

Elevated plasma interleukin-18 is a marker of insulin-resistance in type 2 diabetic and non-diabetic humans

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Abstract

Elevated plasma IL-18 is present in several conditions sharing insulin-resistance as common trait, but the association with insulin-resistance per se is not established. Plasma/serum IL-6, IL-18, TNF- α , soluble TNF receptor II (sTNFR2), and C-reactive protein (CRP) were measured in 97 patients with type 2 diabetes (DM) and 84 non-diabetic controls (CON). The association with insulin-resistance—estimated using the homeostasis model assessment (HOMA-IR)—was analyzed using multivariate linear and logistic regression. Compared to CON, DM demonstrated higher plasma levels of IL-18 ($P = 0.001$), IL-6 ($P < 0.001$), sTNFR2 ($P = 0.005$), and CRP ($P < 0.001$), while TNF- α was lower ($P = 0.017$). Plasma IL-18 increased across HOMA-IR quartiles in both DM and CON, both with and without adjustment for confounders (all $P < 0.05$). In contrast, neither IL-6, TNF- α , sTNFR2, nor CRP was associated with HOMA-IR in CON when adjusting for confounders. Accordingly, 50% increase of IL-18 corresponded to a marked increase of HOMA-IR in both DM and CON (DM: 26%, $P = 0.014$; CON: 25%, $P = 0.003$) after adjustment for confounders. Our results show that plasma IL-18 was associated with HOMA-IR independent of obesity and type 2 diabetes.

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Introduction

It is recognized that the pathogenesis of insulin-resistance—the key feature of type 2 diabetes—includes low-grade inflammation [1]. Indeed, elevated concentrations of various inflammatory markers in the circulation have been reported in humans with insulin-resistance, e.g., interleukin-

6 (IL-6) [2]; tumor necrosis factor α (TNF- α) [3]; soluble TNF receptors (sTNFR1, sTNFR2) [4]; and C-reactive protein (CRP) [5]. In addition to the production by immunocompetent cells, adipose tissue contributes significantly to the levels of, e.g., IL-6 [6] and TNF- α [7] in the circulation. Accordingly, obesity is associated with elevated circulating levels of IL-6 [8], TNF- α [3], sTNFR2 [9], as well as CRP [10]. Therefore, elevated plasma levels of different inflammatory markers may sometimes reflect obesity rather than insulin-resistance per se [11,12], though obesity is playing an important role in insulin-resistance as well type 2 diabetes.

One decade ago, the cytokine interleukin-18 (IL-18) was first described as an interferon- γ (INF- γ) inducing member of the interleukin-1 (IL-1) superfamily [13,14], but unlike IL-1, IL-18 does not induce fever [15]. The human IL-18

Abbreviations: CRP, C-reactive protein; DBP, diastolic blood pressure; HDL, high-density lipoprotein; HOMA-IR, homeostasis assessment of insulin-resistance; IL-18, interleukin-18; IL-6, interleukin-6; TNF- α , tumor necrosis factor- α ; sTNFR2, soluble tumor necrosis factor receptor II; OR, odds ratio; SBP, systolic blood pressure; TAG, triacylglyceride; TZD, thiazolidinediones.

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gene is located on chromosome 11q22.2–22.3 [16]. A number of hemopoietic as well as non-hemopoietic cell lines express IL-18 [17], which initially is synthesized as a 24-kDa inactive precursor subsequently cleaved by the IL-1 β -converting enzyme (caspase-1) in order to generate the biologically active 18-kDa IL-18 [18]. Known immunological stimuli are lipopolysaccharide (LPS), which induces IL-18 via a caspase-1-dependent conversion of the precursor, pro-IL-18 [18], and the Fas ligand, which stimulates IL-18 in a caspase-1-independent manner [19]. Currently, IL-18 is characterized as a pro-inflammatory cytokine involved in apoptosis and tissue destruction [20], thus playing a role in the host defense against infections and neoplasms [21]. In addition, elevated plasma IL-18 predicts cardiovascular mortality in patients with coronary atherosclerosis [22]. Also, a role for IL-18 in the autoimmune β -cell destruction leading to type 1 diabetes has been proposed [23–25]. Patients with systemic juvenile idiopathic arthritis demonstrate extremely high plasma IL-18 levels [26]. However, recent studies also report that plasma IL-18 levels are associated with type 2 diabetes [27–29], obesity [30], metabolic syndrome [31], polycystic ovary syndrome [32], and HIV lipodystrophy [33]. Since a common feature of these otherwise dissimilar diseases is insulin-resistance, we hypothesized that circulating IL-18 is an inflammatory marker of insulin-resistance. Accordingly, plasma IL-18 should reflect insulin-resistance not only in patients with established type 2 diabetes, but also in non-diabetic controls. In addition, we hypothesized that plasma levels of IL-18 would be a superior marker of insulin-resistance when compared to IL-6, TNF- α , sTNFR2, and CRP.

Materials and methods

Subjects

Using a cross-sectional case-control design, 97 patients with type 2 diabetes (DM) and 84 healthy, age-matched, non-diabetic controls (CON) participated in the present study. The purpose of the study, possible risks, and discomforts were explained to the subjects before obtaining written consent. The study protocol was approved by the local ethical committee, and was performed in accordance with the Declaration of Helsinki.

The patients with type 2 diabetes were recruited using an outpatient clinic database (diabetesRASK) at the Department of Medicine, Roskilde County Hospital, K \ddot{o} ge. Initially, 720 consecutive records of type 2 diabetic patients were evaluated. Primary exclusion criteria were treatment with insulin ($n = 265$), recent or ongoing infection, history of cancer disease or treatment with anti-inflammatory drugs ($n = 249$). Of the remaining 206 patients, 122 patients volunteered for the study. The non-diabetic controls ($n = 89$) were recruited among relatives to the patients and employees at the hospital.

To verify correct diagnosis, the World Health Organization (WHO) diagnostic criteria for diabetes were employed [34]. Thus, 97 patients with fasting venous plasma glucose concentration 7.0 mmol or higher, and 84 controls with fasting venous plasma glucose concentration lower than 7.0 mol/l were included in the final analysis.

Sampling

Each participant attended the laboratory after an overnight fast, which included intake of any medication. A general health examination including interview regarding lifestyle was performed. The body weight and height was determined in order to calculate the body mass index (BMI). Sphygmomanometric measurement of the brachial arterial blood pressure was performed on the participant resting in the sitting position. Blood samples were obtained from the antecubital vein between 07:30 and 10:00 in the morning. The following were analyzed immediately on the Department of Clinical Biochemistry at Roskilde County Hospital using routine methods: plasma glucose was measured using a hexokinase assay (Hitachi 917 analyzer, Roche Diagnostics, Mannheim, Germany), while cholesterol content of lipoprotein fractions and triacylglycerides (TAG) were measured using enzymatic methods (Hitachi 917 analyzer, Roche Diagnostics). Plasma was obtained by drawing blood into ethylenediaminetetraacetic acid (EDTA)-containing glass tubes, while serum was obtained by drawing blood into glass tubes containing a clot-inducing plug. The tubes were subsequently centrifuged at $2200 \times g$ for 15 min at 4°C, and the supernatants were stored at -80°C until further analysis.

Plasma IL-18, IL-6, TNF- α , sTNFR2, serum CRP, and plasma insulin concentrations were measured using ELISA (IL-18: #7620, Medical and Biological Laboratories, Nagoya, Japan; IL-6: #HS600B, R&D Systems, Minneapolis, Minnesota; TNF- α : #HSTA00C, R&D Systems; sTNFR2: #DRT200, R&D Systems; CRP: #1000, Alpha Diagnostic, San Antonio, Texas; insulin: #K6219, DakoCytomation, St. Charles, Missouri). The detection limits were 12.5 pg/ml for IL-18, 0.1 pg/ml for IL-6, 0.3 pg/ml for TNF- α , 2.3 pg/ml for sTNFR2, 0.4 $\mu\text{g/l}$ for CRP, and 10 pmol/l for insulin. The intra-assay and inter-assay CV were less than 5% and 14% for IL-18, 5% and 20% for IL-6, 7% and 8% for TNF- α , 5% and 7% for sTNFR2, 9% and 9% for CRP, and 7% and 14% for insulin, respectively.

To minimize the effect of inter-assay variation, samples from diabetes patients and controls were equally represented on each ELISA plate. All samples were analyzed in duplicates, and the mean of the duplicates was used for the statistical analysis.

Calculations and statistics

Based on the fasting plasma concentrations of glucose and insulin, the level of insulin-resistance was calculated using the homeostasis model assessment (HOMA) version

from 1996 (available at www.ocdem.ox.ac.uk), since the original HOMA described by Matthews in 1985 [35] tends to overestimate insulin-resistance as well as β -cell function [36].

For all parameters, distribution of the data was compared with the normal distribution using histograms and probability plots. Thus, plasma levels of glucose, insulin, HDL, TAG, IL-18, IL-6, TNF- α , sTNFR2, CRP, and HOMA-IR demonstrated left-skewed distributions, and were therefore \log_{10} -transformed before normal distribution was obtained. Differences between groups were tested using Fisher's exact test for categorical variables and Student's two-sample *t* test for continuous variables. By subdivision of the DM and CON groups into quartiles of HOMA-IR ($Q_{\text{HOMA-IR}}$), the association between HOMA-IR and each of the inflammatory markers was initially investigated via 2-way ANOVA (model: $\log_{10}(\text{IM}) = \text{diagnosis} + Q_{\text{HOMA-IR}} + \text{diagnosis} \times Q_{\text{HOMA-IR}}$), followed by calculation of the linear trend across quartiles, with or without age, gender, BMI, and current smoking as covariates.

Associations between plasma IL-18 and possible confounders as well as the other inflammatory markers were tested by subdivision of the DM and CON groups into IL-18 quartiles. Via logistic regression, the odds ratio (OR) for high insulin-resistance (defined as HOMA-IR > 75th percentile) within each group was estimated using the 75th percentile as cutoff level for each inflammatory markers. The OR was adjusted for age, gender, obesity (defined as BMI > 30 kg/m²), and current smoking. In addition, the magnitude of the associations between HOMA-IR and each of the inflammatory markers were evaluated using the following multivariate linear regression model:

$$\log_{10}(\text{HOMA-IR}) = \beta_0 + \beta_1 \log_{10}(\text{IM}) + \beta_2 \text{age} + \beta_3 \text{gender} + \beta_4 \text{BMI} + \beta_5 \text{smoking} \quad (1)$$

using age and BMI as continuous variables, and gender and smoking as categorical variables. Accordingly, if θ represents the fold change in HOMA-IR corresponding to the ϕ fold change in concentration of a specific IM, the regression coefficient β_1 describes the relative relation between the selected inflammatory marker and HOMA-IR as follows:

$$\log_{10}(\theta \text{HOMA-IR}) = \beta_0 + \beta_1 \log_{10}(\phi \text{IM}) \Rightarrow \log_{10}(\theta) = \beta_1 \log_{10}(\phi) \Leftrightarrow \theta = \text{antilog}_{10}[\beta_1 \log_{10}(\phi)] \quad (2)$$

All ANOVA and regression models were evaluated in accordance with the following criteria: (1) normal distribution of the residuals using probability plots and the Kolmogorov–Smirnov one-sample test of goodness of fit, (2) no discernible pattern when plotting residuals against the fitted values. If outliers were detected during the initial

analysis, the regression model was reevaluated without these before conclusions were made. *P* values lower than 0.05 were considered significant. All statistical analyses were computed using SAS statistical software (release 8.02, SAS Institute Inc., Cary, North Carolina, USA).

Results

The two groups (DM and CON) were similar with respect to age and height (Table 1). In both groups, the majority of the subjects were men (76% in DM and 56% in CON). Of note, gender was not associated with HOMA-IR (2-way ANOVA: effect of diagnosis *P* < 0.001; effect of gender *P* = 0.896, effect of diagnosis \times gender *P* = 0.866). Compared to CON, DM demonstrated significantly higher mean weight, BMI, systolic blood pressure, P-glucose, P-insulin, HOMA-IR, P-TAG, while P-HDL was lower than in CON (all *P* < 0.05). Of note, 98% of the type 2 diabetic patients were treated with antidiabetic medication (75% sulfonylurea, 67% biguanides, 3% thiazolidinediones), while 41% received treatment with statins (versus none in CON). In addition, the DM group was characterized by markedly higher levels of IL-18 (*P* = 0.001), IL-6 (*P* <

Table 1
General characteristics

	CON	DM	Difference
<i>n</i> (M/F)	84 (47/37)	97 (74/23)	
Age (years)	56.4 (54.1–58.6)	58.9 (56.8–61.0)	
Current smoker (%)	24	35	
Statins (%)	0	41	***
Biguanides (%)	0	67	***
Sulfonylurea (%)	0	75	***
TZD (%)	0	3	***
Height (cm)	172.5 (170.6–174.5)	174.3 (172.5–176.1)	
Weight (kg)	78.0 (74.7–81.2)	90.9 (86.9–94.8)	***
BMI (kg/m ²)	26.1 (25.2–27.0)	29.7 (28.7–30.8)	***
WHR	0.89 (0.87–0.92)	0.96 (0.95–0.98)	***
SBP (mm Hg)	138 (134–141)	146 (143–149)	***
DBP (mm Hg)	85 (83–87)	86 (85–88)	
P-HDL (mmol/l)	1.51 (1.42–1.61)	1.24 (1.18–1.30)	***
P-TAG (mmol/l)	1.24 (1.11–1.39)	1.99 (1.81–2.20)	***
P-glucose (mmol/l)	5.2 (5.1–5.4)	9.7 (9.2–10.2)	***
P-insulin (pmol/l)	39 (33–46)	68 (58–78)	***
HOMA-IR	0.88 (0.74–1.04)	1.83 (1.57–2.13)	***
P-IL-18 (pg/ml)	258 (240–278)	307 (286–329)	***
P-IL-6 (pg/ml)	1.4 (1.1–1.6)	2.3 (2.0–2.6)	***
P-TNF- α (pg/ml)	2.4 (1.9–3.0)	1.7 (1.5–2.0)	*
P-sTNFR2 (ng/ml)	1.9 (1.8–2.0)	2.1 (2.0–2.2)	***
S-CRP (mg/l)	1.2 (0.9–1.6)	2.3 (1.9–2.8)	***

DM, type 2 diabetic patients; CON, non-diabetic controls; F, female subjects; M, male subjects; TZD, thiazolidinediones; BMI, body mass index; WHR, waist-to-hip ratio; SBP, systolic blood pressure; DBP, diastolic blood pressure; HOMA-IR, homeostasis model assessment of insulin-resistance; HDL, high-density lipoproteins; TAG, triacylglycerides. Categorical variables are shown as prevalence, continuous variables shown as geometric means with 95% CIs. Differences between CON and DM: **P* < 0.05; ****P* < 0.0005.

0.001), sTNFR2 ($P = 0.005$), and CRP ($P < 0.001$), while TNF- α was lower ($P = 0.017$), when compared to CON.

Figs. 1a–e show the relationship between HOMA-IR and each of the inflammatory markers, when the DM and CON groups were divided into subgroups according to HOMA-IR quartiles: an overall effect of HOMA-IR quartile was observed for IL-18 ($P = 0.001$) and CRP ($P < 0.001$), while HOMA-IR quartiles failed to explain the variation of

plasma IL-6, TNF- α , and sTNFR2. Importantly, the linear trend across HOMA-IR quartiles for plasma IL-18 (Fig. 1a) remained significant when adjusting for age, gender, BMI, and current smoking in DM ($P < 0.001$) as well as in CON ($P = 0.010$). In contrast, serum CRP (Fig. 1e) was associated with HOMA-IR quartiles in DM but not in CON, when including the covariates.

The associations between plasma IL-18 and possible confounders as well as the other inflammatory markers were tested by subdivision of the DM and CON groups into IL-18 quartiles as shown in Table 2: in neither DM nor CON was IL-18 associated with age, BMI, or percentage current smokers. Neither was IL-18 associated with level of self-reported physical activity. In addition, there was no trend for statins, biguanides, or sulfonylurea users across IL-18 quartiles, indicating no or minimal effect of the medication on plasma IL-18. In accordance, we found no effect of statins, biguanides, or sulfonylurea on HOMA-IR in DM when using 2-way ANOVA (not shown, model $\log\text{HOMA-IR} = \log\text{IL18} + \text{medication} + \log\text{IL18} \times \text{medication}$), indicating no or minimal effect of the medication taken the day before the blood sampling. Plasma IL-18 was also associated with plasma insulin in both CON and DM (adjusted trend $P = 0.035$ and $P = 0.015$ for CON and DM, respectively) but not with plasma glucose in any of the groups. Accordingly, plasma insulin appeared to be the more essential component of the association between HOMA-IR and plasma IL-18. Though only IL-18 was associated with HOMA-IR, there was a marked correlation between IL-18 and the other inflammatory markers (Table 2), especially in the DM group.

Fasting plasma TAG concentration decreased across IL-18 quartiles in DM (adjusted trend $P = 0.043$), and tended to decrease in CON (crude trend $P = 0.055$), while plasma HDL tended to increase across IL-18 quartiles in CON (crude trend $P = 0.051$). The associations between IL-18 on one hand and HDL and TAG on the other hand were, however, markedly less consistent than the associations between IL-18 and HOMA-IR. Accordingly, plasma IL-18 >75th percentile (CON: >306 pg/ml; DM: >403 pg/ml) was associated with HOMA-IR >75th percentile (Fig. 2a) in both CON and DM (CON: adjusted OR 3.3, 95% CI 1.0–10.9, $P = 0.048$; DM: adjusted OR 4.1, 95% CI 1.2–14.1, $P = 0.023$). In DM but not CON, plasma IL-6 >75th percentile (>3.2 pg/ml) was associated with HOMA-IR >75th percentile ($P = 0.045$). Neither of the other markers were associated with HOMA-IR >75th percentile in either CON or DM.

To evaluate the potential clinical significance of the association between plasma IL-18 and HOMA-IR, the magnitude of the associations between HOMA-IR and each of the inflammatory markers was estimated separately for DM and CON (Fig. 2b) using the multivariate regression model [2] and equation [3]. Accordingly, a 50% increase of plasma IL-18 was associated with 26% (95% CI 5–50%, $P = 0.014$) increase of HOMA-IR in CON and 25% (95% CI 8–44%, $P = 0.003$) increase in

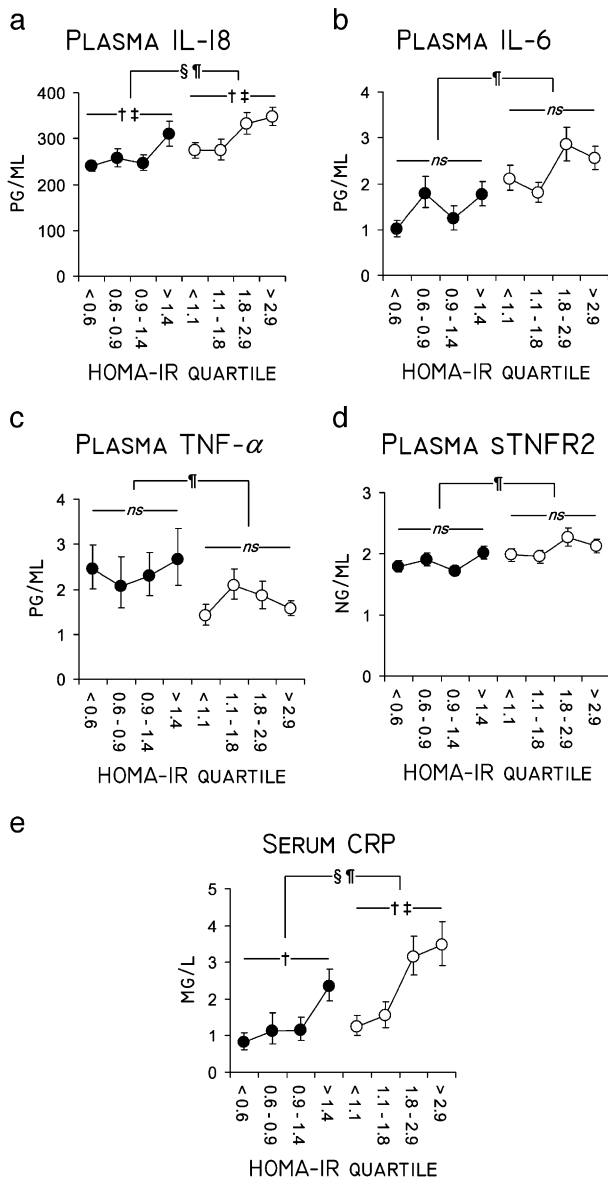


Fig. 1. Concentrations of IL-18, IL-6, TNF- α , sTNFR2, and CRP according to HOMA-IR. Panels A–E show the plasma/serum concentrations of IL-18 (a), IL-6 (b), TNF- α (c), sTNFR2 (d), and CRP (e) as geometric means \pm geometric SEM, when each group (DM and CON) was subdivided using HOMA-IR quartiles (the limits are shown on the abscissa). Closed circles represent CON, while open circles represent DM. †Overall effect of the diagnosis “type 2 diabetes” (2-way ANOVA, $P < 0.05$); ‡overall effect of HOMA-IR quartile (2-way ANOVA, $P < 0.05$); †crude linear trend ($P < 0.05$) across quartiles; ‡linear trend ($P < 0.05$) across quartiles adjusted for age, gender, BMI, and current smoking; ns, not significant.

Table 2
Effect of plasma IL-18 quartiles

Group quartile	CON				Trend	DM				Trend
	Q1	Q2	Q3	Q4		Q1	Q2	Q3	Q4	
<i>n</i> (M/F)	21 (13/8)	21 (9/12)	21 (11/10)	21 (14/7)		24 (13/9)	24 (18/6)	25 (22/3)	24 (21/3)	
P-IL18 (pg/ml)	178 (167–190)	224 (219–230)	274 (268–281)	404 (363–449)		199 (186–214)	267 (259–275)	347 (335–359)	478 (449–508)	
Age (years)	55.7 (52.2–59.1)	56.0 (51.6–60.3)	58.4 (53.7–63.0)	54.7 (49.7–59.6)		56.1 (51.9–60.4)	59.8 (55.2–64.3)	58.1 (54.7–61.6)	61.8 (57.5–66.1)	
Current smoker (%)	29	19	10	29		42	38	16	46	
Statins (%)	0	0	0	0		42	54	44	25	
Biguanides (%)	0	0	0	0		58	83	60	67	
Sulfonylureas (%)	0	0	0	0		58	83	84	75	
BMI (kg/m ²)	25.8 (24.2–27.3)	24.8 (23.0–26.7)	26.1 (23.9–28.3)	27.6 (25.7–29.5)		30.1 (27.6–32.6)	28.6 (26.6–30.5)	30.9 (29.3–32.6)	29.4 (27.4–31.3)	
P-HDL (mmol/l)	1.64 (1.47–1.83)	1.59 (1.39–1.82)	1.51 (1.34–1.71)	1.38 (1.19–1.59)		1.25 (1.16–1.35)	1.34 (1.19–1.52)	1.18 (1.09–1.28)	1.19 (1.06–1.34)	
P-TAG (mmol/l)	1.11 (0.95–1.30)	1.06 (0.86–1.31)	1.13 (0.93–1.38)	1.47 (1.17–1.84)		1.88 (1.57–2.25)	1.80 (1.52–2.14)	2.07 (1.72–2.48)	2.24 (1.76–2.85)	‡
P-glucose (mmol/l)	5.3 (5.1–5.5)	5.2 (5.0–5.4)	5.1 (4.9–5.3)	5.2 (4.9–5.5)		9.0 (8.1–10.0)	9.6 (8.7–10.5)	10.4 (9.2–11.7)	9.8 (8.9–10.8)	
P-insulin (pmol/l)	36 (27–48)	26 (18–39)	39 (29–51)	62 (49–80)	†,‡	62 (47–82)	61 (46–80)	65 (51–84)	86 (60–122)	‡
P-IL-6 (pg/ml)	1.0 (0.7–1.4)	1.2 (0.8–1.8)	1.5 (1.0–2.2)	1.9 (1.3–2.6)	†,‡	1.7 (1.4–2.1)	2.4 (1.9–3.0)	2.5 (2.0–3.2)	2.7 (2.1–3.4)	†,‡
S-CRP (mg/l)	0.8 (0.5–1.3)	1.1 (0.5–2.4)	1.4 (0.7–2.5)	1.4 (0.9–2.1)		1.5 (1.0–2.3)	2.4 (1.6–3.6)	2.9 (1.9–4.2)	2.7 (1.8–4.1)	†,‡
P-TNF-α (pg/ml)	2.7 (1.5–4.6)	1.6 (1.1–2.4)	2.7 (1.8–4.1)	2.6 (1.9–3.5)		1.4 (1.1–1.7)	1.5 (1.1–2.1)	1.9 (1.4–2.5)	2.2 (1.6–3.1)	†,‡
P-sTNFR2 (ng/ml)	1.8 (1.6–2.0)	1.9 (1.7–2.1)	1.8 (1.7–2.0)	1.9 (1.7–2.2)		1.8 (1.6–1.9)	2.0 (1.8–2.3)	2.2 (2.0–2.4)	2.4 (2.1–2.7)	†,‡

The non-diabetic controls (CON) as well as the type 2 diabetic patients (DM) divided according to plasma IL-18 quartiles. M, males; F, females; BMI, body mass index; LDL, low-density lipoproteins; HDL, high-density lipoproteins; TAG, triacylglycerides. Categorical variables are shown as prevalence, continuous variables shown as geometric means with 95% CIs. †Crude linear trend ($P < 0.05$); ‡adjusted linear trend ($P < 0.05$) with age, BMI, gender, and current smoking as obligatory explanatory variables in each analysis.

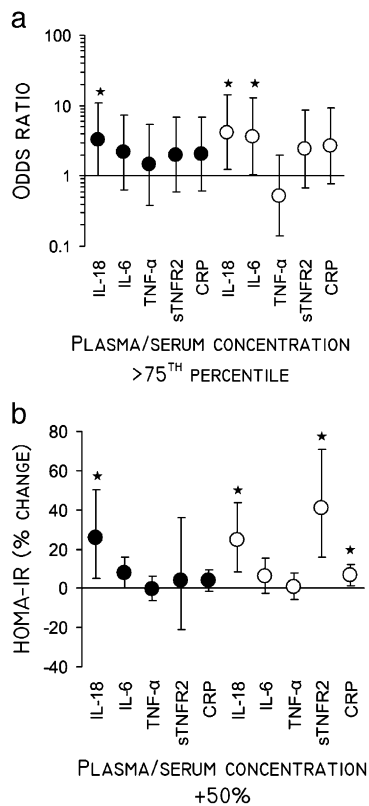


Fig. 2. Odds ratio for high HOMA-IR, and estimated changes of HOMA-IR corresponding to changes of IL-18, IL-6, TNF- α , sTNFR2, or CRP. (a) shows the odds ratio (OR) of HOMA-IR >75th percentile in CON and DM, respectively, if plasma/serum concentration of IL-18, IL-6, TNF- α , sTNFR2, or CRP was >75th percentile, adjusted for age, gender, obesity (BMI >30 kg/m²), and current smoking. (b) shows the magnitude of the association between HOMA-IR and plasma/serum concentrations of IL-18, IL-6, TNF- α , sTNFR2, and CRP for DM and CON, adjusted for age, gender, BMI, and current smoking. The percentage change of HOMA-IR (shown as mean with 95% CI) associated with a 50% increase of each inflammatory marker was calculated using the multivariate model Eqs. (1) and (2), described under the Materials and methods section. Thus, a 50% increase of plasma IL-18 was associated with 26% (95% CI 5–50%, $P = 0.014$) increase of HOMA-IR in CON and a 25% (95% CI 8–44%, $P = 0.003$) increase of HOMA-IR in CON. Presented as means with 95% CIs, closed circles represent CON, while open circles represent DM. * $P < 0.05$.

DM (after adjustment for age, gender, BMI, and current smoking). A 50% increase in plasma sTNFR2 was associated with an increase in HOMA-IR only in DM (41%, 95% CI 16–71%, $P = 0.001$). In contrast, a 50% increase of plasma IL-6, TNF- α , or CRP was not associated with an increase of HOMA-IR in either DM or CON.

Discussion

Our results demonstrate that plasma IL-18 is closely associated with insulin-resistance independent of obesity. Importantly, IL-18 was associated with HOMA-IR in both

patients with type 2 diabetes as well as in non-diabetic controls, even when adjusting for age, gender, BMI, and current smoking.

Though elevated levels of IL-18 in plasma is observed in a number of various conditions associated with insulin-resistance [27–33], a clear relationship between insulin-resistance per se and circulating IL-18 has not been demonstrated previously. Simple correlations between plasma IL-18 and fasting plasma glucose have been shown in patients with type 2 diabetes [27], and between plasma IL-18 and fasting plasma insulin in obese women [30,37]. The relationship between plasma IL-18 and insulin-resistance in healthy subjects has not been investigated previously. While IL-18 was associated with HOMA-IR in both CON and DM with and without adjusting for confounders and using different statistical models, IL-6 and TNF- α were not generally associated with insulin-resistance in neither DM nor CON. Serum CRP was associated with insulin-resistance in DM but not in CON when adjusting for confounders, and high CRP (>75th percentile) was not associated with high HOMA-IR when adjusting for confounders. Considering that IL-18 is linked to loss of β -cell function in type 1 diabetes [24], it could be argued that decreased insulin secretion contributed to insulin-resistance observed in both CON and DM. However, plasma insulin was positively correlated with plasma IL-18 in both CON and DM. Also, β -cell function calculated using HOMA [36] increased with increasing levels of IL-18 in CON (not shown), while no association between β -cell function and IL-18 was apparent in DM. Thus, in non-type 1 diabetic humans, IL-18 appears to be associated with insulin-resistance but not loss of β -cell function.

In contrast to the clear association between plasma IL-18 and HOMA-IR, we observed only weak associations between IL-18 and indices of obesity and dyslipidemia: IL-18 was not associated with BMI, associated with plasma TAG in DM and insignificantly in CON, and insignificantly associated with plasma HDL in only CON. Olusi et al. reported that plasma IL-18 is associated with plasma TAG and fasting serum glucose [38], though only in the obese or diabetic subjects, but not in the non-obese or non-diabetic subjects. And despite the larger number of subjects investigated in the study by Olusi et al., the relationship between insulin-resistance and IL-18 was not addressed specifically. Escobar-Morreale et al. [32] observed an overall relationship between obesity and plasma IL-18 in polycystic ovary syndrome, as well as between insulin-resistance and plasma IL-18. The latter, however, did not remain significant when including covariates in a multivariate analysis, which may be due to the limited number of subjects. Recently, Hung et al. [31] found that elevated plasma IL-18 is associated with the metabolic syndrome in non-diabetic humans ($n = 955$) even when adjusting for obesity and plasma insulin. Of note, Hung et al. did not investigate the relationship between IL-18 and insulin-resistance per se, but reported that the odds ratio for

metabolic syndrome according to plasma IL-18 tertiles was markedly reduced when adjusting for plasma insulin and obesity. If more subjects had been included in our study, a stronger association between IL-18 and indices of obesity and dyslipidemia may have appeared, though most probably still less apparent than the association between plasma IL-18 and HOMA-IR.

When considering the limitations of the present study, it should be noted that the majority of the subjects in the present study were men; thus, our conclusion regarding the association between IL-18 and insulin-resistance may be limited to the male gender. Of note, the association between IL-18 and HOMA-IR remained significant even when adjusted for age, BMI, and smoking, if all females were excluded from the analysis (not shown). In addition, an effect of prior medication on the observed associations was possible, since all medications were paused only during an overnight fast. However, neither use of sulfonylurea, biguanides, nor statins had a general effect on the overall association between plasma IL-18 and HOMA-IR. Thus, we conclude that medication used the day before the sampling had no or minimal effect on the general association between IL-18 and HOMA-IR.

In our study, the source of IL-18 remains obscure, since only plasma concentrations were measured. However, we agree with the point of view [30] that—at least in relation to insulin-resistance—adipose tissue may be a major source of circulating IL-18. Recently, our laboratory has shown that adipose tissue expresses IL-18 mRNA, with elevated levels in HIV-associated lipodystrophy [39]. Though we in the present study find that obesity is poorly associated with elevated plasma IL-18, an increased production of IL-18 within adipose tissue due to infiltration of macrophages [40] is possible. The mechanism by which IL-18 is linked to insulin-resistance include (1) a direct effect of IL-18 on insulin signaling, (2) a secondary response to insulin-resistance, or (3) an indirect effect of IL-18 on insulin signaling via induction of TNF- α in tissue. We support the latter theory, since IL-18 can induce TNF- α , which is capable of stimulating the suppressor of cytokine signaling, SOCS-3 [41], and inhibiting the insulin receptor substrate 1 (IRS-1) tyrosine phosphorylation [42]. Of note, TNF- α in the circulation not closely correlated the expression of TNF- α in adipose tissue expression, especially in non-obese subjects [9].

Importantly, plasma IL-18 concentration may be modulated: plasma IL-18 is acutely increased by hyperglycemia [43], while intervention with diet alone [30] or in combination with physical exercise [37] leading to a moderate weight loss decreases plasma IL-18 by 25–50%. This suggests that plasma IL-18 is highly sensitive to simple lifestyle changes. In the present study, we show that a 50% decrease of the plasma IL-18 concentration was associated with a ~25% decrease in HOMA-IR in both type 2 diabetic patients and non-diabetic controls. Taken together, these findings suggest that specific modulation of

IL-18 may exert dramatic effects on insulin sensitivity. Of note, both IL-18 [44] and specific antibodies targeting IL-18 [45] are presently being tested for clinical use. The IL-18 binding protein provides another potential target of IL-18 modulation [46].

In conclusion, we have observed a clear association between plasma IL-18 and insulin-resistance in patients with type 2 diabetes as well as in non-diabetic, age-matched controls. This association was independent of obesity, age, and gender. In contrast, we observed only weak associations between plasma IL-18 and indices of dyslipidemia in either group.

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