

Original Investigation

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Plasma interleukin 8 concentrations in obese subjects with impaired glucose tolerance.

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Abstract

Background: Interleukin 8 (IL-8) is a cytokine with atherogenic properties. In vitro studies revealed that it is produced and secreted by human adipocytes. We recently reported that plasma IL-8 is increased in obese subjects with normal glucose tolerance (NGT). The aim of the present study was to measure plasma IL-8 concentrations in subjects with impaired glucose tolerance (IGT).

Methods: A total of 44 subjects with marked overweight or obesity (BMI > 27.8 kg/m²), 27 with NGT and 17 with IGT, were recruited for the present study. Plasma IL-8 levels were measured in fasting state, after an oral glucose tolerance test (OGTT) and after euglycemic hyperinsulinemic clamp.

Results: The studied groups did not differ in fasting IL-8 concentrations. Both OGTT and clamp resulted in a significant increase in plasma IL-8. The change in IL-8 after clamp was similar in both groups. In contrast, after OGTT plasma IL-8 levels (IL-8_{OGTT}) were markedly higher in IGT individuals. In IGT, but not NGT group, IL-8_{OGTT} was positively related to postload glucose and negatively to insulin sensitivity.

Conclusion: Plasma IL-8 concentrations after glucose load are increased in obese IGT subjects in comparison to normoglycemic weight-matched individuals. Increase in plasma IL-8 might be both insulin-mediated (during clamp) and glucose-mediated (during OGTT).

Background

Type 2 diabetes is associated with accelerated atherogenesis, this relationship may be observed already in the pre-diabetic states, i.e. in impaired glucose tolerance (IGT) [1]. Also, obesity itself is recognized as an independent

risk factor for cardiovascular disease [2]. Precise mechanisms linking those conditions are not fully understood. In recent years, theories about the role of chronic low-grade inflammation in the pathogenesis of both type 2 diabetes [3] and atherosclerosis [4] have been developed.

Table 1: Basal clinical characteristics of the studied groups.

	NGT group (n = 27)	IGT group (n = 17)	p value
Age (yr)	40.89 ± 11.76	42.41 ± 5.79	0.62
BMI (kg/m ²)	33.62 ± 3.19	33.93 ± 3.99	0.78
WHR	0.89 ± 0.08	0.88 ± 0.05	0.81
Percent body fat	39.98 ± 10.05	39.00 ± 6.71	0.72
FM (kg)	41.17 ± 16.79	37.02 ± 10.42	0.37
FFM (kg)	58.72 ± 7.28	56.53 ± 7.13	0.33

Data are presented as mean ± S.D. NGT, normal glucose tolerance; IGT, impaired glucose tolerance; BMI, body mass index; WHR, waist-to-hip ratio; FM, fat mass; FFM, fat-free mass

Interleukin-8 (IL-8) is one of the proinflammatory cytokines, which might also have atherogenic properties. Through its multiple actions, IL-8 might promote intimal thickening and atherosclerosis. Those actions include recruitment of neutrophils and T lymphocytes into the sub-endothelial space, monocyte adhesion to endothelium [5] and migration of vascular smooth muscle cells [6]. Macrophage-derived human foam cells contain high amounts of IL-8 [7]. This cytokine is also able to increase the instability of atherosclerotic plaque through inhibition of tissue inhibitor of metalloproteinase expression, which results in the increased release of matrix-degrading metalloproteinases [8].

Two studies of Bruun et al [9,10] reported that IL-8 is produced and secreted *in vitro* by human adipocytes. We recently demonstrated that plasma IL-8 concentrations are increased in obese subjects with normal glucose tolerance (NGT) and are related to body mass index (BMI), waist-to-hip ratio (WHR), percent body fat and fat mass and tumor necrosis factor- α (TNF α) system [11]. We also observed that circulating IL-8 increases both after an oral glucose tolerance test (OGTT) and euglycemic hyperinsulinemic clamp. An increase after OGTT was similar in lean and obese subjects, while after clamp it was present only in the obese [11].

Elevated circulating IL-8 levels were also reported in type 1 and type 2 diabetic patients [12], it was hypothesized that this cytokine could be involved in the pathogenesis of diabetic macroangiopathy. However, no data are available about IL-8 levels in IGT individuals.

The aim of the present study was to evaluate plasma IL-8 concentrations in obese subjects with IGT in fasting state, after OGTT and after euglycemic hyperinsulinemic clamp.

Methods

A total of 44 subjects with marked overweight or obesity (BMI > 27.8 kg/m²), without previously diagnosed disturbances of glucose tolerance, were included in the present

study. On the basis of OGTT, 27 had NGT (12 men and 15 women) and 17 had IGT (7 men and 10 women) according to WHO criteria. Subjects with NGT were part of the obese group reported in our previous study [11]. Basal clinical characteristics of the studied groups are given in Table 1. Individuals with diabetes were excluded from the study. The participants had no cardiovascular disease, hypertension, infections or any other serious medical problems. Before entering the study, physical examination and resting electrocardiography were performed. Laboratory analyses were performed after an overnight fast. The study protocol was approved by the Ethics Committee of Medical Academy, Białystok. All the subjects gave written informed consent before entering the study.

The BMI was calculated according to Quetelet's formula. The waist-to-hip ratio (WHR) was estimated. The waist circumference was 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), fat mass (FM) and fat-free mass (FFM) were calculated.

Insulin sensitivity was evaluated by the euglycemic hyperinsulinemic clamp technique as described by DeFronzo et al [13]. 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 for blood sampling, that hand was heated to approximately 60°C. Insulin (Actrapid HM, Novo Nordisk, Copenhagen, Denmark) was given as a primed-continuous intravenous infusion for 2 hours at 50 mU × kg⁻¹ × h⁻¹, resulting in constant hyperinsulinemia of approximately 550 pmol/l. Arterialized blood glucose was obtained every 5 minutes and 40% dextrose (2.22 mol/l) infusion was adjusted to maintain plasma glucose levels at 5.0 mmol/l. The glucose infusion rate approached stable values during final 40 minutes of the study and the rate of whole-body

Table 2: Biochemical parameters in the studied groups.

	NGT group (n = 27)	IGT group (n = 17)	p value
Fasting glucose (mmol/l)	5.41 ± 0.49	6.10 ± 0.65	0.00027
Postload glucose (mmol/l)	6.01 ± 1.35	9.20 ± 0.89	0.000001
Fasting insulin (pmol/l)	138.37 ± 113.23	154.44 ± 38.65	0.57
Postload insulin (pmol/l)	505.79 ± 415.38	772.35 ± 198.48	0.017
HbA1c (%)	5.99 ± 0.92	6.48 ± 0.55	0.053
TC (mmol/l)	5.51 ± 1.12	5.83 ± 1.49	0.41
TG (mmol/l)	1.94 ± 1.16	2.23 ± 1.11	0.42
HDL-C (mmol/l)	1.27 ± 0.26	1.10 ± 0.44	0.18
LDL-C (mmol/l)	3.44 ± 0.97	3.71 ± 1.24	0.43
M/FFM (μmol × kg ⁻¹ × min ⁻¹)	31.62 ± 14.81	21.99 ± 8.94	0.02

Data are presented as mean ± S.D. NGT, normal glucose tolerance; IGT, impaired glucose tolerance; HbA1c, glycated hemoglobin; TC, total cholesterol; TG, triglycerides; HDL-C, HDL-cholesterol; LDL-C; LDL-cholesterol; M/FFM, whole-body glucose uptake normalized for fat-free mass

glucose uptake (M value) was calculated as the mean glucose infusion rate from 80 to 120 min, corrected for glucose space and normalized per kilogram of fat-free mass (M/FFM).

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 and IL-8. Plasma IL-8 concentration was also estimated at the end (at 120 minute) of the OGTT and the clamp. For the determination of plasma IL-8, samples were frozen at -70°C.

Plasma glucose was measured immediately by the enzymatic method using glucose analyzer. 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 inter-assay coefficients of variation (CVs) were below 5.5% and 10%, respectively. In this method, human and animal proinsulins present no cross-reaction. HbA1c were measured by the high-performance liquid chromatography method (Bio-Rad, Muenchen, Germany). Plasma total cholesterol (TC), triglycerides (TG) and HDL-cholesterol (HDL-C) were assessed by the enzymatic methods (Cormay, Warsaw, Poland). LDL-cholesterol (LDL-C) was calculated with the Friedewald's formula.

Plasma IL-8 concentration was assessed with the human ultrasensitive ELISA kit (BioSource International) with the standard curve range of 0.39 to 25.0 pg/ml and the detection limit of the method below 100 fg/ml. The intra-assay and inter-assay CVs were below 7.5% and 9.0%, respectively.

The statistics were performed with the STATISTICA 5.0 program (StatSoft, Krakow, Poland). Differences between

the groups were assessed with the unpaired Student's t-test. Differences between fasting and postload IL-8 levels in respective groups were evaluated using the paired Student's t-test. Relationships between variables were estimated with the simple and multiple regression analysis. The level of significance was accepted at p value less than 0.05.

Results

Subjects with IGT had higher levels of plasma glucose ($p < 0.0005$) and postload insulin ($p < 0.02$) and lower M/FFM value ($p < 0.05$) (Table 2). The difference in HbA1c was of borderline significance ($p = 0.053$). Plasma lipids did not differ between the studied groups (Table 2).

There was no significant difference in fasting IL-8 concentrations between NGT and IGT group (Table 3). In both groups fasting IL-8 was related to BMI (NGT, $r = 0.38$, $p < 0.05$; IGT, $r = 0.68$, $p < 0.005$), percent body fat (NGT, $r = 0.48$, $p < 0.02$; IGT, $r = 0.54$, $p < 0.05$) and FM (NGT, $r = 0.49$; IGT, $r = 0.62$, both $p < 0.01$), and in NGT group also to WHR ($r = 0.46$, $p < 0.02$). There was no significant correlations between fasting IL-8 and glucose, insulin, HbA1c and insulin sensitivity. Correlations in NGT group are similar to those reported previously [11].

Euglycemic hyperinsulinemic clamp resulted in a significant increase in plasma IL-8, similar in both groups (NGT, $p < 0.002$; IGT, $p < 0.02$). IL-8 values after clamp were still not different between the groups (Table 3). In both groups, the change in IL-8 during clamp ($\Delta\text{IL8}_{\text{clamp}}$) was related to FM (NGT, $r = 0.49$, $p < 0.01$; IGT, $r = 0.49$, $p < 0.05$) and steady-state insulin concentrations (NGT, $r = 0.41$, $p < 0.05$; IGT, $r = 0.57$, $p < 0.02$).

Plasma IL-8 levels increased in both groups also after OGTT (both $p < 0.005$). In that case, an increase in IL-8

Table 3: Plasma IL-8 concentrations in the studied groups.

	NGT group (n = 27)	IGT group (n = 17)	p value
Fasting IL-8 (pg/ml)	4.27 ± 1.61	4.22 ± 1.54	0.92
IL-8 after clamp (pg/ml)	6.23 ± 3.82	6.05 ± 3.05	0.86
IL-8 after OGTT (pg/ml)	5.02 ± 1.60	6.30 ± 2.52	0.044

Data are presented as mean ± S.D. NGT, normal glucose tolerance; IGT, impaired glucose tolerance; IL-8, interleukin 8; OGTT, oral glucose tolerance test

(Δ IL8_{OGTT}) in IGT individuals was markedly higher than in NGT subjects ($p < 0.02$). Also, IL-8 concentrations after OGTT were markedly higher in IGT than in NGT group ($p < 0.05$) (Table 3). Δ IL8_{OGTT} was not related to insulin response during OGTT and not to anthropometric parameters in NGT and IGT subjects, while it was associated with postload glucose in IGT ($r = 0.51$, $p < 0.05$), but not in NGT group ($r = 0.25$, $p = 0.21$). Plasma IL-8 concentrations after OGTT were positively related to postload glucose level ($r = 0.59$, $p < 0.02$), and negatively to insulin sensitivity ($r = -0.49$, $p < 0.05$) in IGT, but not in NGT group.

Relationship between postload glucose and IL-8 values was independent of insulin sensitivity ($\beta = 0.51$, $p < 0.02$). In contrast, correlation between M/FFM and IL-8 level after OGTT was of borderline significance after adjustment for postload glucose ($\beta = -0.38$, $p = 0.07$).

There were no differences in plasma IL-8 concentrations in all the conditions studied between men and women in the whole examined group and also within subgroups of NGT and IGT individuals.

Discussion

Plasma IL-8 concentrations in the present study are similar to values found in our previous report [11] and in other observations [14].

In the present study, fasting IL-8 levels were similar in obese subjects with NGT and IGT and were determined mostly by body fat content and not by glucose levels. These data indicate that similar processes are involved in regulation of fasting plasma IL-8 levels in obese NGT and IGT subjects. Our results suggest, that increase in plasma IL-8 levels might be both insulin-mediated (during clamp) and glucose-mediated (during OGTT). Acute hyperinsulinemia up-regulated circulating IL-8 levels in the same manner in both groups. Our previous study revealed that this effect of insulin is dependent on obesity [11].

It was demonstrated that glucose at high concentrations is able to stimulate IL-8 production and secretion from cul-

tured endothelial cells [15]. Plasma IL-8 levels were increased and related to the degree of metabolic control in type 1 and type 2 diabetic subjects [12]. Recently it was reported, that urinary levels of IL-8 are increased in patients with diabetic nephropathy. Urinary IL-8 was significantly related to HbA1c [16]. Subjects with IGT do not exhibit prolonged overt hyperglycemia, this is the probable explanation of the unchanged fasting IL-8 levels in comparison to their weight-matched counterparts.

However, we demonstrated increased IL-8 concentrations after oral glucose load in IGT group. Plasma IL-8 levels after OGTT were related to postload glucose concentrations. Our previous study revealed that oral glucose load is able to increase circulating IL-8 even in normoglycemic individuals and that this effect is probably independent of obesity, as lean and obese subjects exhibited similar increase despite different baseline IL-8 values [11]. The present study indicates, that this effect of glucose load is exaggerated in glucose intolerant individuals and postload IL-8 concentrations are increased in IGT group. Our finding might give an additional explanation of the accelerated atherogenesis observed in prediabetic states.

In the previous study from our center, conducted on men referred for coronary arteriography, a significance of postload glycemia as predictor for coronary atherosclerosis was demonstrated. Marked correlation between postload glycemia and number of involved vessels was observed in men referred for coronary arteriography without previously known disturbances of glucose tolerance [17]. Association of postload IL-8 and glucose values might indicate the potential mechanism for the important role of postload glucose. It should be noted that this relationship is present at glucose values higher than normal, as it was not found in normoglycemic individuals.

Also, an inverse correlation between insulin sensitivity and postload, but not fasting, IL-8 levels, was present only in glucose intolerant individuals. Relationship between IL-8 and insulin action was not reported previously. Brun et al [9] observed a decrease in IL-8 adipose tissue expression after incubation with insulin sensitizing agents,

thiazolidinedione ciglitazone and biguanide metformin. It should be noted, that in our study the relationship between M/FFM and IL-8 level after OGTT was neither significant in IGT subjects after controlling for postload glucose nor it was present in NGT individuals. Also, our results do not reveal any causality. Probably this association might be important only in markedly insulin resistant individuals, as was IGT group in the present study.

Conclusions

We conclude that plasma IL-8 concentrations after glucose load are increased in obese IGT subjects in comparison to normoglycemic weight-matched individuals. Our findings also indicate, that an increase in plasma IL-8 might be both insulin-mediated (during clamp) and glucose-mediated (during OGTT).

Competing interests

none declared

Authors' contributions

Marek Straczkowski and Irina Kowalska planned the study, performed clinical examinations, clamps and statistical analysis and wrote the paper; Agnieszka Nikolajuk participated in clamp studies and performed immunoassays; Stella Dzienis-Straczkowska participated in clinical examinations and clamp studies; Malgorzata Szlachowska participated in clinical part of the study; Ida Kinalska participated in design and coordination of the study. All authors read and approved final manuscript.

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