Arginase: Biological and Therapeutic Implications in Diabetes Mellitus and Its Complications

Yuanyuan Ren,1,2 Zhuozhuo Li,1,2 Wenqing Li,1 Xiaobin Fan,3 Feifei Han,4 Yaoyao Huang,1 Yi Yu,1,2 Lu Qian,1,3 and Yuyan Xiong1,2

1Xi’an Key Laboratory of Cardiovascular and Cerebrovascular Diseases, Xi’an No.3 Hospital, The Affiliated Hospital of Northwest University, Faculty of Life Sciences and Medicine, Northwest University, Xi’an, Shaanxi, China
2Key Laboratory of Resource Biology and Biotechnology in Western China, Ministry of Education, School of Medicine, Northwest University, Xi’an, Shaanxi, China
3Department of Obstetrics and Gynecology, Xi’an No.3 Hospital, The Affiliated Hospital of Northwest University, Northwest University, Xi’an, Shaanxi, China
4Department of Endocrinology, Xi’an No.3 Hospital, The Affiliated Hospital of Northwest University, Northwest University, Xi’an, Shaanxi, China

Correspondence should be addressed to Yi Yu; yiyu@nwu.edu.cn, Lu Qian; 2640933799@qq.com, and Yuyan Xiong; yuyan.xiong@nwu.edu.cn

Received 18 August 2022; Accepted 18 October 2022; Published 26 October 2022

Academic Editor: Liang-Jun Yan

Copyright © 2022 Yuanyuan Ren et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Arginase is a ubiquitous enzyme in the urea cycle (UC) that hydrolyzes L-arginine to urea and L-ornithine. Two mammalian arginase isoforms, arginase1 (ARG1) and arginase2 (ARG2), play a vital role in the regulation of β-cell functions, insulin resistance (IR), and vascular complications via modulating L-arginine metabolism, nitric oxide (NO) production, and inflammatory responses as well as oxidative stress. Basic and clinical studies reveal that abnormal alterations of arginase expression and activity are strongly associated with the onset and development of diabetes mellitus (DM) and its complications. As a result, targeting arginase may be a novel and promising approach for DM treatment. An increasing number of arginase inhibitors, including chemical and natural inhibitors, have been developed and shown to protect against the development of DM and its complications. In this review, we discuss the fundamental features of arginase. Next, the regulatory roles and underlying mechanisms of arginase in the pathogenesis and progression of DM and its complications are explored. Furthermore, we review the development and discuss the challenges of arginase inhibitors in treating DM and its related pathologies.

1. Introduction

Diabetes mellitus (DM), one of the most prevalent chronic metabolic diseases, which leads to life-threatening, disabling, and costly complications and compromises life expectancy [1]. There are two primary forms of DM: type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM). Fundamental pathogenic differences exist in the two types of DM, of which T1DM is insulin-dependent [2]. T2DM is the most common type of diabetes, accounting for more than 90% and is characterized by insulin resistance (IR) and/or β-cell dysfunction [3]. Intriguingly, abnormalities in NO production, inflammatory responses, and oxidative stress have all been implicated in the development and progression of DM and its complications [4]. In the past few decades, researchers have been working to interpret the underlying mechanisms of DM pathogenesis and progression in order to seek effective and efficient therapeutic targets. Nevertheless, the exact mechanism remains elusive.

Arginase is a binuclear manganese-containing metalloenzyme that catalyzes the conversion of L-arginine to L-ornithine and urea in the last reaction of the urea cycle (UC) [5]. Available data have demonstrated the pathophysiological importance of aberrant arginase expression in
hypertension [6], obesity [7], aging [8], diabetes [9], etc. Currently, arginase is being considered by the scientific community as a potential biomarker for the progression and severity of these diseases. In type 2 diabetic patients, plasma arginase activity was increased by 50% in diabetic versus control group [10]. Accumulating studies have revealed that arginase may contribute to the progression of DM and its complications, owing to its regulatory role in β-cell functions [11], IR [12], and vascular dysfunction [13] via mediating L-arginine metabolism, inflammatory responses, and oxidative stress. Alterations of arginase expression and activity have been confirmed in experimental and clinical investigations as a diagnostic tool for the progression of DM and its complications [9, 10, 14–17]. Therefore, arginase may represent an appealing and prospective pharmacological target for the treatment of DM and its complications.

Spurred by this context, here we conducted a thorough review of arginase’s biological functions in diabetes-related pathological processes, as well as its mechanism of action in DM complications. The developmental progress and challenges of arginase inhibitors in treating DM and related complications are also highlighted. Moreover, we offer several potential approaches to tackle the issues concerning the clinical application of arginase as the diagnostic tool and therapeutic target for DM and its complications. We hope this knowledge will help us better understand the functions of arginase in DM pathogenesis and provide a reference for future clinical development of arginase in DM therapy.

2. The Features of Arginase and Its Roles in DM Pathology

2.1. Arginase Isoforms. Arginase, a ubiquitous metalloenzyme with L-arginine hydrolase activity, which has been found in bacteria, yeasts, plants, invertebrates, and vertebrates, plays a critical role in both physiological and pathological conditions [18]. In mammals, there are two distinct isoforms of arginases, arginase1 (ARG1) and arginase2 (ARG2). Despite the fact that both isoforms are present throughout the body and present similar physicochemical properties, they differ in encoding genes, expression patterns, and physiological activities as well as molecular regulation [19]. ARG1, a cytoplasmic enzyme mainly expressed in the liver and also exists in extrahaepatic tissues, is located on chromosome 6q23 and encodes a 322 amino acid protein [20]. Whereas ARG2, a mitochondrial enzyme widely expressed in the kidney and some extrahepatic tissues (such as the heart, blood vessels, prostate, gastrointestinal tract, muscle, and endocrine tissues), is found on chromosome 14q24.1 and encodes a 354 amino acid protein [21]. The different biochemical environments of tissues favor the complementary roles of these two isomers in the body. ARG1 primarily functions in the UC to remove toxic ammonia and fight inflammation. ARG2 has been shown to modulate cellular L-arginine metabolism, polyamine synthesis, NO homeostasis, and proinflammation as well as oxidative stress [22].

2.2. L-Arginine Metabolism. Arginase catalyzes the conversion of L-arginine to L-ornithine and urea to dispose of toxic ammonia in the last step of UC. L-ornithine is further metabolized by ornithine decarboxylase (ODC) to synthesize polyamines (putrescine, spermidine, and spermine) which are involved in β-cell dysfunction, IR, and proinflammation, or catalyzed by ornithine aminotransferase (OAT) to form L-proline that mediates β-cell dysfunction and IR [23] (Figure 1). In both diabetic rats [24–27] and human patients [15, 28, 29], plasma arginine concentrations were markedly decreased, which might be positively correlated with the upregulation of arginase in DM [30]. Experimental and clinical data confirmed that L-arginine supplementation might be helpful in improving insulin secretion [31] and insulin sensitivity [32] as well as glucose tolerance [33]. Urea, another metabolite of arginase-mediated L-arginine metabolism, has been demonstrated that it is implicated in β-cell dysfunction, insulin sensitivity reduction, and glucose intolerance [34, 35]. A clinical study found that elevated blood urea nitrogen (BUN) levels significantly increased the risk of incident T2DM in humans [36]. Compared to healthy subjects, the level of salivary urea was elevated in diabetic patients [37]. Moreover, increased serum levels of urea were observed to be significantly associated with the severity of diabetic retinopathy (DR) [38]. In mammals, L-ornithine is a crucial precursor for polyamines and L-proline biosynthesis. Elevated ornithine was recently reported to be specifically correlated with an increased risk of T2DM [39]. In T2DM patients with dysregulated polyamine metabolism, serum putrescine and spermine levels are significantly elevated [40]. Accumulated polyamines have been shown to promote the pathogenesis of T1DM via inducing β-cell dysfunction and enrichment of proinflammatory immune cells [41, 42]. Furthermore, impaired glucose-stimulated insulin secretion was also observed in transgenic mice with hyperactivation of the polyamine catabolic pathway [43]. Increased polyamine synthesis exacerbates DM complications in the kidney [44] and liver [45] of diabetes animal models. L-proline can be partially synthesized from L-ornithine. It has also been discovered to be elevated in T2DM patients [46], and excessive L-proline contributes to β-cell dysfunction [47] and insulin resistance (IR) [48]. L-citrulline, a product of nitric oxide synthase (NOS) catalyzing L-arginine, has been shown to improve IR, which is associated with enhanced insulin sensitivity [49]. In rat β-cells, L-citrulline at a physiologic concentration increased glucose-stimulated insulin release [50]. Considering the essential role of arginase in the metabolism of L-arginine, abnormal arginase activity and expression are doomed to influence the progression of DM.

2.3. Arginase and NO Production. L-arginine also serves as a specific substrate for nitric oxide synthase (NOS), which metabolizes explicitly L-arginine to produce L-citrulline and NO [51] (Figure 1). Consequently, under conditions of excessive arginase activity, it will compete with NOS for L-arginine, eventually leading to NOS uncoupling, producing less NO and more superoxide [52, 53]. Arginase-mediated removal of L-arginine is also able to suppress inducible
Physiologically, NO is essential for maintaining insulin secretion, improving insulin sensitivity, and promoting vascular health. Whereas, under pathological conditions, NO has been implicated in the development of insulin resistance, insulin sensitivity reduction, and glucose intolerance. Meanwhile, NOS metabolizes arginine into L-citrulline and NO. Physiologically, NO is essential for maintaining insulin secretion, improving insulin sensitivity, and promoting vascular health. Whereas, under pathological conditions, NO has been implicated in the development of β-cell dysfunction, IR, vascular dysfunction, proinflammatory responses, etc. L-citrulline has also been shown to protect β-cell function and improve IR. ODC: ornithine decarboxylase; OAT: ornithine aminotransferase; IR: insulin resistance; NOS: nitric oxide synthase; NO: nitric oxide; CAT: cationic amino acid transporter.

2.4. Arginase Mediates Inflammation. DM and its complications are inflammatory diseases [66]. Over the past few years, increasing solid evidence has demonstrated that arginase is involved in mediating pro- and anti-inflammatory responses linked to the pathology of DM and its complications. ARG1, mainly expressed in M2-like macrophages, protects inflammatory tissue from damage and clears pathogens by decreasing intracellular iNOS bioavailability of L-arginine [67]. In rat β-cells and RINm5F cells, inhibition of ARG1 expression resulted in aggravation of insulinitis, which is an inflammatory lesion and a pathologic hallmark of T1DM [68, 69]. Transactivation of macrophage ARG1 drives an anti-inflammatory M2 phenotype, which lowers inflammation, promotes white adipose tissue (WAT) beiging, and maintains metabolic homeostasis in WAT, thereby reducing the risk of obesity-related DM [70] (Figure 2). Whereas, the elevation of ARG1 in ECs induces eNOS uncoupling that limits NO production and enhances reactive oxygen species (ROS) generation, resulting in a proinflammatory response [71] (Figure 2). In high-fat-high sucrose- (HFHS-) stimulated obesity mice, endothelial-specific ARG1 knockout attenuates obesity-induced adipose tissue inflammation via maintaining endothelial NO levels [72]. By contrast, ARG2 appears to function as a proinflammatory M1-like phenotype [67]. Our previous studies in vitro and in vivo showed that targeted disruption of the ARG2 gene prevents high-fat diet- (HFD-) induced IR.
by suppressing the proinflammatory response of macrophage in mice [73] (Figure 2). In the aging-associated T2DM mice model, ARG2 is mainly expressed in acinar cells and upregulated with aging, which promotes tumor necrosis factor-α (TNF-α) release from pancreatic acinar cells, ultimately resulting in β-cell apoptosis and subsequent reduction of insulin secretion [11]. In adipose tissue and ECs, disruption of ARG2 reduces aging-related inflammation [74, 75]. In mice model, ARG2 deletion prevents HFHS-induced collagen deposition and visceral adipose tissue (VAT) inflammation, enhances adipocyte metabolism, and improves IR [76] (Figure 2). Moreover, ARG2 deficient mice have been shown
to protect HFD-induced DM complication (hepatic steatosis) via inhibition of liver macrophage-mediated proinflammatory responses [77]. As L-arginine displays anti-inflammatory effects, aberrant arginase expression and activity induces the dysregulation of intracellular L-arginine, which is essential for pancreatic β-cell functional integrity, metabolism, and defense from an inflammatory challenge, thereby modulating insulin sensitivity and secretion [78]. These findings uncover that the two isoforms of arginase exert different functions in regulating inflammatory responses, contributing to the pathological progression and prognosis of DM and its complications.

2.5. Arginase and Reactive Oxygen Species (ROS). Reactive oxygen species (ROS) is thought to be one of the culprits to the induction and progression of DM and its complications, owing to an excess of ROS that causes oxidative stress, which promotes β-cell dysfunction, IR, and vascular dysfunction by activating multiple cellular stress-sensitive signaling pathways [66, 79]. To date, the emerging evidence suggests that arginase regulates ROS generation upon various pathological stimuli, which further modulates the progression of DM and its complications in particular. In streptozotocin (STZ-) induced diabetic rat model, significantly increased arginase activity and ROS levels were observed. In contrast, suppression of arginase by almond treatment remarkably ameliorated blood glucose levels and vasculogenic erectile dysfunction via the reduction of ROS production [80]. Red blood cells (RBCs) from T2DM patients display higher levels of arginase activity and ARG1 protein expression, which can induce endothelial but not smooth muscle cell dysfunction in both healthy rat aortas and human internal mammary arteries through a ROS-dependent manner [81]. Diabetic mice and retinal ECs treated with high glucose (HG) or H₂O₂, showed prominent increases in ROS formation and ARG1 expression and activity, which lead to ECs premature senescence [82]. Our previous study discloses that obesity-induced ARG2 upregulation enhances mitochondrial ROS production, subsequently accelerating the development of obesity-associated IR [73] (Figure 2). Urea, as a crucial metabolite of arginase-catalyzed arginine, is infused in normal mice. Urea, as a crucial metabolite of arginase-catalyzed arginine, is infused in normal animals has been shown to induce IR and elevation of IR-related adipokines as a consequence of excessive ROS generation [83]. Additionally, arginase inhibition boosting endogenous NO production helps to dissipate ROS and promote β-cell survival, leading to the amelioration of insulin release [84]. However, overproduced NO may react with ROS to generate peroxynitrite, which triggers β-cell dysfunction and death [85], contributing to the onset of DM in non-obese diabetic (NOD) mice [86]. In this context, the delicate interaction between arginase and ROS may represent a novel mechanism of DM and its complications pathogenesis.

3. Roles of Arginase in the Regulation of β-Cell Function and IR

3.1. Arginase and β-Cell Function. Destruction or dysfunction of insulin-producing pancreatic β-cells persists throughout the pathological course of T1DM and T2DM. Accumulating evidence demonstrates that arginase is implicated with DM development via the mediation of β-cell functions [87]. Immunohistochemical analysis of mice pancreas showed that two arginase activities were indeed present in the pancreas. ARG1 but not ARG2 was detected in islets, and ARG2 was moderately expressed in acini [88]. Constitutive arginase activity and ARG1 are detected in freshly isolated rat islets of Langerhans and RINm5F cells [89]. However, compared to ARG1, ARG2 is dominantly expressed in human pancreatic islets [90]. In various models, arginase has been suggested to directly or indirectly modulate β-cells function through regulating inflammatory response, NO production, and L-arginine metabolism. For example, ARG1 has been shown to modulate proinflammatory cytokines (IL-1 and IFN-γ) induced β-cells apoptosis and dysfunction via the excessive NO production from iNOS activation [69, 90, 91] (Figure 2). In our previous study, upregulated ARG2 expression in acinar cells during aging activates p38 MAPK, which induces the release of paracrine TNF-α, resulting in the β-cell apoptosis and insufficient insulin secretion, contributing to the aging-associated glucose intolerance [11] (Figure 2). Fu et al. found that in arginase-mediated ureagenesis diminishes arginase utilization for producing NO, which protects β-cells from inflammation and death [92]. Polyamines, the metabolite of arginase-catalyzed arginine, were found to be restricted to the insulin-producing β-cells; its deletion in mouse models of STZ-induced T1DM can protect islet β-cell from inflammation-induced dysfunction and destruction [93, 94] (Figure 2). Recently, β-cells regeneration is expected to offer a novel therapy for DM. In alloxan-induced diabetic rats, targeting neuronal nitric oxide synthase (nNOS) in arginine metabolite pathway ameliorates blood insulin and glucose levels in a manner of stimulating β-cell neogenesis via activating pancreas duodenum homeobox-1 (PDX-1) and nuclear factor-kappa-B (NF-κB) [95] (Figure 2). Besides, inhibiting polyamine biosynthesis by either α-difluoromethylornithine (DFMO) (NCT02384889) or imatinib (NCT01781975) could also enhance β-cell regeneration in the setting of DM [96]. This compelling evidence reveals the important implications of arginase in the regulation of β-cell mass and function.

3.2. Arginase and Insulin Resistance. Insulin resistance (IR), also known as damaged insulin sensitivity, is a fundamental aspect of the etiology of T2DM and is also linked to obesity [97]. Over the past decade, arginase has been verified to be implicated in the development of IR. In epididymal white adipose tissue (eWAT), abnormal ARG1 expression induced by an imbalance of M1- and M2-macrophage proportions is able to provoke adipose tissue dysfunction and obesity-related IR [58]. In HFD mice, upregulation of ARG1 reduces infiltration of macrophages in adipose tissue and facilitates polarization of macrophages to M2, thus alleviating obesity and improving insulin sensitivity [98]. Additionally, exosomes from adipose-derived stem cells (ADSCs) facilitate immune and metabolic homeostasis in WAT through the transactivation of ARG1 by exosome-carried active STAT3, thereby relieving obesity-related IR [70] (Figure 2). ARG2, also has been found to be upregulated in obesity mice, which
contributes to IR via the promotion of hydrogen peroxide production and proinflammatory responses. Furthermore, ARG2-deficient mice showed lower fasting blood glucose and improved glucose tolerance and insulin sensitivity [73]. In obese Zucker rats (ZR) with IR, arginase inhibition enhances insulin sensitivity [12]. More important, in clinical practice, elevated arginase activity is detected in the plasma of T2DM patients, while IR causes a decrease in NOS activity through producing methylated arginine [10]. These studies indicate that arginase may represent a promising therapeutic target for ameliorating obesity-associated IR. Nevertheless, the underlying mechanisms of ARG1/ARG2 modulate IR still require further investigation.

4. Arginase in DM Complications

DM, not a single disease, is also strongly associated with both microvascular and macrovascular complications, including macrovascular diseases (cardiovascular disease, CVD) and microvascular diseases (diabetic nephropathy, retinopathy, and wound-healing disorder), leading to the major cause of morbidity and mortality in individuals with DM [99]. To date, etiologies of DM vascular complications have not yet been fully elucidated. Most notably, both ARG1 and ARG2 have been identified as crucial modulators in the pathogenesis of DM complications [52, 100–102], and targeting arginase is capable of improving macrovascular and microvascular complications in DM patients [15, 16, 103, 104] (Table 1) (Figure 3).

4.1. Arginase and Diabetic Cardiovascular Disease. CVD increases 2-4 times in adults with DM, and the risk increases dramatically with worsening glycemic control. Increased activity and expression of arginase have been reported to exacerbate pathological diabetic CVD, such as coronary artery disease (CAD), ischemia-reperfusion (I/R) injury, and hypertension, by lowering NO generation, boosting ROS production, and proinflammation [21, 105]. Clinically, ARG1 is found in the walls of coronary arterioles in T1DM or T2DM patients but not in the nondiabetic group [15]. In the diabetes-related HG model, upregulated ARG1 induced eNOS uncoupling through the sequential activation of RhoA/Rho kinase (ROCK) and p38 mitogen-activated protein kinases (p38 MAPK) in mouse aortic and bovine aortic endothelial cells (BAECs), contributing to the development of diabetes/hyperglycemia-induced vascular endothelial dysfunction [106, 107] (Figure 2). In addition, sequential activation of low-density lipoprotein receptor-1 (LOX-1), c-Jun N-terminal kinase (JNK), and ARG1 induces ROS-dependent oxidative stress and impairs coronary arteriolar function during DM [108] (Figure 2). In STZ-induced diabetic Wistar rats, activation of p38 MAPK promotes DM-induced endothelial dysfunction via selectively upregulating the expression of ARG1 in coronary arteries and the expression of ARG2 in mesenteric arteries [109]. Our group also found that increase of ARG2 promoted eNOS uncoupling and vascular dysfunction via the activation of p38 MAPK in HFD-induced obesity mice, which could be prevented by ARG2 gene knockout [110]. ARG2 expression is significantly enhanced in the aorta and myocardium of Goto-Kakizaki (GK) rats with T2DM. Disrupting ARG2 activity by arginase inhibitor restores coronary microvascular function through a mechanism related to the increased NO availability [111]. Importantly, a clinical study showed that arginase inhibition improved isolated coronary dilation and protected against endothelial dysfunction caused by I/R in DM patients with CAD [17]. Hypertension is also commonly associated with DM. Increased vascular ARG1 expression and arginase activity have been associated with higher blood pressure in numerous experimental models of hypertension [112]. STZ-induced DM is accompanied by the elevation in systolic and diastolic blood pressure and arginase activity. In contrast, arginase inhibition mitigates DM-induced hypertension through preventing the impairment of endothelial-dependent relaxation and NO production [113]. Therefore, arginase might be considered as a novel marker for the diagnosis of DM vascular complications.

4.2. Arginase and Diabetic Nephropathy. Diabetic nephropathy (DN) is one of the terrifying chronic microvascular complications of DM and the leading cause of end-stage renal disease (ESRD) [114]. Inflammation and mitochondrial dysfunction have been identified as the key pathogenic factors in DN development [115]. ARG1 is reduced in STZ-administered diabetic kidneys. Inducing ARG1 expression in renal macrophages can prevent the progression of DN via alleviating inflammation and mitochondrial dysfunction in tubular epithelial cells (TECs) [116]. Macrophage-specific deletion of ARG1 reduces macrophage infiltration but does not affect albuminuria as an early DN marker in STZ-induced DM [117]. On the contrary, after 6 and 18 weeks of STZ administration, kidney arginase activity and ARG2 exhibited significant elevation in wild-type (WT) mice, which was associated with a reduction in renal medullary blood flow and diabetic renal injury [118] (Figure 3). ARG2 expression was also increased in the renal cortex of HFD-induced obese mice. Inhibition of ARG2 was able to protect mouse kidneys from proinflammatory responses to ameliorate DN [119]. Significantly, pharmacological blockade or genetic deficiency of ARG2 reduced proteinuria levels and renal histopathological changes and lowered blood urea nitrogen and macrophage recruitment, thereby slowing down the development of DN [118, 120] (Figure 3). Further research disclosed that arginase inhibition protects renal tissue in DN via an eNOS-dependent mechanism while simultaneously having an eNOS-independent effect on renal macrophage recruitment [121]. Thus, targeting arginase, particularly ARG2, could be a new potential therapeutic intervention for DN treatment.

4.3. Arginase and Diabetic Retinopathy. Diabetic retinopathy (DR) is a common microvascular disorder of DM and a leading cause of blindness. The bulk of accumulating studies suggest that arginase is involved in the mediation of DR pathophysiological progression. Recently, a clinical study claims that ARG1 rs2781666 single nucleotide polymorphism (SNP) is substantially linked to DR susceptibility in T2DM patients [122]. Retinal ECs senescence under HG
condition is the main pathomechanism of DR. Retinal ECs treated with HG or H₂O₂ showed prominent increases in arginase expression and activity, which evoked retinal ECs senescence through a mechanism related to NADPH oxidase-2 (NOX2-) generated ROS and decrease in NO bioavailability, hastening the onset of DR [82]. In a mice model, STZ-induced DM promoting the increase in ARG1 expression accelerated retinal ECs senescence, which could be prevented by ARG1 gene deletion or pharmacological inhibition [123] (Figure 3). Elms et al. also found that the diabetes-induced vascular dysfunction was markedly attenuated in mice with heterogeneous ARG1 gene deletion (ARG1+/-) and in mice treated with arginase inhibitors [124]. Retinal ARG2 was similarly upregulated in HFHS

Table 1: The dysregulated expression of arginase in DM complications.

| DM complications | Location | Arginase expression | Species/region | Effect of arginase | Inducer or activator | Refs. |
|------------------|----------|---------------------|----------------|-------------------|---------------------|-------|
| Diabetic Cardiovascular disease | Peripheral blood leukocytes | ARG1 | Patients with DM | Increased susceptibility to diabetic retinopathy | ARG1 rs2781666 single nucleotide polymorphism (SNP) | [122] |
| | Retinal EC | ↑ARG1 | Mice retinal vessels | Accelerated retinal ECs senescence | Increased in DM | [123] |
| | Central retinal artery | ↑ARG1 | Rat CRA | Impaired endothelial-dependent vasodilation responses | – | [124] |
| | Retinal EC | ↑ARG2 | BREC | Increased retinal oxidative stress and inflammation | Increased in HFHS-diet | [125] |
| Diabetic Nephropathy | Macrophages | ↓ARG1 | Mouse renal tissues | Promote inflammation and mitochondrial dysfunction | – | [116, 117] |
| | Renal cortex | ↑ARG2 | Mouse mesangial | Promote inflammation | Increased in obese | [119] |
| | Kidney tissue | ↑ARG2 | Mice kidney | Increased blood urea nitrogen and macrophage recruitment | – | [118] |
| Diabetic Retinopathy | Epidermal keratinocytes | ↓ARG1 | Mice wound tissue | Induced wound healing impairment | Increased in DM | [133] |
| | Cavernosal tissue | ↑ARG2 | Human cavernosal tissue | Decreased NO generation and CC relaxation | – | [138] |
| | | ↑ARG2 | Mice cavernosal tissue | Decreased CC relaxation | Activated by ERK pathway | [158] |
diet-induced retinopathy mice model, and depletion of ARG2 was protected against the western diet-induced retinopathy via the suppression of retinal oxidative stress and inflammation [125]. Researchers studying DR patients’ metabolomics found that arginine and proline dysregulated metabolism was associated with proliferative diabetic retinopathy (PDR) [126, 127]. Besides, spermine, as an arginase-modulated metabolite, is dramatically elevated in vitreous samples from patients with PDR [128]. Studies by Narayanan et al. and Liu et al. disclosed that diabetes-induced upregulation of spermine oxidase (SMOX) leads to the oxidation of spermine to spermidine, resulting in the increase in reactive aldehydes and H₂O₂, which are further converted to acrolein, resulting in retinal neuronal damage and dysfunction [129, 130]. Thus, arginase mediated the metabolism of arginine and proline, and polyamine metabolism might also contribute to the pathogenesis of DR. However, the underlying mechanism that arginase-related metabolites regulate DR development still requires further investigations.

4.4. Arginase and Diabetic Wound-Healing Disorder. Diabetic wound healing disorder, e.g., diabetic foot ulcer, is a severe complication of DM with significant morbidity and mortality, as wound healing or skin repair impairment occurs at the diabetic wound sites [131]. Arginase is expressed in a variety of wound-healing cell types, including epithelial cells, fibroblasts, polymorphonuclear cells, and macrophages [132, 133]. Convincing clinical studies showed the considerable elevation of arginase activity and protein expression in diabetic ulcers, which influences the characteristic callus formation around these ulcers [134] (Figure 3). In db/db and ob/ob diabetic mice with severe wound healing disorders, both ARG1 and ARG2 isoforms mRNA expressions and arginase activity were strongly upregulated upon injury, which paralleled the expressional and activity kinetics of the iNOS. Conversely, leptin administration reduced the overall arginase activity in healing wounds, which causes a readjustment of arginases and iNOS at the wound site, improving healing [133]. After surgery, wound closure is accelerated by inhibiting arginase activity using an arginase inhibitor via hastening re-epithelialization and localization of myoblasts beneath the wound epithelium [135]. Notably, subcutaneous injection of arginine into the foot ulcer of diabetic patients improved local blood circulation and promoted wound healing by increasing NO-dependent blood flow and nutrient supply [136]. These studies substantially support the notion that arginase plays a vital role in

![Diagram of DM complications and risk factors associated with upregulation of arginase](image)
regulating DM-associated wound healing through the regulation of NO production, inflammatory responses, or L-arginine metabolism.

4.5. Arginase and Diabetic Erectile Dysfunction. Erectile dysfunction (ED), another complication of diabetic vascular dysfunction, has a three-fold increased risk in people with diabetes compared to healthy men [137]. Both ARG1 and ARG2 are expressed in the corpus cavernosum (CC); their expression and arginase activities appear to be dysregulated in CC of diabetic individuals with ED [138–140]. Increased ARG2 but not ARG1 expression in DM patients’ CC tissue, along with decreased NO generation and CC relaxation, has been found to contribute to ED [138] (Figure 3). In the animal model, the CC of WT diabetic mice displayed the enhanced arginase activity and ARG2 protein expression and the reduced phospho-eNOS at Ser-1177, while deletion of the ARG2 gene or pharmacological inhibition of arginase dramatically improved the nitricergic and endothelium-dependent relaxation in CC of diabetic mice [139]. Mechanistically, increased arginase activity caused the reduction in NO production in the cavernous tissue of DM, leading to the impairment of endothelial function and nitrogen function [141]. Additionally, activated RhoA/Rho kinase (ROCK) mediates diabetes-induced elevation of arginase activity and activity, which contributes to impaired CC relaxation probably through the activation of p38 MAPK [140]. Undeniably, targeting arginase, particularly ARG2, may represent a new approach to preventing diabetic ED [142].

5. Arginase Inhibitors for DM and Its Complications Therapy

Arginase inhibitors mainly comprise chemical and natural compounds. Their effects have been evaluated in DM and its complications, among which chemical arginine inhibitors include N-omega-hydroxy-L-arginine (NOHA) and its analog, 2(S)-amino-6-boronohexanoic acid (ABH), S-(2-boron)methyl-cysteine (BEC), and α-difluoromethylornithine (DFMO), of which natural arginine inhibitors comprise amino acids, polyphenolic compounds, and traditional Chinese medicine (TCM) herbs (Table 2).

5.1. Chemical Arginine Inhibitors. NOHA and nor-NOHA, hydroxy derivatives of arginine, are both reversible and competitive inhibitors of arginase. NOHA, a transition intermediate of NO from arginine catalyzed by NOS, is a competitive inhibitor with $K_i$ of 3.6 μM (pH 8.5) for human ARG1 [143] and with $K_i$ of 1.6 μM (pH 7.5) for human ARG2 [144]. In diabetic patients, arginase suppression with NOHA markedly improved coronary endothelium-dependent vasodilation [15]. nor-NOHA, a derivate of NOHA, with a longer half-life and higher affinity for arginase [145], binds to human ARG1 with $K_i$ of 0.517 μM (pH 8.5) [143] and inhibits human ARG2 with $K_i$ of 51 nM (pH 7.5) [144].

In the obese Zucker rats (ZR) model, arginase inhibition by nor-NOHA ameliorates obese-induced IR and prevents the development of hypertension, while L-arginine administration only attenuates hypertension [12]. Administration of nor-NOHA in RBCs from T2DM patients has been shown to reduce ROS generation and cardiac injury postischemia-reperfusion in db/db mice [146]. Treatment with nor-NOHA for 24 days, the citrulline-NO pathway was upregulated, while the incidence of autoimmune diabetes was reduced in elderly diabetic female NOD mice [147]. Similarly, nor-NOHA administration protects I/R-induced cardiac impairment in T1DM [148]. In the registered clinical trial (NCT02009527), nor-NOHA administration suppresses the elevated arginase activity in coronary artery disease (CAD) patients with T2DM remarkably improved endothelial function following I/R, and no side effects were reported [17]. DM impairs endothelium-dependent dilation of retinal arterioles, while nor-NOHA significantly improves endothelial function of retinal arterioles in the STZ-induced diabetes pig model [149].

ABH and BEC, two boronic acid analogs, are highly selective and competitive arginase inhibitors that bind to the active manganese cluster site of arginase [150], with $K_i$ value of 0.11 mM and 0.4–0.6 mM, respectively [151]. ABH inhibits human ARG1 with IC50 of 1.54 μM and 2.55 μM on human ARG2 [152]. BEC binds to human ARG1 with $K_i$ of 270 nM and $K_i$ of 30 nM for human ARG2 [153, 154]. In T2DM patients, plasma arginase activity is significantly elevated and accompanied by reduced NO production and impaired vasorelaxation, while inhibition of arginase by ABH (100 μmol/L) restored these alternations to normal [155]. Administration with ABH for 18 hours prevented endothelium-dependent relaxation (EDR) injury induced by T2DM erythrocytes in rat aorta [81]. Inhibition of ARG1 with ABH therapy avoided the decline of NO and significantly reduced the incidence of diabetes and obesity-induced bone complications [156]. In the diabetic mouse model, BEC treatment markedly improved endothelium-dependent vasorelaxation of the aortas [157]. Of note, BEC administration can also prevent the progression of established DN through the eNOS-dependent mechanism [118, 120, 121]. For DR, ABH and BEC have been shown in vivo and in vitro studies to reduce oxidative stress and alleviate diabetes-induced retinal blood flow impairment [124]. Besides, BEC was able to improve cavernosal relaxation in STZ-diabetic mice [158].

DFMO, an irreversible mixed inhibitor of arginase and ODC, has an inhibitory effect on arginase ($K_i$ of 3.9 ± 1.0 mM on HT-29 homogenate arginase) [159]. Its administration significantly improved the diabetic endothelial-dependent vasodilatory response via inhibiting arginase activity [52].

5.2. Natural Arginine Inhibitors. A portion of the natural amino acids have been discovered to effectively decrease arginase activity, preventing diabetes and its complications. L-citrulline, an amino acid present in watermelon [160], has been reported to be an allosteric inhibitor of bovine liver arginase with 53% inhibition at 20 mM [161]. L-citrulline administered hepatoma H4IIE cells, and SHRSP.Z-Leprf/a/IzmDmcr rats presented the improvement in insulin sensitivity [49]. Clinically, T2DM patients taking L-citrulline supplements (2000 mg/day) for one month have been shown to
Table 2: Interventional studies with arginase inhibitors in DM and DM complications.

| Chemical arginase inhibitors | Arginase inhibitors | Chemical class | Dose range | Models | Effects | Refs. |
|------------------------------|---------------------|----------------|------------|--------|---------|-------|
| **NOHA**                     |                     |                |            |        |         |       |
|                              |                     |                | 10 μmol/L, 30 min (ex vivo) | Patients with DM | Restoration of endothelium-dependent agonist-induced dilation in coronary arterioles of patients with DM | [15] |
|                              |                     |                | 25 mg/kg/day | Zucker rats with obese | Prevention in the development of hypertension | [12] |
|                              |                     |                | 4 weeks (Ip) | | Reduction in body weight and insulin resistance | [12] |
|                              |                     |                | 1 and 3 mmol/L, 20 min (ex vivo) | RBCs from diabetes mice | Improvement in postischemic-myocardial function | [153] |
|                              |                     |                | 30 mg/kg, 24 days (Ip) | Female mouse with T1DM | Reduction in the incidence of autoimmune diabetes | [154] |
| nor-NOHA                     |                     | NO-OH-based arginine analog | 0.1 mg/min, 20 min (Ia) | Male patients with CAD or CAD+T2DM | Improvement in endothelial function following ischemia-reperfusion | [17] |
|                              |                     |                | 0.1 mmol/L, 90 minutes intraluminal | Pigs with DM | Improvement in endothelial function of retinal arterioles | [149] |
|                              |                     |                | 100 mg/kg, 15 min (Iv) | Rat with T2DM | Improvement in myocardial microvascular function | [111] |
|                              |                     |                | 1 mL/min, 2 h (Ia) | Patients with type 2 diabetes +CAD | Improvement in endothelial function irrespective of glucose-lowering regimen | [16] |
| ABH                          |                     | Boronic acid-based arginine analog | 0.1 mg/min, 2 h (Ia) | Patients with T2DM and microvascular dysfunction | Improvement in endothelium-dependent microvascular dilatation | [104] |
|                              |                     |                | 8 mg/kg, 5 days (Sc) | Mouse with T1DM | Improvement in retinal endothelial function | [124] |
| Bec                          |                     |                | 10 mg/kg/day, 1 month (Po) | Mice with obesity and T2DM | Prevention in diabetic bone complications | [156] |
|                              |                     |                | 50 μmol/L, 30 minutes (ex vivo) | Mouse with T1DM | Improvement in endothelial function | [139] |
|                              |                     |                | 2.3 mg/kg/day, 6-12 weeks (Sc) | Mouse with T1DM | Protection of kidney tissue by eNOS-dependent | [120, 121] |
|                              |                     |                | 100 μmol/L, 45 min (ex vivo) | Mouse and rat with DM | Recruitment in kidney macrophage by eNOS-independent | [121] |
|                              |                     |                | 10-4 mol/L (ex vivo) | Rat with T1DM | Improvement in retinal vascular endothelial function | [124] |
| DFMO                         |                     | L-ornithine analog | 50 μmol/L, 1 h (ex vivo) | Rat with T1DM | Improvement in cavernosal relaxation | [158] |
| **Natural arginase inhibitors** |                     |                | 250 μmol/L, 1 h (ex vivo), 2 g/kg/day, 8 weeks (Po) | H4IE cell and SHRSP Z-Lprfα/ZmDmcr rats | Improvement in insulin sensitivity | [49] |
| L-citrulline                 |                     |                | 2000 mg/day, 1 month (Po) | Patients with T2DM | Improvement in H1Ac levels | [162] |
|                              |                     |                | 1 mmol/L (ex vivo) | Rat with T1DM | Improvement in endothelial function | [52] |
| L-norvaline                  | L-valine analog     |                | 20 mg/kg/day, continuing every third day for five weeks (Ip) | Mice with HFD/DM | Reduction in hypertension | [113] |
|                              |                     |                | 10 mg/kg, 30 days (Ip) | Adult male rats with DM | Reduction in blood glucose levels | [166] |
| Quercetin                    | Polyphenolic compounds | Moringa oleifera | 5% of Moringa concentrate (MC), 12 weeks | Mice with VHFD | Improvement in the diabetic sexual impairment | [168] |
|                              |                     |                | 4% of Moringa oleifera, 14 days | Male rats with DM | Remission in diabetic retinopathy | [173] |
|                              |                     |                |                        |                    | Improvement in glucose tolerance and insulin sensitivity | [175] |
|                              |                     |                |                        |                    | Improvement in diabetic-induced ED | [176] |
| Arginase inhibitors | Chemical class         | Dose range                        | Models            | Effects                                                   | Refs. |
|---------------------|------------------------|-----------------------------------|-------------------|-----------------------------------------------------------|-------|
| Semen cuscutae      | Traditional Chinese medicine | 0.5–10 μg/mL (ex vivo)            | Rats with HFD    | ↓ Reduction in hepatic lipid metabolism and systemic adiposity | [182] |
| HGWWD               |                        | 60 g/kg/d, 2 weeks (gavage)       | Mice with T1DM   | ↑ Improvement in vascular dysfunction                     | [183] |
| XSF                 |                        | 3 g/kg/d, 6 weeks (gavage)        | Mice with T1DM   | ↓ Prevention in diabetic kidney damage                    | [184] |

Ia: intra-arterial; Ic: Intracoronar; Iv: intravenous; Ip: intraperitoneal; Sc: subcutaneous; Po: peros.
decrease arginase activity by 21% and meanwhile improve glycated hemoglobin (HbA1c) levels and plasma NO production (NCT03358264) [162]. In vitro and vivo studies, treatment with the arginase inhibitor L-citrulline (1 mmol/L) effectively blocked the HG-induced increase in arginase activity and superoxide formation in bovine coronary endothelial cells (BCECs) and reversed diabetes-impaired coronary endothelial cell-dependent vasorelaxation in the STZ-induced diabetic rats model [52]. Excitingly, clinical studies have confirmed that amino acids are capable of producing the minimum side effects compared to other medical treatments [163]. L-norvaline is also a powerful arginase inhibitor and a unique compound with a wide range of biological characteristics [164]. Because of its structural similarities to ornithine, it inhibits NO synthesis via a negative feedback mechanism and significantly increases NO production rate [165]. In HFD/STZ-induced diabetic mice, L-norvaline treatment reduced fasting blood glucose levels by 27.1% when compared with untreated HFD/STZ mice [166]. In fructose-induced metabolic syndrome, L-norvaline administration reduced hyperinsulinaemia and hypertriglyceridaemia without affecting hyperuricaemia or hypercholesterolaemia associated with metabolic syndrome [167]. Recently, it also has been reported to improve vascular function in diabetics by decreasing arginase activity in cavernous tissue and raising NO levels [168]. L-norvaline has minimal side effects, but because of its high water solubility and high half-maximal inhibitory concentration (IC50 of 5.6 mM on rat arginase), its application in blocking the arginase pathway is still unsatisfactory [169]. High water solubility can lead to burst or uncontrolled release, while high IC50 requires high drug loading content of L-norvaline to satisfy high dosage.

Plant-derived molecules that inhibit arginase activity have also been extensively investigated. Quercetin, a bioactive plant flavonol compound, exhibits a competitive arginase inhibitory activity and inhibits Leishmania arginase with IC50 of 3.8 μM [170]. In cultured skeletal muscle cells, it stimulated glucose uptake through an insulin-independent mechanism involving the activation of adenosine monophosphate-activated protein kinase (AMPK) signaling pathway [171], which is consistent with our previous study that overexpressed ARG2 inhibited the AMPK phosphorylation in ECs [172]. Treatment with a nanoformulation of quercetin for 21 days alleviated DR in zebrafish by reducing arginase activity [173]. *Moringa oleifera*, an important natural source of phenolic compounds that can inhibit rat arginase with IC50 of 159.59 μg/mL [174], is an effective dietary food for the prevention and treatment of obesity and T2DM [175]. Supplementation of 5% *Moringa* in a very high-fat diet (VHFD) fed C57BL/6L mice significantly improved glucose tolerance and insulin sensitivity compared to VHFD-fed mice [175]. In addition, treatments of diabetic rats with *Moringa oleifera* had beneficial effects on the management of ED caused by DM [176]. Mechanistically, *Moringa oleifera* inhibiting arginase activity promotes the production of NO in penile tissue. Moreover, the clinical trials evaluating the effects of *Moringa oleifera* in patients with T2DM are undergoing [177–179].

With a history of over 2000 years, traditional Chinese medicine (TCM) has developed into a unique system for treating various diseases; TCM herbs show protective effects against DM and its complications by modulating arginase expression and activity. These herbs contain multiple biological molecules, which interact with each other and produce synergistic effects that strengthen therapeutic efficacy and lower the toxicity of individual herbs [180]. *Semen cuscutae* (SC), a well-known Chinese medicine extracted from the mature dried seeds of *Cascuta chinensis* Lam, owns various biological properties, including antioxidant and anti-inflammation [181]. In HFD-induced obese mice, SC treatment remarkably inhibits HFD-induced increases in arginase activity and weights of liver and visceral fat tissue in a dose-dependent manner to reduce hepatic lipid metabolism and systemic adiposity via the suppression of hepatic arginase [182]. HuangqiGuizhiWuwu Decoction (HGWWD), commonly used for the treatment of diverse cardiovascular and cerebrovascular diseases in mice, was reported to lessen STZ-induced impairment of velocity and pulsatility of left femoral arteries; aortic pulse wave velocity and vascular relaxation enhance NO production in the aorta and plasma, as well as blunt endothelial arginase activity and aortic ARG1 expression [183]. In the type 1 DN mice model, Xiao-Shen-Formula (XSF) treatment improved STZ-induced renal hyperfiltration, glomerulosclerosis, and renal microvascular remodeling and prevented the increased of oxidative stress and inflammatory cytokines releases by ablating the increased levels of ARG2 protein and arginase activity, which was comparable to that of ABH treatment alone [184].

### 6. Concluding Remarks

Overall, dysregulated arginase expression and activity play a critical role in the onset and development of DM and its complications via the modulation of insulin release, IR, L-arginine metabolism, and oxidative stress as well as immune response. Therefore, monitoring the alterations of arginase activity and expression and targeting arginase offer a promising approach to diagnosing and treating DM and its complications. Nevertheless, there are still some limitations and challenges waiting for the translation of preclinical findings into therapeutic applications.

First of all, substantial clinical and experimental studies suggest that arginase could be a biomarker and diagnostic parameter for DM and its complications. However, there is no clinical definition standard of arginase activity or ARG1/2 expression levels in blood or tissues for diagnosing DM and its complications. For this purpose, it is feasible to build an artificial intelligence- (AI-) based prediction model through the deep learning of clinical data of patients with DM or DM complications, including the arginase activity values, expression levels, and patient information, to evaluate the potential risk of DM and its complications quickly. Secondly, arginase activity is indispensable for normal cellular physiological function since ARG1 exerts as the final enzyme of UC to detoxify ammonia and ARG2 is required for urine concentration in the kidney and smooth muscle cell proliferation [185, 186]. Concerning safety considerations, to lower the toxicity of arginase inhibitors, it is necessary to take into account the inhibition potency of inhibitors...
on ARG1 and ARG2 and which isoform of arginase dominantly contributes to the pathogenesis of DM and its complications in different individuals. Thirdly, as the distinct roles of ARG1 and ARG2 in the pathogenesis of DM and its complications, developing isoform-specific arginase inhibitors is a novel strategy to improve the therapeutic efficacy. In contrast, the high homology of the active enzymatic sites between human ARG1 and ARG2 frustrates this progress. Presently, high-resolution crystallographic structures of the enzyme, molecular and computational modeling have provided a possible route to developing hyperactive arginase inhibitors with specific properties [112]. Finally, due to the molecular diversity and low toxicity of nature arginase inhibitors, their extraction from natural medicinal plants or TCM herbs appears to be a promising approach, which not only provides new structures references for designing pharmaceutical arginase inhibitors but may also allow dietary therapy to treat DM and its complications.

Abbreviations

UC: Urea cycle
ARG1: Arginase1
ARG2: Arginase2
IR: Insulin resistance
NO: Nitric oxide
DM: Diabetes mellitus
T1DM: Type 1 diabetes mellitus
T2DM: Type 2 diabetes mellitus
ODC: Ornithine decarboxylase
OAT: Ornithine aminotransferase
BUN: Blood urea nitrogen
DR: Diabetic retinopathy
NOS: Nitric oxide synthase
iNOS: Inducible nitric oxide synthase
eNOS: Endothelial nitric oxide synthase
ECs: Endothelial cells
VSMCs: Vascular smooth muscle cells
WAT: White adipose tissue
ROS: Reactive oxygen species
HFHS: High fat-high sucrose
DR: High fat diet
TNN-α: Tumor necrosis factor-α
VAT: Visceral adipose tissue
STZ: Streptozotocin
RBCs: Red blood cells
HG: High glucose
NOD: Nonobese diabetic
nNOS: Neuronal nitric oxide synthase
PDX-1: Pancreas duodenum homeobox-1
NF-kB: Nuclear factor-kappa-B
DFMO: α-Difluoromethylornithine
eWAT: Epididymal white adipose tissue
ADSCs: Adipose-derived stem cells
ZR: Zucker rats
CVD: Cardiovascular disease
CAD: Coronary artery disease
I/R: Ischemia-reperfusion
ROCK: RhoA/Rho kinase

p38 MAPK: p38 Mitogen-activate protein kinases
BAECs: Bovine aortic endothelial cells
LOX-1: Lipoprotein receptor-1
JNK: c-Jun N-terminal kinase
DN: Diabetic nephropathy
ESRD: End-stage renal disease
TECs: Tubular epithelial cells
WT: Wild-type
DR: Diabetic retinopathy
SNP: Single nucleotide polymorphism
NOX2: NADPH oxidase-2
PDR: Proliferative diabetic retinopathy
SMOX: Spermine oxidase
ED: Erectile dysfunction
CC: Corpus cavernosum
NOHA: N-omega-hydroxy-L-arginine
nor-NOHA: N-omega-hydroxy-nor-L-arginine
ABH: 2(S)-amino-6-boronoheptanoic acid
BEC: S-(2-boronoethyl)-l-cysteine
DFMO: Difluoromethylornithine
TCM: Traditional Chinese medicine
EEDR: Endothelium dependent relaxation
BCECs: Bovine coronary endothelial cells
AMPK: Adenosine monophosphate-activated protein kinase
VHFD: Very high fat diet
SC: Semen cuscutae
XSF: Xiao-Shen-Formula
HGWW: HuangqiGuizhiWuwu Decoction
Al: Artificial intelligence
CAT: Cationic amino acid transporter
IL-1: Interleukin-1
IFN-γ: Interferon-γ.

Data Availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethical Approval

There was no involvement of humans or animals in this study.

Conflicts of Interest

The authors declare that they have no competing interests.

Authors’ Contributions

Y.R. and Y.X. collected the literatures and drafted the manuscript; Z.L., X.F., F.H., and Y.H. participated in the design of the review; L.Q., Y.Y., and Y.X. initiated the study and revised and finalized the manuscript. All authors read and approved the final manuscript.
Acknowledgments

This work was supported by National Natural Science Foundation of China (grant no. 82103589), Natural Science Foundation of Shaanxi Province (grant no. 2021Q-458, 2022JM-608), Special Scientific Research Plan for Emergency Public Health Safety 2020 of Shaanxi Provincial Department of Education (grant no. 20JG034), and Key Research and Development Program of Shaanxi Province (grant no. 2021SF-277, 2022SF-514).

References

[1] A. H. Heald, M. Stedman, M. Davies et al., “Estimating life years lost to diabetes: outcomes from analysis of national diabetes audit and office of national statistics data,” Cardiovascular Endocrinology & Metabolism, vol. 9, no. 4, pp. 183–185, 2020.
[2] D. L. Eizirik, L. Pasquali, and M. Cnop, “Pancreatic beta-cells in type 1 and type 2 diabetes mellitus: different pathways to failure,” Nature Reviews Endocrinology, vol. 16, no. 7, pp. 349–362, 2020.
[3] X. Wang and C. X. Hai, “ROS acts as a double-edged sword in the pathogenesis of type 2 diabetes mellitus: is Nrf2 a potential target for the treatment?” Mini Reviews In Medicinal Chemistry, vol. 11, no. 12, pp. 1082–1092, 2011.
[4] A. Magenta, S. Greco, M. C. Capogrossi, C. Gaetano, and F. Martelli, “Nitric oxide, oxidative stress, and p66Shc interplay in diabetic endothelial dysfunction,” Biomed Research International, vol. 2014, Article ID 193095, p. 16, 2014.
[5] G. Wu and S. M. Morris Jr., “Arginine metabolism: nitric oxide and beyond,” Biochemical Journal, vol. 336, no. 1, pp. 1–17, 1998.
[6] D. L. Michell, K. L. Andrews, and J. P. Chin-Dusting, “Endothelial dysfunction in hypertension: the role of arginase,” Frontiers in Bioscience-Scholar, vol. 3, no. 3, pp. 946–960, 2011.
[7] T. Ito, M. Kubo, K. Nagao, et al., “Early obesity leads to increases in hepatic arginase I and related systemic changes in nitric oxide and L-arginine metabolism in mice,” Journal Of Physiology And Biochemistry, vol. 74, no. 1, pp. 9–16, 2018.
[8] J. Moretto, C. Girard, and C. Demougeot, “The role of arginase in aging: a systematic review,” Experimental Gerontology, vol. 116, pp. 54–73, 2019.
[9] S. Wang, F. Fang, W. B. Jin, X. Wang, and D. W. Zheng, “Assessment of serum arginase I as a type 2 diabetes mellitus diagnosis biomarker in patients,” Experimental and Therapeutic Medicine, vol. 8, no. 2, pp. 585–590, 2014.
[10] S. R. Kashyap, A. Lara, R. Zhang, Y. M. Park, and R. A. DeFronzo, “Insulin reduces plasma arginase activity in type 2 diabetic patients,” Diabetes Care, vol. 31, no. 1, pp. 134–139, 2008.
[11] Y. Xiong, G. Yepuri, S. Necetin, J. P. Montani, X. F. Ming, and Z. Yang, “Arginase-II promotes tumor necrosis factor-alpha release from pancreatic acinar cells causing beta-cell apoptosis in aging,” Diabetes, vol. 66, no. 6, pp. 1636–1649, 2017.
[12] K. J. Peyton, X. M. Liu, A. R. Shehib, F. K. Johnson, R. A. Johnson, and W. Durante, “Arginase inhibition prevents the development of hypertension and improves insulin resistance in obese rats,” Amino Acids, vol. 50, no. 6, pp. 747–754, 2018.
[13] J. Pernow and C. Jung, “The emerging role of arginase in endothelial dysfunction in diabetes,” Current Vascular Pharmacology, vol. 14, no. 2, pp. 155–162, 2016.
[14] H. Zhang, J. Liu, D. Qu et al., “Serum exosomes mediate delivery of arginase 1 as a novel mechanism for endothelial dysfunction in diabetes,” Proceedings of the National Academy of Sciences, vol. 115, no. 29, pp. E6927–E6936, 2018.
[15] T. Beleziani, A. Feher, D. Spielvogel, S. L. Landsman, and Z. Bagi, “Arginase 1 contributes to diminished coronary arteriolar dilation in patients with diabetes,” American Journal of Physiology-Heart and Circulatory Physiology, vol. 300, no. 3, pp. H777–H783, 2011.
[16] A. Shemyakin, O. Kovamees, A. Rafnsson et al., “Arginase inhibition improves endothelial function in patients with coronary artery disease and type 2 diabetes mellitus,” Circulation, vol. 126, no. 25, pp. 2943–2950, 2012.
[17] O. Kovamees, A. Shemyakin, and J. Pernow, “Effect of arginase inhibition on ischemia-reperfusion injury in patients with coronary artery disease with and without diabetes mellitus,” PLoS One, vol. 9, no. 7, article e103260, 2014.
[18] J. M. Dzik, “Evolutionary roots of arginase expression and regulation,” Frontiers In Immunology, vol. 5, p. 544, 2014.
[19] C. P. Jenkinson, W. W. Grody, and S. D. Cederbaum, “Comparative properties of arginases,” Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology, vol. 114, no. 1, pp. 107–132, 1996.
[20] R. S. Sparkes, G. J. Dizikes, I. Klisak et al., “The gene for human liver arginase (ARG1) is assigned to chromosome band 6q23,” American Journal Of Human Genetics, vol. 39, no. 2, pp. 186–193, 1986.
[21] R. W. Caldwell, P. C. Rodriguez, H. A. Toque, S. P. Narayan, and R. B. Caldwell, “Arginase: a multifaceted enzyme important in health and disease,” Physiological Reviews, vol. 98, no. 2, pp. 641–665, 2018.
[22] S. M. Morris Jr., “Arginine metabolism in vascular biology and disease,” Vascular Medicine, vol. 10, Supplement 1, pp. S83–S87, 2005.
[23] R. C. Blantz, J. Satriano, F. Gabbai, and C. Kelly, “Biological effects of arginine metabolites,” Acta Physiologica Scandinavica, vol. 168, no. 1, pp. 21–25, 2000.
[24] P. Persson, A. Fasching, T. Terefink, P. Hansell, and F. Palm, “Cellular transport of L-arginine determines renal medullary blood flow in control rats, but not in diabetic rats despite enhanced cellular uptake capacity,” American Journal of Physiology-Renal Physiology, vol. 312, no. 2, pp. F278–F283, 2017.
[25] F. Palm, D. G. Buerk, P. O. Carlsson, P. Hansell, and P. Liss, “Reduced nitric oxide concentration in the renal cortex of streptozotocin-induced diabetic rats - effects on renal oxygenation and microcirculation,” Diabetes, vol. 54, no. 11, pp. 3282–3287, 2005.
[26] G. M. Pieper and L. A. Dondlinger, “Plasma and vascular tissue arginine are decreased in diabetes: acute arginine supplementation restores endothelium-dependent relaxation by augmenting cGMP production,” Journal of Pharmacology and Experimental Therapeutics, vol. 283, no. 2, pp. 684–691, 1997.
[27] J. T. Brosnan, K. C. Man, D. E. Hall, S. A. Colbourne, and M. E. Brosnan, “Interorgan metabolism of amino acids in streptozotocin-diabetic ketoacidotic rat,” American Journal of Physiology-Endocrinology and Metabolism, vol. 244, no. 2, pp. E151–E158, 1983.
L-arginine administration improves peripheral and hepatic glycemia and insulin secretion in chronic kidney disease, *Diabetes Care*, vol. 24, no. 5, pp. 875–880, 2001.

C. Clemmensen, S. Smajilovic, and M. Lindqvist, “Oral L-arginine stimulates GLP-1 secretion to improve glucose tolerance in male mice,” *Metabolism*, vol. 41, no. 1, pp. 28–32, 1992.

J. Moon, O. Y. Kim, G. Jo, and M. J. Shin, “Alterations in circulating amino acid metabolite ratio associated with arginine activity are potential indicators of metabolic syndrome: the Korean genome and epidemiology study,” *Nutrients*, vol. 9, no. 7, p. 740, 2017.

E. Adeghate, A. S. Ponery, T. El-Sharkawy, and H. Parvez, “L-arginine stimulates insulin secretion from the pancreas of normal and diabetic rats,” *Amino Acids*, vol. 21, no. 2, pp. 205–209, 2001.

P. M. Piatti, L. D. Monti, G. Valsecchi et al., “Long-term oral L-arginine administration improves peripheral and hepatic insulin sensitivity in type 2 diabetic patients,” *Diabetes Care*, vol. 24, no. 5, pp. 875–880, 2001.

C. Clemmensen, S. Smajilovic, E. P. Smith et al., “Oral L-arginine stimulates GLP-1 secretion to improve glucose tolerance in male mice,” *Endocrinology*, vol. 154, no. 11, pp. 3978–3983, 2013.

L. Koppe, E. Nyam, K. Vivot et al., “Urea impairs β cell glycolysis and insulin secretion in chronic kidney disease,” *The Journal Of Clinical Investigation*, vol. 126, no. 9, pp. 3598–3612, 2016.

S. J. Allison, “Urea inhibits insulin secretion in CKD,” *Nature Reviews Nephrology*, vol. 12, no. 10, p. 581, 2016.

Y. Xie, B. Bowe, T. T. Li, H. Xian, Y. Yan, and Z. Al-Aly, “Higher blood urea nitrogen is associated with increased risk of incident diabetes mellitus,” *Kidney International*, vol. 93, no. 3, pp. 741–752, 2018.

M. Shiri-Zaify, F. Heidari, Z. Dalirsani, and J. Dehghan, “Estimation of salivary sodium, potassium, calcium, phosphorus and urea in type II diabetic patients,” *Diabetes & Metabolic Syndrome: Clinical Research & Reviews*, vol. 9, no. 4, pp. 332–336, 2015.

S. Saxena, S. Ruia, S. Prasad et al., “Increased serum levels of urea and creatinine are surrogate markers for disruption of retinal photoreceptor external limiting membrane and inner segment ellipsoid zone in type 2 diabetes mellitus,” *Retina*, vol. 37, no. 2, pp. 344–349, 2017.

S. M. Chen, S. Akter, K. Kuwahara et al., “Serum amino acid profiles and risk of type 2 diabetes among Japanese adults in the Hitachi health study,” *Scientific Reports*, vol. 9, no. 1, p. ???, 2019.

J. C. Fernandez-Garcia, A. Delpino-Rius, I. Samarra et al., “Type 2 diabetes is associated with a different pattern of serum polyamines: a case-control study from the PREDIMED-Plus trial,” *Journal Of Clinical Medicine*, vol. 8, no. 1, pp. 71, 2019.

A. Kulkarni, C. M. Anderson, R. G. Mirmira, and S. A. Teryey, “Role of polyamines and hypusine in β cells and diabetes pathogenesis,” *Metabolites*, vol. 12, no. 4, p. 344, 2022.

C. Karacay, B. Prietl, C. Harer et al., “The effect of spermidine on autoimmunity and beta cell function in NOD mice,” *Scientific Reports*, vol. 12, no. 1, 2022.
[58] D. Kumar, K. Shankar, S. Patel et al., “Chronic hyperinsulinemia promotes meta-inflammation and extracellular matrix deposition in adipose tissue: implications of nitric oxide,” Molecular and Cellular Endocrinology, vol. 477, pp. 15–28, 2018.

[59] J. Y. Kim, E. H. Song, H. J. Lee et al., “Chronic Ethanol Consumption-induced Pancreatic β-Cell Dysfunction and Apoptosis through Glucokinase Nitration and Its Down-regulation,” Journal of Biological Chemistry, vol. 285, no. 48, pp. 37251–37262, 2010.

[60] D. Eckersten and R. Heningsson, “Nitric oxide (NO) - production and regulation of insulin secretion in islets of freely fed and fasted mice,” Regulatory Peptides, vol. 174, no. 1-3, pp. 32–37, 2012.

[61] A. Mollsten, M. Lajer, A. Jorsal, and L. Tarnow, “The endothelial nitric oxide synthase gene and risk of diabetic nephropathy and development of cardiovascular disease in type 1 diabetes,” Molecular Genetics And Metabolism, vol. 97, no. 1, pp. 80–84, 2009.

[62] P. Tessari, “Nitric oxide in the normal kidney and in patients with diabetic nephropathy,” Journal Of Nephrology, vol. 28, no. 3, pp. 257–268, 2015.

[63] R. Opatrilova, P. Kubatka, M. Caprnda et al., “Nitric oxide in the pathophysiology of retinopathy: evidences from preclinical and clinical researches,” Acta Ophthalmologica, vol. 96, no. 3, pp. 222–231, 2018.

[64] M. J. Malone-Povolny, S. E. Maloney, and M. H. Schoenfisch, “Nitric oxide therapy for diabetic wound healing,” Advanced Healthcare Materials, vol. 8, no. 12, article e1801210, 2019.

[65] B. Musicki and A. L. Burnett, “Constitutive NOS uncoupling and NADPH oxidase upregulation in the penis of type 2 diabetic men with erectile dysfunction,” Andrology, vol. 5, no. 2, pp. 294–298, 2017.

[66] J. L. Evans, I. D. Goldfine, B. A. Maddux, and G. M. Grodsky, “Are oxidative stress-activated signaling pathways mediators of insulin resistance and beta-cell dysfunction?,” Diabetes, vol. 52, no. 1, pp. 1–8, 2003.

[67] Z. Yang and X. F. Ming, “Functions of arginase isoforms in macrophage inflammatory responses: impact on cardiovascular diseases and metabolic disorders,” Frontiers In Immunology, vol. 5, p. 533, 2014.

[68] K. Rieneck, L. F. Bovin, K. Josefsen, K. Buschard, M. Svenson, and K. Bendtzen, "Massive parallel gene expression profiling of RINm5F pancreatic islet β-cells stimulated with interleukin-1β," Apmis., vol. 108, no. 12, pp. 855–872, 2000.

[69] A. K. Cardozo, M. Kruhoffer, R. Leeman, T. Orntoft, and D. L. Eizirik, "Identification of novel cytokine-induced genes in pancreatic beta-cells by high-density oligonucleotide arrays," Diabetes, vol. 50, no. 5, pp. 909–920, 2001.

[70] H. Zhao, Q. W. Shang, Z. Z. Pan et al., "Exosomes from adipose-derived stem cells attenuate adipose inflammation and obesity through polarizing M2 macrophages and beiging in white adipose tissue," Diabetes, vol. 67, no. 2, pp. 235–247, 2018.

[71] E. Shosha, A. Y. Fouda, S. P. Narayanan, R. W. Caldwell, and R. B. Caldwell, "Is the arginase pathway a novel therapeutic avenue for diabetic retinopathy?," Journal Of Clinical Medicine, vol. 9, no. 2, 2020.

[72] A. Bhatta, L. Yao, Z. Xu et al., "Obesity-induced vascular dysfunction and arterial stiffening requires endothelial cell arginase 1," Cardiovascular Research, vol. 113, no. 13, pp. 1664–1676, 2017.

[73] X. F. Ming, A. G. Rajapakse, G. Yepuri et al., "Arginase II promotes macrophage inflammatory responses through mitochondrial reactive oxygen species, contributing to insulin resistance and atherogenesis," Journal of the American Heart Association, vol. 1, no. 4, 2012.

[74] G. Yepuri, S. Velagapudi, Y. Y. Xiong et al., "Positive crosstalk between arginase-II and S6K1 in vascular endothelial inflammation and aging," Aging Cell, vol. 11, no. 6, pp. 1005–1016, 2012.

[75] J. Huang, C. Liu, X. F. Ming, and Z. Yang, "Inhibition of p38mapk reduces adipose tissue inflammation in aging mediated by arginase-II," Pharmacology, vol. 105, no. 9-10, pp. 491–504, 2020.

[76] R. T. Atawia, H. A. Toque, M. M. Meghil et al., "Role of arginase 2 in systemic metabolic activity and adipose tissue fatty acid metabolism in diet-induced obese mice," International Journal of Molecular Sciences, vol. 20, no. 6, p. 1462, 2019.

[77] C. Liu, A. G. Rajapakse, E. Riedo et al., "Targeting arginase-II protects mice from high-fat-diet-induced hepatic steatosis through suppression of macrophage inflammation," Scientific Reports, vol. 6, no. 1, pii: 74016, 2016.

[78] M. Krause, N. H. McClennaghan, P. R. Flatt, J. R. Krause, C. Murphy, and P. Newsholme, "L-arginine is essential for pancreatic beta cell functional integrity, metabolism and defence from inflammatory challenge," Diabetologia, vol. 54, p. S206-S, 2011.

[79] S. K. Panigraphy, R. Bhatt, and A. Kumar, "Reactive oxygen species: sources, consequences and targeted therapy in type 2 diabetes," Journal Of Drug Targeting, vol. 25, no. 2, pp. 93–101, 2017.

[80] A. A. Adebayo, G. Oboh, and A. O. Ademosun, "Effect of dietary inclusion of almond fruit on sexual behavior, arginase activity, pro-inflammatory, and oxidative stress markers in diabetic male rats," Journal of Food Biochemistry, vol. 45, no. 3, article e13269, 2021.

[81] Z. Zhou, A. Mahdi, Y. Tratsiakovitch et al., "Erythrocytes from patients with type 2 diabetes induce endothelial dysfunction via arginase I," Journal of the American College of Cardiology, vol. 72, no. 7, pp. 769–780, 2018.

[82] M. Rojas, T. Lemtalsi, H. A. Toque et al., "NOX2-induced activation of arginase and diabetes-induced retinal endothelial cell senescence," Antioxidants (Basel), vol. 6, no. 2, p. 43, 2017.

[83] M. D’Apolito, X. L. Du, H. H. Zong et al., "Inhibition of nitric oxide (NO) - production, arginase activity, pro-inflammatory, and oxidative stress markers in diabetic men with erectile dysfunction, arginase-II in vascular endothelial dysfunction requires endothelial cell arginase 1," FEBS Letters, vol. 394, no. 3, pp. 300–306, 1996.

[84] W. L. Suarez-Pinzon, J. G. Mabley, K. Strynadka, R. F. Power, C. A. Delaney, B. Tyrberg, L. Bouwens, H. Vaghef, B. Hellman, and D. L. Eizirik, "Sensitivity of human pancreatic islets to peroxynitrite-induced cell dysfunction and death," FEBS Letters, vol. 543–544, 2007.

[85] C. A. Delaney, D. Tyrberg, L. Bouwens, H. Vaghef, B. Hellman, and D. L. Eizirik, "Sensitivity of human pancreatic islets to peroxynitrite-induced cell dysfunction and death," FEBS Letters, vol. 394, no. 3, pp. 300–306, 1996.

[86] W. L. Suarez-Pinzon, J. G. Mabley, K. Strynadka, R. F. Power, C. A. Delaney, and D. L. Eizirik, "Sensitivity of human pancreatic islets to peroxynitrite-induced cell dysfunction and death," FEBS Letters, vol. 394, no. 3, pp. 300–306, 1996.
endothelial dysfunction in diabetic rats,” *Frontiers in Immunology*, vol. 4, 2013.

[103] O. Kovamees, A. Shemyakin, and J. Pernow, “Amino acid metabolism reflecting arginase activity is increased in patients with type 2 diabetes and associated with endothelial dysfunction,” *Diabetes And Vascular Disease Research*, vol. 13, no. 5, pp. 354–360, 2016.

[104] O. Kovamees, A. Shemyakin, A. Checa et al., “Arginase inhibition improves microvascular endothelial function in patients with type 2 diabetes mellitus,” *The Journal of Clinical Endocrinology & Metabolism*, vol. 101, no. 11, pp. 3952–3958, 2016.

[105] J. Pernow and C. Jung, “Arginase as a potential target in the treatment of cardiovascular disease: reversal of arginine steal?,” *Cardiovascular Research*, vol. 98, no. 3, pp. 334–343, 2013.

[106] S. Chandra, D. J. R. Fulton, R. B. Caldwell, R. W. Caldwell, and H. A. Toque, “Hyperglycemia-impaired aortic vasorelaxation mediated through arginase elevation: role of stress kinase pathways,” *European Journal Of Pharmacology*, vol. 844, pp. 26–37, 2019.

[107] S. Mazrouei, F. Sharifipanah, R. W. Caldwell et al., “Regulation of MAP kinase-mediated endothelial dysfunction in hyperglycemia via arginase I and eNOS dysregulation,” *Biochimica et Biophysica Acta (BBA)-Molecular Cell Research*, vol. 1866, no. 9, pp. 1398–1411, 2019.

[108] T. W. Hein, X. Xu, Y. Ren et al., “Requisite roles of LOX-1, NKF, and arginase in diabetes-induced endothelial vasodilator dysfunction of porcine coronary arterioles,” *Journal Of Molecular And Cellular Cardiology*, vol. 131, pp. 82–90, 2019.

[109] J. Pernow, A. Kiss, Y. Tratsiakovich, and B. Climent, “Tissue-specific up-regulation of arginase I and II induced by p38 MAPK mediates endothelial dysfunction in type 1 diabetes mellitus,” *British Journal Of Pharmacology*, vol. 172, no. 19, pp. 4684–4698, 2015.

[110] Y. Yu, A. G. Rajapakse, J. P. Montani, Z. H. Yang, and X. F. Ming, “p38 mitogen-activated protein kinase is involved in arginase-II-mediated eNOS uncoupling in obesity,” *Cardiovascular Diabetology*, vol. 13, 2014.

[111] J. Gironros, C. Jung, J. O. Lundberg, R. Cerrato, C. G. Osten- son, and J. Pernow, “Arginase inhibition restores in vivo coronary microvascular function in type 2 diabetic rats,” *Journal of Physiology-Heart and Circulatory Physiology*, vol. 300, no. 4, pp. H1174–H1181, 2011.

[112] T. Bagnost, L. Ma, R. F. da Silva et al., “Cardiovascular effects of arginase inhibition in spontaneously hypertensive rats with fully developed hypertension,” *Cardiovascular Research*, vol. 87, no. 3, pp. 569–577, 2010.

[113] H. M. El-Bassossy, R. El-Fawal, and A. Fahmy, “Arginase inhibition alleviates hypertension associated with diabetes: effect on endothelial dependent relaxation and NO production,” *Vascular Pharmacology*, vol. 57, no. 5-6, pp. 194–200, 2012.

[114] Y. Cao, Z. Yang, Y. Chen et al., “An overview of the posttranslational modifications and related molecular mechanisms in diabetic nephropathy,” *Frontiers in Cell and Developmental Biology*, vol. 9, 2021.

[115] R. Lindblom, G. Higgins, M. Coughlan, and J. B. de Haan, “Targeting mitochondria and reactive oxygen species-driven pathogenesis in diabetic nephropathy,” *The Review Of Diabetic Studies: RDS*, vol. 12, no. 1-2, pp. 134–156, 2015.
Mesenchymal stem cells prevent the progression of diabetic nephropathy by improving mitochondrial function in tubular epithelial cells,” *Experimental & Molecular Medicine*, vol. 51, no. 7, pp. 1–14, 2019.

S. M. Morris Jr., H. You, T. Gao, J. Vacher, T. K. Cooper, and A. S. Awad, “Distinct roles of arginases 1 and 2 in diabetic nephropathy,” *American Journal of Physiology-Renal Physiology*, vol. 313, no. 4, pp. F899–F905, 2017.

S. M. Morris Jr., T. Gao, T. K. Cooper, D. Kepta-Lenhart, and A. S. Awad, “Arginase-2 mediates diabetic renal injury,” *Diabetes*, vol. 60, no. 11, pp. 3015–3022, 2011.

E. S. Lee, J. S. Kang, H. M. Kim et al., “Dehydrozingerone inhibits renal lipotoxicity in high-fat diet-induced obese mice,” *Journal Of Cellular And Molecular Medicine*, vol. 25, no. 18, pp. 8725–8733, 2021.

H. You, T. Gao, T. K. Cooper, S. M. Morris Jr., and A. S. Awad, “Arginase inhibition: a new treatment for preventing progression of established diabetic nephropathy,” *American Journal of Physiology-Renal Physiology*, vol. 309, no. 5, pp. F447–F455, 2015.

H. N. You, T. Gao, T. K. Cooper, S. M. Morris, and A. S. Awad, “Arginase inhibition mediates renal tissue protection in diabetic nephropathy by a nitric oxide synthase 3-dependent mechanism,” *Kidney International*, vol. 84, no. 6, pp. 1189–1197, 2013.

M. Buraczynska and I. Zakrocka, “Arginase gene polymorphism increases risk of diabetic retinopathy in type 2 diabetes mellitus patients,” *Journal of Clinical Medicine*, vol. 10, no. 22, 2021.

E. Shosha, Z. M. Xu, S. P. Narayanan et al., “Mechanisms of diabetes-induced endothelial cell senescence: role of arginase 1,” *International Journal of Molecular Sciences*, vol. 19, no. 4, 2018.

S. C. Elms, H. A. Toque, M. Rojas, Z. Xu, R. W. Caldwell, and R. B. Caldwell, “The role of arginase 1 in diabetes-induced retinal vascular dysfunction in mouse and rat models of diabetes,” *Diabetologia*, vol. 56, no. 3, pp. 654–662, 2013.

R. T. Atawia, K. L. Bunch, A. Y. Fouda et al., “Role of arginase 2 in murine retinopathy associated with western diet-induced obesity,” *Journal of Clinical Medicine*, vol. 9, no. 2, 2020.

L. P. Paris, C. H. Johnson, E. Aguilar et al., “Global metabolomics reveals metabolic dysregulation in ischemic retinopathy,” *Metabolomics*, vol. 12, no. 1, 2016.

X. R. Zhu, F. Y. Yang, J. Lu et al., “Plasma metabolomic profiling of proliferative diabetic retinopathy,” *Nutrition & Metabolism*, vol. 16, p. 37, 2019.

R. Nicoletti, I. Venza, G. Ceci, M. Visalli, D. Teti, and A. Reibaldi, “Vitreous polyamines spermidine, putrescine, and spermine in human proliferative disorders of the retina,” *British Journal Of Ophthalmology*, vol. 87, no. 8, pp. 1038–1042, 2003.

S. P. Narayanan, E. Shosha, and C. D. Palani, “Spermine oxidase: a promising therapeutic target for neurodegeneration in diabetic retinopathy,” *Pharmacological Research*, vol. 147, article 104299, 2019.

F. Liu, A. B. Saul, P. Chavaram et al., “Pharmacological inhibition of spermine oxidase reduces neurodegeneration and improves retinal function in diabetic mice,” *Journal of Clinical Medicine*, vol. 9, no. 2, 2020.

D. Baltzis, I. Effthmeriadou, and A. Veves, “Pathogenesis and treatment of impaired wound healing in diabetes mellitus: new insights,” *Advances In Therapy*, vol. 31, no. 8, pp. 817–836, 2014.

J. E. Albina, C. D. Mills, A. Barbul et al., “Arginine metabolism in wounds,” *American Journal of Physiology-Endocrinology And Metabolism*, vol. 254, no. 4-1, pp. E459–E467, 1988.

H. Kampfer, J. Pfelsschifer, and S. Frank, “Expression and activity of arginase isoenzymes during normal and diabetes-impaired skin repair,” *Journal of Investigative Dermatology*, vol. 121, no. 6, pp. 1544–1551, 2003.

E. B. Jude, A. J. Boulton, M. W. Ferguson, and I. Appleton, “The role of nitric oxide synthase isoforms and arginase in the pathogenesis of diabetic foot ulcers: possible modulatory effects by transforming growth factor beta 1,” *Diabetologia*, vol. 42, no. 6, pp. 748–757, 1999.

S. L. Kavalukas, A. R. Uzgare, T. J. Bivalacqua, and A. Barbul, “Arginase inhibition promotes wound healing in mice,” *Surgery*, vol. 151, no. 2, pp. 287–295, 2012.

V. Arana, Y. Paz, A. Gonzalez, V. Mendez, and J. D. Mendez, “Healing of diabetic foot ulcers in L-arginine-treated patients,” *Biomedicine & Pharmacotherapy*, vol. 58, no. 10, pp. 588–597, 2004.

A. Castela and C. Costa, “Molecular mechanisms associated with diabetic endothelial-erectile dysfunction,” *Nature Reviews Urology*, vol. 13, no. 5, pp. 266–274, 2016.

T. J. Bivalacqua, W. J. Hellstrom, P. J. Kadowitz, and H. C. Champion, “Increased expression of arginase II in human diabetic corpus cavernosum: in diabetic-associated erectile dysfunction,” *Biochemical And Biophysical Research Communications*, vol. 283, no. 4, pp. 923–927, 2001.

H. A. Toque, R. C. Tostes, L. Yao et al., “Arginase II deletion increases corpora cavernosa relaxation in diabetic mice,” *The Journal Of Sexual Medicine*, vol. 8, no. 3, pp. 722–733, 2011.

H. A. Toque, K. P. Nunes, L. Yao et al., “Activated Rho kinase mediates diabetes-induced elevation of vascular arginase activation and contributes to impaired corpora cavernosa relaxation: possible involvement of p38 MAPK activation,” *The Journal Of Sexual Medicine*, vol. 10, no. 6, pp. 1502–1515, 2013.

H. A. Toque, K. P. Nunes, L. Yao et al., “Akita spontaneously type 1 diabetic mice exhibit elevated vascular arginase and impaired vascular endothelial and nitricergic function,” *PloS One*, vol. 8, no. 8, article e72277, 2013.

H. A. Toque and R. W. Caldwell, “New approaches to the design and discovery of therapies to prevent erectile dysfunction,” *Expert Opinion on Drug Discovery*, vol. 9, no. 12, pp. 1447–1469, 2014.

L. Di Costanzo, M. Ilies, K. J. Thorn, and D. W. Christianson, “Inhibition of human arginase I by substrate and product analogues,” *Archives Of Biochemistry And Biophysics*, vol. 496, no. 2, pp. 101–108, 2010.

D. M. Colleluori and D. E. Ash, “Classical and slow-binding inhibitors of human type II arginase,” *Biochemistry*, vol. 40, no. 31, pp. 9356–9362, 2001.

R. B. Caldwell, H. A. Toque, S. P. Narayanan, and R. W. Caldwell, “Arginase: an old enzyme with new tricks,” *Trends In Pharmacological Sciences*, vol. 36, no. 6, pp. 395–405, 2015.

J. Yang, X. Zheng, A. Mahdi 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 to Translational Science*, vol. 3, no. 4, pp. 450–463, 2018.
Oxidative Medicine and Cellular Longevity

[147] L. F. Hernandez, P. Buchwald, and M. H. Abdulreda, “Effect of arginase-1 inhibition on the incidence of autoimmune diabetes in NOD mice,” Current Research In Diabetes & Obesity Journal, vol. 5, no. 3, 2018.

[148] Y. Tratsiakovich, A. Kiss, A. T. Gonon, J. Yang, P. O. Sjoquist, and J. Pernow, “Inhibition of Rho kinase protects from ischaemia–reperfusion injury via regulation of arginase activity and nitric oxide synthase in type 1 diabetes,” Diabetes & Vascular Disease Research, vol. 14, no. 3, pp. 236–245, 2017.

[149] T. W. Hein, T. Omae, W. Xu, A. Yoshida, and L. Kuo, “Role of arginase in selective impairment of endothelium-dependent nitric oxide synthase-mediated dilation of retinal arterioles during early diabetes,” Investigative Ophthalmology & Visual Science, vol. 61, no. 5, p. 36, 2020.

[150] J. D. Cox, E. Cama, D. M. Colleluori et al., “Mechanistic and metabolic inferences from the binding of substrate analogues and products to arginase,” Biochemistry, vol. 40, no. 9, pp. 2689–2701, 2001.

[151] N. N. Kim, J. D. Cox, R. F. Baggio et al., “Probing erectile function: S-(2-boronoethyl)-L-cysteine binds to arginase as a transition state analogue and enhances smooth muscle relaxation in human penile corpus cavernosum,” Biochemistry, vol. 40, no. 9, pp. 2678–2688, 2001.

[152] M. C. Van Zandt, G. E. Jagdmann, D. L. Whitehouse et al., “Discovery of N-substituted 3-amino-4-(3-boronopropyl)-pyrrolidine-3-carboxylic acids as highly potent third-generation inhibitors of human arginase I and II,” Journal Of Medicinal Chemistry, vol. 62, no. 17, pp. 8164–8177, 2019.

[153] E. Cama, D. M. Colleluori, F. A. Emig et al., “Human arginase II: crystal structure and physiological role in male and female sexual arousal,” Biochemistry, vol. 42, no. 28, pp. 8445–8451, 2003.

[154] L. Di Costanzo, G. Sabio, A. Mora et al., “Crystal structure of human arginase I at 1.29-angstrom resolution and exploration of inhibition in the immune response,” Proceedings of the National Academy of Sciences, vol. 102, no. 37, pp. 13058–13063, 2005.

[155] A. Shatanawi and M. S. Momani, “Plasma arginase activity is elevated in type 2 diabetic patients,” BioMed Research International, vol. 28, no. 9, 2017.

[156] A. Bhatta, R. Sangani, R. Kolhe et al., “Deregulation of arginase induces bone complications in high-fat/high-sucrose diet diabetic mouse model,” Molecular And Cellular Endocrinology, vol. 422, pp. 211–220, 2016.

[157] L. Yao, S. Chandra, H. A. Toque et al., “Prevention of diabetes-induced arginase activation and vascular dysfunction by Rho kinase (ROCK) knockout,” Cardiovascular Research, vol. 97, no. 3, pp. 509–519, 2013.

[158] K. P. Nunes, H. A. Toque, R. B. Caldwell, R. W. Caldwell, and R. C. Webb, “Extracellular signal-regulated kinase (ERK) inhibition decreases arginase activity and improves corpora cavernosal relaxation in streptozotocin (STZ)-induced diabetic mice,” Journal of Sexual Medicine, vol. 8, no. 12, pp. 3335–3344, 2011.

[159] S. M. Morris Jr., “Recent advances in arginine metabolism: roles and regulation of the arginases,” British Journal Of Pharmacology, vol. 157, no. 6, pp. 922–930, 2009.

[160] S. R. Reddy and T. G. Baby, “The inhibition of arginase from the hepatopancreas of a terrestrial snail by amino acids,” Archives Internationales de Physiologie et de Biochimie, vol. 84, no. 4, pp. 759–766, 1976.

[161] J. L. Boucher, J. Custot, S. Vadot et al., “N-omega-hydroxyl-L-arginine, an intermediate in the L-arginine to nitric oxide pathway, is a strong inhibitor of liver and macrophage arginase,” Biochemical And Biophysical Research Communications, vol. 203, no. 3, pp. 1614–1621, 1994.

[162] A. Shatanawi, M. S. Momani, R. Al-Aqtaash, M. H. Hamdan, and M. N. Gharaibeh, “L-citrulline supplementation increases plasma nitric oxide levels and reduces arginase activity in patients with type 2 diabetes,” Frontiers In Pharmacology, vol. 11, article 584699, 2020.

[163] S. B. Solerte, M. Fioravanti, E. Locatelli et al., “Improvement of blood glucose control and insulin sensitivity during a long-term (60 weeks) randomized study with amino acid dietary supplements in elderly subjects with type 2 diabetes mellitus,” The American Journal Of Cardiology, vol. 101, no. 11, pp. 82E–88E, 2008.

[164] R. Rognstad, “Sources of ammonia for urea synthesis in isolated rat liver cells,” Biochimica et Biophysica Acta (BBA)-General Subjects, vol. 496, no. 2, pp. 249–254, 1977.

[165] C. L. Chang, J. C. Liao, and L. Kuo, “Arginase modulates nitric oxide production in activated macrophages,” American Journal Of Physiology-Heart and Circulatory Physiology, vol. 274, no. 1, pp. H342–H348, 1998.

[166] H. Javrushyan, E. Nadiryan, A. Grigoryan, N. Avandilyan, and A. Maloyan, “Antihyperglycemic activity of L-norvaline and L-arginine in high-fat diet and streptozotocin-treated male rats,” Experimental and Molecular Pathology, vol. 126, article 104763, 2022.

[167] H. M. El-Bassossy, R. El-Fawal, A. Fahmy, and M. L. Watson, “Arginase inhibition alleviates hypertension in the metabolic syndrome,” British Journal Of Pharmacology, vol. 169, no. 3, pp. 693–703, 2013.

[168] A. De, M. F. Singh, V. Singh, V. Ram, and S. Bisht, “Treatment effect of L-norvaline on the sexual performance of male rats with streptozotocin induced diabetes,” European Journal Of Pharmacology, vol. 771, pp. 247–254, 2016.

[169] H. H. Deng, X. Q. Shi, H. P. Peng et al., “Gold nanoparticle-based photoluminescent nanoswitch controlled by host-guest recognition and enzymatic hydrolysis for arginase activity assay,” ACS Applied Materials & Interfaces, vol. 10, no. 6, pp. 5358–5364, 2018.

[170] E. R. da Silva, C. do Carmo Maquiaveli, and P. P. Magalhães, “The leishmanicidal flavonoids quercetin and quercitrin target Leishmania (Leishmania) amazonensis arginase,” Experimental Parasitology, vol. 130, no. 3, pp. 183–188, 2012.

[171] H. M. Eid, A. Nachar, F. Thong, G. Sweeney, and P. S. Hadad, “The molecular basis of the antidiabetic action of quercetin in cultured skeletal muscle cells and hepatocytes,” Pharmacognosy Magazine, vol. 11, no. 41, pp. 74–81, 2015.

[172] Y. Xiong, G. Yepuri, M. Forbith et al., “ARG2 impairs endothelial autophagy through regulation of MTOR and PRKAA/AMPK signaling in advanced atherosclerosis,” Autophagy, vol. 10, no. 12, pp. 2223–2238, 2014.

[173] S. Wang, S. Du, W. Wang, and F. Zhang, “Therapeutic investigation of quercetin nanomedicine in a zebrafish model of diabetic retinopathy,” Biomedicine & Pharmacotherapy, vol. 130, article 110573, 2020.

[174] G. Ohoh, A. O. Ademiluyi, A. O. Ademuson et al., “Phenolic extract from Moringa oleifera leaves inhibits key enzymes linked to erectile dysfunction and oxidative stress in rats’ penile tissues,” Biochemistry Research International, vol. 2015, Article ID 175950, p. 8, 2015.
[175] C. Waterman, P. Rojas-Silva, T. B. Tumer et al., “Isothiocyanate-rich Moringa oleifera extract reduces weight gain, insulin resistance, and hepatic gluconeogenesis in mice,” *Molecular Nutrition & Food Research*, vol. 59, no. 6, pp. 1013–1024, 2015.

[176] S. I. Oyeleye, O. R. Ojo, and G. Oboh, “Moringa oleifera leaf and seed inclusive diets influenced the restoration of biochemicals associated with erectile dysfunction in the penile tissue of STZ-induced diabetic male rats treated with/without Acarbose drug,” *Journal of Food Biochemistry*, vol. 45, no. 3, article e13323, 2021.

[177] S. A. Adefegha, G. Oboh, A. E. Iyoha, and A. A. Oyagbemi, “Comparative effects of horseradish (Moringa oleifera) leaves and seeds on blood pressure and crucial enzymes relevant to hypertension in rat,” *PharmaNutrition*, vol. 9, 2019.

[178] M. A. Hassan, T. Xu, Y. Tian et al., “Health benefits and phenolic compounds of Moringa oleifera leaves: a comprehensive review,” *Phytotherapy Research*, vol. 93, article 153771, 2021.

[179] S. I. Oyeleye, T. A. Olasehinde, A. O. Ademosun, A. J. Akinyemi, and G. Oboh, “Horseradish (Moringa oleifera) seed and leaf inclusive diets modulates activities of enzymes linked with hypertension, and lipid metabolites in high-fat fed rats,” *Pharmanutrition*, vol. 7, 2019.

[180] N. D. Zhang, T. Han, B. K. Huang et al., “Traditional Chinese medicine formulas for the treatment of osteoporosis: implication for antiosteoporotic drug discovery,” *Journal Of Ethnopharmacology*, vol. 189, pp. 61–80, 2016.

[181] J. H. Liu, S. C. Ho, T. H. Lai, T. H. Liu, P. Y. Chi, and R. Y. Wu, “Protective effects of Chinese herbs on D-galactose-induced oxidative damage,” *Methods And Findings In Experimental And Clinical Pharmacology*, vol. 25, no. 6, pp. 447–452, 2003.

[182] J. Moon, M. J. Ha, M. J. Shin et al., “Semen cuscutae administration improves hepatic lipid metabolism and adiposity in high fat diet-induced obese mice,” *Nutrients*, vol. 11, no. 12, 2019.

[183] H. Cheng, T. Lu, J. Y. Wang et al., “HuangqiGuizhiWuwu decoction prevents vascular dysfunction in diabetes via inhibition of endothelial arginase 1,” *Frontiers In Physiology*, vol. 11, 2020.

[184] X. F. An, M. X. Zhang, S. S. Zhou, T. Lu, Y. J. Chen, and L. Yao, “Xiao-Shen-Formula, a Traditional Chinese medicine, improves glomerular hyper-filtration in diabetic nephropathy via inhibiting arginase activation and heparanase expression,” *Frontiers In Physiology*, vol. 9, 2018.

[185] Y. Y. Xiong, Y. Yu, J. P. Montani, Z. H. Yang, and X. F. Ming, “Arginase-II induces vascular smooth muscle cell senescence and apoptosis through p66Shc and p53 independently of its L-arginine ureahydrolase activity: implications for atherosclerotic plaque vulnerability,” *Journal of the American Heart Association*, vol. 2, no. 4, 2013.

[186] J. Huang, J. P. Montani, F. Verrey, E. Feraillé, X. F. Ming, and Z. Yang, “Arginase-II negatively regulates renal aquaporin-2 and water reabsorption,” *The FASEB Journal*, vol. 32, no. 10, pp. 5520–5531, 2018.