **1995**, *80*, 2198–2202.

242. De Caterina, R.; Libby, P.; Peng, H.B.; Thannickal, V.J.; Rajavashisth, T.B.; Gimbrone, M.A., Jr.; Shin, W.S.; Liao, J.K. Nitric oxide decreases cytokine-induced endothelial activation. Nitric oxide selectively reduces endothelial expression of adhesion molecules and proinflammatory cytokines. *J. Clin. Investig.* **1995**, *96*, 60–68.
243. Sperone, A.; Dryden, N.H.; Birdsey, G.M.; Madden, L.; Johns, M.; Evans, P.C.; Mason, J.C.; Haskard, D.O.; Boyle, J.J.; Paleolog, E.M.; et al. The transcription factor Erg inhibits vascular inflammation by repressing NF-kappaB activation and proinflammatory gene expression in endothelial cells. *Arterioscler. Thromb. Vasc. Biol.* **2011**, *31*, 142–150.
244. Gambardella, J.; Santulli, G. Integrating diet and inflammation to calculate cardiovascular risk. *Atherosclerosis* **2016,** 253, 258–261.
245. Huo, Y.; Ley, K. Adhesion molecules and atherogenesis. *Acta Physiol. Scand.* **2001**, *173*, 35–43.
246. Blum, A.; Miller, H.; Blum, A.; Miller, H. The effects of L-arginine on atherosclerosis and heart disease. *Int. J. Cardiovasc. Intervent.* **1999**, *2*, 97–100.
247. Adams, M.R.; Jessup, W.; Hailstones, D.; Celermajer, D.S. L-arginine reduces human monocyte adhesion to vascular endothelium and endothelial expression of cell adhesion molecules. *Circulation* **1997**, *95*, 662–668.
248. Adams, M.R.; McCredie, R.; Jessup, W.; Robinson, J.; Sullivan, D.; Celermajer, D.S. Oral L-arginine improves endothelium-dependent dilatation and reduces monocyte adhesion to endothelial cells in young men with coronary artery disease. *Atherosclerosis* **1997**, *129*, 261–269.
249. Ceremuzynski, L.; Chamiec, T.; Herbaczynska-Cedro, K. Effect of supplemental oral L-arginine on exercise capacity in patients with stable angina pectoris. *Am. J. Cardiol.* **1997**, *80*, 331–333.
250. Lerman, A.; Burnett, J.C., Jr.; Higano, S.T.; McKinley, L.J.; Holmes, D.R., Jr. Long-term L-arginine supplementation improves small-vessel coronary endothelial function in humans. *Circulation* **1998**, *97*, 2123–2128.
251. Chen, C.; Mattar, S.G.; Lumsden, A.B. Oral administration of L-arginine reduces intimal hyperplasia in balloon-injured rat carotid arteries. *J. Surg. Res.* **1999**, *82*, 17–23.
252. Yin, W.H.; Chen, J.W.; Tsai, C.; Chiang, M.C.; Young, M.S.; Lin, S.J. L-arginine improves endothelial function and reduces LDL oxidation in patients with stable coronary artery disease. *Clin. Nutr.* **2005**, *24*, 988–997.
253. Walker, H.A.; McGing, E.; Fisher, I.; Boger, R.H.; Bode-Boger, S.M.; Jackson, G.; Ritter, J.M.; Chowienczyk, P.J. Endothelium-dependent vasodilation is independent of the plasma L-arginine/ADMA ratio in men with stable angina: Lack of effect of oral L-arginine on endothelial function, oxidative stress and exercise performance. *J. Am. Coll. Cardiol.* **2001**, *38*, 499–505.
254. Padilla, F.; Garcia-Dorado, D.; Agullo, L.; Inserte, J.; Paniagua, A.; Mirabet, S.; Barrabes, J.A.; Ruiz-Meana, M.; Soler-Soler, J. L-Arginine administration prevents reperfusion-induced cardiomyocyte hypercontracture and reduces infarct size in the pig. *Cardiovasc. Res.* **2000**, *46*, 412–420.
255. Rector, T.S.; Bank, A.J.; Mullen, K.A.; Tschumperlin, L.K.; Sih, R.; Pillai, K.; Kubo, S.H. Randomized, double-blind, placebo-controlled study of supplemental oral L-arginine in patients with heart failure. *Circulation* **1996**, *93*, 2135–2141.
256. Bednarz, B.; Jaxa-Chamiec, T.; Gebalska, J.; Herbaczynska-Cedro, K.; Ceremuzynski, L. L-arginine supplementation prolongs exercise capacity in congestive heart failure. *Kardiol. Pol.* **2004**, *60*, 348–353.
257. Rodrigues-Krause, J.; Krause, M.; Rocha, I.; Umpierre, D.; Fayh, A.P.T. Association of l-Arginine Supplementation with Markers of Endothelial Function in Patients with Cardiovascular or Metabolic Disorders: A Systematic Review and Meta-Analysis. *Nutrients* **2018**, *11*, 351–353
258. Hambrecht, R.; Hilbrich, L.; Erbs, S.; Gielen, S.; Fiehn, E.; Schoene, N.; Schuler, G. Correction of endothelial dysfunction in chronic heart failure: Additional effects of exercise training and oral L-arginine supplementation. *J. Am. Coll. Cardiol.* **2000**, *35*, 706–713.
259. Weyrich, A.S.; Ma, X.L.; Lefer, A.M. The role of L-arginine in ameliorating reperfusion injury after myocardial ischemia in the cat. *Circulation* **1992**, *86*, 279–288.
260. Huk, I.; Nanobashvili, J.; Orljanski, W.; Neumayer, C.; Punz, A.; Holzaepfel, A.; Fuegl, A.; Mittlboeck, M.; Polterauer, P.; Roth, E. L-arginine treatment in ischemia/reperfusion injury. *Cas. Lek. Cesk.* **1998**, *137*, 496–499.

261. Takeuchi, K.; McGowan, F.X.; Danh, H.C.; Glynn, P.; Simplaceanu, E.; del Nido, P.J. Direct detrimental effects of L-arginine upon ischemia--reperfusion injury to myocardium. *J. Mol. Cell Cardiol.* **1995**, *27*, 1405–1414.
262. Kubo, S.H.; Rector, T.S.; Bank, A.J.; Williams, R.E.; Heifetz, S.M. Endothelium-dependent vasodilation is attenuated in patients with heart failure. *Circulation* **1991**, *84*, 1589–1596.
263. Hirooka, Y.; Imaizumi, T.; Tagawa, T.; Shiramoto, M.; Endo, T.; Ando, S.; Takeshita, A. Effects of L-arginine on impaired acetylcholine-induced and ischemic vasodilation of the forearm in patients with heart failure. *Circulation* **1994**, *90*, 658–668.
264. Doutreleau, S.; Mettauer, B.; Piquard, F.; Rouyer, O.; Schaefer, A.; Lonsdorfer, J.; Geny, B. Chronic L-arginine supplementation enhances endurance exercise tolerance in heart failure patients. *Int. J. Sports Med.* **2006**, *27*, 567–572.
265. Parnell, M.M.; Holst, D.P.; Kaye, D.M. Augmentation of endothelial function following exercise training is associated with increased L-arginine transport in human heart failure. *Clin. Sci.* **2005**, *109*, 523–530.
266. Chin-Dusting, J.P.; Kaye, D.M.; Lefkovits, J.; Wong, J.; Bergin, P.; Jennings, G.L. Dietary supplementation with L-arginine fails to restore endothelial function in forearm resistance arteries of patients with severe heart failure. *J. Am. Coll. Cardiol.* **1996**, *27*, 1207–1213.
267. Schulman, S.P.; Becker, L.C.; Kass, D.A.; Champion, H.C.; Terrin, M.L.; Forman, S.; Ernst, K.V.; Kelemen, M.D.; Townsend, S.N.; Capriotti, A.; et al. L-arginine therapy in acute myocardial infarction: The Vascular Interaction With Age in Myocardial Infarction (VINTAGE MI) randomized clinical trial. *JAMA* **2006**, *295*, 58–64.
268. Shu, J.; Santulli, G. Update on peripheral artery disease: Epidemiology and evidence-based facts. *Atherosclerosis* **2018**, *275*, 379–381.
269. Azab, S.M.; Zamzam, A.; Syed, M.H.; Abdin, R.; Qadura, M.; Britz-McKibbin, P. Serum Metabolic Signatures of Chronic Limb-Threatening Ischemia in Patients with Peripheral Artery Disease. *J. Clin. Med.* **2020**, *9*, 1877.
270. Misra, S.; Shishehbor, M.H.; Takahashi, E.A.; Aronow, H.D.; Brewster, L.P.; Bunte, M.C.; Kim, E.S.H.; Lindner, J.R.; Rich, K.; American Heart Association Council on Peripheral Vascular, D.; et al. Perfusion Assessment in Critical Limb Ischemia: Principles for Understanding and the Development of Evidence and Evaluation of Devices: A Scientific Statement From the American Heart Association. *Circulation* **2019**, *140*, e657-e672.
271. Boger, R.H.; Bode-Boger, S.M.; Thiele, W.; Junker, W.; Alexander, K.; Frolich, J.C. Biochemical evidence for impaired nitric oxide synthesis in patients with peripheral arterial occlusive disease. *Circulation* **1997**, *95*, 2068–2074.
272. Maxwell, A.J.; Cooke, J.P. Cardiovascular effects of L-arginine. *Curr. Opin. Nephrol. Hypertens.* **1998**, *7*, 63–70.
273. Maxwell, A.J.; Anderson, B.E.; Cooke, J.P. Nutritional therapy for peripheral arterial disease: A double-blind, placebo-controlled, randomized trial of HeartBar. *Vasc. Med.* **2000**, *5*, 11–19.
274. Wilson, A.M.; Harada, R.; Nair, N.; Balasubramanian, N.; Cooke, J.P. L-arginine supplementation in peripheral arterial disease: No benefit and possible harm. *Circulation* **2007**, *116*, 188–195.
275. Avogaro, A.; Albiero, M.; Menegazzo, L.; de Kreutzenberg, S.; Fadini, G.P. Endothelial dysfunction in diabetes: The role of reparatory mechanisms. *Diabetes Care* **2011**, *34* (Suppl. 2), S285–S290.
276. Sena, C.M.; Pereira, A.M.; Seica, R., Endothelial dysfunction—A major mediator of diabetic vascular disease. *Biochim. Biophys. Acta* **2013**, *1832*, 2216–2231.
277. Bogdanski, P.; Suliburska, J.; Grabanska, K.; Musialik, K.; Cieslewicz, A.; Skoluda, A.; Jablecka, A. Effect of 3-month L-arginine supplementation on insulin resistance and tumor necrosis factor activity in patients with visceral obesity. *Eur. Rev. Med. Pharmacol. Sci.* **2012**, *16*, 816–823.
278. Eid, H.M.; Reims, H.; Arnesen, H.; Kjeldsen, S.E.; Lyberg, T.; Seljeflot, I. Decreased levels of asymmetric dimethylarginine during acute hyperinsulinemia. *Metabolism* **2007**, *56*, 464–469.
279. Sobrevia, L.; Nadal, A.; Yudilevich, D.L.; Mann, G.E. Activation of L-arginine transport (system y+) and nitric oxide synthase by elevated glucose and insulin in human endothelial cells. *J. Physiol.* **1996**, *490 Pt 3*, 775–781.
280. Mann, G.E.; Yudilevich, D.L.; Sobrevia, L. Regulation of amino acid and glucose transporters in endothelial and smooth muscle cells. *Physiol. Rev.* **2003**, *83*, 183–252.

281. Lembo, G.; Iaccarino, G.; Vecchione, C.; Barbato, E.; Morisco, C.; Monti, F.; Parrella, L.; Trimarco, B. Insulin enhances endothelial alpha2-adrenergic vasorelaxation by a pertussis toxin mechanism. *Hypertension* **1997**, *30*, 1128–1134.
282. Zeng, G.; Nystrom, F.H.; Ravichandran, L.V.; Cong, L.N.; Kirby, M.; Mostowski, H.; Quon, M.J. Roles for insulin receptor, PI3-kinase, and Akt in insulin-signaling pathways related to production of nitric oxide in human vascular endothelial cells. *Circulation* **2000**, *101*, 1539–1545.
283. Vecchione, C.; Aretini, A.; Maffei, A.; Marino, G.; Selvetella, G.; Poulet, R.; Trimarco, V.; Frati, G.; Lembo, G. Cooperation between insulin and leptin in the modulation of vascular tone. *Hypertension* **2003**, *42*, 166–170.
284. Scherrer, U.; Randin, D.; Vollenweider, P.; Vollenweider, L.; Nicod, P. Nitric oxide release accounts for insulin's vascular effects in humans. *J. Clin. Investig.* **1994,** *94*, 2511–2515.
285. Iaccarino, G.; Ciccarelli, M.; Sorriento, D.; Cipolletta, E.; Cerullo, V.; Iovino, G.L.; Paudice, A.; Elia, A.; Santulli, G.; Campanile, A.; et al. AKT participates in endothelial dysfunction in hypertension. *Circulation* **2004**, *109*, 2587–2593.
286. Muniyappa, R.; Montagnani, M.; Koh, K.K.; Quon, M.J. Cardiovascular actions of insulin. *Endocr. Rev.* **2007**, *28*, 463–491.
287. Pieper, G.M.; Siebeneich, W.; Dondlinger, L.A. Short-term oral administration of L-arginine reverses defective endothelium-dependent relaxation and cGMP generation in diabetes. *Eur. J. Pharmacol.* **1996**, *317*, 317–320.
288. Khaidar, A.; Marx, M.; Lubec, B.; Lubec, G. L-arginine reduces heart collagen accumulation in the diabetic db/db mouse. *Circulation* **1994**, *90*, 479–483.
289. Lubec, G.; Bartosch, B.; Mallinger, R.; Adamiker, D.; Graef, I.; Frisch, H.; Hoger, H. The effect of substance L on glucose-mediated cross-links of collagen in the diabetic db/db mouse. *Nephron* **1990**, *56*, 281–284.
290. Lubec, B.; Hayn, M.; Kitzmuller, E.; Vierhapper, H.; Lubec, G. L-Arginine reduces lipid peroxidation in patients with diabetes mellitus. *Free Radic. Biol. Med.* **1997**, *22*, 355–357.
291. Pieper, G.M. Review of alterations in endothelial nitric oxide production in diabetes: Protective role of arginine on endothelial dysfunction. *Hypertension* **1998**, *31*, 1047–1060.
292. De Pirro, R.; Tamburrano, G.; Fusco, A.; Lauro, R. Arginine does not influence insulin binding on circulating monocytes. *Endokrinologie* **1980**, *75*, 243–246.
293. Wascher, T.C.; Graier, W.F.; Dittrich, P.; Hussain, M.A.; Bahadori, B.; Wallner, S.; Toplak, H. Effects of low-dose L-arginine on insulin-mediated vasodilatation and insulin sensitivity. *Eur. J. Clin. Investig.* **1997**, *27*, 690–695.
294. Piatti, P.M.; Monti, L.D.; Valsecchi, G.; Magni, F.; Setola, E.; Marchesi, F.; Galli-Kienle, M.; Pozza, G.; Alberti, K.G. Long-term oral L-arginine administration improves peripheral and hepatic insulin sensitivity in type 2 diabetic patients. *Diabetes Care* **2001**, *24*, 875–880.
295. Huynh, N.T.; Tayek, J.A. Oral arginine reduces systemic blood pressure in type 2 diabetes: Its potential role in nitric oxide generation. *J. Am. Coll. Nutr.* **2002**, *21*, 422–427.
296. Lai, Y.L.; Aoyama, S.; Nagai, R.; Miyoshi, N.; Ohshima, H. Inhibition of L-arginine metabolizing enzymes by L-arginine-derived advanced glycation end products. *J. Clin. Biochem. Nutr.* **2010**, *46*, 177–185.
297. Fiory, F.; Lombardi, A.; Miele, C.; Giudicelli, J.; Beguinot, F.; Van Obberghen, E. Methylglyoxal impairs insulin signalling and insulin action on glucose-induced insulin secretion in the pancreatic beta cell line INS-1E. *Diabetologia* **2011**, *54*, 2941–2952.
298. Polykretis, P.; Luchinat, E.; Boscaro, F.; Banci, L. Methylglyoxal interaction with superoxide dismutase 1. *Redox Biol.* **2020,** 30, 101421.

299. Martina, V.; Masha, A.; Gigliardi, V.R.; Brocato, L.; Manzato, E.; Berchio, A.; Massarenti, P.; Settanni, F.; Della Casa, L.; Bergamini, S.; et al. Long-term N-acetylcysteine and L-arginine administration reduces endothelial activation and systolic blood pressure in hypertensive patients with type 2 diabetes. *Diabetes Care* **2008**, *31*, 940–944.
300. Deveaux, A.; Fouillet, H.; Petzke, K.J.; Hermier, D.; Andre, E.; Bunouf, P.; Lantoine-Adam, F.; Benamouzig, R.; Mathe, V.; Huneau, J.F.; et al. A Slow-Compared with a Fast-Release Form of Oral Arginine Increases Its Utilization for Nitric Oxide Synthesis in Overweight Adults with Cardiometabolic Risk Factors in a Randomized Controlled Study. *J. Nutr.* **2016**, *146*, 1322–1329.