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Differential nitric oxide levels in the blood and skeletal muscle of type 2 diabetic subjects may be consequence of adiposity: a preliminary study

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ABSTRACT

Background and Aims. Nitric oxide (NO·) exerts key regulatory functions including vasodilation and glucose uptake. Thus reduced NO· levels are associated with insulin resistance and hypertension. In this preliminary work we aimed to measure the levels of NO· metabolites in serum and skeletal muscle of obese and non-obese subjects, with or without type 2 diabetes mellitus (T2DM).

Methods. Fifteen sedentary male participants [7 obese controls (C) vs 5 obese and 3 non-obese T2DM; age 54±9 years] were selected according to their BMI (>30 kg/m^2 for obese and 23–27 kg/m^2 for non-obese participants) and evaluated for fasted values of blood glucose, HbA1c, lipid profile, serum CRP (C-reactive protein), erythrocyte glutathione (GSH) metabolism, plasma adiponectin, leptin and cytokines (TNF-α and INFγ), serum and skeletal muscle nitric oxide metabolites (nitrite and nitrates; tNOx) and skeletal muscle nNOS and iNOS expression. Body composition was measured by whole body DEXA and muscle microbiopsy was performed in the vastus lateralis.

Results. We found that serum tNOx (total nitrite/nitrate; μmol/L) was lower in obese T2DM group (12.7±3.5) when compared with their controls (21.1±2.4), although the non-obese group presented higher concentration of tNOx (33.8±7.2). Skeletal muscle nNOS was higher in obese controls, lower in non-obese T2DM and undetected in obese T2DM. On the other hand, expression of iNOS had an inverse relationship with nNOS, showing higher expression in obese T2DM, decrease in non-obese T2DM and absence in obese control group. tNOx levels (μmol/mg protein) were decreased in the non-obese T2DM group (12.07±0.59) when compared with the obese control (21.68±6.2) and the obese T2DM group (26.3±7.26).

Abbreviations: GSH, Reduced Glutathione; GSSG, Glutathione Disulfide; tNOx, Nitric oxide metabolites; T2DM, type 2 diabetes mellitus; CRP, C-reactive protein.

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Conclusion. We conclude that the decreased serum NO· production in obese T2DM patients seems to be associated with adipose mass as lower adiposity was associated with normal NO· which was reduced in the skeletal muscle of the non-obese T2DM patients. We suggest that the lower adiposity (and higher adiponectin) in non-obese T2DM could be responsible for differential levels of NO· production and insulin resistance.

1. Introduction

Diminished insulin sensitivity is a characteristic feature of various pathological conditions such as the metabolic syndrome, Type 2 diabetes mellitus (T2DM), cardiovascular disease and hypertension [1]. Individuals with essential hypertension are more prone than those that are normotensive, to develop diabetes. This propensity may reflect decreased ability of insulin to promote relaxation in vascular tissue and glucose transport in skeletal muscle, due to insulin resistance [2], where insulin signalling is impaired in target cells and tissues, indicating common molecular signals.

The free radical nitric oxide (NO·) is a common signalling molecule regulating blood flow, blood pressure and immune function and may participate in cellular and organ functions across the body. Physiologic levels of NO· produced by endothelial cells are essential for relaxation and proliferation of vascular smooth muscle cells, leukocyte adhesion, platelet aggregation, angiogenesis, and thrombosis [1]. In addition, NO· produced by neurons serves as a neurotransmitter, and NO· produced by activated macrophages is an important mediator of inflammation [3]. However, as an oxidant and as an inhibitor of proteins containing an iron–sulfur center, excess production of NO· may exert detrimental effects in sensitive cells and tissues, as well as cardiovascular function [4].

Nitric oxide is required for glucose uptake during exercise, an effect exaggerated in T2DM [5]. Moreover, it has been shown that NO· donors, such as sodium nitroprusside (SNP), increased glucose uptake in primary human skeletal muscle cells (HSkMC) derived from both healthy individuals and patients with T2DM [6]. Thus, in the skeletal muscle and other insulin-sensitive tissues, inhibition of NO· production will culminate in blunted glucose transport and subsequently in insulin resistance [1].

The role of NO· in the cardiovascular system and regulation of blood pressure has been extensively studied [7]. NO· is synthesized from the amino acid L-arginine in endothelial cells by the constitutive calcium–calmodulin-dependent enzyme nitric oxide synthase (NOS). The principal physiologic stimulus for nitric oxide synthesis and release from the endothelium is shear stress associated with blood flow over the surface of the vessel by a nonreceptor-dependent mechanism. NO·, released from the endothelium as a gas or attached to other molecules, stimulates soluble guanylyl-cyclase in vascular smooth muscle underlying the endothelium, producing increased concentrations of cyclic GMP and, thus activating cGMP-dependent kinases which promote relaxation [8]. Endothelial dysfunction, which is defined by decreased endothelium-dependent vasodilatation, is associated with an increased number of cardiovascular events. Nitric oxide bioavailability is reduced by altered endothelial signal transduction or increased formation of reactive oxygen species (ROS) reacting with NO· to produce peroxynitrite, so depleting bioavailable NO· [1]. Endothelial dysfunction is therapeutically reversible and physical exercise, calcium channel blockers, angiotensin converting enzyme (ACE) inhibitors, and angiotensin receptor antagonists improve flow-evoked endothelium-dependent vasodilation in patients with hypertension and diabetes [9].

NO· not only is essential for normal skeletal muscle and endothelial function, but also is required for the insulin secretion from pancreatic β-cells [10]. It is widely accepted that L-arginine, the immediate precursor of NO·, is a potent secretagogue for β-cell insulin release [11], while L-arginine deficiency is associated with insulinopenia and failure of glucose stimulated insulin secretion [12]. Hence, although NO· may be cytotoxic for β-cells at high concentrations [10], in most conditions L-arginine-derived NO· is essential mediator for the secretagogue action of L-arginine [13].

In summary, decreased production of NO· causes detrimental effects, resulting in cell dysfunction that leads to insulin resistance, hypertension, pancreatic β-cell dysfunction [14] and diabetes. In this preliminary work, we aimed to measure the levels of nitric oxide oxidative metabolites (total nitrite and nitrates; tNOx) in serum and skeletal muscle of obese and non-obese subjects, with or without diabetes. We also compared the redox state, hormones and intracellular proteins that could have influence on the production of nitric oxide in the same populations.

2. Materials and methods

2.1. *Participants characteristics*

Fifteen sedentary non-smoking male participants (54±9 years old) volunteered for this study (7 obese controls vs 5 obese T2DM and 3 non-obese T2DM). Participants were selected according to their BMI (>30 kg/m^2 for obese and 23–27 kg/m^2 for non-obese participants). Informed consent was obtained from all participants prior to the study. Research assessments and protocols were approved by the UCD Human Research Ethics Committee–Sciences (Ref: LS-08-106) and Institute of Technology Tallaght Research Ethics Committee (Ref: REC-A6-09).

Participants were free from diabetic complications such as retinopathy, neuropathy, nephropathy or vascular disease at the time of recruitment. Participants attended the laboratory in the morning after an overnight fast. All participants were assessed for biochemical variables such as fasted glycaemia, lipid profile (LDL, HDL, total cholesterol and triglycerides) and HbA1c (glycated haemoglobin). Participants' biochemical and physiological characteristics are listed on Table 1.