Original Article

Plasma Interleukin-6 Levels Are Independently Associated With Insulin Secretion in a Cohort of Italian-Caucasian Nondiabetic Subjects

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We have investigated the relationships between plasma interleukin-6 (IL-6) levels and insulin sensitivity and insulin secretion in a cohort of Italian-Caucasian glucose-tolerant subjects. Insulin sensitivity was assessed by euglycemic-hyperinsulinemic clamp, and first-phase insulin secretion was measured by intravenous glucose tolerance test. Fasting plasma IL-6 concentration was negatively correlated with the rate of insulin-stimulated glucose disposal (M) ($P = 0.001$). The correlation remained statistically significant, while attenuated, after adjusting for sex, age, and BMI ($P < 0.03$); after an additional adjustment for free fatty acids (FFAs), a further attenuation was observed, but statistical significance was maintained ($P < 0.044$). Fasting plasma IL-6 concentration was positively correlated with first-phase insulin secretion assessed as acute insulin response (AIR) ($P = 0.001$). The correlation remained significant after adjusting for sex, age, and BMI ($P = 0.003$). To estimate the independent contribution of plasma IL-6 levels to AIR, we carried out forward stepwise linear regression analysis in a model that included sex, age, BMI, waist-to-hip ratio, FFAs, and insulin-stimulated glucose disposal. Only insulin sensitivity and plasma IL-6 concentration were independently associated with AIR, accounting, respectively, for 19.0 and 5.2% of its variation. These data indicate that IL-6 is associated in a reciprocal manner with the two pathophysiological components of type 2 diabetes, i.e., insulin resistance and insulin secretion. *Diabetes* 55:2021–2024, 2006

The pathogenesis of type 2 diabetes is characterized by a combination of insulin resistance at the level of skeletal muscle, fat, and liver and failure of pancreatic β-cells to compensate for the enhanced insulin demand. A body of evidence has accumulated over the past decade supporting the concept that insulin resistance and type 2 diabetes are related to a chronic, low-grade, inflammatory state (1,2). Cross-sectional studies in type 2 diabetic patients or in individuals with impaired glucose tolerance/impaired fasting glucose have shown that acute-phase markers are elevated in these subjects compared with nondiabetic control subjects (1–3). Several studies have shown that proinflammatory cytokines and acute-phase reactants are correlated with clinical features of the metabolic syndrome, including measures of insulin resistance/plasma insulin concentration, BMI/waist circumference, and circulating triglyceride and HDL cholesterol concentration (2,4–6). In addition, many prospective studies in different human populations have identified proinflammatory cytokines, acute-phase proteins, and several indirect markers of inflammation as predictors of type 2 diabetes and glucose disorders (7,8).

Interleukin-6 (IL-6), a major regulatory proinflammatory cytokine, is produced by a variety of cells, including leukocytes, adipocytes, and endothelial cells, and acts on the liver to stimulate the production of a number of acute-phase proteins. Circulating IL-6 levels have been reported to be elevated in subjects with type 2 diabetes (1) and to correlate with direct and indirect measures of insulin resistance (6,9–11). However, while the relationship between insulin resistance and circulating IL-6 levels is well established, there is little information on an independent association between plasma IL-6 levels and insulin secretion (11). Conflicting results have been also reported from in vitro studies, showing that IL-6 has stimulatory (12–14), neutral (15), or inhibitory (16,17) effects on insulin secretion from pancreatic β-cells, likely as a result of a wide variability in experimental conditions.

The aim of the present study was to examine the relationship between fasting plasma IL-6 levels and insulin secretion in a cohort of 80 Italian-Caucasian glucose-tolerant subjects.

RESEARCH DESIGN AND METHODS

All subjects were Caucasian and were consecutively recruited at the Department of Internal Medicine of the University of Rome-Tor Vergata and at the Department of Experimental and Clinical Medicine of the University "Magna Graecia" of Catanzaro. Subjects were excluded if they had chronic gastrointestinal diseases associated with malabsorption, chronic pancreatitis, history of any malignant disease, history of alcohol or drug abuse, liver or kidney failure, and treatments able to modify glucose metabolism. The study was approved by institutional ethics committees, and informed consent was obtained from each subject in accordance with principles of the Declaration of Helsinki.

Anthropometric measurements and oral glucose tolerance test. After 12 h fasting, all subjects underwent anthropometrical evaluation, and a 75-g oral glucose tolerance test was performed with 0, 30, 60, 90, and 120 min sampling

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Received for publication 13 January 2006 and accepted in revised form 13 April 2006.

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AIR, acute insulin response; FFA, free fatty acid; IL-6, interleukin-6; RIAD, Risk Factors in Impaired Glucose Tolerance for Atherosclerosis and Diabetes.

DOI: 10.2337/db06-0063

TABLE 1
Anthropometric and biochemical characteristics of the study subjects

Characteristics	
Sex (M/F)	23/57
Age (years)	35.0 ± 10 (18–56)
BMI (kg/m^2)	29.0 ± 8.4 (18.4–45.7)
Waist-to-hip ratio	0.84 ± 0.09 (0.67–1.1)
SBP (mmHg)	119 ± 14 (90–150)
DBP (mmHg)	77 ± 10 (57–100)
Total cholesterol (mg/dl)	196 ± 38 (119–299)
HDL cholesterol (mg/dl)	56 ± 12 (34–87)
Triglyceride (mg/dl)	105 ± 58 (35–211)
FFA (mEq/l)	0.5 (0.2–2.1)
Fasting glucose (mgl/dl)	84 ± 9 (65–99)
2-h glucose (mg/dl)	103 ± 22 (68–138)
Fasting insulin (μU/ml)	10 ± 5 (2.8–27.0)
2-h insulin (μU/ml)	54 ± 34 (5.4–220)
IL-6 (pg/ml)	1.3 (0.12–10.5)
Glucose disposal (mg · kg^{-1} · min^{-1})	8.4 ± 2.9 (2.2–15.6)
AIR (μU · ml^{-1} · min^{-1})	233 (75–1,064)

Data are means ± SD (range) or median (range). DBP, diastolic blood pressure; SBP, systolic blood pressure.

for plasma glucose and insulin. A total of 80 subjects had normal glucose tolerance according to the American Diabetes Association criteria (18).

Hyperinsulinemic-euglycemic clamp and intravenous glucose tolerance test. Subjects were subjected to intravenous glucose tolerance test and to hyperinsulinemic-euglycemic clamp. At 8 A.M., after a 12-h overnight fast, an intravenous catheter was placed in the antecubital vein for the infusion of glucose. Another cannula for blood sampling was inserted into a wrist vein surrounded by a heated box. After baseline blood collection, a bolus of glucose (300 mg/kg in a 50% solution) was given (within 30 s) into the antecubital vein to acutely increase the blood glucose level. Samples for the measurement of blood glucose and plasma insulin were drawn at 2, 4, 6, 8, and 10 min. Acute insulin response (AIR) was calculated as the mean increment in the plasma insulin concentration above basal during the first 10 min after glucose administration by the trapezoidal method according to the formula [(Ins10′ + Ins8′) + (Ins8′ + ivIns6′) + (Ins6′ + Ins4′) + (Ins4′ + Ins2′) (Ins2′ + Ins0′) × 2]. The degree of insulin sensitivity was evaluated with the euglycemic-hyperinsulinemic clamp technique. Subjects received insulin (Humulin; Eli Lilly, Indianapolis, IN) as a primed-continuous infusion targeted to produce plasma insulin levels of ~420 pmo/l. The insulin infusion rate was fixed at 40 mU/m^2 per min. Blood glucose level was maintained constant throughout the study by infusing 20% glucose at varying rates according to blood glucose measurements performed at 5-min intervals. The rate of total insulin-stimulated glucose disposal (M) was calculated for the last 60 min of the insulin infusion.

Biochemical assays. Plasma insulin concentration was determined by radioimmunoassay (Adaltis, Italy). IL-6 concentration was measured by an enzyme-linked immunosorbent assay (Quantikine kit; R&D Systems, Minneapolis, MN). All others metabolites were measured by standard methods.

Statistical analysis. The Kolmogorov-Smirnov test was used to test the normality of distribution, and non–normally distributed variables were natural log transformed. Continuous variables are expressed as means ± SD or as median (range). Relationships between variables were determined by Pearson's correlation coefficient (r). Relationships between variables were sought by stepwise multivariate linear regression analysis with forward selection to assess the magnitude of their individual effect on insulin secretion. For all analyses, a P value ≤0.05 was considered to be statistically significant. All analyses were performed using SPSS software version 12.0 for Windows.

RESULTS

Anthropometric and biochemical characteristics of the study subjects are shown in Table 1. Fasting plasma IL-6 concentration was positively correlated with BMI, fasting and 2-h postload insulin concentrations, and fasting free fatty acid (FFA) levels (Table 2). These correlations remained significant after adjusting for sex and age but were no longer significant after adjustment for BMI, with the exception of the correlation between plasma IL-6 concentration and 2-h postload insulin concentration ($P = 0.04$). Fasting plasma IL-6 concentration was negatively correlated with the rate of insulin-stimulated glucose disposal (M) ($P = 0.001$). The correlation remained statistically significant, while attenuated, after adjusting for sex, age, and BMI ($P < 0.03$). Adjustment for FFAs in addition to sex, age, and BMI resulted in further attenuation of the significant correlation between plasma IL-6 concentration and insulin-stimulated glucose disposal ($P < 0.044$).

Fasting plasma IL-6 concentration was positively correlated with first-phase insulin secretion assessed as AIR ($P = 0.001$) (Table 2). The correlation remained significant after adjusting for sex, age, and BMI ($P = 0.003$). Adjustment for insulin-stimulated glucose disposal (M) in addition to sex, age, and BMI resulted in attenuation of the significant correlation between plasma IL-6 concentration and insulin-stimulated glucose disposal ($P < 0.01$). To estimate the independent contribution of plasma IL-6 levels to insulin-stimulated glucose disposal (M), we carried out forward stepwise linear regression analysis in a model that included sex, age, BMI, waist-to-hip ratio, and FFAs. The results of the multivariate analysis revealed that only two variables were independently associated with insulin-stimulated glucose disposal (M) (Table 3); BMI was the strongest, accounting for 37.5% of its variation, whereas plasma IL-6 concentration accounted for 4.3% of the variation. The model accounted for 41.8% of the total variation in AIR.

To estimate the independent contribution of plasma IL-6 levels to AIR, we carried out forward stepwise linear regression analysis in a model that included sex, age, BMI, waist-to-hip ratio, FFAs, and insulin-stimulated glucose disposal. The results of the multivariate analysis revealed that only two variables were independently associated with AIR (Table 3); insulin sensitivity was the strongest, accounting for 19.0% of its variation, whereas plasma IL-6 concentration accounted for 5.2% of the variation. The model accounted for 24.2% of the total variation in AIR.

TABLE 2
Univariate correlations between plasma IL-6 concentration and anthropometric and biochemical variables

	IL-6	
	r	P
Age (years)	−0.10	0.33
BMI (kg/m^2)	0.32	0.003
Waist-to-hip ratio	0.16	0.16
SBP (mmHg)	0.03	0.84
DBP (mmHg)	0.17	0.13
Total cholesterol (mg/dl)	−0.15	0.18
HDL cholesterol (mg/dl)	−0.11	0.30
Triglyceride (mg/dl)	−0.04	0.71
FFA (mEq/l)	0.21	0.05
Fasting glucose (mgl/dl)	−0.07	0.52
2-h glucose (mg/dl)	0.15	0.18
Fasting insulin (μ U/ml)	0.23	0.03
2-h insulin (μ U/ml)	0.25	0.02
Glucose disposal (mg · kg^{-1} · min^{-1})	−0.37	0.001
AIR (μU · ml^{-1} · min^{-1})	0.37	0.001

DBP, diastolic blood pressure; SBP, systolic blood pressure.

TABLE 3
Independent predictors of insulin-stimulated glucose disposal rate (*M*) and AIR after forward stepwise linear regression analysis

	Partial r^2 (%)	Total r^2 (%)	β	*t*	*P*
Insulin-stimulated glucose disposal					
Variables entered					
BMI (kg/m^2)	37.5	37.5	−0.545	−5.694	0.0001
IL-6 (pg/ml)	4.3	41.8	−0.193	−2.013	0.03
Variables excluded					
Sex			−0.068	−0.746	0.458
Age (years)			0.062	0.663	0.509
Waist-to-hip ratio			−0.128	−1.328	0.188
FFA (mEq/l)			−0.008	−0.085	0.933
AIR					
Variables entered					
M clamp (mg · kg^{-1} · min^{-1})	19.0	19.0	−0.343	−3.211	0.0001
IL-6 (pg/ml)	5.2	24.2	0.247	2.308	0.02
Variables excluded					
Sex			0.102	1.021	0.311
Age (years)			0.056	0.561	0.576
Waist-to-hip ratio			−0.018	−0.145	0.885
FFA (mEq/l)			0.044	0.432	0.667

DISCUSSION

The most important finding of the present study is the association between plasma IL-6 levels and first-phase insulin secretion and that this relationship is independent of confounding factors such as insulin sensitivity, age, sex, BMI, and waist-to-hip ratio. Only one study (11), to the best of our knowledge, has investigated the relationship between circulating IL-6 levels and direct measures of insulin secretion. The authors reported that, in a cohort of 44 Pima Indians with normal glucose tolerance, first-phase insulin secretion, assessed as AIR during an intravenous glucose tolerance test, was not significantly correlated with plasma IL-6 levels ($r = 0.13$, $P = 0.33$) (11). The differing results between the present study and the previous one may be due to ethnic, demographic, or clinical differences. In addition to the obvious difference in ethnicity existing between the two populations studied, BMI was higher in the Pima Indians group than in subjects of the present study. Mean fasting insulin levels and 2-h glucose concentrations were markedly higher in Pima Indians group than those values in our study population, denoting a condition of greater insulin resistance and initial β-cell decompensation. Another study has investigated the relationship of inflammatory markers regulated by IL-6 such as C-reactive protein and fibrinogen with insulin secretion in a cohort of 396 subjects from the follow-up of the Risk Factors in Impaired Glucose Tolerance for Atherosclerosis and Diabetes (RIAD) study, who were at high risk for type 2 diabetes (5). The authors did not observe association between subclinical inflammation markers and insulin secretion, evaluated by several indexes from oral glucose tolerance test. However, the divergent results between the RIAD study and the present study could in part be due to differences in subject selection. For instance, the RIAD included subjects with type 2 diabetes, a condition known to be associated with elevated IL-6 levels and marked impairment in insulin secretion. Furthermore, it is possible that the effects of IL-6 on pancreatic β-cell function are direct rather than mediated by inflammatory molecules partly, but not exclusively, regulated by IL-6. In this respect, some (12–14) but not all (15–17) studies have demonstrated that IL-6 directly stimulates insulin secretion in cultured insulinoma cells and rat pancreatic islets. It is possible that these discrepancies are due to the experimental conditions used. Indeed, in experiments utilizing physiological concentrations of IL-6, ranging from 1 to 100 pg/ml, a stimulatory effect of IL-6 has been observed on both basal and glucose-stimulated insulin secretion. By contrast, neutral or inhibitory effects of IL-6 have been reported in experiments employing high pharmacological concentrations of IL-6, ranging from 500 to 25,000 pg/ml (15–17). The mechanisms by which IL-6 may modulate insulin secretion are not clear, although some evidence suggest that it may increase insulin secretion and preproinsulin mRNA expression via a Ca^{2+}-dependent mechanism (14). Among the variables included in the multivariate stepwise analysis only insulin sensitivity and IL-6 concentration were independently associated with AIR, accounting for 24.2% of its variation. However, it is highly plausible that other cytokine-related effects, such as an increase in other proinflammatory cytokines; for example, tumor necrosis factor-α may contribute to variation in insulin secretion. In the present study, we provide further evidence of the link between plasma IL-6 levels and insulin resistance or related components of the metabolic syndrome such as high blood pressure, overweight/obesity, and low HDL levels. According to some (10,11,19) but not all (9) studies the relationship between fasting plasma IL-6 concentration and insulin sensitivity was attenuated after adjustment for BMI, likely due to the fact that adipose tissue secretes a considerable amount of IL-6, ranging from 15 to 35% of total circulating IL-6, and that increased release of IL-6 dependent from increasing adiposity may be involved in obesity-related insulin resistance (20). IL-6 may contribute to insulin resistance indirectly by stimulating lipolysis in adipocytes, thus resulting in an increase in circulating FFAs, which would impair insulin action (21). It has been demonstrated that acute rhIL-6 infusion increased circulating FFA concentration and that the relationship between plasma IL-6 levels and insulin sensitivity occurred in parallel to increases in plasma FFAs (9,22,23). According to this view, we found that fasting FFA levels were associated with plasma IL-6 concentration and that the inclusion of FFAs in a multivariate regression model, also including age and sex, attenuated

the capability of IL-6 levels to predict insulin-stimulated glucose disposal.

In conclusion, fasting plasma IL-6 levels are positively related to first-phase insulin secretion and negatively related to insulin sensitivity in a cohort of Italian-Caucasian glucose-tolerant subjects. The relationship between IL-6 and insulin secretion appears to be independent of modulators of insulin secretion such as age, sex, BMI, and insulin sensitivity. The relationship between IL-6 and insulin action seems to be partially mediated through adiposity.

ACKNOWLEDGMENTS

This study was supported in part by grants from European Community's FP6 EUGENE2 no. LSHM-CT-2004-512013 (to G.S.) and Progetto di Ricerca Finalizzata-Ministero della Sanità (to G.S.).

We are grateful to Cristina Ricasoli for her skillful technical assistance.

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