Serum Interleukin-6 Correlates With Endothelial Dysfunction in Healthy Men Independently of Insulin Sensitivity

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OBJECTIVE — Interleukin (IL)-6 is a proinflammatory cytokine that is implicated in the pathogenesis of atherosclerosis and insulin resistance. Both endothelial dysfunction and insulin resistance are among the earliest abnormalities that can be detected in people at risk for cardiovascular events. We aimed to evaluate whether increased serum IL-6 concentrations associated with endothelial dysfunction are independent of insulin sensitivity in apparently healthy men.

RESEARCH DESIGN AND METHODS — Association studies were performed in well-characterized nondiabetic Caucasian men ($n = 99$) recruited for energy balance studies. Insulin sensitivity (minimal model) and brachial vascular reactivity (high-resolution external ultrasound) were assessed. Circulating IL-6 concentrations were measured by enzyme-linked immunosorbent assay.

RESULTS — Serum IL-6 was an independent contributor to the variance of endothelium-dependent vasodilatation after adjusting for age, BMI, smoking status, LDL cholesterol, systolic blood pressure, diastolic blood pressure, and insulin sensitivity ($P = 0.001$). In fact, circulating IL-6 was negatively associated with endothelium-dependent vasodilatation ($r = -0.247$, $P = 0.014$) and insulin sensitivity ($r = -0.262$, $P = 0.011$) and correlated positively with age ($r = 0.241$, $P = 0.016$), BMI ($r = 0.240$, $P = 0.017$), systolic blood pressure ($r = 0.299$, $P = 0.003$), diastolic blood pressure ($r = 0.295$, $P = 0.003$), and triglycerides ($r = 0.212$, $P = 0.035$). No significant associations were observed between endothelium-independent vasodilatation and serum IL-6 concentrations.

CONCLUSIONS — Circulating IL-6 is linked to endothelial dysfunction independently of insulin sensitivity in apparently healthy men.

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Chronic inflammation is linked to endothelial dysfunction, atherosclerosis, and insulin resistance (1,2). Plasma concentrations of proinflammatory cytokines, such as interleukin (IL)-18, IL-6, and tumor necrosis factor (TNF)-α, and of several other inflammatory markers are increased in patients with ischemic heart disease (1,3–6). Men who have been exposed to increased inflammation-sensitive plasma proteins have higher fatality in future coronary events even after adjustment for traditional risk factors (5,6). Circulating cytokines also are elevated in type 2 diabetes, obesity, and insulin resistance syndrome and play a central role in the pathogenesis of these disorders (1).

IL-6 is a mediator of the inflammatory response, and it is linked to dyslipidemia, type 2 diabetes, and risk of myocardial infarction (1,7–9). IL-6 is secreted by a variety of different cell types, including lymphoid and endothelial cells, fibroblasts, skeletal muscle, and adipose tissue. Circulating IL-6 levels correlate with obesity and insulin resistance and may predict the development of type 2 diabetes (9–12).

Endothelial dysfunction is regarded as a causal factor in the development of atherosclerosis (13). It is one of the earliest abnormalities that can be detected in people at risk for cardiovascular events, and it is linked to insulin resistance and type 2 diabetes (14,15). Cytokines have an important role in the endothelial injury induced by inflammation. The vascular endothelium is involved in the inflammatory response to atherosclerosis (13–16), and changes in endothelium function could underlie the association between cardiovascular disease and inflammation. It is difficult to ascertain whether cytokines induce endothelial injury directly or at least in part through insulin resistance–associated inflammation. Recently, an independent association between insulin resistance and vascular dysfunction has been described in 81 patients with type 2 diabetes (15). Insulin resistance was accompanied by a decreased endothelium-dependent vasodilation and increased low-grade inflammation. In type 2 diabetes, a characteristic sensitivity of endothelium to insulin resistance also has been suggested (15). Nevertheless, we have found no studies in which insulin resistance and parameters of inflammation have been studied simultaneously in healthy subjects.

The aim of this study was to evaluate the relationship among serum IL-6 levels, insulin sensitivity, and endothelial function in healthy subjects. Due to the close association between inflammation and insulin resistance, we especially tried to evaluate if this association was independent of insulin sensitivity.

RESEARCH DESIGN AND METHODS

— Ninety-nine consecutive apparently healthy men were randomly selected from consecutive, unselected (except for inclusion and exclusion criteria) Caucasian subjects, who

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Abbreviations: IL, interleukin; TNF, tumor necrosis factor.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

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were participants in an ongoing prospective study of insulin sensitivity in northeastern Spain. All subjects were of Caucasian origin and reported that their body weight had been stable for at least 3 months before the study. None of the subjects was taking any medication or had any evidence of metabolic disease other than obesity. Inclusion criteria were *1*) BMI <40 kg/m^2, *2*) absence of any systemic disease, and *3*) absence of any infections or anti-inflammatory treatment in the previous month. Diabetic patients were excluded. Diabetes was diagnosed according to fasting glycemia ≥126 mg/dl (7 mmol/l) or a 2-h postload glucose ≥200 mg/dl (11.1 mmol/l) during a 75-g oral glucose tolerance test . The oral glucose tolerance test was performed in subjects who had a fasting glycemia ≤126 mg/dl to rule out type 2 diabetes.

Informed written consent was obtained after the purpose, nature, and potential risks of the study were explained to the subjects. The ethics committee of the Hospital of Girona approved the experimental protocol. All subjects were interviewed, and a medical history was recorded. Smoking was defined as more than one cigarette per day in the previous 6 months. BMI was calculated using the following formula: kg (weight)/m^2 (height). Blood pressure was measured in the supine position on the right arm after a 10-min rest; a standard sphygmomanometer of appropriate cuff size was used and the first and fifth phases were recorded. Values used in the analysis are the average of three readings taken at 5-min intervals.

Analytical methods

Blood samples were obtained after a 12-h fast. Total serum cholesterol was measured through the reaction of cholesterol estearase/cholesterol oxidase/peroxidase. Total serum triglycerides were measured through the reaction of glycerol-phosphate-oxidase and peroxidase. HDL cholesterol was quantified after precipitation with polyethylene glycol at room temperature. Plasma glucose level was measured in duplicate by the glucose oxidase method, with a coefficient of variation (CV) <2%.

Serum insulin levels during the frequently sampled intravenous glucose tolerance test were measured in duplicate by monoclonal immunoradiometric assay (IRMA; Medgenix Diagnostics, Fleunes, Belgium). Intra- and interassay CVs were similar to those previously reported (17).

Serum IL-6 concentrations were measured using a solid-phase, enzyme-labeled, chemiluminescent sequential immunometric assay (Immulite 2000; DPC DIPESA S.A., Madrid, Spain). Analytical intra-assay sensitivity was 0.5 pg/ml. The intra- and interassay CVs were 11.6 and 5.1%, respectively. No cross-reactivity with other cytokines was evident.

Study of insulin sensitivity

Insulin sensitivity was measured by the frequently sampled intravenous glucose tolerance test. In brief, the experimental protocol started between 8:00 and 8:30 A.M. after an overnight fast. A butterfly needle was inserted into an antecubital vein, and patency was maintained with a slow saline drip. Basal blood samples were drawn at −30, −10, and −5 min, after which glucose (300 mg/kg body wt) was injected over 1 min starting at time 0, and insulin (0.03 units/kg Actrapid; Novo, Bagsvaerd, Denmark) was administered at time 20 min. Additional samples were obtained from a contralateral antecubital vein up to 180 min, as previously described (17).

Brachial artery vascular reactivity

High-resolution external ultrasound (128×P/10 mainframe with a 7.5-MHz linear array transducer, Toshiba SSH-140A; Toshiba, Tokyo, Japan) was used to measure changes in brachial artery diameter. The lumen diameter of the artery was defined as the distance between the leading edge of the echo of the near wall–lumen interface to the leading edge of the far wall–lumen interface echo. All scans were taken electrocardiogram-triggered coincident with the *R* wave end diastolic. All images were recorded with a S-VHS videotape (Panasonic MD-830AG). Endothelial-dependent vasodilatation was elicited by induced hyperemia following inflation of a pneumatic tourniquet placed around the forearm, distal to the scanned part of the artery, up to a pressure of 300 mmHg for 5 min, followed by sudden deflation. This maneuver is recognized to raise shear stress on the endothelial cells, which in turn release nitric oxide (NO)-producing vasodilatation, which allows testing for endothelial function. Reactive hyperemia is calculated as the percentage of change between the maximum flow recorded in the first 15 s after cuff deflation and the flow during the resting scan. Endothelial-dependent vasodilatation is expressed as the percentage of change in the arterial diameter 1 min after hyperemia. Endothelial-independent vasodilatation is induced after sublingual administration of a 400-μg metered dose of glyceryl trinitrate, an exogenous NO donor (Solinitrina spray; Almirall Prodesfarma, Barcelona, Spain), and expressed as the percentage of change in the arterial diameter 3 min later.

A first scan was recorded after 10 min of resting in a quiet room in the supine position. Then the tourniquet was inflated for 5 min. A second scan was recorded during 90 s beginning 10 s before cuff deflation. After at least 10 more min of rest, a new control scan was recorded. A last scan was recorded from 2 min after GTN administration during 70 s. All images were registered on an S-VHS tape and later analyzed by two independent observers blinded to the metabolic status of the subject and the stage of the experiment. Each observer analyzed the arterial diameter for three cardiac cycles for each condition, and these measurements were averaged.

Before the initiation of the study, validation of this technique was performed through the evaluation of inter- and intraobserver reproducibility in 22 healthy subjects (12 men and 10 women, mean age 30.1 years [95% CI 27.1–33.2], BMI 22.6 kg/m^2 [21.3–23.8]). Measurements were performed by two independent observers (A and B). Intraclass coefficient of correlation of fixed effects between observers A and B was 0.90. CV between means obtained by observers A and B was 9%. The CV obtained by a same observer was 3%. The repeatability (95% CI) was 0.27 mm (observer A). In observer B, the CV was 4%, with repeatability (95% CI) of 0.39 mm. The study of the variability of the means by the same observer in 5 consecutive days showed a CV of 6% (observer A) and 2% (observer B).

Statistical methods

Statistical analyses were performed using SPSS statistical package version 10.0. Descriptive results of continuous variables are expressed as means ± SD or median (1st–3rd quartile). Before statistical analyses, normal distribution and homogeneity of the variances were tested. Parameters that did not fulfill normal distribution (i.e., triglycerides, insulin sensitivity, and IL-6) were log transformed for subsequent analyses. For a given value of $P = 0.05$, the study had a 78% power to detect significant correlations between endothelial-dependent or -independent vasodilation and IL-6 concentrations

Table 1—*Anthropometric and biochemical characteristics of subjects*

Variables	
n	99
Age (years)	52.53 ± 10.66
BMI (kg/m^2)	27.59 ± 3.18
Glucose (mg/dl)	97.8 ± 10.05
Triglycerides (mg/dl)	90 (67–120)
HDL cholesterol (mg/dl)	52.38 ± 11.63
LDL cholesterol (mg/dl)	137.6 ± 38.24
Systolic blood pressure (mmHg)	126.46 ± 14.25
Diastolic blood pressure (mmHg)	81.21 ± 9.86
IL-6 (ng/l)	0.99 (0.72–1.4)
Insulin sensitivity ($10^{-4} \cdot min^{-1} \cdot mU \cdot l^{-1}$)	2.28 (1.36–3.36)
Endothelium-dependent vasodilatation (%)	4.61 ± 5.08
Endothelium-independent vasodilatation (%)	16.02 ± 11.96
Smoking (no/yes)	79/20

Data are means ± SD unless otherwise indicated. Parameters that did not fulfill normal distribution are shown as median (interquartile range).

(Pearson coefficient of at least 0.24). The relationships between variables were analyzed by simple correlation (Pearson's *r*) and multiple regressions in a stepwise manner. Levels of statistical significance were set at $P < 0.05$.

RESULTS — Anthropometric and clinical characteristics of subjects are shown in Table 1. Serum IL-6 correlated negatively with insulin sensitivity ($r = -0.262$, $P = 0.011$) and endothelium-dependent vasodilatation ($r = -0.247$, $P = 0.014$) (Fig. 1). Interestingly, we observed that among the 62 subjects with strictly normal fasting glucose (<100 mg/dl), according to American Diabetes Association 2006 criteria, the negative association between IL-6 levels and endothelium-dependent vasodilation was strenghtened ($r = -0.40$, $P < 0.001$). IL-6 levels also were positively associated with BMI ($r = 0.241$, $P = 0.017$), serum triglycerides ($r = 0.212$, $P = 0.035$), systolic blood pressure ($r = 0.299$, $P = 0.003$), and diastolic blood pressure ($r = 0.295$, $P = 0.003$). Endothelium-dependent vasodilation did not correlate with either insulin sensitivity or other parameters. (Table 2)

Multiple regression analysis models were constructed to predict serum endothelium vasodilatation, taking into account age, BMI, smoking status, LDL cholesterol, systolic and diastolic blood pressure, insulin sensitivity, and serum IL-6 concentrations as independent variables (Table 3). Serum IL-6 and diastolic blood pressure were shown to independently contribute to endothelium-dependent vasodilatation. The association between insulin sensitivity and IL-6 levels was lost after adjusting for BMI. Finally, no relationship was found between endothelium-independent vasodilatation and circulating IL-6 levels.

CONCLUSIONS — We found a negative correlation between IL-6 levels and endothelium-dependent vasodilation, which remained significant after adjusting for insulin sensitivity and other risk factors in apparently healthy men. In this study, we analyzed healthy men in order to minimize the confounding effect of other risk factors or treatments that could interfere in the interpretation of our results.

There are different studies that confirm a close relationship between inflammation and atherosclerosis in an independent way or through different risk factors like dyslipidemia, diabetes, hypertension, and insulin resistance (7,18,19). Ridker et al. (7) found an independent correlation between IL-6 levels and coronary risk in healthy men. IL-6 has been speculated to play a key role in the development of coronary disease though a number of metabolic, endothelial, and procoagulant mechanisms (9,20). On the other hand, serum IL-6 is associated with insulin action in human subjects (1). Circulating IL-6 was associated with insulin sensitivity evaluated by clamp technique or minimal model approach and has been described to predict the development of type 2 diabetes (1).

In a previous study, we found that the positive association between circulating IL-6 and systolic blood pressure was stronger in women than men (21). In the present study, serum IL-6 concentration independently contributed to endothelium-dependent vasodilation in men after adjusting for systolic and diastolic blood pressure. It remains to be tested whether these associations are strengthened in women. Chan et al. (22) performed an analysis by structural equation modeling in 107 nondiabetic subjects (59 men) using fasting insulin as a surrogate of insulin resistance and circulating markers of endothelial activation as a measure of endothelial function. They found that obesity, dyslipidemia, and cytokines (IL-6 and TNF-α) were the principal explanatory variables for the various components of the metabolic syndrome, with IL-6 and TNF-α having different explanatory variables and effects. The complex interrelationships were, in part, mediated by hyperinsulinemia and endothelial activation (22).

Endothelial and smooth muscle cells have been shown to produce IL-6 (23). Inflammation may produce endothelial dysfunction by different mechanisms. Inflammation has the capacity to impair flow-mediated vasodilatation by increasing vasoconstriction or by reducing endothelium-derived vasodilators (24). Cytokines may induce vasoconstriction through different pathways, such us induced synthesis of endothelin-1, decreased expression of endothelial NO synthase or by reducing the bioavailability of NO (2).

In the Framinghan Offspring Study (25), an inverse correlation between IL-6 concentrations and endothelial function was shown. This relationship was attenuated after adjusting for traditional risk factors. The relationship between IL-6 and flow-mediated vasodilation also has been described in subjects with acute coronary syndrome and hypercholesterolemia (26,27). In these studies, despite the close relationship between metabolic syndrome and cardiovascular risk, the effect of insulin resistance on endothelium function was not assessed. Due to the close association between inflammation and insulin resistance, it is possible that cytokines could contribute, at least in part, to impaired vascular reactivity through decreased insulin sensitivity.

Insulin resistance syndrome is associated with an increased risk for cardiovascular disease and type 2 diabetes in both sexes (28). Decreased insulin sensitivity is related with coronary artery disease. Part

Table 2—*Simple correlation analysis between variables*

$n = 99$	Log10 insulin sensitivity (min · mU^{-1} · 0.1^{-1})	EDVD (%)	EIVD (%)	Age (years)	BMI (Kg/m^2)	Log triglycerides (mg/dl)	HDL cholesterol (mg/dl)	Systolic blood pressure (mmHg)	Diastolic blood pressure (mmHg)	LDL cholesterol (mg/dl)
Log10 IL-6 (ng/l)	−0.262	−0.247	−0.038	−0.183	0.240	0.212	−0.05	0.299	0.295	−0.184
	0.011	0.014	0.754	0.078	0.017	0.035	0.579	0.003	0.003	0.068
Log10 insulin sensitivity (min · mU^{-1} · l^{-1})		−0.018	0.105	−0.183	−0.563	−0.377	0.190	−0.108	−0.195	−0.087
		0.866	0.400	0.078	0.000	0.000	0.066	0.298	0.060	0.403
EDVD (%)			−0.009	0.053	0.061	−0.025	0.097	0.025	−0.106	0.046
			0.942	0.603	0.552	0.803	0.340	0.807	0.301	0.654
EIVD (%)				−0.182	−0.211	0.131	−0.708	−0.129	−0.143	−0.50
				0.128	0.078	0.277	0.518	0.286	0.239	0.679
Age (years)					0.135	−0.006	0.162	0.456	0.301	0.102
					0.182	0.951	0.110	0.000	0.003	0.314
BMI (kg/m^2)						0.201	−0.239	0.167	0.325	0.056
						0.046	0.017	0.101	0.001	0.584
Log triglycerides (mg/dl)							−0.369	−0.011	0.141	0.127
							0.000	0.914	0.166	0.209
HDL cholesterol (m/dl)								0.161	0.097	−0.07
								0.114	0.343	0.946
Systolic blood pressure (mmHg)									0.645	0.110
									0.000	0.281
Diastolic blood pressure (mmHg)										0.091
										0.375

EIVD, endothelium-independent vasodilatation; EDVD, endothelium-dependent vasodilatation.

of the association is accounted for by dyslipidemia, hypertension, diabetes, and obesity (29). Insulin action, measured by the euglycemic-hyperinsulinemic clamp technique (*M* index), correlated strongly and negatively with endothelium-dependent vasodilatation in obese men (30). The compensatory hyperinsulinemia present in insulin resistance also has a direct effect on endothelial function, independently of other metabolic abnormalities (31). Insulin-sensitizing drugs improve endothelial function in nondiabetic hypertensive individuals with insulin resistance, and the improvement was associated with the amelioration of insulin resistance (32). In the same direction, pioglitazone is shown to improve vascular reactivity in the same manner that it reduces IL-6 levels, suggesting a role of this cytokine on the effect of insulin resistance on vascular function (33).

The close links between inflammation, insulin resistance, and risk factors associated with the metabolic syndrome make it difficult to separate and explain the results of different studies. Only one previous study has simultaneously measured insulin resistance and endothelial function by direct and reliable methods. In 81 type 2 diabetic patients, inflammation contributed to endothelial dysfunction, but this effect was additive to that of insulin resistance (15). In this study, endothelial dysfunction was significantly linked with insulin resistance, in association with an obesity index (BMI or waist-to-hip ratio) or a cytokine. In other studies, the direct association between insulin sensitivity and endothelial dysfunction was not observed in either patients with obesity or healthy subjects (34,35). We also failed to demonstrate this association in our group of healthy subjects. As it has been suggested, a special sensitivity of endothelium in subjects with diabetes, or the lower degree of insulin resistance in healthy subjects compared with type 2 diabetic patients, could underlie the different results of these studies. Nevertheless, in our healthy subjects, IL-6 clearly contributed to endothelial injury, whereas insulin sensitivity did not. In this regard, the association between serum IL-6 levels and endothelium-dependent vasodilation was especially significant in subjects with strictly normal fasting glucose. Other factors and cytokines in the context of hyperglycemia and insulin resistance may blunt the relationship between IL-6 and endothelial dysfunction when fasting glucose increases.

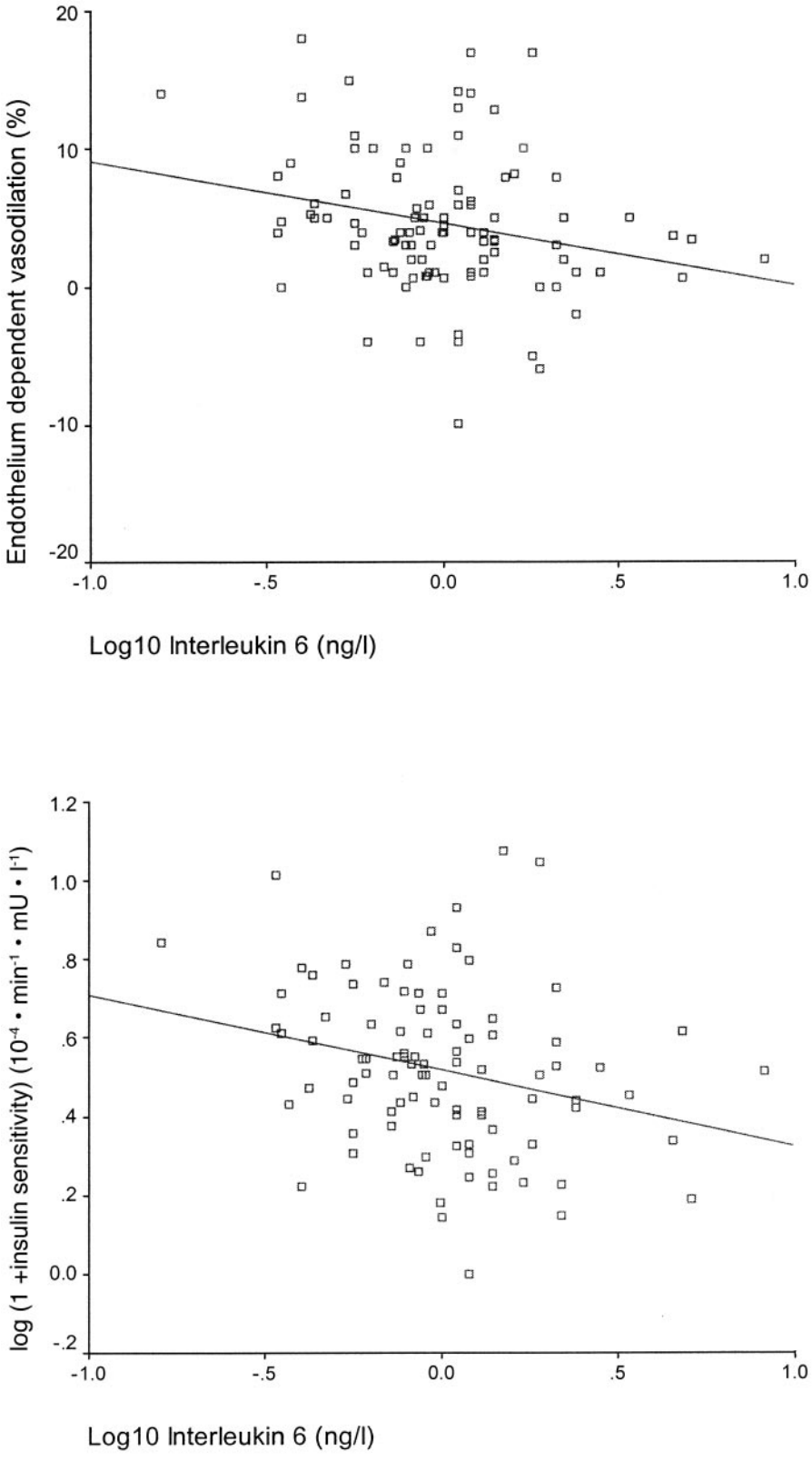

Figure 1—*Linear correlation between log-transformed IL-6 levels and endothelium-dependent vasodilatation and insulin sensitivity. The association between insulin sensitivity and IL-6 levels was lost after adjusting for BMI.*

Another explanatory hypothesis is that a low-grade inflammation (increased IL-6) could constitute a first hit—a first aggression that would exert a central role in the cluster inflammation/insulin resistance by inducing predominantly endothelial dysfunction. In a second subsequent hit, the effect of insulin resistance over endothelium would turn out to be

Table 3—*Linear multiple regression of endothelium-dependent vasodilatation as dependent variable*

	Endothelium-dependent vasodilation				
	Independent variables				
	Unstandardized coefficients		Standardized		
Dependent variables	β	SE	β	T	*P*
Log10 IL-6 (ng/l)	−6.994	7.801	−0.393	−3.355	0.001
Age (years)	3.756E-02	0.052	0.082	0.721	0.473
BMI (kg/m^2)	0.379	0.209	0.228	1.817	0.073
Smoking (yes/no)	2.432	1.304	0.197	1.865	0.066
Log 10 insulin sensitivity ($10^{-4} \cdot min^{-1} \cdot mU \cdot l^{-1}$)	−0.446	2.963	−0.018	−0.150	0.881
LDL cholesterol (mg/dl)	−1.297E-02	0.013	−0.100	−0.967	0.336
Systolic blood pressure (mmHg)	5.783E-02	0.049	0.164	1.174	0.244
Diastolic blood pressure (mmHg)	−0.141	0.069	−0.281	−2.050	0.044

more important per se, playing the central role of the metabolic abnormalities associated with vascular dysfunction. Prospective studies simultaneously analyzing inflammation, insulin sensitivity, and endothelial function will be necessary to better understand the underlying mechanism of these processes.

Our results might be applicable only to middle-aged, apparently healthy men, in whom endothelium-independent vasodilation did not correlate with inflammation, insulin sensitivity, or other risk factors. Only in severe degrees of insulin resistance does endothelium-independent function seem to be affected (15). The apparently higher insulin sensitivity of our healthy subjects could explain the results.

It should be recognized that the chronic inflammatory response in the context of insulin resistance and endothelium dysfunction concern not only IL-6 but also probably a myriad of other factors, mainly other proinflammatory cytokines such as TNF-α and IL-1-β. Moreover, IL-6 also has an anti-inflammatory activity regarding the interaction with TNF-α. In future studies, it will be interesting to sort out whether IL-6 is more important than other circulating cytokines and factors.

In summary, our results confirmed an early and negative relationship between circulating IL-6 and flow-mediated vasodilation in apparently healthy men. To our knowledge, this is the first study evaluating simultaneously circulating IL-6, insulin sensitivity, and endothelium function. The association between IL-6 and endothelium function was found to be independent of insulin sensitivity and other risk factors in healthy men.

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