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Nitrite increases glucose-stimulated insulin secretion and islet insulin content in obese type 2 diabetic male rats

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ABSTRACT

Purpose: Reduced bioavailability of nitric oxide (NO) is associated with pathogenesis of type 2 diabetes. Nitrite can act as a substrate for generation of systemic NO. The aim of this study was to examine the effects of nitrite administration on glucose-stimulated insulin secretion (GSIS) and islet insulin content in obese type 2 diabetic rats.

Methods: Male rats were divided into 4 groups: Control, control + nitrite, diabetes, and diabetes + nitrite. Sodium nitrite (50 mg/L in drinking water) was administered for 8 weeks. Diabetes was induced using high-fat diet and low-dose of streptozotocine. Serum levels of fasting glucose, insulin, and lipid profile were measured and the insulin resistance/sensitivity indices were calculated every 2 weeks. Glycated hemoglobin (HbA_{1C}) was measured every month. At the end of the study, tissue levels of glucose transporter 4 (GLUT4) protein and serum interleukin-1 beta (IL-1β) were measured as well as glucose and insulin tolerance test were done. GSIS from isolated pancreatic islets and islet insulin content were also determined.

Results: Nitrite administration significantly increased insulin secretion in both control and diabetic rats in presence of 16.7 mM glucose. Nitrite also significantly increased islet insulin content by 27% and 39% in both control and diabetic rats, respectively. Nitrite decreased elevated serum IL-1β in diabetic rats (4.0 ± 0.2 vs. 2.9 ± 0.2 pg/mL, $P = 0.001$). In diabetic rats, nitrite also significantly increased tissue levels of GLUT4 by 22% and 26% in soleus muscle and epididymal adipose tissue, respectively. In addition, nitrite significantly improved glucose and insulin tolerance, insulin sensitivity, lipid profile, and decreased fasting glucose and insulin, but had no effect on HbA_{1C}.

Conclusions: Long-term nitrite administration increased both insulin secretion and insulin content in obese type 2 diabetic rats. In addition, nitrite therapy had favorable effects on glucose tolerance, insulin resistance, inflammation, and dyslipidemia in type 2 diabetic rats.

1. Introduction

Prevalence of diabetes is increasing worldwide [1]. Type 2 diabetes is characterized by insulin resistance and dysfunction of pancreatic beta cells [2]. Decreased NO bioavailability has been reported in obesity and diabetes in both animal [3,4] and human [5,6] studies. In addition, NO may have a unifying role in the clustering of metabolic syndrome components [7–9].

Nitrate/nitrite, which enhances NO generation and bioactivity, has beneficial effects in diabetes and obesity and could therefore potentially be used for nutrition-based therapy in type 2 diabetes [10–12]. It has been shown that nitrate/nitrite-mediated NO

Abbreviations: NO, nitric oxide; GSIS, glucose-stimulated insulin secretion; QUICKI, quantitative insulin sensitivity check index; HOMA-IR, homeostasis model assessment of insulin resistance; GLUT4, glucose transporter type 4; eNOS, endothelial nitric oxide synthase; GTT, glucose tolerance test; ITT, insulin tolerance test; HFD, high-fat diet; I.P, intraperitoneal; STZ, streptozotocin; NO_X, nitrite + nitrate; TG, triglycerides; TC, total cholesterol; LDL-C, low-density lipoprotein-cholesterol; HDL-C, high-density lipoprotein-cholesterol; CV_S, coefficient of variations; AUC, area under the curve; cGMP, cyclic guanosine monophosphate; IDE, insulin-degrading enzyme; IL-1β, interleukin-1 beta; HbA_{1C}, glycated hemoglobin.

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production can improve glucose tolerance in both young [13] and aged [14] rodents with hyperglycemia, improve insulin resistance [11], increase translocation of insulin-sensitive glucose transporter (GLUT4) to plasma membrane [15], reverse some features of metabolic syndrome in eNOS (endothelial nitric oxide synthase)-deficient mice [3], and prevent inflammation [16].

The role of NO in insulin secretion is disputable and NO can both inhibit [17] and stimulate [18,19] insulin secretion depending on its concentrations [17] and on the isoform of enzyme involved in its production [19,20]. Injection of sodium nitrite 10 min before the glucose tolerance test (GTT) increases insulin secretion from isolated islets and serum insulin concentrations in normal rats, which could be due to microcirculation redistribution and also to the direct insulinotropic effects of nitrite [20].

To the best of our knowledge, there is no documented study addressing the long-term effects of nitrate/nitrite administration on insulin secretion and content in diabetes. The aim of this study was to determine the effects of oral sodium nitrite administration on insulin secretion and insulin content from isolated pancreatic islets in obese type 2 diabetic rats; in addition, its effects on glucose tolerance, insulin resistance, insulin sensitivity, glycated hemoglobin (HbA_{1C}), serum interleukin-1 beta (IL-1β), lipid profile, and protein levels of GLUT4 in soleus muscle and epididymal adipose tissue were also assessed.

2. Materials and methods

2.1. Animals and diets

Male Wistar rats (190–210 g) were housed under controlled room temperature 23 ± 2 °C, and relative humidity of 50 ± 6%, with a 12/12-hour light-dark cycle. The protocol of animal care was approved by the ethics committee of the Research Institute for Endocrine Sciences, affiliated to the Shahid Beheshti University of Medical Sciences.

Rats had free access to water and a normal rat pellet diet, the macronutrient contents of which were carbohydrates (72.1%), proteins (22.1%), and lipids (5.7%) with a total caloric value of ~3100 kcal/kg, or a high-fat diet (HFD) consisting carbohydrates (27.5%), proteins (14.5%), and lipids (58.8%) with a total caloric value of ~4900 kcal/kg. According to the American Institute of Nutrition [21], we chose casein as the source of protein because its amino acid composition is adequate. Shortage of sulfur amino acids, particularly cystine/cysteine is however the major limitation of casein. This defect was overcome by adding DL-methionine to the diet, which is converted to cysteine in vivo [21]. The composition of the HFD is shown in Table 1.

2.2. Induction of diabetes

Diabetes was induced by feeding HFD for two weeks followed by a single intraperitoneal (I.P) injection of streptozotocin (STZ, 25 mg/kg dissolved in 0.1 mM citrate buffer, pH 4.5; Sigma Aldrich, Hamburg, Germany). One week after STZ injection, serum glucose was measured and rats with fasting glucose levels ≥150 mg/dL were considered to be diabetic. The rats continued their respective diets until the end of the study.

Table 1
Composition of high-fat diet.

Ingredients	Diet (g/kg)
Normal pellet diet	585.4
Butter	310.9
Casein	73.2
Mineral mix	24.6
Vitamin mix	4.1
DL-methionine	1.8

2.3. Experimental design

Rats were randomly divided into 4 groups of 10 rats each: A control group consumed tap water (C); control rats consumed 50 mg/L sodium nitrite in drinking water for 8 weeks (CN); diabetic rats consumed tap water (D) and diabetic rats consumed 50 mg/L sodium nitrite in drinking water for 8 weeks (DN). Body weight was recorded every 3 days (using A&D Scale, EK-300i, Japan; sensitivity 0.01 g), as were water consumption (mL/day), food intake (g/day), and calorie intake (kcal/day). Fasting serum levels of nitrite + nitrate (NO_x), glucose, insulin, total cholesterol (TC), triglycerides (TG), low-density lipoprotein-cholesterol (LDL-C), and high-density lipoprotein-cholesterol (HDL-C) were measured and homeostasis model assessment of insulin resistance (HOMA-IR) as well as quantitative insulin-sensitivity check index (QUICKI) were calculated every 2-week. HbA_{1C} was measured every month.

Intraperitoneal GTT was performed one week after STZ injection, and also at the end of the study. Intraperitoneal insulin tolerance test (ITT) was done 1 week after latest GTT. Glucose-stimulated insulin secretion (GSIS) was measured from isolated pancreatic islets at two concentrations of glucose (5.6 and 16.7 mM); in addition, islet insulin content was measured after isolation. Protein levels of GLUT4 in soleus muscle and epididymal adipose tissue and serum IL-1β were measured at the end of the study. The experimental design is shown in Fig. 1.

2.4. Biochemical analyses of serum parameters

After 12–14 h fasting, blood samples were collected and centrifuged at 5000 *g* for 10 min. Serum insulin concentration was measured using a rat ELISA kit (Rat insulin ELISA; Mercodia, Uppsala, Sweden); intra-and interassay coefficient of variations (CVs) were 8.4% and 10.3%, respectively. The sensitivity of the assay was ≤0.15 μg/L (26.1 pmol/L). Serum levels of glucose, TC, TG, LDL-C, and HDL-C were measured using commercial kits (Pars Azmoon, Tehran, Iran). Intra-assay CVs for glucose, TC, TG, LDL-C, and HDL-C were 1.4%, 5.6%, 2.3%, 1.6%, and 2.1%, respectively and inter-assay CVs were 2.2%, 6.2%, 2.8%, 4.3%, and 4.1%, respectively. Serum IL-1β was measured using a rat ELISA kit (ZellBio GmbH, Germany); intra-assay CV was 2.5% and the sensitivity of the assay was 0.1 pg/mL.

Serum levels of total NO_x and nitrite were measured using the Griess method with slight modification [22]. In brief, sera were deproteinized with zinc sulfate (15 mg/mL) and NaOH (3.72 M), centrifuged at 10,000 *g* for 10 min, and supernatants were removed for measurement of NO_x and nitrite levels. For NO_x measurement, nitrate was reduced to nitrite by adding vanadium trichloride (8 mg/mL prepared in 1M HCl), after which 2% sulfanilamide, dissolved in 5% HCl, and 0.1% *N*-(1-naphthyl) ethylenediamine, in ddH2O, were added to deproteinated serum samples. Samples were incubated for 30 min at 37 °C, and optical density was read at 540 nm by a microplate reader (BioTek, MQX2000R2, USA). NO_x concentrations in the samples were measured using a standard calibration curve of 2.5–100 μM of sodium nitrate (Supplementary Fig. 1A). Nitrite was measured in a similar manner, except that samples and nitrite standards (2.5–20 μM; Supplementary Fig. 1B) were only exposed to sulfanilamide, *N*-(1-naphthyl) ethylenediamine and 1M HCl instead of vanadium trichloride. Serum nitrate concentration was calculated by subtracting nitrite values from NO_x concentrations. Intra-assay CVs for NO_x and nitrite were 1.9% and 1.7%, respectively and inter-assay CVs were 7.4% and 9.2%,