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The influence of obesity on the oxidative stress status and the concentration of leptin in type 2 diabetes mellitus patients

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ABSTRACT

The aim of this study was to determinate both the oxidative stress/anti-oxidative defense status and the concentration of leptin in obese, overweight and normal weight type 2 diabetes mellitus patients to seek possible association between oxidative stress and hyperleptinemia.

Oxidative stress status parameters [thiobarbituric acid-reacting substances (TBARS), superoxide anion ($O_2^{\bullet -}$), superoxide dismutase (SOD) activity and total sulphydryl groups] and the concentration of leptin were measured in 312 subjects (178 patients and in 134 control subjects). Obese patients had a significantly higher concentration of leptin compared to obese subjects in the control population ($P < 0.001$). They also had significantly higher plasma concentrations of TBARS, $O_2^{\bullet -}$ and SOD activity in combination with a lower sulphydryl group concentration when compared to control subjects. Obese patients had significantly higher concentrations of both TBARS and $O_2^{\bullet -}$ and increased SOD activity compared to normal weight patients. The odds ratio for the degree of association between oxidative stress status parameters and hyperleptinemia was strongest for TBARS [odds ratio 2.66, 95% CI (1.02–6.94), $P = 0.045$]. The observed positive correlation between TBARS and leptin ($\rho = 0.29$, $P < 0.01$) in obese patients suggests that increased oxidative stress and hyperleptinemia, both consequences of obesity, may play a role in type 2 diabetes mellitus development.

1. Introduction

Obesity and the onset of diabetes are two closely linked medical phenomena particularly prevalent in the Western world. Leptin, the obese (*ob*) gene product produced by adipocytes, is thought to play an important role both in the regulation of body weight and general metabolism in obese individuals [1]. The concentration of plasma leptin is proportional to the amount of adipose tissue and is markedly increased in obese individuals as well as in animals with dietary-induced obesity [2]. It has been suggested that hyperleptinemia may be involved in the pathogenesis of

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obesity-related diseases such as type 2 diabetes mellitus. The pathogenesis of various diseases may also be regulated by local or whole body perturbations in the anti-oxidative defense system [3]. One of the many diabetic phenotypes is an increase in oxidative stress characterised in part by increased production of free radicals and a reduced capacity of the anti-oxidative defense system. Increased oxidative stress in type 2 diabetes mellitus impairs glucose uptake in muscle and fat [4] and decreases insulin secretion from the pancreatic β cells [5]. In addition, obesity *per se* may induce systemic oxidative stress such as increased production of free radicals in accumulated fat [6]. To assess the role of obesity-induced hyperleptinemia in the pathogenesis of type 2 diabetes mellitus and the possible inter-relationship of leptin with a number of oxidative stress status parameters [lipid peroxidation measured as thiobarbituric acid-reacting substances (TBARS), superoxide anion ($O_2^{\bullet -}$), superoxide dismutase activity (SOD) and sulphydryl groups content], we studied normal weight, overweight and obese patients diagnosed with type 2 diabetes and compared them with appropriately matched control groups.

2. Materials and methods

2.1. Subjects

Two populations were studied: the patient group consisted of 178 subjects diagnosed with type 2 diabetes mellitus according to the National Diabetes Data Group criteria [7], the control group consisted of 134 subjects that had no clinical signs of type 2 diabetes mellitus or impaired glucose tolerance (IGT). Twenty-six patients (15%) were undergoing dietary control (1400–1600 kcal/day). The others (74%) were receiving oral hypoglycaemic agents (46% sulphanylurea agents, 10% biguanidin agents and 18% a combination of sulphanylurea + biguanidin) in addition to dietary control. Only 20 patients (11%) were receiving insulin in combination with oral hypoglycaemic therapy. None of the patients had microvascular complications (diabetic nephropathy or retinopathy). The study protocol included height and weight measurements for determination of body mass index (BMI) [measured as weight/(height)^2] and waist and hip measurements for waist-to-hip ratio (WHR). Individuals were considered to be of normal weight when their BMI was $\leq$25 kg/m^2, overweight when their BMI was $>$25 kg/m^2 and $<$30 kg/m^2 and obese when their BMI was $\geq$30 kg/m^2 [8]. Three groups within the type 2 diabetes mellitus patient population were defined: 32 (18%) registered a normal weight, 88 (50%) were overweight and 58 (32%) were classified as obese. In the control population 58 (43%) registered a normal weight, 46 (35%) were overweight and 30 (22%) were classified as obese. To determine patient's body fat distribution, we assessed their WHR. Intra-abdominal (or android) obesity existed if WHR $\geq$ 1.0 for males, WHR $\geq$ 0.86 for females and if the BMI $\geq$ 30 kg/m^2 for both sexes [9,10]. In the control obese subgroup (BMI $>$ 30), only four individuals (13%) had intra-abdominal obesity. However, in the type 2 diabetes obese subgroup, 42 (72%) were classified as intra-abdominally obese.

At the point of study entry, all individuals underwent a complete clinical and biochemical investigation revealing age, gender, blood pressure and the assessment of risk factors (including familial history of myocardial infarction, arterial hypertension, smoking, hyperlipidemia, current medication and other socioeconomic variables). Replies to a standard questionnaire were collected in-person by trained interviewers. Among the type 2 diabetes patients 12 (7%) had stable angina and 4 (2%) had experienced a previous myocardial infarction. Study participants were deemed hypertensive if they had a systolic pressure $\geq$130 mm Hg and/or a diastolic pressure $\geq$85 mm Hg or were already taking any form of anti-hypertensive medication. The criterion for a ''hyperlipidemic status'' was if the low-density lipoprotein cholesterol (LDL-C) concentration was above 3.36 mmol/L and/or the triglyceride (TG) concentration was above 1.69 mmol/L [11].

To avoid confounding factors we excluded patients that had experienced any of the following conditions during the 4 weeks prior to initiating the study: recent clinical infection (as a consequence of concurrent major renal, hepatic or malignant disease), myocardial infarction, surgery or major trauma. Patients who were being treated with anti-oxidant supplementation or lipid-lowering drugs were initially excluded from our study in order to avoid influence of medication on unique oxidative system status and lipid profile. The whole study was planned according to the ethical standards detailed in the Declaration of Helsinki (as revised in 1983) and according to institutional guidelines. The local institutional review committee approved the research proposal and informed consent was obtained from all individuals involved in the study.

2.2. Sample collection

Two samples (each of 4 mL) of venous blood were drawn from the antecubital vein after night-time fasting ($>$10 h). The blood was collected into one EDTA sample tube (for plasma) and one serum sample tube before immediate centrifugation at 1500 $\times$ *g* for 10 min at 4 °C. Plasma and serum samples were stored at −80 °C in aliquots until analysis. The concentration of leptin in plasma was determined using a Leptin (sandwich) ELISA Kit (DRG Instruments, Marburg, Germany). The ELISA detected human leptin with sensitivity (lowest detectable level) of 1.0 ng/mL. The intra-assay coefficient of variation was 7% and inter-assay coefficient of variation was 9% at 5.63 ng/mL. The TBARS concentration was measured using its molar absorption coefficient of $1.56 \times 10^5\ mol^{-1}\ cm^{-1}$ at 535 nm, previously described by Girotti et al. [12]. For the measurement of $O_2^{\bullet -}$, plasma from heparinised blood samples was used immediately. The rate of nitroblue tetrazolium (NBT) reduction was used to measure the rate of $O_2^{\bullet -}$ generation, as described by Auclair and Voisin [13]. Plasma SOD activity was measured according to the previously published method by Misra and Fridovich [14]. We monitored SOD-mediated inhibition of adrenalin auto-oxidation to adrenochrome. The concentration of sulphydryl groups in plasma was determined using 0.2 mmol/L 5.5′-dithiobis(2-nitrobenzoic acid) (DTNB) reported by Ellman [15].

Fasting glucose levels, glycosylated haemoglobin (HbA_{1c}), lipid status parameters [total cholesterol (t-C), LDL-C, high-density lipoprotein cholesterol (HDL-C), TG, apolipoprotein A_1 (Apo A-I) and apolipoprotein B (Apo B)] were measured using a Hitachi 912 autoanalyser using commercial kits (Roche Diagnostics, Mannheim, Germany).