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Nitric Oxide Bioavailability and Insulin Resistance: An Overview

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ABSTRACT

The aim of this review are to discusses the mechanisms by which insulin resistance develops in the presence of increased adiposity, to summarize the causative relationship between impaired NO bioavailability and insulin resistance, and also to show the implications of life-style changes to prevent insulin resistance. Obesity with increased visceral adiposity is an inflammatory condition that leads to insulin resistance. Because the insulin signalling pathway is linked to endothelial nitric oxide synthase (eNOS) activation, insulin resistance is always associated with decreased nitric oxide (NO) bioavailability. Recently, accumulating evidence has suggested that physical exercise and dietary nitrate/nitrite diets rich in vegetables improve insulin resistance by enhancing NO bioavailability, and thus provide potential preventive and therapeutic options for these patients with insulin resistance.

Keywords: Nitric oxide (NO); NO bioavailability; life-style-related disease; insulin resistance; nitrite; nitrate.

1. INTRODUCTION

The prevalence of obesity has recently increased, which has had a significant influence on global health [1]. In developed nations, obesity is a significant economic burden and the cause of a pre-diabetic condition [2]. Therefore, daily lifestyle adjustments linked to nutrition and exercise are strongly advised for obese people before turning to costly medication therapy [3]. A growing body of research has shown that vascular endothelial dysfunction may be a common mechanism underlying lifestyle-related diseases like insulin resistance, hypertension, and atherosclerosis, and that nitrate/nitrite-rich diets and exercise training can improve the characteristics of these pre-diabetic states by increasing the bioavailability of nitric oxide (NO) in both animal models and humans [3-5]. The aim of this review is to summarize the causal relationship between impaired NO bioavailability and insulin resistance, and to show the molecular-based

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mechanisms and the preventive effects of exercise and nitrate/nitrite rich-diets on insulin resistance.

2. OBESITY IS AN INFLAMMATORY STATE LEADING TO INSULIN RESISTANCE

Insulin resistance is a characteristic feature of obese patients with type 2 diabetes mellitus (DM) and/or metabolic syndrome. In particular, visceral obesity plays an important role in the development of insulin resistance [6]. The total number of adipocytes is thought to be determined in childhood and adolescence. Thus, young people can exhibit adipose hyperplasia due to the generation of de novo adipocytes, however, adults consuming a large number of calories and high-fat diets store the excess lipids in preexisting adipocytes due to their lower capacity for adipogenesis, resulting in adipocyte hypertrophy and visceral obesity [7].

Although adipose tissue is necessary for the normal secretion of leptin and adiponectin to enhance insulin sensitivity, impaired secretion of such adipokines, as is observed in lipodystrophy of humans and mice, results in insulin resistance [8,9]. In contrast, hypertrophic adipocytes produce other kinds of adipokines, such as monocyte chemoattractant protein-1 (MCP-1) and tumor necrosis factor-α (TNF-α), which lead to the adhesion and infiltration of macrophages into muscle and adipose tissues and increased production of inflammatory mediators. Increased visceral adiposity induces lipolysis in adipose tissues and releases free fatty acids (FFAs) into the systemic circulation via the portal vein [6]. It has been well documented that toxic lipid metabolites such as long-chain fatty acyl CoAs, diacylglycerol and ceramides play an important role in the pathogenesis of insulin resistance in skeletal muscle and liver [10,11]. In particular, saturated fatty acids, induce toll-like receptor 4 (TLR4)-mediated inflammatory responses in macrophages, which then express and secret pro-inflammatory cytokines, including interleukin-1B (IL-1B), interleukin-6 (IL-6), TNF-α, and MCP-1 through transcription factor-mediated signaling pathways including the IkkB/NF-κB and JNK/AP-1 pathways [12-14]. These inflammatory mediators activate a number of kinases, which phosphorylate the serine residues of insulin receptor substrate-1 (IRS-1), leading to the inhibition of insulin signaling and thereby causing insulin resistance (Fig. 1) [10].

In addition, excessive mitochondrial production of reactive oxygen species (ROS) accounts for another mechanism underlying the dysregulation of signaling and insulin resistance [15]. In particular, superoxide anion formed by leaked electrons and oxygen following excessive nutrient intake rapidly reacts with NO, and reduces the bioavailability of NO due to the formation of a potent oxidant, peroxynitrite. The ROS emitted in the mitochondria of insulin-targeted cells [15] disrupts the delicate redox balance that is normally regulated by the phosphorylation/dephosphorylation of molecules in the insulin signaling cascade, including insulin receptor (IRS1/2), leading to insulin resistance [16,17] (Fig.1).

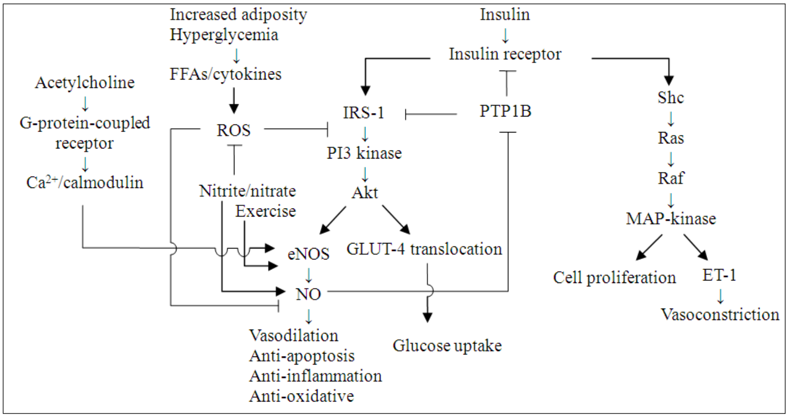

Fig. 1. The insulin signaling pathway

Insulin signaling starts with insulin binding to its receptor. The activation of the insulin receptor results in tyrosine phosphorylation of IRS and Shc, followed by the activation of two parallel pathways, the PI3K-Akt pathway, and the Ras/Raf/MAP kinase pathway. The PI3K-Akt pathway is intricately regulated by the redox balance and its disruption easily leads to insulin resistance and endothelial dysfunction (NO availability). However, the Ras/Raf/MAP kinase pathway is generally preserved even in the presence of insulin resistance, and subsequently produces ET-1 and exerts mitogenic effects leading to endothelial dysfunction

ROS: reactive oxygen species, FFAs: free fatty acids, IRS-1: insulin receptor substrate-1, PI3 kinase: phosphatidylinositol 3 kinase, NO: nitric oxide, eNOS: endothelial nitric oxide synthase. GLUT4: glucose transporter 4: MAP-kinase: mitogen-activated protein kinase, PTP1B: protein tyrosine phosphatase 1B, ET-1: endothelin 1

3. IMPAIRED NO BIOAVAILABILITY UNDER CONDITIONS OF OBESITY AND INSULIN RESISTANCE

The bioavailability of NO is diminished by reduced nitric oxide synthase (NOS) expression, impaired NOS enzymatic activity and NO quenching by reactions with reactive species (e.g., superoxide). The recent reports regarding cause-and-effect relationship between impaired NO bioavailability and diabetic states are listed in Table 1. Pro-inflammatory cytokines such as TNF-α downregulate the expression of endothelial NOS (eNOS) [18-20] by decreasing the stability of eNOS mRNA [21]. Valerio et al. demonstrated that TNF-α reduces the eNOS expression in the adipose tissues and skeletal muscles of obese rodents, and also showed that genetic deletion of TNF receptor 1 in this obese model restores the eNOS expression. These animals exhibit less body weight gain than the wild type control [22]. eNOS knockout animal models exhibit a number of features of insulin resistance and hypertension even in the absence of obesity [23-27]. Recent evidence also suggests that polymorphisms in the eNOS gene are associated with the susceptibility to insulin resistance and metabolic syndrome in humans [28-31]. Cook reported that mice with partial eNOS deficiency (eNOS+/-)

exhibit insulin resistance and hypertension only when challenged with a high-fat diet [32], suggesting a causative role for genetic variations in the eNOS gene in the development of insulin resistance in animal models and humans [27].

The details of the cause-and-effect relationship between impaired NO availability and insulin resistance are also well described in several review articles [16,43,48,55,110,111].

The enzymatic activity of eNOS is post-translationally regulated [33]. The insulin signal is transmitted downstream in the phosphatidylinositol 3-kinase (PI3K)-Akt-eNOS pathway, and activates eNOS through the phosphorylation of serine 1177 (S1177). Besides insulin, eNOS S1177 is also phosphorylated by Akt and AMP kinases activated by multiple mediators including shear stress, vascular endothelial growth factor, estrogen, statins, leptin, and adiponectin. Therefore, eNOS phosphorylation at S1177 is a crucial step in regulating eNOS activity and glucose uptake [34]. The interaction of eNOS with heat shock protein 90 (HSP 90) and its localization in the caveolae are also important for eNOS phosphorylation and activation. High-fat diets and obesity decrease eNOS activity by downregulating caveolin-1 [35] and disrupting the interaction of the eNOS-Akt complex with HSP 90 [36], thus impairing the assembly of the eNOS phosphorylation complex. In obese individuals, FFAs induce TLR4-mediated production of inflammatory cytokines and ROS, which inhibit the insulin-stimulated PI3K-Akt-eNOS pathway and eNOS phosphorylation [37], resulting in decreased NO bioavailability and insulin resistance [38-43]. Other mechanisms of insulin resistance associated with the phosphorylation of eNOS or IRS1/2 in diabetic states have also been reported [6,44,45].

The activity of NOS is also dependent on its proper dimerization (coupling). In particular, a reducing cofactor, tetrahydrobiopterin (BH_4), is critical for its activity [46]. In the case of obesity and diabetic states, the excessive oxidative stress leads to a decrease in the level of BH_4 and an increase in the level of its oxidized form (BH_2), which lead to eNOS uncoupling, resulting in the production of more superoxide rather than NO [47,48]. Superoxide rapidly reacts with NO to produce more potent oxidant peroxynitrite leading to endothelial dysfunction and atherosclerosis by oxidizing membrane lipids and low density lipoprotein cholesterol (LDL).

Another intriguing system post-translationally regulating eNOS activity is the dimethyl arginine dimethyl aminohydrolase (DDAH)/ asymmetric dimethyl arginine (ADMA)/NOS pathway [49]. ADMA is an endogenous NOS inhibitor, which is causally associated with insulin resistance [50]. Razny et al. demonstrated that the transgenic mice overexpressing DDAH, which degrades ADMA, increased NO bioavailability and attenuated high-fat diet-induced metabolic alterations including insulin resistance [27].

As shown in the Table 1, decreased NO bioavailability leads to a number of features of the diabetic state and might be an important molecular mechanism underlying the development of insulin resistance [51, 52].

Table 1. The cause-and-effect relationship between impaired NO availability and insulin resistance

Subjects/animals	Cause	Effects	References
Mice	eNOS/nNOS knockout	increased insulin resistance	[23]
Mice	eNOS knockout	insulin resistance, hyperlipidemia, hypertension	[24]
Humans	insulin resistance	increased expression of endogenous NOS inhibitor (ADMA)	[50]
Mice	diabetic mice	decreased BH_4 and endothelial dysfunction	[47]
Mice	eNOS knockout	increased cardiovascular risk, mimicking metabolic syndrome	[25]
Humans	eNOS polymorphism	increased insulin resistance, type 2 diabetes	[29]
Mice	partial gene deletion of eNOS	insulin resistance and hypertension when challenged with a high-fat diet	[32]
Humans	eNOS polymorphism	susceptible to metabolic syndrome	[28]
Humans	type 2 DM	impaired NOS activity	[37]
Rodent	obese rodent model	enhanced TNF-α downregulates eNOS expression	[22]
Humans	insulin resistance/type 2 DM	decreased nNOS protein expression in skeletal muscle	[95]
Mice	diet-induce obesity	reduced NO availability in isolated heart	[105]
Humans	eNOS polymorphism	increased susceptibility to metabolic syndrome	[31]
Humans	eNOS polymorphism	increased susceptibility to metabolic syndrome	[30]
Humans	obesity in juveniles	reduced NO availability	[106]
Mice	high fat diet-induced obesity	insulin resistance and reduced NO production	[60]
Mice	high fat diet-induced obesity	reduced NO-cGMP signaling, vascular inflammation and insulin resistance	[65]
Human	obesity/type 2 DM	reduced eNOS expression and insulin resistance	[107]
Mice	high-fat diet and transgenic mice (DDAH overexpressing)	increased insulin resistance, DDAH (degrades ADMA) increased NO availability and decreased insulin resistance	[26]
mice	high-fat diet/eNOS transgenic mice	decreased diet-induced obesity	[61]
humans	obese/decreased insulin sensitivity	reduced eNOS expression in skeletal muscle	[108]
humans	insulin resistance	decreased NO production	[109]
mice	iNOS knockout	increased insulin resistance and improved with nitrite supplementation	[104]

4. NO PROTECTS AGAINST INSULIN RESISTANCE

As mentioned above, the insulin receptor, which regulates the glucose homeostasis of insulin-responsive cells in the liver, muscle and adipose tissue, is associated with signaling pathways linked to the activation of eNOS [43,53-57] (Fig. 1). This might be a mechanism regulating the postprandial blood flow and nutrient disposition to peripheral tissues. Because insulin contributes to the coupling of metabolic (glucose uptake) and hemodynamic (endothelial function) homeostasis in normal subjects (as shown in Fig. 1), impairments upstream in the insulin signaling pathway are always accompanied by metabolic and endothelial dysfunction, consequently leading to insulin resistance, hypertension and atherosclerosis [43]. Biasucci et al. reported that endothelial dysfunction was found to occur in morbidly obese humans only when insulin resistance is present [58]. Assar et al. also showed that, unless insulin resistance is present, the vascular endothelial function can be preserved [59]. These lines of evidence suggest that a reciprocal relationship exists between insulin resistance and endothelial dysfunction [55,60]. Therefore, enhancing the bioavailability of NO should be a promising treatment strategy for the patients with insulin resistance [61].

Recent evidence suggests that NO plays suppressive roles in the development of insulin resistance at various levels, including effects on insulin secretion [27,62,63], mitochondrial function [64], modulation of inflammation [65], insulin signaling [66], and glucose uptake [27,67]. For example, insulin-stimulated NO production has physiological consequences resulting in capillary recruitment and increased blood flow in skeletal muscle for efficient glucose disposal [57]. NO suppresses the TLR4-mediated inflammation and ROS production by inactivating IkB kinase-β/nuclear factor-κB (IκκB/NF-κβ) [68,69]. Because NF-κB is an important trigger for the subsequent induction of a number of proinflammatory cytokines such as TNF-α and IL-1β, the suppression of this transcription factor could reduce metabolic disorders and the complications occurring in diabetics [70]. NO has been also shown to inhibit mitochondrial ROS overproduction by the *S*-nitrosation of mitochondrial respiratory chain complex 1 enzyme and to improve the efficiency of oxidative phosphorylation in mitochondria [5].

Accumulating evidence has suggested that the defect responsible for insulin resistance lies mostly at the post-receptor level of insulin signaling [71] (Fig. 1). Many kinases and phosphatases associated with the insulin signaling pathways are intricately regulated and balanced by protein phosphorylation/dephosphorylation and nitrosation [17]. Increased adiposity causes an oxidative shift in the intracellular redox environment [69], and impairs the early steps of the insulin signaling pathway [72]. Wang et al recently indicated that NO mediates *S*-nitrosation of protein-tyrosine phosphatase 1B (PTPB1) and enhances the effects of insulin [57]. Because PTPB1 dephosphorylates the insulin receptor and its substrates, attenuating the effects of insulin, its phosphatase activity tends to be suppressed by eNOS-mediated *S*-nitrosation. In contrast, once the vascular eNOS activity is impaired, PTPB1 suppresses the downstream signaling to PI3K/Akt, leading to insulin resistance (Fig. 1). Therefore, NO might act as a regulatory factor for the downstream signaling molecules linking GLUT4 translocation and glucose uptake [66,73]. In addition, Jiang recently reported that the NO-dependent nitrosation of GLUT4 facilitates GLUT4 translocation to the membrane for glucose uptake and improves insulin resistance [27,74].

5. EXERCISE ENHANCES THE BIOAVAILABILITY OF NO

Among the three isoforms of NOS, skeletal muscle expresses nNOSμ, an alternatively spliced isozyme of nNOS. eNOS is also expressed in skeletal muscle, but is mainly associated with the vascular endothelium. iNOS is not expressed in healthy skeletal muscle [75]. Many studies using animal models and studies in humans have demonstrated that exercise increases the expression of both the nNOS and eNOS proteins in skeletal muscle [76-79], but nNOS was the primary source of NO in skeletal muscle during contraction in a mouse model [80]. Muscle contraction increases the intracellular Ca^{2+} released from the sarcoplasmic reticulum and induces nNOS activation by causing the post-translational phosphorylation of the nNOS protein and producing NO in skeletal muscle during acute exercise [33,81]. In addition, shear stress on the vascular endothelium is an important stimulus that regulates the eNOS mRNA and protein expression levels in vitro [82-84] and in vivo [85,86].

Exercise training usually increases the heart rate, and enhances the blood flow and vascular shear stress [87]. Animal studies have demonstrated that exercise training increases the eNOS gene expression and improves the NO-mediated endothelial functions (flow-mediated dilatation study) [88,89]. During exercise as well as resting, the vascular endothelium senses mechanical stimulation from pulsatile and laminar blood flow, which is followed by signal transduction involving c-Src-tyrosine kinase and the subsequent activation of NF-κB, which then increases eNOS transcription and leads to the long-term stabilization of eNOS mRNA [90].

While the exercise-induced up-regulation of constitutive NOS expression is favorable for increasing the blood flow and energy efficiency during acute exercise in healthy subjects, it is also useful for allowing skeletal muscle to increase the bioavailability of NO in obese and insulin resistant subjects. Gomes et al. reported that while human subjects with metabolic syndrome exhibited lower plasma levels of nitrite and cGMP and increased ROS production compared with healthy subjects, a three-month exercise training program increased the plasma levels of nitrite and cGMP, and decreased the ROS production and plasma levels of an endogenous NOS inhibitor, ADMA [91].

Because skeletal muscle is an important target organ for the activities of insulin, enhanced NOS activity might play an important role in improving the glucose metabolism [92,93]. Kingwell reported that intra-arterial administration of L-NMMA, a NOS inhibitor, to type 2 DM and control groups significantly reduced the glucose uptake during exercise in both groups, but the type 2 DM groups exhibited a greater reliance on NO for glucose uptake during exercise than the control group [94].

In contrast, Bradley et al. examined the nNOS protein level in the vastus lateralis muscles of patients with impaired glucose homeostasis and low levels of muscle nNOS, and found that physical exercise improved the insulin sensitivity without influencing the nNOS protein levels in the muscle. They proposed that a reduction of upstream inflammatory mediators, including iNOS, following exercise might be responsible for improving insulin sensitivity in obese and type 2 diabetic patients [76,95]. Eghbalzadeh et al. have recently published a review article regarding the beneficial effects of physical exercise on the altered NO metabolism in the skeletal muscle of obese diabetic patients [76]. Because there have been few studies to date that have dealt with the effects of physical exercise on NOS-mediated NO metabolism in the skeletal muscle of subjects in diabetic states, further studies will be necessary to determine the detailed mechanism underlying the impact of exercise on these disorders.

6. NITRATE/NITRITE-RICH DIETS IMPROVE INSULIN RESISTANCE BY ENHANCING THE BIOAVAILABILITY OF NO

In addition to the NO produced by NOS, NO and NO-like activities can be also endogenously produced through the NOS-independent nitrate-nitrite-NO pathway. The mechanism by which nitrate/nitrite is reduced to NO is simple protonation, and this is enhanced during hypoxia and acidosis. There are a number of catalytic factors in blood and tissues which reduce nitrate/nitrite to NO, but a detailed discussion of these is beyond the scope of this review [96]. Contrary to the NOS-dependent mechanism, which requires oxygen and substrate arginine, the nitrate-nitrite-NO pathway serves as a back-up system to produce NO when the NOS function is impaired, as occurs in atherosclerosis with vascular endothelial dysfunction [97].

Nitrite and nitrate are rich in green leafy vegetables such as lettuce, spinach and beetroot [98]. Vegetables account for 60-80% of the daily nitrate intake in a Western diet [99]. One serving of such a vegetable contains more nitrate than what is endogenously generated by all three NOS isoforms during a 24-hour period in humans [56]. The ingested nitrate is absorbed in the upper gastrointestinal tract, and approximately 25% of the absorbed nitrate is concentrated in the salivary gland and secreted in saliva, followed by the reduction to nitrite by commensal anaerobic bacteria on the tongue [96]. In the acidic gastric milieu of stomach, part of this swallowed nitrite is immediately protonated to nitrous acid ($NO_2^- + H^+ \rightarrow HNO_2$), then decomposed to form a variety of nitrogen oxides such as NO, nitrogen dioxides (NO_2), dinitrogen trioxide (N_2O_3) ($2HNO_2 \rightarrow N_2O_3 + H_2O$, $N_2O_3 \rightarrow NO + NO_2$) and *S*-nitrosothiols (e.g. *S*-nitrosoglutathione and S-nitrosocysteine) [96]. Substantial elevations in plasma nitrite and the S-nitrosothiols can occur by increasing the dietary nitrate intake [100, 101], and can serve as a substrate or a source for NO generation in muscle and adipose tissue.

The therapeutic potential of dietary nitrate/nitrite has been supported by recent studies describing the improvements in insulin resistance and metabolic syndrome in human and animal experiments by enhancing the NO bioavailability in plasma and tissues [51,74,102-104].

7. CONCLUSION

In conclusion, endogenous NO defects underlie the development of insulin resistance. Life-style changes, including changes in diet and physical activity, might improve the features of insulin resistance and provide an inexpensive and easily practicable method to enhance the bioavailability of NO for patients.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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Research and Academic Experience: He is currently a full-time Professor of Department of Clinical Dietetics and Human Nutrition, Faculty of Pharmaceutical Science, Josai University in Japan. He graduated from the Hamamatsu University School of Medicine in Japan in 1981, and has worked in hospitals as a paediatric cardiologist for 20 years. In parallel with his clinical works, he obtained his PhD degree at the Hamamatsu University School of Medicine in 1996. He got interested in the basic study on cardiovascular pathophysiology after his three years' research work for Dr. Marlene Rabinovitch in the Hospital for Sick Children in Toronto, Canada. After returning to Japan, he started his own research work in 2001, and currently researching preventive and therapeutic significance of dietary nitrate/nitrite particularly for lifestyle-related diseases with cardiac and vascular remodelling. So far, he and his colleagues have demonstrated that the therapeutic effects of nitrate/nitrite, rich in vegetables such as spinach, lettuce and beetroot, on hypertension, diabetes, dyslipidaemia, and metabolic syndrome in experimental animal models. He is also now trying to reveal the evidence that the decrease in endogenous vascular endothelial nitric oxide (NO) production with ageing and vascular endothelial dysfunction might underlie these disease status, and dietary nitrate/nitrite easily achievable by the daily intake of vegetables could provide a NO backup system as prophylactic/on-demand NO donor.

Research Area: His research area is in Nitric oxide, cardiology, molecular biology.

Number of Published Papers: He has published 120 papers in national and international journal of repute.

Special Award: He received award of Minister of Health, Labour and Welfare for Nutrition-related Achievement in 2021.

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