L-Arginine in Nutrition: Multiple Beneficial Effects in the Etiopathology of Diabetes

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Abstract: L-arginine is a nutritionally important amino acid that controls a wide spectrum of cellular functions and physiological processes, acting by itself or through its various metabolites. There are several factors that determine overall L-arginine homeostasis: dietary supplementation, endogenous de novo synthesis, whole-body protein turnover and its extensive metabolism. The destiny of L-arginine is determined by the complex network of enzymes and pathways differentially expressed according to health and disease status. Diabetes is characterized by reduced concentrations of L-arginine in plasma and many tissues, and failure of its metabolic effects. Emerging data suggest that oral supplementation of L-arginine exerts multiple beneficial effects on the complex etiological and pathophysiological basis of diabetes including: i) β-cell function and mass and ii) obesity and peripheral insulin resistance. This review emphasizes important aspects of L-arginine action which classifies this amino acid as a promising therapeutic approach in the treatment of diabetes.

Keywords: L-arginine, diabetes, β-cells, insulin resistance, obesity.

1. INTRODUCTION

Research into L-arginine has a rich history, and started with its isolation from lupin seedlings in 1886 [1], through the clarification of its metabolic pathways as well as nutritional needs, to the discovery that this amino acid is a precursor for nitric oxide (NO) [2], and the recognition of NO as an endothelium-derived relaxing factor [3, 4]. Due to the 100-year long physiological and nutritional studies on L-arginine, today we are aware that this amino acid is involved in multiple areas of human physiology and metabolism.

The availability of L-arginine for physiological functions is determined by its endogenous sources, including its de novo synthesis and whole-body protein turnover, and its exogenous source, i.e. dietary intake. With respect to endogenous sources, whole-body protein turnover is the main contributor, while de novo synthesis accounts for only 5-15% of endogenous arginine flux in adult animals and humans [5]. Interestingly, arginine biosynthetic pathways do not have the capacity to compensate for depletion or inadequate dietary supply in adult rats and humans to meet metabolic demand [6-8]. Thus, with regard to dietary requirements, L-arginine is classified as a semi-essential or conditionally essential amino acid [9, 10]. Dietary intake of L-arginine remains the primary determinant of plasma arginine levels and may be of special importance in L-arginine homeostasis disturbances.

Diabetes is a complex metabolic disorder caused by a defect in insulin secretion and/or insulin action. Diabetes is associated with disturbances in the metabolism of the main energetic substrates, glucose and lipid. In addition, disturbances in protein and L-arginine metabolism [11] result in reduced concentrations of L-arginine in plasma and in many tissues [12]. Such changes in L-arginine homeostasis can be improved by L-arginine supplementation which has been shown to increase plasma urea and protein [13] as well as arginine levels [14] in diabetes. Many of the positive effects of arginine in diabetes, obtained from experimental models of diabetes type 1 and type 2 as well as in diabetic patients [15-17], are thought to be mediated by its effects on endothelium-dependent relaxation and vascular disturbances. However, important progress in L-arginine research suggests that this amino acid and its metabolites play a more complex role in the regulation of whole-body energy homeostasis, and thus dietary supplementation of L-arginine could have multiple beneficial effects in the treatment of this disease.

In view of the growing evidence regarding the beneficial effects of L-arginine in diabetes, the aim of
this review is to highlight the effects of L-arginine on: (1) β-cells function and population in relation to their role in the etiology and progression of diabetes type 1 and type 2 and (2) peripheral insulin sensitivity in terms of insulin resistance and type 2 diabetes-associated obesity.

2. L-ARGININE HOMEOSTASIS - REGULATORY PATHWAYS

2.1. L-Arginine in Dietary Sources: Nutraceutical Aspects

The relative amounts of L-arginine in various proteins range from 3% to 15% [18]. Foods rich in L-arginine include seafood, watermelon juice, nuts, seeds, algae, meats, rice, and soy [19-21]. Differing dietary habits between populations may account for differences in L-arginine plasma levels in various parts of the world. It has been reported that about 5 g of L-arginine is ingested each day in the average Western diet [22].

Oral L-arginine supplementation is hampered by its extensive metabolism. In adult humans, pigs and rats about 40% of dietary L-arginine is degraded by the small intestine [23, 24]. Absorbed L-arginine is transported to the liver where the major portion is utilized by the hepatic urea cycle. The dietary L-arginine that passes the liver has multiple metabolic fates determined by a complex network of factors including arginine transporters in plasma and mitochondrial membranes and several key enzymatic pathways differentially expressed according to the cell type, age and developmental stage, diet and health and disease status. Thus, multiple endogenous and exogenous aspects need to be considered when L-arginine is ingested each day in the average Western diet [22].

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by the gradual increased capacity of the kidney to synthesize arginine from citrulline, extracted from the blood after release from the small intestine. Thus, in adults the kidneys take over the role of about 60% of net arginine synthesis [6, 58]. In contrast, in the liver there is no or little net production of arginine due to high arginase activity in hepatocytes [59]. For details of L-arginine metabolism see Figure 1 and the excellent review articles by Wu and Moriss [5] and Wu and coworkers [60].

2.3. L-Arginine Interconversion Pathways

The importance of L-arginine from the physiological and nutritional aspects arises from the fact that, in addition to L-arginine per se, products of its metabolism play significant roles in the regulation of fundamental cellular processes and physiological functions. Arginine is metabolized through multiple inter-related metabolic pathways that show complex compartmentalization and interactions at the cellular, tissue and systemic levels. Not only is it metabolically interconvertible with the amino acids proline and glutamate, but it also serves as a precursor for the synthesis of protein, NO, creatine, polyamines, agmatine and urea. Key enzymes catalyzing these pathways are arginase, NO synthases (NOSs), arginine:glycine amidinotransferase and arginine decarboxylase.

In mammals, the arginase pathway is quantitatively most important for arginine catabolism. Arginase exists in two distinct forms (type I and type II), encoded by separate genes, that are immunologically distinguishable and have specific subcellular localizations and tissue distribution [61]. Arginase I is a cytosolic enzyme located primarily in the liver of ureotelic organisms where it serves as one of the key enzymes of the urea cycle [59]. Arginase II is located

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**Figure 1: General pathways of L-arginine metabolism.** Major players in L-arginine de novo synthesis and catabolism are shown. Central place in the scheme belongs to the Krebs bicycle (consisting of urea cycle, citric acid cycle and aspartate-argininosuccinate shunt) that represents meeting point for the majority of L-arginine metabolic pathways, both anabolic and catabolic. L-arginine and its metabolites are highly important in maintaining the structural and functional integrity of cells (especially in the context of cell signaling). Arginase-catalyzed pathway provides the highest rate of L-arginine turnover (to ornithine and, further, to polyamines) but the biological effects of this amino acid are far more visible through its conversion into nitric oxide by enzyme nitric oxide synthase. In the scheme, only enzymes that convert L-arginine into its key metabolites are identified.
within the mitochondrion and has a widespread tissue distribution, with the highest levels of expression in the kidney, prostate, and brain and lower levels in a variety of other tissues including the pancreas [62-64]. The products of arginase activity are polyamines, putrescine, spermidine and spermine. Polyamines play important roles in the differentiation, growth and proliferation of cells [65, 66]. They interact with various cell components including nucleic acids, and their intracellular concentration increases in certain cell phases [67].

Furthermore, the significant role of arginase in the regulation of L-arginine metabolic effects may be ascribed to the fact that arginase effectively competes with NOSs for L-arginine [5]. Therefore, it determines the availability of L-arginine for NO synthesis and NO-dependent biological processes. In diabetes, increased liver arginase activity causes a decrease in L-arginine plasma level and NO-mediated effects [11].

2.3.1. NO-Producing System

Another pathway of arginine metabolism is its oxidation catalyzed by NOS, which leads to NO and citrulline production. The large majority of the physiological effects of exogenous L-arginine is due to its metabolism by NOS, despite the fact that there is a low level of NO production compared to the overall arginine catabolism. The reason for this lies in the chemical nature of NO; as a free radical, NO possesses high reactivity. This classifies NO as a signaling molecule with significant miscellaneous potential. NO mediates signaling either through cGMP-dependent or independent pathways. The former involves activation of soluble guanylate cyclase and cGMP-signaling [68], while the latter comprises NO interactions with various intracellular molecules ranging from small redox molecules (superoxide and glutathione) to large biomolecules (proteins - membrane receptors, intracellular kinases and phosphatases and transcriptional factors).

In mammals, three isoforms of NOS have been identified: neuronal (nNOS or NOSI) and endothelial (eNOS or NOSIII), originally described in neuronal tissue and endothelial cells, respectively, and inducible NOS (iNOS and NOSII), originally found in macrophages [69, 70]. All three isoforms identified in mammals are heme-containing proteins that are dimeric in native conditions; dimerization of NOS is supported by L-arginine, suggesting its structural role, in addition to a substrate role for NOS [71]. The initially established paradigm that nNOS and eNOS are constitutively expressed, while iNOS is solely an inducible form is being challenged by the increasing number of reports demonstrating that all three isoforms can be induced by different stimuli and expressed in various tissues and cells [69, 70]. However there are clear differences between the NOS isoforms in relation to the level of NO production; iNOS produces higher NO levels (μM-mM) and remains active for a longer time period compared to nNOS and eNOS (level of NO production from nM-μM).

The effect of NO depends on its local concentration, which is determined by the rate of its synthesis, and by the cellular redox milieu [69]. It is important to mention that dietary supplementation of arginine increases NO synthesis within the physiological range in various tissues [70]. Besides, the effects of NO depend on the intracellular place of its production. To accomplish both issues, NOSs are tightly regulated at the transcriptional and translational levels, through co- and post-translational modification, by substrate and cofactors availability and subcellular compartmentalization [70, 72]. NO synthases are localized at specific cellular sites, where they are in close proximity to their target molecules. This functional localization is defined by lipid modifications and protein-protein interactions [72]. A perfect example of spatial dependence of NOS function has been demonstrated in β-cells, where the specific localization of nNOS in secretory vesicles enables this enzyme to control insulin secretion, as it is discussed below [73].

2.3.2. L-Arginine and NOS: Arginine Paradox

The intracellular concentration of L-arginine is in the range of 100-800 μM in cells and from 40-100 μM in plasma [74]. Therefore, it seems that there is saturation level of L-arginine for NO synthesis since the Km of L-arginine for nNOS is 1.4-2.2 μM and for eNOS is ~2.9 μM [75]. However, it has been shown that an external supply of L-arginine increases cellular NO production [76]. This phenomenon is known as the “arginine paradox”. In other words, this shows that exogenous L-arginine application in vivo causes additional NO-mediated biological effects despite the fact that NOSs are theoretically saturated with the substrate. Supplementation of L-arginine has been shown repeatedly to exert beneficial effects on endothelium-dependent vasodilatation in vivo in a dose-dependent manner [77]. This phenomenon remains to be explained, but may be related to endogenous NOS inhibitors [75], receptor-mediated activation of NOS by
exogenous L-arginine [78] and compartmentalization of intracellular L-arginine [79, 80].

3. BENEFICIAL EFFECTS OF L-ARGININE IN DIABETES

3.1. β-Cells in Diabetes

Diabetes is the result of an inadequate mass of functional β-cells. In type 1 diabetes, the immune system attacks and destroys β-cells by mechanisms still incompletely understood [81]. Type 2 diabetes is a progressive disorder which begins with peripheral insulin resistance and ends with the failure of pancreatic β-cells. To compensate for peripheral insulin resistance, pancreatic β-cells increase in mass and secrete more insulin, leading to hyperinsulinemia. However, at a certain point, β-cells can no longer compensate for peripheral insulin resistance, and plasma glucose levels rise. Increased glucose levels, along with increased levels of free fatty acids due to insulin resistance, can damage β-cells (gluco- and lipotoxicity), leading to progressive loss of these cells [82]. Thus, it seems likely that the replacement of β-cells could allow physiological control of glycemia in both types of the disease. Human islets transplantation is successful in restoring normal glycemia, but it is limited by the need for toxic immunosuppressive drugs, the scarcity of donors, and graft failure usually within a few years [83]. Alternative ways of replacing β-cells are needed and may theoretically be obtained by: (a) replication of the remaining β-cells; (b) neogenesis and (c) transplantation of β-cells derived from stem or somatic precursor cells. In adults, the capacity of highly differentiated β-cells to replicate is very low [84, 85]. Thus, neogenesis represents an attractive approach to restore functional β-cell population from non-insulin progenitor cells in the pancreas occurring in two phases, extensive proliferation of the precursor cells followed by their differentiation [85]. The source of new β-cells may be the pancreas itself (acinar, ductal and endocrine cells) or extra-pancreatic tissue such as the liver. Understanding the molecular mechanisms that trigger and control neogenesis as well as the nature of precursor cells has been the focus of diabetes research in the last decade and is central in the therapeutic approaches to induce meaningful β-cell neogenesis in diabetes.

3.1.1. L-Arginine Induces β-Cell Regeneration

The β-cell regenerative capacity of L-arginine and the underlying mechanisms of its action in the diabetic pancreas have recently been reported [86]. We reported that 12 days of oral supplementation with L-arginine had multiple beneficial roles in the diabetic pancreas resulting in endocrine pancreatic cell neogenesis (Figure 2). Initially, this included activation of pancreatic duodenum homeobox-1 (PDX-1), a specific transcription factor that determines β-cell phenotype including the expression of insulin, glucose transporter 2 (GLUT2) and hexokinase IV [87]. Strong coexpression of PDX-1 with insulin was observed in exocrine pancreas suggesting that precursors of new β-cells reside here. Surprising plasticity and capacity of differentiated acinar and ductal cells to transdifferentiate in insulin-producing cells has been shown previously under various conditions when the endocrine function of this gland was challenged [88-90]. Our results suggest that L-arginine could be one of the stimuli that induce the transition of exocrine cells into endocrine β-cells in the diabetic pancreas [86]. This process appears to involve proliferation of the exocrine cells, since L-arginine induces nuclear translocation of proliferating cell nuclear antigen (PCNA), positive regulators of cell growth and proliferation as well as nuclear factor-kB (NF-kB), a ubiquitous transcription factor, whose specific function in the pancreas is activation of transcription in so-called “regenerative” genes [91, 92]. It seems likely that the observed effects of L-arginine on β-cell regeneration are NO-dependent as strong nNOS expression was observed after L-arginine treatment in diabetic animals. This suggests that nNOS-derived NO plays a more complex role in the regulation of β-cell population dynamics in addition to its role in the regulation of insulin secretion (discussed below).

A similar beneficial effect of the L-arginine/NO pathway in the pancreas was recently reported after an L-arginine rich diet in rats with type 1 diabetes [93]. Namely, coconut kernel protein, which is rich in L-arginine, reduced diabetes-related pancreatic damage, in parallel with an increase in plasma NOS activity.

There is also experimental evidence that L-arginine causes an increase of polyamines levels in the diabetic pancreas suggesting that the effects of L-arginine in recovery of the β-cell population may be NO-independent [94, 95].

3.1.2. L-Arginine Regulates β-Cell Metabolism and Insulin Secretion

In addition to restoring β-cell mass, the beneficial effects of L-arginine on these cells could be due to its interference with insulin synthetic and secreting
pathways (Figure 3). The role of L-arginine in the stimulation of insulin secretion has been shown in physiological conditions and in the diabetic state [96, 97]. The effect of L-arginine on insulin secretion is more complex than its action as a cationic amino acid on membrane depolarization [98, 99], but could also be mediated by the products of its metabolism in pancreatic β-cells.

It has been shown that L-arginine supplementation in diabetic animals increases pancreatic arginase activity and polyamine levels [94]. Polyamines play a role in insulin biosynthesis and β-cell replication [66]. They are localized in the secretory granules of β-cells and their concentration increases in response to β-cell mitogens, such as glucose and growth hormone [66].

The effects of L-arginine on insulin secretion could also be mediated by NO. NO acts as a physiological modulator of islet hormone release through S-nitrosylation of certain thiol-dependent enzymes and regulatory proteins involved in the insulin-releasing pathway, as well as derangement of the reduced/oxidized GSH equilibrium [100]. However, many studies in which NOS activity was manipulated by NO donors and NOS substrates produced controversial data regarding the influence of NO on islet hormone release. Data suggest that L-arginine-derived NO may mediate insulin secretion via stimulation of guanylate cyclase and cGMP formation [101], while some authors reported inhibitory effects of NO on insulin release [102]. Evidence from these studies suggest that the effects of NO depend on the concentration and indicates that analysis of NOSs is necessary for defining the influence of NO on islet hormone release.

All three isoforms of nitric oxide synthase, nNOS, eNOS and iNOS, are expressed in the pancreas. Specific tissue and cellular localization of the NOS isoform determines its role in the regulation of pancreatic function [103]. nNOS is usually considered to be the major NOS isoform with multifunctional properties in pancreatic tissue. Besides ganglion cells and nerve fibers, nNOS is also expressed in pancreatic exocrine and islet cells. Furthermore, Lajoix et al. [73] found that nNOS and insulin were colocalized mainly in insulin secretory granules in β-cells. In addition, Rizzo and Piston [104] reported that the regulation of hexokinase IV localization and activity in β-cells is directly related to NO production and that the association of hexokinase IV with secretory granules occurs through its interaction with nNOS. These data suggest that the nNOS isoform is more conveniently situated to control insulin release than iNOS, which is localized in the cytoplasm [105]. We recently found that L-arginine treatment differentially affected nNOS immunopositivity in normal and diabetic pancreas, and thus probably insulin secretion [86]. In non-diabetic rats, faint nNOS immunopositivity was accompanied by a decrease in insulin plasma concentration, while in diabetic rats there was strong nNOS expression associated with restored insulin level in plasma and pancreatic tissue. These observations favor the assumption that NO acts as a positive modulator of insulin release and that nNOS mediates the effects of supplemented L-arginine in the diabetic pancreas.

The broad spectrum of beneficial effects due to L-arginine in β-cells includes its action on intracellular protective mechanisms and energy state, both very important for β-cell structural and functional integrity.
Oral supplementation with L-arginine improves the glutathione-dependent part of antioxidative defense in the diabetic pancreas [86]. Krause et al. [106] showed in vitro that increased provision of L-arginine to clonal ß-cells resulted in enhanced synthesis of glutathione and antioxidative defense and a protective response against inflammatory challenge. Given that insulin-secreting cells have very low basal expression levels of antioxidant enzymes such as catalase and glutathione peroxidase [107, 108], the improvement in antioxidative defense by L-arginine could make these cells less prone to oxidative stress in the environment typical of type 1 and type 2 diabetes [109-112].

Direct evidence for the role of L-arginine in the regulation of ß-cell energy metabolism has been provided recently by Krause et al. [106]. The authors reported that L-arginine increases glucose consumption and intermediary metabolism in ß-cells. These results suggest that L-arginine could improve ß-cell glucose responsiveness, one of the primary goals in the treatment of ß-cells in diabetes [113].

Furthermore, the effects of L-arginine supplementation on insulin secretion in vivo may be more complex, related to its systemic actions not directly related to ß-cells. This includes the effects on blood flow [114, 115], neurotransmission [116, 117] and exocrine secretion [118], all of which could affect insulin release itself.

3.2. Insulin Resistance: Link with Obesity

In addition to ß-cell failure, type 2 diabetes is characterized by peripheral insulin resistance. In most
cases, peripheral insulin resistance, defined as the attenuated response to insulin in fat tissue, liver and skeletal muscle, appears long before the development of hyperglycemia [82]. The etiology of insulin resistance is multifactorial including genetic and exogenous factors, first of all low physical activity and obesity. Obesity characterizes approximately 60-80% cases of diabetes type 2. It causes features of metabolic dysfunction in adipose tissue including reduced insulin-stimulated glucose transport, reduced expression of glucose transporter 4 (GLUT4), altered expression of adipokines and adipocyte hypertrophy. Hypertrophy of adipose tissue develops when dietary intake of energy exceeds whole-body energy expenditure. In the case where the capacity of adipose tissue to store fatty acids is exhausted, the level of free fatty acids in blood increases and their mobilization and ectopic accumulation in skeletal muscle and liver occur. This triggers resistance to insulin in liver and skeletal muscle [119]. Resistance to the insulin-mediated suppression of hepatic gluconeogenesis and glycogenolysis increases glucose output from the liver, while in skeletal muscle insulin resistance leads to reduced glucose uptake. All these lead to hyperglycemia and further progression to type 2 diabetes. Therefore, type 2 diabetes is a particularly challenging clinical condition to treat because of the complex pathophysiological basis. The main research effort in the last few years in the prevention and treatment of diabetes type 2 has been to find ways to increase lipid metabolism in adipose tissue and regulate energy partitioning, i.e. treat obesity and/or to directly improve glucose uptake and metabolism.

3.2.1. L-Arginine Reduces Obesity: Regulation of Fat Metabolism

Positive effects of L-arginine on adipose tissue reduction have been documented in obese and non-obese rats and humans [120-124]. Fu et al. [120] showed that dietary L-arginine supplementation reduced body weight and epididymal and retroperitoneal fat weight in adult Zucker diabetic obese rats, a genetically obese model of diabetes type 2. Similar changes were observed after supplementation of citrulline-rich watermelon to these animals [19] and after supplementation of L-arginine to diet-induced obese rats [122, 125]. To date, only one clinical trial has confirmed the effect of L-arginine in reducing fat mass in obese diabetic subjects. Lucotti et al. [123] showed that L-arginine-induced a decrease in body weight was due to fat mass reduction when L-arginine was added to a hypocaloric diet and exercise in obese, insulin-resistant type 2 diabetic patients. In addition, a number of experimental and clinical studies have reported the beneficial effects of L-arginine on the serum lipid profile in diabetes including total cholesterol, low-density lipoproteins and triglyceride levels [125-127].

The outlined effects of L-arginine on fat mass are a result of its role in the regulation of adipocyte lipid metabolism. In vitro studies have revealed that arginine stimulates lipolysis in adipocytes and promotes the oxidation of long-chain fatty acids in insulin-sensitive tissues [121, 128, 129]. An in vivo study also showed that along with a reduction in fat mass, arginine induced an increase in the oxidation of octanoate in abdominal and epididymal adipose tissue in obese rats [120]. In contrast, L-arginine decreased the incorporation of palmitate and glucose into triglycerides in human adipocytes suggesting decreased lipogenesis [122, 129]. The molecular mechanisms underlying such a metabolic shift in white fat cells from lipid storage toward lipid mobilization and fatty acid utilization after L-arginine dietary supplementation have been suggested. These include the regulation of gene expression and activity of the enzymes involved in lipid and glucose metabolism. Specifically, arginine supplementation down-regulates the expression of lipogenic genes, such as lipoprotein lipase (LPL), acetyl-CoA carboxylase (ACC) alpha, but up-regulates the expression of genes involved in lipolysis in porcine white adipose tissue, including hormone-sensitive lipase (HSL) [130]. Furthermore, L-arginine up-regulates the expression of genes involved in fatty acid oxidation: nNOS, heme oxygenase-3 and peroxisome proliferator-activated receptor-γ coactivator 1α (PGC-1α), as has been shown in the adipose tissue of obese diabetic rats [120]. An increase in nNOS suggests that the effects of dietary L-arginine supplementation on lipid metabolism in white adipose tissue are mediated by NO. However, in vitro studies on the effect of NO donors on lipid metabolism in adipocytes produced controversial data, some of which confirmed the stimulatory effect, and some showed the opposite effect of NO on lipolysis. Such contradictory results could be due to differences in NO donors used and concentration of NO, tissue origin, intracellular redox state etc. In vivo studies provided more consistent results demonstrating that the physiological level of NO, which is achieved with L-arginine supplementation, increases lipolysis in rat adipose tissue [128].

In addition to the direct effect of L-arginine on white fat lipid metabolism, it can also regulate the level of the
adipocyte-derived hormones, adipokines. With respect to the role that adipokines play in the central regulation of food intake and peripheral regulation of energy expenditure and insulin sensitivity, alterations in their synthesis and plasma levels contribute to diabetes-associated disturbances in whole-body energy homeostasis. It has been shown that increased plasma level of leptin, coupled with leptin resistance, is associated with fat accumulation [131]. Stingl et al. [132] found that L-arginine infusion transiently decreased the plasma level of leptin in insulin-deficient and hyperinsulinemic diabetic patients. A similar effect was observed after long-term dietary L-arginine supplementation in obese, insulin-resistant type 2 diabetic patients; L-arginine decreased leptin level and the leptin/adiponectin ratio [123]. Oral L-arginine supplementation can also induce a decrease in resistin expression in white adipose tissue, as we recently observed in L-arginine-treated animals (unpublished data). Such effect of L-arginine on the level of resistin could be beneficial in respect to pathology of type 2 diabetes, since the increase in resistin level positively correlate with increase in adiposity and decreased insulin sensitivity [133-135].

3.2.2. Brown Adipose Tissue Stimulation: Strategy for Increasing Energy Expenditure in Diabetes

Brown adipose tissue (BAT) is specialized for adaptive thermogenesis, the energy expenditure induced by cold exposure or diet. Fatty acid oxidation and heat production by BAT are due to intense metabolic activity because of the presence of a large number of mitochondria, and the expression of uncoupling protein 1 (UCP1), which uncouples ATP synthesis from the respiratory process to generate heat [136]. The main fuel, fatty acids, is provided both by BAT and white adipose tissue. Therefore, it is not surprising that in the last few years there has been particular interest in finding a way to stimulate BAT function or to induce the conversion from white to brown adipocytes as a strategy to control fat mass and obesity (conversion from energy storage to energy expenditure). This research is especially encouraging as the evidences for functional BAT in adult humans emerge [137, 138]. Furthermore, it has been shown that BAT activity is reduced in adult overweight or obese humans and is positively correlated with resting metabolic rate [139].

There is growing evidence to show that L-arginine has the potential to induce BAT function in physiological states and diabetes. Vasilijevic et al. [140] showed that L-arginine supplementation restored diabetes-induced disturbances in the molecular basis of thermogenesis (including UCP1 expression) in BAT of animals with type 1 diabetes. In addition, the stimulatory effects of L-arginine on BAT mass have been reported in diet-induced obese diabetic rats [141]. Our extensive research on BAT physiology has shown that the L-arginine/NO action on BAT involves structural, metabolic and molecular remodeling of this tissue, in terms of its functional activation. In that context, L-arginine supplementation to cold-acclimated rats induced BAT hyperplasia, proliferation and differentiation of brown adipocytes, increased tissue vascularization and innervation as well as mitochondriogenesis [142-145]. Such structural changes in BAT are accompanied by L-arginine-induced recruitment of the molecular program including key thermogenesis-related regulatory and effectors molecules: PGC-1α, peroxisome proliferator-activated receptor γ (PPARγ), vascular endothelial growth factor (VEGF), and UCP1 [146]. The effect of L-arginine on BAT functional activation involves improving the oxidative metabolism of this tissue through the regulation of key enzymes for glucose and fatty acid metabolism and energy state sensors (5'-AMP-activated protein kinase α - AMPKα and hypoxia-inducible factor 1α - HIF-1α) [147].

3.2.3. L-Arginine Improves Insulin Resistance: Regulation of Glucose Uptake and Metabolism

The favorable effects of exogenous L-arginine in diabetes type 2 can also be attributed to its direct role in improving insulin sensitivity and glucose uptake and metabolism. It has been shown that dietary L-arginine supplementation ameliorates insulin sensitivity in an experimental model of diabetes as well as in diabetic patients [127, 148-150]. Data from the well-known insulin sensitivity tests clearly show that L-arginine increases glucose uptake and metabolism in insulin sensitive tissues and suggest the involvement of the L-arginine-NO producing pathway. The improvement in glucose metabolism by L-arginine could be a consequence of increased blood flow and tissue substrate supply due to NO-induced vasodilatation [128]. Vasodilatatory effects of NO could be mediated by NO itself or through its interference with the insulin-signaling pathway in the endothelium. Baron et al. [151, 152] showed that insulin-mediated vasodilatation is largely dependent on the action of insulin on NO release, whereas Petrie et al. [153] showed that endothelial NO synthesis and insulin sensitivity are positively correlated in healthy individuals. In type 2
diabetic patients, L-arginine administration decreases vascular resistance and increases blood flow [149].

However, the increase in NO availability after L-arginine supplementation could improve systemic insulin sensitivity independently of its vasodilatation effects. In support of this, insulin-sensitive tissues express NOS: skeletal muscles express all three isoforms while adipose tissue mainly expresses eNOS and iNOS [128, 154-156]. In addition, in vitro studies showed that NO per se affects glucose uptake: NO donors increase [157], while NOS inhibitors decrease glucose transport in isolated skeletal muscle [158].

The direct effect of NO on glucose uptake could be related to insulin signaling in skeletal muscle and adipose tissue. There is direct in vivo evidence that insulin stimulation of glucose uptake in skeletal muscle and adipose tissue is NO dependent. Roy et al. [159] showed that infusion of the NOS inhibitor, Nω-monomethyl-L-arginine (L-NMMA), in rats inhibited insulin-mediated glucose uptake in muscles and various adipose tissue depots, while Marliss et al. [160] reported that the increase in plasma level of this NOS inhibitor correlates with insulin resistance in obese and aging subjects.

Furthermore, it has been shown that NO stimulates glucose uptake in skeletal muscle and adipose tissue in an insulin-independent manner [149, 161, 162]. These effects of NO imply GLUT4 translocation independent of phosphorylation of the insulin receptor substrate and activation of phosphatidyl inositol 3 kinase. It has been shown that exercise-stimulated GLUT4 activation and glucose uptake is mediated by NO, and the existence of two different pools of GLUT4 transporter in skeletal muscles (exercise- and insulin- sensitive) has been demonstrated [163, 164]. McConell et al. [165] showed that L-arginine infusion increases glucose clearance during prolonged exercise independently of its effects on blood flow and insulin level. The potential of L-arginine to increase glucose uptake independently of insulin could be of special importance in terms of its pharmacological potential in diabetes treatment.

In addition to increasing glucose uptake, NO can improve overall glucose metabolism acting on glucose oxidation in insulin-sensitive tissues. Sodium nitroprusside, a well-known NO donor, significantly increased lactate release and glucose oxidation in isolated skeletal muscle [166, 167], while L-NNAME treatment of cold-acclimated animals decreased lactate dehydrogenase activity in skeletal muscle in vivo (unpublished data). Fu et al. [120] showed that dietary L-arginine supplementation enhanced glucose oxidation in the fat tissues of obese rats. It seems likely that L-arginine is also able to enhance glucose metabolism in the liver. Supplementation with dietary coconut kernel protein, which is rich in L-arginine, resulted in reversal of diabetes-induced decreased activities of hexokinase and pyruvate kinase in the liver of diabetic rats [168]. In addition, L-arginine increased glucokinase activity in cultured rat hepatocytes [169].

4. CENTRAL MEDIATOR OF THE METABOLIC EFFECTS OF L-ARGININE IN DIABETES - AMPKα

AMPKα is recognized as an important sensor of cellular energy status and as an effector in maintaining energy balance at the whole body level. It is activated by an increase in the AMP/ATP ratio, and acts by switching on ATP-consuming pathways and switching off ATP-consuming pathways [170]. Thus, it plays an important role in regulation of the metabolism of energy substrates, glucose and fatty acids, in many tissues. In skeletal muscle, AMPKα increases glucose oxidation and glucose uptake, by increasing the expression and translocation of GLUT4 [171-175]. AMPKα signaling is also crucial in the regulation of fatty acid oxidation in liver and skeletal muscle [171, 176]. Acting through the inhibition of ACC, AMPKα favors fatty acids oxidation and suppresses their synthesis. In addition it has been shown that activation of AMPKα signaling inhibits gluconeogenesis in the liver [177].

The regulatory role of AMPKα on lipid and glucose metabolic pathways, along with previously discussed metabolic problems in diabetes, suggests that AMPKα could be a promising pharmaceutical target in the treatment of diabetes and associated metabolic disturbances. The anti-hyperglycemic drug, metformin, which is already incorporated in the therapy of diabetes type 2, activates AMPKα both in intact cells and in vivo [178]. Furthermore, it has been shown that activation of AMPKα by this anti-diabetic agent is mediated by NO, specifically with peroxynitrite (ONOO−) [179]. However, the hypoglycemic effect of metformin is mainly due to its inhibitory effect on glucose synthesis in the liver, as the concentration of the drug in the peripheral circulation (as opposed to the portal vein, which supplies the liver direct from the gut) may not be sufficiently high to yield significant AMPKα activation in other tissues, including skeletal muscle and adipose tissue. In contrast, it has been shown that oral supplementation of L-arginine induces the expression and/or activation of AMPKα in adipose tissue [120].
skeletal muscle [175, 180] and liver [181]. Thus, L-arginine may have an advantage over metformin in the regulation of whole-body energy metabolism. It seems that AMPKα may be a unique mediator in the beneficial effects of dietary L-arginine supplementation in obesity and insulin resistance in diabetes type 2. This hypothesis is supported by the data reported by Fu et al. [120], which showed that along with decreased fat mass in diabetic obese rats, dietary L-arginine supplementation also increased gene expression of AMPKα in these tissues. In addition, Linden et al. [179] reported recently that the stimulatory effect of L-arginine on glucose clearance rate included activation of AMPKα signaling in skeletal muscle.

Furthermore, the positive effect of L-arginine on β-cell dysfunction may be mediated by AMPKα. Krause et al. [106] showed that L-arginine induces activation of AMPKα in clonal β-cells and mouse islets. The role of AMPKα in β-cell stimulus-secretion coupling is unclear [182], but as AMPKα triggers energy-producing pathways, its activation could positively interfere with insulin secretion. It has also been suggested that activation of AMPKα may contribute to β-cell functional integrity [183] and consequently serve to counter β-cell glucolipotoxicity.

5. SUMMARY AND PERSPECTIVES

The understanding that diabetes is a chronic multifactorial disease, which is poorly treated with the available therapeutic approaches, has stimulated a renewed interest in the development of novel safe and effective therapies for this disease. Dietary components represent an attractive source of potential therapeutics for diabetes due to their wide availability, natural metabolism and low cost. This paper aimed to review the already known facts on L-arginine supplementation as a potentially useful nutritional strategy for the prevention and management of diabetes, and to emphasize and open new issues for examination in coming years.

The discovery that oral supplementation of L-arginine has an amazing capacity to increase the β-cell population in diabetes is highly important as the main effort in regenerative medicine is to stimulate β-cell regeneration in vivo. As the L-arginine/NO pathway positively interferes with insulin synthesis and secretion

Figure 4: Overview of multiple beneficial effects of L-arginine/NO pathway in diabetes; regulation of β-cell function, energy expenditure and insulin sensitivity. Cellular processes and metabolic pathways that are targets of L-arginine action are represented. The symbol “↑” denotes up-regulatory, while the symbol “↓” indicates down-regulatory effects of L-arginine. Abbreviation: WAT, white adipose tissue; BAT, brown adipose tissue.
pathways in β-cells, this amino acid may be used, along with insulin therapy, to normalize insulin levels in diabetes.

The vast majority of the anti-diabetic actions of L-arginine originate from its role in the regulation of the metabolism of the key energy substrates: lipid and glucose. Due to the effects of L-arginine in improving lipid and glucose metabolism as well as glucose uptake, this amino acid may be important in the control of the complex pathophysiological basis of type 2 diabetes, including obesity and insulin resistance. These multilevel actions may provide L-arginine with an advantage over the existing anti-diabetic drugs (metformin) and suggest that L-arginine could be an excellent nutritional support to increase energy expenditure (exercise) or reduce energy intake (diet) in the treatment of diabetes type 2 and associated pathology (Figure 4).

In addition, it is hoped that the presented data on the effects of L-arginine in diabetes will stimulate and provide a good basis for further research into the anti-diabetic potential of L-arginine–rich food. With respect to dietary intake, several issues require to be fully elucidated including L-arginine absorption, its tissue distribution and bioavailability, and tissue-specific metabolic pathways. Also, the physiological consequences of oral L-arginine supplementation in diabetes should be reconsidered in light of growing knowledge on the complex signaling pathways and molecular targets of its metabolic products, particularly NO. Some aspects of L-arginine action in diabetes are only experimentally proved, therefore clinical studies are needed to support our expectations regarding this nutritionally important amino acid in diabetes prevention and treatment.

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