Association between insulin resistance, body mass and neopterin concentrations

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Abstract

Obesity is frequently associated with insulin resistance. Recently an important role of the cytokine tumor necrosis factor-α in mediating insulin resistance of obesity through its overexpression in fat tissue has been reported. In order to examine the relation of insulin resistance to obesity and to serum neopterin, as a parameter of immune activation, we studied 1234 otherwise healthy outpatients, who visited the physician's office for a medical health check-up. 7% showed elevated glucose concentrations, 34% elevated body mass indices. There were significant correlations between glucose concentrations and body mass indices and of the latter with serum neopterin concentrations. Neopterin concentrations were significantly higher in patients with elevated body mass indices (Mann-Whitney test, $U = 131\ 358$, $p = 0.0003$) and elevated glucose concentrations (Mann-Whitney test, $U = 35\ 350$ $p = 0.02$). The data may indicate that moderate immune stimulation plays a role in the development of insulin resistance, and an influence of tumor necrosis factor-α seems to be probable. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Non-insulin-dependent diabetes mellitus; Insulin resistance; Tumor necrosis factor-α; Interferon-γ; Neopterin; Body mass

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1. Introduction

Non-insulin-dependent diabetes mellitus (NIDDM) is among the most common metabolic disorders in the industrial world. Although genetic factors are highly likely to have a role in this disease, obesity is the most common factor in insulin resistance [1]. The association between obesity and insulin resistance in this disease is poorly understood. Recent data from multiple experimental models of obesity suggested a key role of tumor necrosis factor-α (TNF-α) in insulin resistance of NIDDM. From the data it seems likely that elevated TNF-α produced by adipocytes of the obese state may inhibit the activity of tyrosine kinase of the insulin receptor β-subunit, specifically in muscle and fat tissue [2,3]. TNF-α is mainly produced by endotoxin-stimulated macrophages. It plays a key role in the immune system due to its tumoricidal effects and its capacity to mediate inflammatory mechanisms in normal immunosurveillance and in pathologic conditions.

Upon stimulation by interferon-γ (IFN-γ), a typical Th1-cytokine [4], preferentially human monocytes/macrophages produce and release large amounts of neopterin [5,6], 6-D-erythro-1′,2′,3′-trihydroxypropylpterin, which is synthesised from guanosine triphosphate (GTP) by GTP-cyclohydrolase I (EC 3.5.4.16). In humans, elevations of neopterin in serum and urine have been found in viral infections including human immunodeficiency virus type 1, various malignant disorders, autoimmune diseases and during allograft rejection episodes [5–10]. Significant associations between enhanced neopterin and IFN-γ production have been obtained also in patients [11], and monitoring of neopterin concentrations has turned out to be a sensitive and useful marker to estimate the activation of Th1-cells [6]. A link of neopterin to the TNF system was found in various diseases, when concentrations of soluble TNF-α receptors correlated strongly with neopterin concentrations in serum or urine [12].

The present study was designed to examine the possible relation of insulin resistance to obesity and serum neopterin levels in a population of normal weight and overweight persons, respectively.

2. Materials and methods

2.1. Subjects

One thousand two hundred and thirty four otherwise healthy outpatients, 473 males and 761 females, who visited the physician′s office for a medical health check-up were studied. From these, some patients showed elevated erythrocyte sedimentation rate (ESR) (above 20 mm after 2 h) or elevated leukocyte counts (above 10^9/l) or decreased blood hemoglobin concentrations (Table 1). Al-

Table 1
Baseline clinical characteristics of the study subjects

Characteristics	n	First quartile	Median value	Third quartile	Range	[a]Out of normal range	
						below n (%)	above n (%)
Age, years	1234	34	42	54	16–91		
Plasma glucose, mmol/l	1169	3.94	4.44	4.88	2.72–12.49	9 (0.8)	77 (6.6)
(mg/dl)		(71)	(80)	(88)	(49)–(225)		
BMI, kg/m^2	1157	20.9	23.4	26.1	15.6–50.0	83 (7.2)	398 (34.4)
Serum neopterin, nmol/l	1234	4.6	5.5	6.9	1.6–36.8		151 (12.2)
Hemoglobin, g/l	1204	129	139	150	80–178	77 (6.4)	6 (0.5)
Leukocytes, 10^9/l	1204	6.3	7.4	8.7	2.5–16.4	9 (0.7)	143 (11.9)
ESR after 2 h (mm)	1199	11	15	21	2–100		352 (29.4)

n, number of observations; BMI, body mass index ESR, erythrocyte sedimentation rate.

[a] Normal ranges: Glucose, 3.05–5.55 mmol/l; BMI, 19–25 kg/m^2; Neopterin > 13.5 (< 19 years), > 8.7 (19–75 years), > 19.0 (> 75 years) nmol/l; Hemoglobin, males 130–180, females 120–160 g/l; Leucocytes, 4×10^8–10^9/l; ESR after 2 h, > 20 mm.

though in such cases acute inflammatory diseases cannot be ruled out, none of them were excluded from the study. Patients' age varied from 16–91 years (median age: 42 years). With exception of contraceptives in some females, all patients were under no medications. Patients' body mass was classified by body mass indices (BMI) as described earlier [13]. Three hundred and ninety eight subjects (34%) were classified as overweight (BMI above 25 kg/m^2), 83 (7%) as underweight (BMI below 19 kg/m^2), whereas the majority of 676 (59%) showed normal weight (BMI 19–25 kg/m^2). Not all data sets were complete (Table 1) resulting in slightly different numbers of measurements when groups were compared.

2.2. Blood collections and measurements

Blood samples were drawn after an overnight fast; for glucose determinations collection tubes contained fluoride EDTA in order to prevent glucose degradation by glycolysis until analysis. Plasma glucose was measured by conventional hexokinase method with a Hitachi 711 autoanalyzer and a commercial kit (Boehringer Mannheim, Vienna, Austria), the laboratory's normal range being 3.05–5.55 mmol/l (55–100 mg/dl). Hemoglobin and leukocyte counts were determined on a Coulter STKS hematology analyzer (Beckman Coulter, Inc., Vienna, Austria). Serum neopterin was measured by a commercially available radioimmunoassay kit (HENNINGtest Neopterin, BRAHMS Diagnostica, Berlin, FRG) with a sensitivity of 1 nmol/l neopterin and an interassay coefficient of variation ranging from 4.7–8.5%. Upper limits of the normal (95th percen-

tiles) are depending on age ranging from 8.7 nmol/l (19–75 years) to 13.5 nmol/l (below 18 years) and 19.0 nmol/l (above 75 years) as described earlier [14].

2.3. Statistical analysis

Correlation between variables were assessed by the non-parametric Spearman's rank correlation technique, since the distributions of observed values were generally non-Gaussian. Differences in distributions of laboratory variables among patient groups were tested for significance by the non-parametric Mann-Whitney test. The effect of the three factors serum neopterin concentrations, BMI and age on plasma glucose concentrations was assessed by a three-way analysis of variance (ANOVA). Thereby the factors neopterin and BMI were dichotomized by the third quartile point, the factor age by the median of the observed distribution (for values see Table 1). Since variances in the eight subgroups formed on the basis of neopterin, BMI and age were different, a reciprocal transformation of glucose concentrations was done before analysis. The success of transformation was confirmed by Bartlett's test for equal variances (test statistic $= 13.45$; $p > 0.05$) as implemented in the program GraphPad Prism (GraphPad Software, Inc., San Diego, CA). To determine whether the response of glucose concentrations to one factor depend on the level of the second or the third factor, the interaction terms were also tested for significance. ANOVA was calculated by the program BMDP2V (BMDP Statistical Software, 1990 edition, University of California Press).

3. Results

Table 1 reports characteristics of the study subjects. Notably, only 7% of the studied patients showed elevated glucose concentrations, but 34% had elevated BMI. Correlations between the investigated variables assessed by Spearman's rank correlation coefficients are shown in Table 2. There was a highly significant correlation ($p < 0.0001$) of age versus glucose concentrations, age versus BMI or age versus neopterin concentrations and glucose concentrations versus BMI. Similarly, a weaker, but still highly significant correlation was found between neopterin concentrations and BMI ($p = 0.0009$) or neopterin concentrations and glucose levels ($p = 0.0018$).

As shown in Fig. 1, there was also a statistically significant increase of serum neopterin concentrations in patients with increasing BMI (Mann-Whitney test, $U = 131\ 358$, $p = 0.0003$) indicated by increasing median values (BMI ≤ 25 kg/m^2: 5.4 nmol/l; BMI > 25 kg/m^2: 5.7 nmol/l). In the same manner, patients with glucose levels above 5.55 mmol/l (100 mg/dl) showed higher neopterin

Table 2
Spearman correlations of Investigated characteristics

	n	Spearman's rank correlation coefficient			
		value	95% confidence	interval	p-value
BMI vs. age	1157	0.359	0.306	0.409	< 0.0001
Neopterin vs. age	1234	0.277	0.223	0.329	< 0.0001
Glucose vs. age	1169	0.214	0.157	0.270	< 0.0001
Glucose vs. BMI	1106	0.131	0.070	0.190	< 0.0001
BMI vs. neopterin	1157	0.097	0.038	0.156	0.0009
Glucose vs. neopterin	1169	0.091	0.032	0.150	0.0018

n, number of pairs; BMI, body mass index.

concentrations (median: 5.8 nmol/l) than those with glucose levels equal or less than 5.55 mmol/l (100 mg/dl) (median: 5.5 nmol/l) (Mann-Whitney test, $U = 35\ 349.5$, $p = 0.0194$) (Fig. 2). To test the relationship between serum neopterin, plasma glucose concentrations, BMI and a further common factor like age, the effect of serum neopterin concentrations, BMI and age on plasma concentrations was calculated by three-way ANOVA. Thereby the factors neopterin and BMI were dichotomized by the third quartile point, the factor age by the median of the observed distribution. Untransformed mean values and standard deviations of the formed subgroups are shown in Fig. 3. All the three
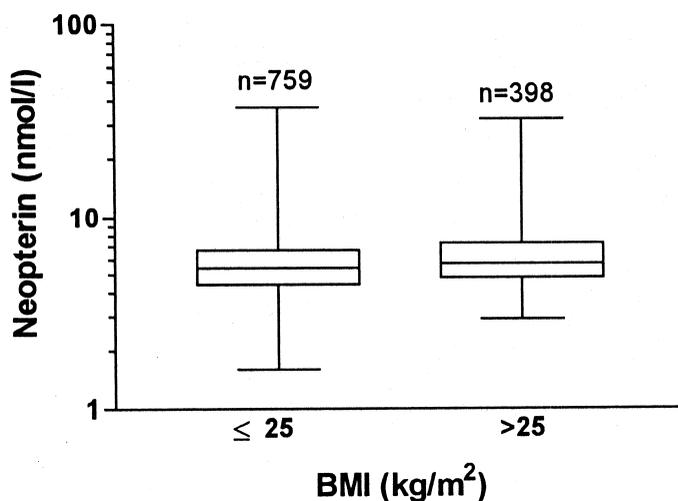

Fig. 1. Box and Whiskers plots of serum neopterin concentrations of patients with different body mass indices (BMI). The box extends from the 25th percentile to the 75th percentile, with a horizontal line at the median (50th percentile). The median neopterin concentration of the group with BMI above 25 kg/m is statistically significantly higher ($p = 0.0003$) compared to the group with BMI equal or below 25 kg/m^2.
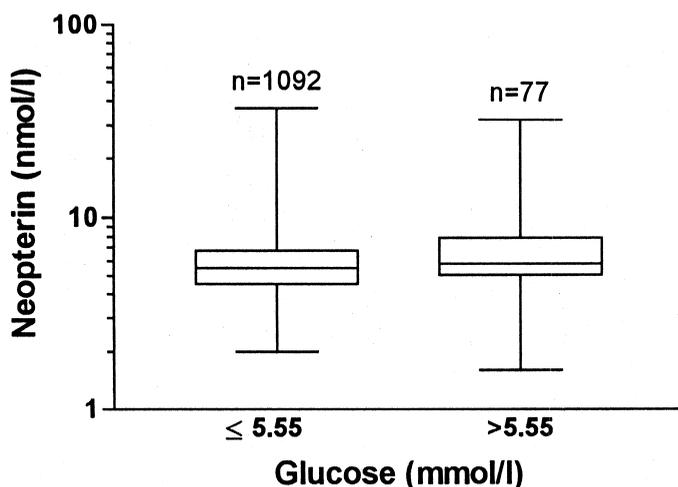
Fig. 2. Box and Whiskers plots of serum neopterin concentrations of patients with different glucose concentrations. The box extends from the 25th percentile to the 75th percentile, with a horizontal line at the median (50th percentile). The median neopterin concentration of the group with plasma glucose above 5.55 mmol/l (100 mg/dl) is statistically significantly higher ($p = 0.019$) compared to to the group with glucose concentrations equal or below 5.55 mmol/l (100 mg/dl).
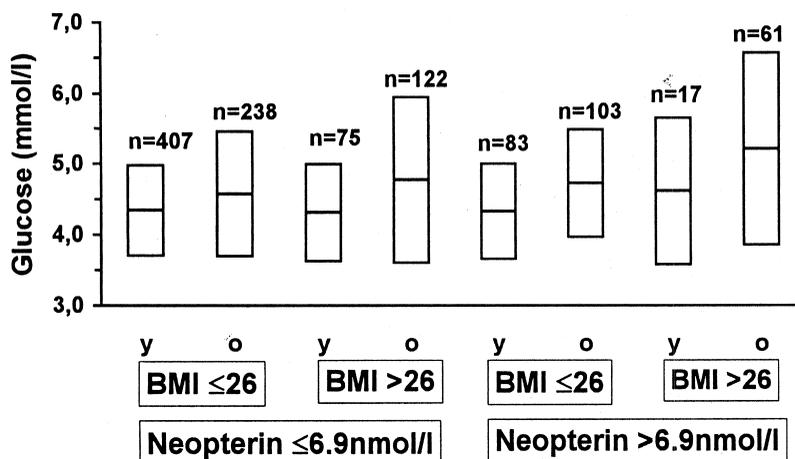

Fig. 3. Box and Whiskers plots of plasma glucose concentrations of patients with different serum neopterin concentrations, BMI and age ('*y*' indicates below or equal 42 years, '*o*' indicates above 42 years). The box extends from the mean-one standard deviation to the mean + one standard deviation, with a horizontal line at the mean.

factors, namely neopterin concentrations ($F = 7.86$; $p = 0.005$), BMI ($F = 5.15$; $p = 0.023$) and age ($F = 31.76$; $p < 0.001$) showed a significant effect on plasma glucose concentrations, whereas all the interaction terms (neopterin vs. BMI, neopterin vs. age, BMI vs. age, neopterin vs. BMI vs. age) were statistically not significant ($p > 0.05$), indicating interactions being negligible.

4. Discussion

The significant positive correlation of glucose levels with BMI and of both of these with age in our study is in good agreement with today's understanding of the relationship between obesity, insulin resistance and NIDDM. Notably, the fact that only 7% of the studied population showed elevated glucose concentrations, but 34% elevated BMI, indicates that obesity alone does not unavoidably induce insulin resistance. Other factors, such as genetic predisposition for example in addition to obesity will be necessary to develop NIDDM [1,15]. Recently, TNF-α produced by adipocytes of the obese state has been reported to induce insulin resistance of NIDDM by inhibiting the activity of tyrosine kinase of the insulin receptor β-subunit, specifically in muscle and fat tissue [2,3]. In our study, there was a significant correlation between neopterin concentrations and BMI and also between neopterin and glucose concentrations. But TNF-α alone is unable to induce neopterin production by human monocytes/macrophages [4], at most it may enhance a basic neopterin production by human monocytes/macrophages upon stimulation by IFN-γ, a typical Th1-cytokine [5,6,9]. As shown in various diseases, concentrations of soluble TNF-α receptors correlate strongly with neopterin concentrations in serum [12]. Therefore, more probably a stimulation of the T-cell/macrophage system indicated by increased neopterin formation and accompanied by TNF-α production by monocytes/macrophages seems to be responsible for the positive correlation of neopterin with glucose concentrations and BMI, respectively. In our investigation, there was a highly significant correlation between neopterin values and patients' age, which agrees with several earlier studies [14,16,17], indicating an increased stimulation of the immune system and cytokine production with age. But when age was included in the multivariate statistical analysis, the relationship between neopterin, BMI, and glucose remained as independent associations. Thus, immune activation even if only moderate might be another factor contributing to the development of insulin resistance possibly via the concomitant regulation of TNF-α. In contrast to this, investigations of individuals infected with human immunodeficiency virus show an inverse correlation of soluble TNF-α receptors and neopterin concentrations with BMI [18,19]. In this case, an anorectic effect of TNF-α, associated with cachexia has to be assumed, which might be due to higher levels of TNF-α in combination

with other cytokines than in case of individuals suffering from obesity and insulin resistance [20]. The identification of two distinct receptors with different affinities and different signalling mechanisms provides a potential additional basis for the diversity of TNF-α's action [12]. The association between increased glucose and neopterin levels would also agree with the hypothesis of a role for oxygen free radicals in the pathogenesis of NIDDM [21,22] Increased neopterin levels in individuals indicates activated monocytes/macrophages which are also a prominent source for various reactive compounds such as superoxide anion or nitric oxide. Moreover, a strong correlation was described earlier between the amount of neopterin released by the activated monocytes/ macrophages and their capacity to secrete hydrogen peroxide [23].

From these findings one might conclude that with increasing age and thereby increasing moderate immune stimulation a development of obesity and insulin resistance will be supported, indicated by the correlation of patients' BMI with neopterin concentrations. On the other hand, at higher age the probability to acquire diseases like autoimmune disease, neurodegenerative diseases malignant tumor disease, chronic infectious disease increases, all of them challenging strongly the immune system. This stronger immune activation is accompanied by the development of cachexia, indicated by an inverse correlation of patients' BMI with, e.g., neopterin production like in human immunodeficiency virus infections [12]. Nevertheless, serum neopterin data from our study let us assume that an influence of moderate immune stimulation seems to be probable in the development of obesity and insulin resistance and supports the concept of a pathogenetic role of TNF-α. The direct comparison of the variables of this study with insulin resistance calculated from fasting insulin concentrations could further substantiate this conclusion. However, these data have not been available.

Acknowledgements

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