Plasma YKL-40
A BMI-Independent Marker of Type 2 Diabetes
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OBJECTIVE—YKL-40 is produced by macrophages, and plasma YKL-40 is elevated in patients with diseases characterized by inflammation. In the present study, YKL-40 was examined in relation to obesity, inflammation, and type 2 diabetes.

RESEARCH DESIGN AND METHODS—Plasma YKL-40 and adipose tissue YKL-40 mRNA levels were investigated in 199 subjects who were divided into four groups depending on the presence or absence of type 2 diabetes and obesity. In addition, plasma YKL-40 was examined in healthy subjects during a hyperglycemic clamp, in which the plasma glucose level was kept at 15 mmol/L for 3 h, and during a hyperinsulinemic-euglycemic clamp.

RESULTS—Patients with type 2 diabetes had higher plasma YKL-40 (76.7 vs. 45.1 ng/mL, P = 0.0001) but not higher expression in adipose tissue YKL-40 mRNA (1.20 vs. 0.98, P = 0.2) compared with subjects with a normal glucose tolerance. Within the groups with normal glucose tolerance and type 2 diabetes, obesity subgroups showed no difference with respect to either plasma YKL-40 or adipose tissue YKL-40 mRNA levels. Multivariate regression analysis showed that plasma YKL-40 was associated with fasting plasma glucose (β = 0.5, P = 0.0014) and plasma interleukin (IL)-6 (β = 0.2, P = 0.0303). Plasma YKL-40 was not related to parameters of obesity. There were no changes in plasma YKL-40 in healthy subjects during either hyperglycemic or hyperinsulinemic-euglycemic clamps.

CONCLUSIONS—Plasma YKL-40 was identified as an obesity-independent marker of type 2 diabetes related to fasting plasma glucose and plasma IL-6 levels. Diabetes 57:3078–3082, 2008

YKL-40 (chitinase-3-like-1 [CHI3L1], human cartilage glycoprotein-39), is a heparin-, chitin-, and collagen-binding lectin produced by immunologically active cells such as macrophages (1) and neutrophils (2). YKL-40 is a member of the mammalian chitinase-like proteins and is a phylogenetically highly conserved serum protein (1,3–5). Other cells shown to produce YKL-40 are vascular smooth muscle and endothelial cells (6–8), arthritic chondrocytes (3), cancer cells (9), and embryonic and fetal cells (10). The exact functions of YKL-40 are unknown. Currently, YKL-40 is known to stimulate growth of fibroblast cells (11), activate the AKT and phosphoinositide-3 kinase signaling pathway, exert antiapoptosis (12), and function in angiogenesis (7) and may take part in the innate immune response (13). High plasma concentrations of YKL-40 are found in patients with diseases characterized by inflammation or increased tissue remodeling or with cancer (1,9).

Adipose tissue is recognized as a source of inflammation (14–16). A high BMI is associated with increased levels of proinflammatory cytokines, and obesity is characterized as a state of chronic systemic low-grade inflammation (17). Studies demonstrate an accumulation of activated macrophages and other immune active cells in adipose tissue from obese subjects (17,18) as possible sources of inflammatory cytokines, determining a link between obesity, low-grade inflammation, and insulin resistance, and both obesity and low-grade inflammation have been linked with the development of insulin resistance and type 2 diabetes (19).

One previous study (20) has shown an elevation of serum YKL-40 in type 2 diabetes. In the present study, using plasma and adipose tissue biopsy material from 103 healthy control subjects and 96 patients with type 2 diabetes with a wide range of BMI, we studied the possible relationship between plasma YKL-40 and adipose tissue expression of YKL-40 on the one hand and obesity, insulin resistance, and inflammation on the other.

We further measured the macrophage marker CD68 in adipose tissue. We hypothesized that macrophages in the adipose tissue might secrete YKL-40 and that plasma YKL-40 would represent macrophage infiltration in adipose tissue and serve as a marker of insulin resistance. In order to obtain further information about the regulation of systemic YKL-40, we examined plasma YKL-40 during hyperglycemic and hyperinsulinemic-euglycemic conditions.

RESEARCH DESIGN AND METHODS

Cohort study. Using a cross-sectional, case-control design, the participants in this study were divided into four distinct groups according to BMI (<30 or ≥30 kg/m2) and according to normal glucose tolerance and the diagnosis of type 2 diabetes. To verify correct diagnosis, an oral glucose tolerance test was performed and the World Health Organization diagnostic criteria for diabetes were used. Participants were carefully screened, and exclusion criteria were treatment with insulin, recent or ongoing infection, history of malignant disease, or treatment with anti-inflammatory drugs. Subjects and protocol have been previously described (21,22). Participants (n = 199) were given both oral and written information about the experimental procedures before giving their written informed consent.

Subjects. Participants reported to the laboratory between 8 and 10 A.M. after an overnight fast. Medication was paused for 24 h and oral antidiabetes medication for 1 week before the examination day. A general health examination was performed; blood samples were drawn from an antecubital vein, adipose tissue biopsy was obtained, an oral glucose tolerance test and a...
RESULTS

Subject characteristics. The cohort has previously been described (21). Characteristics of the four main groups included in the present study are shown in Table 1. Plasma YKL-40 within these four groups is shown in Fig. 1A.

Table 1

| Normal glucose tolerance | Type 2 diabetes |
|--------------------------|-----------------|
|                          | Nonobese | Obese | Nonobese | Obese |
| n (male/female)          | 62 (42/20) | 41 (28/13) | 50 (38/12) | 46 (34/12) |
| Age (years)              | 56 ± 2 | 48 ± 2 | 58 ± 2 | 58 ± 1 |
| BMI (kg/m²)              | 25.7 ± 0.4 | 36.7 ± 0.7 | 26.6 ± 0.3 | 35.5 ± 0.7 |
| HOMA2                    | 0.66 (0.60–0.70) | 1.28 (1.09–1.38) | 1.22 (1.01–1.32) | 2.27 (1.97–2.42) |

Data are means ± SE for continuous variables and geometric means (limits for SE of geometric means), unless otherwise indicated. General characteristics of the study population divided into four groups on the basis of obesity and diagnosis of type 2 diabetes. Normal glucose tolerance/nonobese, normal glucose tolerance/obese, type 2 diabetes/nonobese, and type 2 diabetes/obese. Difference between obesity groups within each glycemia group, *P < 0.01; ‡P < 0.001. Difference between glycemia group (normal glucose tolerance versus type 2 diabetes), $P < 0.001. For age and BMI, there was an interaction between glycemia group and obesity.

FIG. 1. A: Plasma concentrations of YKL-40 in the four groups (n = 196): normal glucose tolerance (NGT)/nonobese, NGT/obese, type 2 diabetes (T2DM)/nonobese, and T2DM/obese. B: YKL-40 mRNA/GAPDH mRNA expression level in adipose tissue in the four groups (n = 159). C: CD68 mRNA/GAPDH mRNA expression level in adipose tissue in the four groups (n = 154). Data are presented as geometric means ± SE. Difference between glycemia group (NGT vs. T2DM), *P < 0.001.
Plasma YKL-40 was increased in type 2 diabetic patients compared with subjects with normal glucose tolerance, independently of obesity ($P < 0.0001$). The expression of YKL-40 mRNA and CD68 mRNA in adipose tissue was not different with regard to either glycemia group or obesity (Fig. 1B and C). No interaction between obesity and diabetes were found in plasma or mRNA analyses.

**YKL-40 and type 2 diabetes**

**Plasma YKL-40.** Univariate and multivariate regression analyses with parameters of obesity, type 2 diabetes, and inflammation as the explanatory variables and plasma YKL-40 as the dependent variable, stratified or not according to normal glucose tolerance/type 2 diabetes, are shown in Table 2. In the multivariate analysis, we adjusted for age, sex, fitness level, and either plasma TNF-α or fasting plasma glucose since these parameters were highly associated with YKL-40 in the univariate analysis. No interactions were found between glycemia group and the explanatory variables, indicating that the slopes between YKL-40 and the explanatory variables did not differ between subgroups with normal glucose tolerance and those with type 2 diabetes. Therefore, here we focus on the nonstratified analyses.

In univariate analysis, plasma YKL-40 was positively associated with fasting plasma glucose (Fig. 2A), fasting plasma insulin, HOMA2, A1C, plasma IL-6, and plasma TNF-α. After adjusting for age, sex, fitness level, and either TNF-α or fasting plasma glucose, plasma YKL-40 was positively associated with fasting plasma glucose ($P = 0.0014$) and plasma IL-6 ($P = 0.0303$). There was a tendency toward a positive association between plasma YKL-40 and HOMA2 ($P = 0.0545$). No association with parameters of obesity was found. Age and fitness level showed separate associations with plasma YKL-40 ($\beta = 0.01, P = 0.0001$ and $\beta = -1.0, P = 0.0001$, respectively). No interactions between the various covariates and these confounders were found.

**YKL-40 mRNA.** Univariate and multivariate regression analyses between YKL-40 mRNA in adipose tissue and explanatory variables stratified or not into groups with normal glucose tolerance and groups with type 2 diabetes are presented in Table 3. As for plasma YKL-40, no interaction was found between YKL-40 mRNA and explanatory variables. In the nonstratified univariate analysis, there was an association between adipose tissue YKL-40 mRNA and plasma YKL-40 ($P = 0.0134$), but this was not present after adjustments. There was no association between adipose tissue CD68 mRNA and plasma YKL-40 ($P = 0.75$).

Adipose tissue YKL-40 mRNA showed positive associations with fasting plasma glucose, fasting plasma insulin, HOMA2, and A1C and a tendency with plasma C-reactive protein ($P = 0.0755$). In multiple regression analysis, positive associations were found with fasting plasma insulin ($P = 0.0018$) and HOMA2 ($P = 0.0011$). There was a tendency to a positive association between YKL-40 mRNA and A1C ($P = 0.0645$). No association was found between adipose tissue YKL-40 mRNA expression and parameters of inflammation or parameters of obesity.

**YKL-40 during clamp.** In healthy subjects, 3 h of hyperglycemic clamp conditions or hyperinsulinemic-euglycemic clamp conditions did not change plasma YKL-40 (Fig. 2B).
that adipose tissue contributes to circulating YKL-40. Consequently, adipose tissue YKL-40 mRNA and plasma YKL-40 support the idea that IL-6 is the most important regulator of YKL-40.

Type 2 diabetes may not be characterized by low-grade inflammation, IL-6 may not be the key cytokine in the pathogenesis of type 2 diabetes. In diabetes, plasma IL-6, as found in infections, can exert acute effects by increasing the production of acute phase proteins (e.g., haptoglobin and ceruloplasmin), thereby causing acute changes in plasma YKL-40, whereas in type 2 diabetes, plasma IL-6, as found in infections, can exert acute effects by increasing the production of acute phase proteins (e.g., haptoglobin and ceruloplasmin), thereby causing acute changes in plasma YKL-40. It is possible that acute changes in plasma YKL-40 but no association between YKL-40 and markers of inflammation were found. However, as is evident from Table 2A, plasma YKL-40 is not related to obesity. Ongoing studies in our laboratory show that acute elevation of plasma IL-6 (by intravenous infusion) increase plasma YKL-40 in humans. At first glance, it seems paradoxical that plasma YKL-40 is not related to obesity. However, as is evident from Table 2A, plasma IL-6 can only explain 3% of the variation in plasma YKL-40, indicating that other factors may be more important for the production/release of YKL-40. It is possible that acute changes in plasma IL-6, as found in infections, can exert acute changes in plasma YKL-40, whereas in type 2 diabetes, characterized by low-grade inflammation, IL-6 may not be the most important regulator of YKL-40.

FIG. 2. A: Association between fasting plasma glucose and plasma YKL-40 levels in subjects with normal glucose tolerance (n = 101) (open circles). Logarithmic data are presented. R² = 0.11. B: Changes in plasma YKL-40 in healthy subjects during two different clamp conditions. A, plasma YKL-40 during hyperinsulinemic-euglycemic clamp (n = 7). Data are presented as means ± SE.

DISCUSSION

In the present study, we demonstrated that patients with type 2 diabetes have elevated plasma YKL-40 compared with healthy control subjects. In multivariate regression analysis adjusted for age, sex, fitness, and either plasma TNF-α or fasting plasma glucose, we found significant associations between plasma YKL-40 and fasting plasma glucose and plasma IL-6 but no associations with parameters of obesity.

Plasma IL-6 and obesity (BMI) showed a strong positive association (R² = 0.2, β = 7.9, P = 0.0001). In addition, we found a relationship between plasma IL-6 and plasma YKL-40 but no association between YKL-40 and markers of obesity. Ongoing studies in our laboratory show that acute elevation of plasma IL-6 (by intravenous infusion) increase plasma YKL-40 in humans. At first glance, it seems paradoxical that plasma YKL-40 is not related to obesity. However, as is evident from Table 2A, plasma IL-6 can only explain 3% of the variation in plasma YKL-40, indicating that other factors may be more important for the production/release of YKL-40. It is possible that acute changes in plasma IL-6, as found in infections, can exert acute changes in plasma YKL-40, whereas in type 2 diabetes, characterized by low-grade inflammation, IL-6 may not be the most important regulator of YKL-40.

The positive correlation between subcutaneous adipose tissue YKL-40 mRNA and plasma YKL-40 support the idea that adipose tissue contributes to circulating YKL-40. Given that YKL-40 is produced by macrophages, it is possible that adipose tissue YKL-40 mRNA and plasma YKL-40 support the idea that IL-6 is the most important regulator of YKL-40.
surprising that CD68 mRNA expression in adipose tissue did not correlate with plasma YKL-40. However, YKL-40 may only be produced by a subgroup of macrophages (CD14+ and CD16+) in adipose tissue, as seen in other diseases (1), in contrast with CD68, which is produced by all monocytes and macrophages. Studying adipose tissue biopsies, we were not able to distinguish between the roles of macrophages and, for example, endothelial cells or smooth muscle cells with regard to the production of YKL-40. It cannot be excluded that adipose tissue is a source of YKL-40 production, though the production of YKL-40 may be attributed to different cells. Furthermore, visceral adipose tissue is more inflamed than subcutaneous adipose tissue and is a possible source of plasma YKL-40, but our study design did not allow for the illumination of the role of visceral fat.

The precise role of YKL-40 remains elusive, but our findings suggest that YKL-40 might be involved in metabolism. However, here we demonstrate that YKL-40 levels do not fluctuate with acute changes in plasma glucose or plasma insulin. Studies are needed to determine the role of adipocytes, macrophages, smooth muscle, and endothelial cells as sources of YKL-40 in adipose tissue and to clarify whether YKL-40 is directly involved in the pathophysiology of type 2 diabetes or may be a more general marker of inflammation. In conclusion, we identify plasma YKL-40 as an obesity-independent marker of type 2 diabetes that is positively associated with fasting plasma glucose and plasma IL-6 level.

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