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Original Research

A single dose of sodium nitrate does not improve oral glucose tolerance in patients with type 2 diabetes mellitus

Naomi M. Cermak[a,e], Dominique Hansen[b,f], Imre W.K. Kouw[a], Jan-Willem van Dijk[a,d], Jamie R. Blackwell[c], Andrew M. Jones[c], Martin J. Gibala[e], Luc J.C. van Loon[a,*]

[a] Faculty of Health, Medicine and Life Sciences, Maastricht University, Maastricht, The Netherlands
[b] Faculty of Medicine and Life Sciences, Hasselt University, Diepenbeek, Belgium
[c] Sport and Health Sciences, College of Life and Environmental Sciences, University of Exeter, Exeter, United Kingdom
[d] Institute of Sport and Exercise Studies, HAN University of Applied Sciences, Nijmegen, The Netherlands
[e] Exercise Metabolism Research Group, Department of Kinesiology, McMaster University, Hamilton, Ontario, Canada
[f] Heart Centre Hasselt, Jessa Hospital, Hasselt, Belgium

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ABSTRACT

Dietary nitrate (NO_3^-) supplementation has been proposed as an emerging treatment strategy for type 2 diabetes. We hypothesized that ingestion of a single bolus of dietary NO_3^- ingestion improves oral glucose tolerance in patients with type 2 diabetes. Seventeen men with type 2 diabetes (glycated hemoglobin, 7.3% ± 0.2%) participated in a randomized crossover experiment. The subjects ingested a glucose beverage 2.5 hours after consumption of either sodium NO_3^- (0.15 mmol $NaNO_3^- \cdot kg^{-1}$) or a placebo solution. Venous blood samples were collected before ingestion of the glucose beverage and every 30 minutes thereafter during a 2-hour period to assess postprandial plasma glucose and insulin concentrations. The results show that plasma NO_3^- and nitrite levels were increased after $NaNO_3^-$ as opposed to placebo ingestion (treatment-effect, $P = .001$). Despite the elevated plasma NO_3^- and nitrite levels, ingestion of $NaNO_3^-$ did not attenuate the postprandial rise in plasma glucose and insulin concentrations (time × treatment interaction, $P = .41$ for glucose, $P = .93$ for insulin). Despite the lack of effect on oral glucose tolerance, basal plasma glucose concentrations measured 2.5 hours after $NaNO_3^-$ ingestion were lower when compared with the placebo treatment (7.5 ± 0.4 vs 8.3 ± 0.4 mmol/L, respectively; $P = .04$). We conclude that ingestion of a single dose of dietary NO_3^- does not improve subsequent oral glucose tolerance in patients with type 2 diabetes.

Abbreviations: ANOVA, analysis of variance; BMI, body mass index; DPP-4, dipeptidyl peptidase 4; HbA_{1c}, glycated hemoglobin; HOMA-IR, homeostatic model of insulin resistance; iAUC, incremental area under the curve; ISI-M, Matsuda-DeFronzo insulin sensitivity index; $NaNO_3^-$, sodium nitrate; NO, nitric oxide; NO_2^-, nitrite; NO_3^-, nitrate; OGIS, oral glucose insulin sensitivity; OGTT, oral glucose tolerance test.

* *Corresponding author*. Faculty of Health, Medicine and Life Sciences, Maastricht University, PO Box 616, 6200, MD, Maastricht, The Netherlands. Tel.: +31 43 3881397; fax: +31 43 3670976.
E-mail address: L.vanLoon@maastrichtuniversity.nl (L.J.C. van Loon).

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1. Introduction

Postprandial hyperglycemia has been associated with an increased risk of cardiovascular complications and mortality in patients with type 2 diabetes [1-4]. Therefore, proper management of postprandial blood glucose concentrations is an important goal in the treatment of type 2 diabetes [4]. Despite the administration of oral blood glucose–lowering medication and the consumption of a healthy diet, postprandial hyperglycemia remains a predominant feature in patients with type 2 diabetes [5,6]. Therefore, additional treatment strategies are warranted to improve daily blood glucose homeostasis in patients with type 2 diabetes.

Epidemiological studies indicate that a diet rich in leafy green vegetables can reduce the risk of developing type 2 diabetes [7]. The health benefits from these particular vegetables may be, at least partly, attributed to their high nitrate (NO_3^-) content [8]. Nitrate and nitrite (NO_2^-) have historically been viewed as inactive end products of nitric oxide (NO) metabolism. However, recent work has revealed that a reverse pathway exists whereby NO_3^- and NO_2^- can be reduced back into NO, which is a potent vasodilator and regulator of vascular tone and blood flow [8]. Nitrite has been shown to function as an endocrine reservoir of NO [9] and is generated from the conversion of dietary NO_3^- into NO_2^- by facultative anaerobic bacteria residing in the oral cavity [10]. This recent discovery complements the classical L-arginine–NO synthase pathway and is now identified as a phenomenon that may have major clinical relevance in both health and disease.

Several groups have investigated the potential physiological effects of dietary NO_3^- by supplementing subjects with sodium NO_3^- ($NaNO_3^-$) or foods high in NO_3^- such as beetroot juice. As such, there is now substantial evidence that dietary NO_3^- supplementation in humans improves postprandial endothelial function, microvascular perfusion of various tissues, and mitochondrial function [11-14]. These exciting findings may also be relevant for people with type 2 diabetes who demonstrate impairments in NO-dependent endothelial function [14,15]. In fact, endothelial dysfunction is particularly prominent in the postprandial state in people with type 2 diabetes [16]. Improvements in postprandial endothelial function (eg, vasodilatation and capillary recruitment) may enhance glucose and insulin delivery to skeletal muscle tissue, thereby facilitating improvements in insulin signaling and postprandial glucose uptake [17]. So far, it has not been established whether the physiological benefits induced by dietary NO_3^- ingestion translate to improvements in postprandial glucose metabolism. We hypothesized that ingestion of a single bolus of dietary NO_3^- before an oral glucose load attenuates the postprandial rise in plasma glucose and/or insulin concentrations in patients with type 2 diabetes. For this purpose, we recruited 18 male patients with type 2 diabetes and evaluated the glycemic and insulinemic responses to the ingestion of a glucose load after the administration of a single dose of $NaNO_3^-$ (0.15 mmol $NaNO_3^- \cdot kg^{-1}$ body weight) or a placebo.

2. Methods and materials

2.1. Subjects

Eighteen male patients with type 2 diabetes using oral glucose-lowering medication were included in this randomized crossover study (Fig. 1). Exclusion criteria included self-reported renal failure or liver disease, morbid obesity (body mass index [BMI], >35 kg/m^2), insulin therapy, use of NO_3^--containing medication, severe hypertension (systolic blood pressure >160 or diastolic blood pressure >100 mm Hg), and cardiovascular events within the last year. The use of blood glucose–lowering medication was maintained as normal throughout the entire study. All subjects were informed about the nature and the risks of the experimental procedures before their written informed consent was obtained. The experimental protocol and procedures were approved by the medical ethical committee of the Jessa Hospital in Hasselt, Belgium.

2.2. Screening

After an overnight fast, subjects arrived at the laboratory at 8 AM by car or public transportation. After 20 minutes of supine rest, a venous blood sample was collected by venipuncture from the antecubital vein for the assessment of glycated hemoglobin (HbA_{1c}) content. Thereafter, body mass was measured to the nearest 0.1 kg using an analog weight scale (Tanita model TBF-300; Tanita Corp, Tokyo, Japan), and height was measured to the nearest 0.1 cm. Body mass index was calculated from the ratio of weight (kilograms) to height squared (meter squared). Next, blood pressure (model HEM-907; Omron, Hoofddorp, The Netherlands) was assessed 5 times, and average blood pressure was calculated using the closest 3 values.

2.3. Study design

Subjects participated in a double-blind crossover study, consisting of 2 test days separated by a washout period of at least 14 days. Subjects were tested on the same day of the week for each of the test days. At the start of each test day, subjects ingested a single bolus of $NaNO_3^-$ (0.15 mmol $NaNO_3^- \cdot kg^{-1}$ body weight; ~12.75 mg $NaNO_3^- \cdot kg^{-1}$ body weight) or an equimolar amount of sodium chloride (placebo) dissolved in 250-mL water. The $NaNO_3^-$ and placebo beverages were provided in a randomized order according to a computer-generated randomization scheme. Exactly 2.5 hours after $NaNO_3^-$ ingestion, subjects ingested a glucose beverage (75-g glucose dissolved in 250-mL tap water). The 2.5-hour resting period was selected because plasma NO_2^- concentrations have been shown to peak 2 to 3 hours after dietary NO_3^- ingestion [18]. Plasma glucose and insulin concentrations were collected before and over the 2-hour period after ingestion of the glucose load.

2.4. Study design and protocol

For the 2 main experimental test days, subjects reported to the laboratory at 8 AM after an overnight fast for an oral glucose tolerance test (OGTT). Upon arrival at the laboratory,