CLINICAL STUDY

Plasma adiponectin concentration and tumor necrosis factor-α system activity in lean non-diabetic offspring of type 2 diabetic subjects

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Abstract

Objective: There is growing evidence that adiponectin function is related to the pathogenesis of insulin resistance. Insulin resistance might be present even in lean subjects with a strong family history of type 2 diabetes. The aim of the study was to look for adiponectin’s role in the pathogenesis of insulin resistance in offspring of type 2 diabetic patients, and its relation to the activity of the tumor necrosis factor (TNF)-α system.

Research design and methods: The study was carried out in 23 lean offspring of type 2 diabetic subjects and in 23 controls matched for age, sex and body mass index. The oral glucose tolerance test for glucose and insulin estimations and hyperinsulinemic, euglycemic clamp studies were performed in all patients. The plasma concentration of adiponectin, TNF-α, soluble TNF receptors 1 and 2 (sTNFR1, sTNFR2), HbA1c, total cholesterol, high-density lipoprotein (HDL)-cholesterol, low-density lipoprotein-cholesterol and triglycerides were estimated.

Results: The insulin sensitivity index, normalized for fat-free mass (Mffm) and adiponectin concentrations were markedly decreased in offspring of type 2 diabetic subjects compared with the control group (P = 0.0046 and P = 0.00058 respectively). TNF-α and sTNFR1 concentrations did not differ between the studied groups; however the concentration of sTNFR2 was markedly increased in the offspring of type 2 diabetic patients (P = 0.0002). Adiponectin concentration was positively correlated to the insulin sensitivity index (r = 0.34; P = 0.020) and to HDL-cholesterol (r = 0.29, P = 0.047) and was inversely related to sTNFR2 (r = -0.33, P = 0.027).

Conclusions: The obtained results suggest that adiponectin could play a role in the pathogenesis of insulin resistance in lean offspring of type 2 diabetic subjects.

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Introduction

Adiponectin, a 30 kDa protein, is synthesized exclusively by adipose tissue (1, 2). There is growing evidence that the function of adiponectin is closely related to the pathogenesis of insulin resistance (3, 4). It is well accepted that insulin resistance plays an important role in the etiology of several contemporary diseases including obesity, coronary heart disease, hypertension and type 2 diabetes mellitus (5–8). However, impaired insulin action was already observed in healthy normoglycemic subjects with a strong family history of type 2 diabetes (9). Pratipanawatr et al. showed that insulin-stimulated receptor substrate-1 activation was impaired in skeletal muscle of offspring of type 2 diabetic subjects (9).

In the last few years, great interest has been directed to the secretory protein of white adipose tissue, known as adiponectin or adipocyte complement related protein (ACRP30), AdipoQ or adipose most abundant gene transcript 1 (apM1). The serum concentration of adiponectin is relatively high. As mentioned earlier, adiponectin is synthesized exclusively by adipose tissue and decreased serum concentrations of adiponectin correlate with insulin resistance, which has been observed in experimental (10) and epidemiological studies (11, 12). Low adiponectin concentrations have been observed in human obesity (13), type 2 diabetes mellitus (12), coronary heart disease (14) and hypertension (15). In patients with anorexia nervosa, the concentration of adiponectin was significantly higher in spite of a marked deficiency of adipose tissue (16). In experimental studies, recombinant adiponectin suppressed the development of atherosclerosis (17) and improved insulin sensitivity, mainly by increasing fatty acids oxidation and inhibition of hepatic glucose production.
measured at the smallest circumference between the rib cage and the iliac crest, with the subject in the standing position. The hip circumference was measured at the widest circumference between the waist and the thighs. Percent of body fat was assessed by bioelectric impedance analysis using the Tanita TBF-511 Body Fat Analyzer (Tanita Corp., Tokyo, Japan), and fat mass (FM) and fat-free mass (FFM) were calculated. The clinical characteristics of the studied groups are shown in Table 1.

Analyses were performed after an overnight fast. Subjects underwent an oral glucose tolerance test (OGTT). No subjects presented with disturbances of glucose metabolism according to WHO criteria.

**Insulin sensitivity**

Insulin sensitivity was evaluated by the euglycemic hyperinsulinemic clamp technique as described by DeFronzo et al. (23). On the morning of the study, two venous catheters were inserted into antecubital veins, one for the infusion of insulin and glucose and the other in the contralateral hand (which was heated to approximately 60°C) for blood sampling. Insulin (Actrapid HM; Novo Nordisk, Copenhagen, Denmark) was given as a primed-continuous intravenous infusion for 2 h at 50 mU £ kg⁻¹ £ h⁻¹, resulting in constant hyperinsulinemia of approximately 76.7 mU/l. Arterialized blood glucose was obtained every 5 min and an infusion of 40% dextrose (2.22 mol/l) was adjusted to maintain plasma glucose levels at 5.0 mmol/l. The glucose infusion rate approached stable values during the final 40 min of the study and the rate of whole-body glucose uptake (M value) was calculated as the mean glucose infusion rate from 80 to 120 min, corrected for glucose space. Normalization for kg of FFM was based on the quantity of FFM estimated from total body mass and the percent of body fat measured by a bioimpedance method.

### Subjects and methods

A total of 23 lean offspring of type 2 diabetic patients and 23 control subjects (with no family history of diabetes) matched for sex, age and body mass index (BMI), were recruited for this study. Relatives of type 2 diabetic patients were recruited for the study if both parents had type 2 diabetes or if one parent and one first- or second-degree relative had type 2 diabetes. All subjects were Caucasian, and had no infections or any other serious medical problems. Smokers were not included in the study. Before entering the study, a physical examination and a resting electrocardiography were performed. The study protocol was approved by the Ethics Committee of the Medical University of Białystok. All the subjects gave written informed consent before entering the study.

### Anthropometric measurements

Anthropometric parameters were measured in all subjects. The BMI was calculated as body weight × height⁻² and was expressed in kg/m². The waist-to-hip ratio (WHR) was estimated. The waist circumference was
Biochemical analyses

Fasting blood samples were also taken from the antecubital vein before the beginning of the clamp for the determination of glycated hemoglobin (HbA1c), plasma lipids, TNF-α, soluble TNF receptors 1 and 2 (sTNFR1, sTNFR2) and adiponectin concentration.

Plasma glucose was measured immediately by the enzymatic method using a glucose analyzer (YSI 2300 STAT PLUS). Plasma insulin was measured with the Medgenix EASIA test (BioSource Europe, Nivelles, Belgium). The minimum detectable concentration was 1.05 pg/l and the intra-assay and interassay coefficients of variation were below 5.5% and 10% respectively. In this method, human and animal proinsulins present no cross-reaction. HbA1c was measured by the high-performance liquid chromatography method (Bio-Rad, Muenchen, Germany). Plasma cholesterol and triglycerides (TG) were assessed by enzymatic methods using commercial kits produced by ANALCO-GBG, Warsaw, Poland. Concentration of low-density lipoprotein-cholesterol (LDL-C) was calculated from Friedewald’s formula: LDL-C = total cholesterol − TG/5 (mg/dl).

The concentration of plasma adiponectin was measured using an RIA method (Linco Res., St. Charles, MI, USA). Ultra sensitive plasma TNF-α concentrations were measured by an immunoassay kit (BioSource International, Camarillo, CA, USA) with a minimum detectable concentration 0.09 pg/ml and with intra-assay and interassay coefficients of variation below 6.8% and 10% respectively. Plasma sTNFR1 and sTNFR2 concentrations were determined with EASIA kits (BioSource Europe). The minimum detectable concentration was 0.05 ng/ml for sTNFR1 and 0.1 ng/ml for sTNFR2. The intra-assay and interassay coefficients of variation for both receptors were below 6.5% and 9% respectively. sTNFR1 EASIA does not cross react with sTNFR2 and TNF-α does not interfere with the assay.

Statistical analysis

The statistics were performed with the STATISTICA 5.0 program (StatSoft, Krakow, Poland) using the non-parametric tests, because of the relatively small number of subjects. The differences between the studied groups were estimated with the Mann–Whitney U-test. The simple and multiple stepwise regression analyses were performed to evaluate the relationship between the adiponectin concentration and other parameters. Statistical significance was accepted at a P value of less than 0.05.

Results

The offspring of type 2 diabetic subjects did not differ significantly in the anthropometric parameters from the control group (Table 1). Also, glucose and insulin concentrations during OGTT did not differ significantly (Table 2). No subjects presented with disturbances of glucose metabolism either in the group of patients with a family history of type 2 diabetes, or in the control group. However, the insulin sensitivity index (Mffm), calculated from the clamp studies, was markedly decreased in lean offspring of type 2 diabetic subjects ($P = 0.0046$) (Table 2). Lipid parameters were not significantly different between the studied groups (Table 2).

Plasma adiponectin concentrations were significantly lower in the offspring of type 2 diabetic subjects ($P = 0.00058$) (Table 3). TNF-α and sTNFR1 concentrations were similar in both groups. The concentration of sTNFR2 was markedly increased in the offspring of type 2 diabetic patients ($P = 0.0002$) (Table 3).

Adiponectin concentration was significantly related to the M value ($r = 0.34$, $P = 0.020$) and to high-density lipoprotein (HDL)-cholesterol concentration ($r = 0.29$, $P = 0.047$) (Table 4). An inverse correlation was observed with sTNFR2 ($r = -0.33$, $P = 0.027$) (Table 4). Insulin sensitivity index correlated inversely with sTNFR2 ($r = -0.35$, $P = 0.018$).

We constructed two models of multiple regression analysis in a subgroup of the offspring of type 2 diabetic subjects: the first model had the insulin sensitivity index as the dependent variable, and the second model had adiponectin as the dependent variable. Multiple regression analysis in a stepwise manner,

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Lean offspring of type 2 diabetic patients (n = 23)</th>
<th>Control group (n = 23)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adiponectin (μg/ml)</td>
<td>13.24±1.58</td>
<td>14.54±1.57*</td>
</tr>
<tr>
<td>TNF-α (ng/ml)</td>
<td>1.33±1.75</td>
<td>1.80±2.50</td>
</tr>
<tr>
<td>sTNFR1 (ng/ml)</td>
<td>1.87±0.40</td>
<td>1.86±0.34</td>
</tr>
<tr>
<td>sTNFR2 (ng/ml)</td>
<td>4.84±0.77</td>
<td>3.98±0.52*</td>
</tr>
</tbody>
</table>

* $P < 0.05$.
with the M value as the dependent variable, revealed that only adiponectin independently predicted insulin sensitivity and was responsible for 8% of its variability, with sex, fasting insulin, TG, HDL-cholesterol and sTNFR2 not entering the regression model. In the second model, with adiponectin as the dependent variable, HDL-cholesterol, TG, sex, fasting insulin and sTNFR2 together explained about 50% of adiponectin variability in the studied group.

**Discussion**

Until recently, adipose tissue has been considered to be only a passive tissue for the storage of excess energy in the form of fat. Now, adipose tissue is recognized as an important endocrine organ producing several substances with endocrine, paracrine and autocrine effects on the adipocyte. There is growing evidence that substances secreted by adipocytes are important determinants of insulin resistance through hormonal effects or local effects on the adipocyte.

There are several studies in the literature concerning circulating adiponectin concentrations in first-degree relatives of type 2 diabetic patients that give inconsistent results (19, 27–32). In the present study, we demonstrated the difference in adiponectin plasma concentration between the group at risk of developing type 2 diabetes and the control group, together with a markedly diminished insulin sensitivity in lean, young offspring of type 2 diabetic subjects. Lihn and colleagues did not observe a difference in serum adiponectin concentration; however, they found decreased mRNA expression of adiponectin in white adipose tissue in first-degree relatives (19). The results of clamp studies were similar as in our study (19). In studies from a Swedish population, adiponectin concentrations in first-degree non-obese relatives of type 2 diabetic patients were markedly decreased in comparison with the control group (27). Also, in an Italian population of first-degree relatives, circulating adiponectin concentrations were significantly decreased in comparison with the control group (31). Important results concerning the adiponectin concentration in relatives of type 2 diabetic patients come from the study performed by Civitarese and colleagues (30). They measured not only plasma concentrations of adiponectin, but also the gene expression of adiponectin receptors (AdipoR1 and AdipoR2) in skeletal muscles. The authors found diminished levels of plasma concentrations of adiponectin together with lower expression levels of AdipoR1 and AdipoR2 in skeletal muscles in people with a family history of type 2 diabetes. Moreover, they observed a positive correlation between adiponectin receptors gene expression in skeletal muscles and insulin sensitivity. The authors concluded that the observed abnormalities could play an important role in promoting the development of type 2 diabetes mellitus (30). Other studies did not show any difference in adiponectin concentration in patients with a family history of type 2 diabetes mellitus, despite the lower insulin sensitivity in the studied groups (28, 29, 32). The discrepancies in the obtained results by different groups of researchers are difficult to explain. One of the potential factors could be differences in the selection and the characteristics of the studied groups.

Some explanation about adiponectin production and secretion in first-degree relatives of type 2 diabetic patients is given in genetic studies performed by Stumvoll and colleagues (33). They studied the influence of a silent highly prevalent T/G polymorphism in exon 2 of the adiponectin gene on insulin sensitivity and measures of obesity in a non-diabetic German population (371 individuals), of which 160 subjects had a strong family history of type 2 diabetes. They showed that carriers of the G allele had higher BMI, higher percentage of body fat and impaired insulin sensitivity, but only in the group with no family history of type 2 diabetes, whereas in the subgroup with a family history, the presence of the G allele had absolutely no effect (33). The authors concluded that this polymorphism, although silent, could somehow contribute to a higher obesity risk and, secondarily, to insulin resistance in the non-diabetic population. In individuals with a family history of type 2 diabetes, other genetic factors probably contribute to the development of insulin resistance.

Interesting information about insulin sensitivity and adiponectin concentration come from studies performed by Kern et al. (34). They found a positive
correlation between adiponectin and the insulin sensitivity index calculated from minimal models analysis in groups of non-diabetic patients with varying degrees of obesity. After adjustment for obesity, insulin-sensitive patients demonstrated a twofold higher plasma level of adiponectin (34). Taking into account different adipokines secreted by adipose tissue (leptin, IL-6, TNF-α), the authors showed that the plasma adiponectin concentration as well as adiponectin adipose tissue mRNA expression correlated significantly with TNF-α mRNA expression in white adipose tissue, which was not true for leptin or IL-6. Moreover, patients with higher adiponectin mRNA expression secreted the lowest levels of TNF-α in vitro (34). There are several studies which show the interaction between adiponectin and TNF-α (17, 35–37).

Treatment with recombinant adiponectin caused the reduction of TNF-α expression (17) in Apo-E-deficient mice. Maeda et al. found that TNF-α dose-dependently reduced the expression of adiponectin in adipocytes by suppressing the promoter activity (35). Also, in patients with HIV-associated lipodystrophy (HALS), who demonstrated diminished insulin sensitivity and decreased plasma adiponectin and mRNA expression in white adipose tissue, an inverse correlation between adiponectin and plasma TNF-α and mRNA expression was observed (36). In our study, offspring of type 2 diabetic patients had similar plasma TNF-α concentrations as controls, but significantly higher sTNFR2 concentrations than the control group. Plasma TNF-α concentrations are usually low and do not give precise information about its action.

More information on the quantification of TNF-α mRNA expression in white adipose tissue is needed. Although we did not perform expression studies, we measured the plasma concentrations of the TNF-α receptors sTNFR1 and sTNFR2. The role of TNF-α receptors is not entirely understood. It is proposed that sTNFRs might inactivate TNF-α or stabilize the bioactivity of the cytokine (38, 39). sTNFR2 is considered as the best predictor of local TNF-α activity (40, 41). In the present study, adiponectin concentration was significantly associated with sTNFR2, which supports the hypothesis without proving causality, about the role of TNF-α in modulating adiponectin activity by local influence on the adipocyte. On the other hand, adiponectin has anti-inflammatory properties and hypoadiponecetinemia might be a primary abnormality, and may result in an increase in TNF-α system activity. Fernandez-Real et al. in a recently published study found that patients with circulating adiponectin in the higher quartile had significantly decreased sTNFR2 concentrations (37).

Previous studies suggested that both adiponectin and TNF-α might play a role in modulating insulin sensitivity in the relatives of type 2 diabetic subjects. On the basis of our study, one cannot draw any conclusion on the interrelationship between adiponectin and the TNF-α system and their influence on insulin resistance. Multiple regression analysis results suggest that adiponectin might be an independent predictor of insulin sensitivity in this group, whereas TNF-α may exert its effect through association with adiponectin, but this hypothesis requires further investigation.

The obtained results suggest that adiponectin could play a role in the pathogenesis of insulin resistance in lean offspring of type 2 diabetic subjects.

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