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# Increased Circulating and Visceral Adipose Tissue Expression Levels of YKL-40 in Obesity-Associated Type 2 Diabetes Are Related to Inflammation: Impact of Conventional Weight Loss and Gastric Bypass

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**Context:** Plasma YKL-40 is elevated in patients with type 2 diabetes. The potential role of visceral adipose tissue (VAT) as a significant source of YKL-40 is unknown.

**Objective:** In the study circulating and expression levels of YKL-40 were examined in VAT analyzing the contribution of adipocytes and stromovascular fraction cells (SVFCs). We also explored YKL-40's implication in insulin resistance and inflammation and the effect of weight loss on plasma YKL-40 concentrations.

**Patients and Methods:** Samples obtained from 53 subjects were used in the study. Gene and protein expression levels of YKL-40 were analyzed in VAT as well as in both adipocytes and SVFCs. In addition, circulating YKL-40 concentrations were measured before and after weight loss achieved either by Roux-en-Y gastric bypass (n = 26) or after a conventional dietetic program (n = 20).

**Results:** Circulating concentrations and VAT expression of YKL-40 were increased in obese patients with type 2 diabetes ($P < 0.01$) as well as associated with variables of insulin resistance and inflammation. No differences in YKL-40 expression levels between adipocytes and SVFCs were detected. Monocyte chemoattractant protein-1 and homeostasis model assessment emerged ($P < 0.01$) as independent factors predicting circulating YKL-40. Elevated levels of YKL-40 in obese patients decreased after weight loss following a conventional hypocaloric diet ($P < 0.05$) but not via a surgery-induced negative energy balance mediated by the Roux-en-Y gastric bypass.

**Conclusions:** The association of increased YKL-40 mRNA and protein levels in VAT with its circulating concentrations indicates an important contribution of VAT in YKL-40 regulation. Furthermore, our data suggest a relevant role of glucose metabolism and inflammation on YKL-40 regulation. (*J Clin Endocrinol Metab* **96:** 200–209, 2011)

Abbreviations: BF, Body fat; BMI, body mass index; CRP, C-reactive protein; GFR, glomerular filtration rate; HDL, high-density lipoprotein; HGF, hepatocyte growth factor; HOMA, homeostatic model assessment; LDL, low-density lipoprotein; MCP, monocyte chemoattractant protein; NG, normoglycemic; QUICKI, quantitative insulin sensitivity check index; RYGB, Roux-en-Y gastric bypass; SAT, sc adipose tissue; SVFC, stromovascular fraction cell; T2D, type 2 diabetes; VAT, visceral adipose tissue; vWF, von Willebrand factor; WHR, waist-to-hip ratio.

Abdominal obesity is a well-established metabolic risk factor for the development of insulin resistance and type 2 diabetes (T2D). Compelling evidence points to excess visceral adipose tissue (VAT) accumulation dysregulating the adipokine secretion profile, which results in adverse effects on glucose and lipid metabolism as well as inflammation, with the latter representing a key etiological factor in linking increased adiposity with T2D development (1, 2).

YKL-40, also known as cartilage glycoprotein-39 or chitinase-3-like-1 is a 40-kDa heparin- and chitin-binding lectin member of the mammalian chitinase-like protein family without glycolytic properties (3). YKL-40 is expressed and secreted by several cell types of the innate immune system and by differentiated vascular endothelial and smooth muscle cells (4). A substantial body of evidence indicates that YKL-40 acts not only as an inflammatory marker in relation to both acute and chronic inflammation but also as a growth factor with involvement in extracellular matrix remodeling and angiogenesis (5). In this regard, YKL-40 was found to be associated with various inflammatory conditions such as rheumatoid diseases and cancer (6) and also in early stages of atherosclerosis (5). Noteworthy, YKL-40 is a growth factor with functions in cancer and cell proliferation (4, 7). In this sense, our group has shown increased *YKL40* gene expression levels in VAT of patients with colon cancer (8). In addition, the participation of YKL-40 in vascular processes implies that YKL-40 may play a role in endothelial dysfunction and atherosclerosis (7). A strong association of YKL-40 with the inflammatory cytokines IL-6 and monocyte chemoattractant protein-1 (MCP-1) has been also described (9, 10). Previous studies have reported that patients with T2D exhibit elevated plasma levels of YKL-40 compared with healthy control subjects independently of obesity and a positive association with fasting glucose, insulin, and the homeostatic model assessment (HOMA) index has been also detected (9–11). In this sense, circulating levels of YKL-40 are also reportedly elevated in patients with type 1 diabetes (12).

Subcutaneous adipose tissue (SAT) reportedly exhibits a positive correlation of YKL-40 gene expression levels with its circulating concentrations (9). A large body of evidence shows that increased VAT is associated with a higher risk of developing obesity-related comorbidities than elevated SAT (13, 14). Nonetheless, YKL-40 gene and protein expression levels have not been analyzed before in VAT. The aim of the present study was to determine gene and protein expression levels of YKL-40 in VAT together with plasma concentrations in healthy control subjects, obese normoglycemic (NG) individuals and obese patients with T2D. We also examined the relation of YKL-40 with key genes and circulating proteins involved in inflammation. Elevated YKL-40 concentrations in morbid obesity have been previously shown to decrease after bariatric surgery-induced weight loss (10). The present study assessed and compared the effect of weight loss achieved either by Roux-en-Y gastric bypass (RYGB) or a conventional dietetic program on plasma YKL-40 levels.

## Patients and Methods

For detailed Research Design and Methods, see Supplemental Patients and Methods, published on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org.

### Patient selection

To analyze the effect of obesity and T2D on YKL-40 concentrations, 53 subjects were recruited from healthy volunteers and patients attending the Departments of Endocrinology and Surgery at the Clínica Universidad de Navarra. Furthermore, gene and protein expression levels of YKL-40 and CD68 in VAT were assessed in a subgroup of subjects (n = 29). In addition, a group of 46 obese female patients was selected to investigate the effect of weight loss on circulating concentrations of YKL-40. The weight loss was achieved by either RYGB [n = 26, evaluated after both a short (14 wk) and long term (13 months) period] or by prescription of a diet (n = 20, evaluated after 14 wk) providing a daily energy deficit of 500-1000 kcal/d as calculated from the determination of the resting energy expenditure through indirect calorimetry (Vmax29; SensorMedics Corp., Yorba Linda, CA) and multiplication by the physical activity level factor to obtain the individual's total energy expenditure. Biochemical assays of subjects included in the study as well as transcript and protein levels for YKL-40 were measured as previously described (15).

### Statistical analysis

Data are presented as mean ± SEM. Differences between groups were assessed by one-way ANOVA followed by Bonferroni's *post hoc* tests, two-tailed unpaired Student's *t* tests, and two-tailed paired *t* tests as appropriate. Pearson's correlation coefficients (r) were used to analyze the association between variables. The calculations were performed using the SPSS/Windows version 15.0 statistical package (SPSS, Chicago, IL). $P < 0.05$ was considered statistically significant.

## Results

### YKL-40 levels are elevated in human obesity-associated 2TD

The biochemical and hormonal characteristics of the subjects included in the study are shown in Table 1. Significant differences in circulating YKL-40 concentrations between the three experimental groups were observed (P = 0.002). Plasma YKL-40 levels were significantly increased in obese patients taken as a whole (18.9 ± 5.3 *vs.* 31.9 ± 3.7 pg/ml; P = 0.004) compared with lean subjects.

**TABLE 1.** Anthropometric and biochemical characteristics of subjects included in the study

|  | Lean | Obese NG | Obese T2D |
|---|---|---|---|
| n | 16 | 20 | 17 |
| Age (yr) | 32 ± 4 | 34 ± 2 | 49 ± 3[a] |
| BMI (kg/m$^2$) | 20.1 ± 0.7 | 44.0 ± 1.3[b] | 47.0 ± 2.4[b] |
| Body fat (%) | 25.5 ± 1.8 | 53.8 ± 0.9[b] | 54.1 ± 1.2[b] |
| Waist (cm) | 66.7 ± 1.8 | 112.8 ± 3.6[b] | 130.5 ± 4.7[b,c] |
| WHR | 0.75 ± 0.01 | 0.86 ± 0.02[a] | 0.90 ± 0.02[b] |
| Fasting glucose (mg/dl) | 84.8 ± 5.0 | 90.7 ± 3.2 | 131.2 ± 12.9[b,d] |
| 2-h OGTT glucose (mg/dl) | 101.0 ± 14.1 | 120.0 ± 8.4 | 238.1 ± 37.5[b,d] |
| Fasting insulin (μU/ml) | 6.4 ± 1.2 | 12.8 ± 1.7 | 28.5 ± 4.1[b,d] |
| 2-h OGTT insulin (μU/ml) | 8.5 ± 1.9 | 87.5 ± 13.9 | 132.6 ± 33.5[b,d] |
| HOMA | 1.4 ± 0.3 | 2.9 ± 0.4 | 9.8 ± 2.4[b,d] |
| QUICKI | 0.38 ± 0.02 | 0.33 ± 0.01[b] | 0.29 ± 0.01[b,d] |
| Triglycerides (mg/dl) | 69 ± 10 | 98 ± 8 | 130 ± 14[a] |
| Cholesterol (mg/dl) | 176 ± 10 | 194 ± 10 | 202 ± 11 |
| LDL cholesterol (mg/dl) | 102 ± 11 | 130 ± 9 | 129 ± 12 |
| HDL cholesterol (mg/dl) | 61 ± 4 | 44 ± 2[b] | 48 ± 5[a] |
| Leptin (ng/ml) | 8.3 ± 1.6 | 64.7 ± 7.0[b] | 67.3 ± 7.0[b] |
| Adiponectin (μg/ml) | 21.4 ± 5.4 | 8.1 ± 1.2[a] | 10.0 ± 0.6[a] |
| Uric acid (mg/dl) | 4.0 ± 0.2 | 5.1 ± 0.3 | 5.9 ± 0.4[b] |
| Plasma creatinine (mg/dl) | 0.76 ± 0.02 | 0.71 ± 0.02 | 0.78 ± 0.04 |
| Urinary creatinine (mg/dl) | — | 130.6 ± 10.0 | 146.4 ± 21.8 |
| GFR | 99.2 ± 3.4 | 101.9 ± 4.3 | 88.1 ± 7.4 |
| CRP (mg/liter) | 1.1 ± 0.3 | 8.4 ± 1.3[b] | 13.3 ± 3.9[b] |
| Fibrinogen (mg/dl) | 208 ± 24 | 363 ± 27[b] | 385 ± 25[b] |
| vWF (%) | 65 ± 10 | 97 ± 12 | 125 ± 17[a] |
| Homocysteine (μmol/liter) | 6.8 ± 0.9 | 6.8 ± 0.5 | 8.3 ± 0.6 |
| SAA (μg/ml) | 16.1 ± 4.8 | 54.4 ± 9.1[a] | 33.0 ± 10.4 |
| IL-6 (pg/ml) | 1.6 ± 0.1 | 2.5 ± 0.2[b] | 2.8 ± 0.3[b] |
| IL-8 (pg/ml) | 1.2 ± 0.8 | 1.1 ± 0.1 | 1.2 ± 1.0 |
| TNF-α (pg/ml) | 2.05 ± 0.06 | 2.40 ± 0.16 | 2.67 ± 0.13[b] |
| MCP-1 (pg/ml) | 81.2 ± 10.7 | 101.1 ± 10.9 | 102.1 ± 15.9 |
| HGF (pg/ml) | 235.3 ± 45.8 | 293.1 ± 49.0 | 478.9 ± 65.4[b,c] |
| NGF (pg/ml) | 4.71 ± 0.99 | 10.86 ± 3.10 | 13.38 ± 3.72 |
| ALT (UI/liter) | 7 ± 1 | 20 ± 3 | 33 ± 6[b,c] |
| AST (UI/liter) | 11 ± 1 | 13 ± 1 | 21 ± 5 |
| ALP (UI/liter) | 88 ± 9 | 109 ± 8 | 122 ± 10 |
| γ-GT (UI/liter) | 9 ± 1 | 18 ± 2 | 33 ± 6[b,c] |

Data are mean ± SEM. Differences between groups were analyzed by one-way ANOVA followed by Bonferroni's *post hoc* tests. CRP and SAA levels were logarithmically transformed for statistical analysis due to their non-normal distribution. OGTT, Oral glucose tolerance test; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; γ-GT, γ-glutamyltransferase; SAA, serum amyloid A.

[a] $P < 0.05$ *vs.* lean.

[b] $P < 0.01$ *vs.* lean.

[c] $P < 0.05$ *vs.* obese NG.

[d] $P < 0.01$ *vs.* obese NG.

Furthermore, YKL-40 levels were higher in obese patients with T2D compared with both lean ($P = 0.003$) and obese NG volunteers ($P = 0.013$) (Fig. 1A). Visceral obesity is reflected by an elevated waist-to-hip ratio (WHR). To investigate the relation between circulating YKL-40 concentrations and visceral adiposity, the whole study population was divided into three categories by tertiles of WHR. Patients in the top WHR tertile exhibited significantly higher circulating YKL-40 concentrations compared with patients included in the bottom tertile ($36.9 ± 6.5$ *vs.* $20.7 ± 1.7$ pg/ml; $P = 0.043$). Plasma YKL-40 levels were positively associated ($P < 0.01$) with fasting glucose and

insulin concentrations as well as with the HOMA index and negatively correlated with the quantitative insulin sensitivity check index (QUICKI) (Table 2). Glycosylated hemoglobin levels corresponding to the group of obese T2D patients were $8.3 ± 2.4\%$ (n = 8). No association between circulating concentrations of glycosylated hemoglobin and YKL-40 were detected ($r = 0.31$; $P = 0.503$).

No differences in the circulating and urinary levels of creatinine were detected between groups. Although the glomerular filtration rate (GFR) was lower in patients with T2D, the differences fell out of statistical significance. YKL-40 concentrations were positively associated with
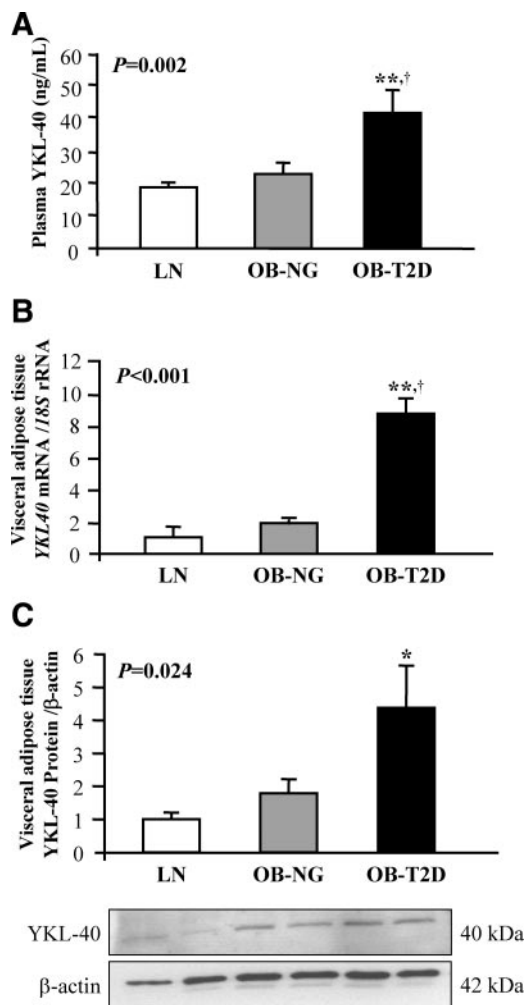
**FIG. 1.** Circulating (A), gene (B), and protein (C) expression of YKL-40 in VAT of lean (LN), obese normoglycemic (OB-NG), and obese diabetic (OB-T2D) volunteers. A, *Bars* represent the mean ± SEM of YKL-40 plasma concentrations (LN: n = 16; OB-NG: n = 20; OB-T2D: n = 17). B, Real-time PCR analysis of *YKL-40*. The mRNA *bars* represent the mean ± SEM of the ratio between the gene expression and *18S* rRNA. The expression level in the LN subjects was assumed to be 1 (LN: n = 9; OB-NG: n = 10; OB-T2D: n = 10). C, Western blot analysis of YKL-40 levels. *Bars* represent the mean ± SEM of the ratio between YKL-40 and *β*-actin. The expression in LN subjects was assumed to be 1 (LN: n = 9; OB-NG: n = 10; OB-T2D: n = 10). Differences between groups were analyzed by one-way ANOVA followed by Bonferroni's tests. *, $P < 0.05$, **, $P < 0.01$ *vs.* LN, and †, $P < 0.05$ *vs.* OB-NG.

plasma creatinine ($P = 0.027$), whereas a negative association with urinary creatinine ($P = 0.018$) as well as with GFR ($P = 0.032$) was observed.

In light of the divergent pathological consequences of adipose tissue distribution, we evaluated the effects of obesity and T2D on gene expression levels of *YKL40* in VAT and SAT. mRNA levels of *YKL40* in VAT were significantly increased in obese T2D patients compared with not only the lean group ($P = 0.009$) but also the obese NG subjects ($P = 0.015$) (Fig. 1B). Gene expression levels of *YKL40* in SAT followed the same trend, being higher in T2D patients. However, no statistically significant differ-

ences were reached ($P = 0.065$) (Supplemental Fig. 1A). Moreover, YKL40 exhibited highest gene expression in VAT compared with that of SAT (Supplemental Fig. 1B). Based on the fact that *YKL40* exhibited the highest gene expression in VAT and given the relevance of this depot in obesity-associated metabolic disturbances, the experiments were focused on YKL40 in this location.

Because the obese T2D group was significantly older, a group of 10 obese NG volunteers with a similar age to that of the obese patients with T2D has been included to investigate the effect of age on the gene expression levels of *YKL40* (Supplemental Table 2). Noteworthy, mRNA levels of *YKL40* in VAT were significantly increased ($P < 0.05$) in the obese T2D patients compared with both groups of obese NG patients (Supplemental Fig. 2). Furthermore, no differences in YKL40 expression levels were detected between both obese NG groups (the former one used who was significantly younger and the older one age matched to the T2D individuals). This finding provides evidence that the increased *YKL40* expression levels are independent of age.

YKL-40 protein levels in VAT followed the same trend than *YKL40* expression levels, being significantly higher in obese T2D patients compared with lean controls ($P = 0.032$) and with obese NG patients. However, no statistically significant differences were reached in the latter comparison ($P = 0.090$) (Fig. 1C). *YKL40* mRNA expression in VAT was positively correlated with both protein expression levels ($P = 0.039$) and circulating concentrations ($P = 0.021$). Analogously to the significant associations observed with YKL-40 circulating concentrations, a strong positive correlation ($P < 0.01$) between gene and protein expression levels with fasting insulin concentrations was found, whereas a negative association with the QUICKI index was evident (Table 2).

## YKL-40 gene expression levels in adipocytes and stromovascular fraction cells (SVFCs)

The presence of YKL-40 in sections of VAT was confirmed by immunohistological analysis (Fig. 2A). Both adipocytes and SVFCs were immunopositive for YKL-40. Because macrophages represent a relevant cell type source in proinflammatory cytokine production and release, the macrophage-specific marker CD68 was used to evaluate the impact of resident macrophages in adipose tissue. As expected, CD68 staining in VAT was localized mainly in macrophages. YKL-40 labeling was detected in CD68-positive cells, but noteworthy a marked staining in mature adipocytes was also observed. As expected, the mRNA of *CD68* was up-regulated in VAT of obese NG compared with LN individuals ($1.00 \pm 0.35$ *vs.* $3.01 \pm 0.54$ arbitrary units; $P = 0.010$) and further increased in obese T2D pa-

**TABLE 2.** Univariate analysis of the correlations between YKL-40 circulating concentrations and its VAT mRNA and protein expression levels with variables of glucose homeostasis and inflammatory markers

| | Plasma YKL-40 | | VAT *YKL-40* mRNA | | VAT YKL-40 protein | |
|---|---|---|---|---|---|---|
| | r | *P* | r | *P* | r | *P* |
| Plasma YKL-40 | — | — | 0.51 | **0.021** | 0.15 | 0.642 |
| VAT *YKL-40* mRNA | 0.51 | **0.021** | — | — | 0.50 | **0.039** |
| VAT YKL-40 protein | 0.15 | 0.642 | 0.50 | **0.039** | — | — |
| Age | 0.44 | **0.002** | 0.02 | 0.994 | 0.16 | 0.551 |
| BMI | 0.20 | 0.184 | 0.39 | 0.059 | 0.18 | 0.493 |
| BF | 0.22 | 0.140 | 0.23 | 0.327 | 0.28 | 0.306 |
| Waist | 0.44 | **0.038** | 0.79 | **0.035** | 0.94 | 0.053 |
| WHR | 0.48 | **0.020** | 0.07 | 0.076 | 0.10 | 0.122 |
| Glucose | 0.53 | **<0.001** | 0.35 | 0.108 | 0.40 | 0.128 |
| Insulin | 0.46 | **0.005** | 0.62 | **0.004** | 0.67 | **0.008** |
| HOMA | 0.59 | **<0.001** | 0.45 | 0.056 | 0.43 | 0.127 |
| QUICKI | −0.47 | **0.006** | −0.63 | **0.004** | −0.52 | 0.054 |
| Plasma creatinine | 0.40 | **0.027** | 0.44 | 0.105 | −0.22 | 0.522 |
| Urinary creatinine | −0.45 | **0.018** | 0.20 | 0.774 | −0.83 | **0.022** |
| GFR | −0.40 | **0.032** | −0.32 | 0.363 | 0.20 | 0.666 |
| CRP | 0.33 | 0.071 | 0.70 | **0.002** | 0.78 | **<0.001** |
| vWF | 0.41 | **0.032** | 0.42 | 0.131 | −0.30 | 0.377 |
| IL-6 | 0.32 | **0.028** | 0.63 | **0.003** | 0.73 | **0.004** |
| TNF-$\alpha$ | 0.67 | **<0.001** | 0.16 | 0.948 | 0.26 | 0.278 |
| MCP-1 | 0.56 | **<0.001** | 0.29 | 0.236 | 0.10 | 0.754 |
| HGF | 0.43 | **0.004** | 0.59 | **0.016** | 0.47 | 0.126 |
| NGF | 0.32 | **0.043** | 0.54 | **0.017** | 0.85 | **<0.001** |

Bold values highlight statistically significant *P* values.

tients (4.05 ± 0.73 arbitrary units; $P = 0.003$ *vs.* LN). A positive association between *YKL40* and *CD68* mRNA levels was also detected ($P < 0.001$).

No differences were found between the gene expression levels of *YKL40* in adipocytes and SVFCs obtained from VAT samples of obese NG and T2D individuals ($P = 0.566$) (Fig. 2B). However, a tendency toward higher mRNA expression levels was observed in adipocytes isolated from patients with T2D compared with fat cells from NG volunteers, although differences did not reach statistical significance (Fig. 2C).

**Circulating and adipose YKL-40 levels are related to the chronic low-grade obesity-associated inflammation**

The inflammatory markers C-reactive protein (CRP), fibrinogen, and IL-6 were significantly increased ($P < 0.01$) in both groups of obese patients compared with lean subjects. In addition, obese patients with T2D exhibited higher von Willebrand factor (vWF), TNF-$\alpha$, and hepatocyte growth factor (HGF) concentrations ($P < 0.01$) than lean volunteers. Circulating concentrations of YKL-40 were positively correlated ($P < 0.01$) with the inflammatory and prothrombotic markers IL-6, TNF-$\alpha$, MCP-1, vWF, HGF, and nerve growth factor (Table 2, Supplemental Table 3). Analogously, gene and protein expression levels in VAT were positively associated ($P < 0.01$) with the inflammatory markers CRP, IL-6, and

nerve growth factor. Gene expression levels of *TNFA* in VAT were up-regulated in obese patients with T2D compared with lean volunteers (1.00 ± 0.49 *vs.* 3.19 ± 0.66 arbitrary units; $P = 0.002$) and a positive correlation with mRNA levels of *YKL40* was found ($P < 0.01$).

Multivariate linear regression analysis was performed including age, HOMA index (as insulin resistance marker) as well as TNF-$\alpha$ and MCP-1 concentrations (as inflammatory markers) in the model due to their highly significant association with the dependent variable YKL-40. Age ($P = 0.019$), HOMA ($P = 0.037$), and MCP-1 ($P = 0.024$) were significant independent determinants of YKL-40, suggesting an influence of factors involved in glucose homeostasis and inflammation on circulating levels of YKL-40.

**Effect of weight loss on the circulating concentration of YKL-40**

To analyze the impact of therapeutic interventions aimed at achieving weight loss in obese patients, the effect on plasma YKL-40 concentrations induced by either bariatric surgery (RYGB) or a conventional lifestyle intervention was examined. Both the short- and long-term effects of weight loss after RYGB were evaluated. After an average 14-wk postsurgical period, RYGB patients experienced a similar decrease in body weight than obese patients following a conventional hypocaloric diet (Table 3). Surgical patients showed a significant decrease ($P < 0.001$) in body weight, body mass index (BMI), body fat
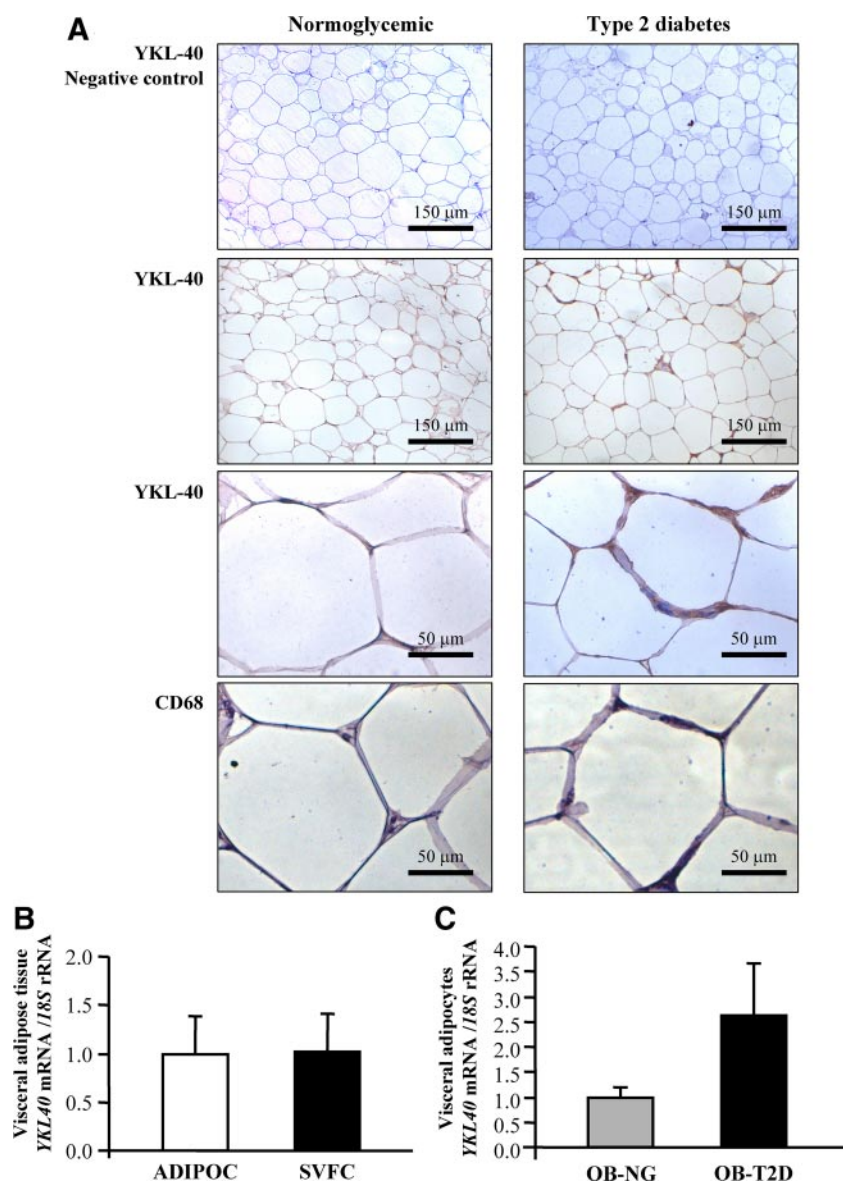
**FIG. 2.** Analysis of YKL-40 in adipocytes and SVFCs. A, Immunohistochemistry of YKL-40 and CD68 in VAT. A strong positivity (*brown staining*) was observed for YKL-40 in both fully mature adipocytes and cells of the SVFCs. No immunoreactivity was found without the primary antibody (negative control). Images are representative of immunostaining in six sections of VAT from obese normoglycemic (OB-NG) and obese diabetic (OB-T2D) subjects (*scale bar*, 50 $\mu$m). B, Comparison of YKL-40 gene expression in adipocytes (ADIPOC) and SVFCs isolated from the VAT of obese patients. *Bars* represent the mean $\pm$ SEM of the ratio between the gene expression and *18S* rRNA. The expression level in adipocytes was assumed to be 1 (adipocytes: n = 9; SVFCs: n = 11). C, Differential expression of *YKL-40* gene in visceral adipocytes obtained from obese patients with normoglycemia (NG) or T2D. *Bars* represent the mean $\pm$ SEM of the ratio between gene expression and *18S* rRNA. The expression level in OB-NG was assumed to be 1 (OB-NG: n = 8; OB-T2D: n = 6). Statistical differences were assessed by a two-tailed unpaired Student's *t* test.

BMI, BF, and waist circumference as well as a significant improvement in the initial glycemia ($P = 0.006$), insulinemia ($P = 0.047$), HOMA ($P = 0.007$), and QUICKI ($P < 0.0001$) indices (Table 3). As expected after the reduction in BF, leptin concentrations were also significantly reduced ($P = 0.012$). Triglycerides and total and low-density lipoprotein (LDL) cholesterol concentrations were significantly lower ($P < 0.0001$), whereas high-density lipoprotein (HDL) cholesterol levels were significantly increased ($P = 0.005$). Our data further show that massive surgery-induced weight loss after RYGB was not accompanied by statistically significant changes in circulating levels of YKL-40 (Fig. 3A). Noteworthy, the differences in YKL-40 concentrations were positively correlated with the reduction in insulin concentrations even after BMI adjustment ($P = 0.002$).

After an average period of 14 wk, obese patients following the conventional hypocaloric diet experimented significant decreases ($P < 0.001$) in weight, BMI, BF, waist circumference, WHR, and triglycerides as well as a significant improvement in glycemia ($P = 0.035$) and the QUICKI ($P = 0.027$) (Table 3). The inflammatory markers CRP and fibrinogen decreased, although differences did not reach statistical significance. However, the diet-induced weight loss produced a statistically significant reduction ($P = 0.040$) in circulating YKL-40 concentrations (Fig. 3B).

## Discussion

VAT secretes a wide range of signaling proteins collectively called adipokines, which are directly involved in the

(BF), and waist circumference. Furthermore, a significant improvement ($P < 0.05$) in glucose and insulin concentrations together with a decrease ($P < 0.01$) in leptin levels was detected. However, no differences in the circulating concentrations of YKL-40 were observed. After an average of 13 months after the RYGB, patients experienced a further significant decrease ($P < 0.0001$) in body weight,

pathogenesis of obesity-related derangements (1). Our results show increased mRNA and protein expression of YKL-40 in human VAT in patients with T2D. To our knowledge, this is the first study describing elevated mRNA and protein expression levels of YKL-40 in VAT in human obesity-associated T2D. Our study further provides evidence that obese patients with T2D exhibit in-

**TABLE 3.** Effects of weight loss in obese patients after RYGB or following a conventional dietetic intervention

| | RYGB | | | Conventional diet | |
|---|---|---|---|---|---|
| | Before WL | After WL (14 wk) | After WL (13 months) | Before WL | After WL (14 wk) |
| n | 26 | 26 | 26 | 20 | 20 |
| Age (yr) | 41 ± 2 | 41 ± 2 | 42 ± 2 | 41 ± 3 | 42 ± 3 |
| Body weight (kg) | 122 ± 5 | 105 ± 4[a] | 85 ± 3[a] | 108 ± 7 | 88 ± 4[a] |
| BMI (kg/m²) | 47.0 ± 1.8 | 40.4 ± 1.5[b] | 33.2 ± 1.2[a] | 36.6 ± 2.1 | 30.1 ± 1.3[a] |
| BF (%) | 54.1 ± 0.8 | 51.7 ± 1.0[b] | 42.0 ± 1.4[a] | 41.9 ± 2.0 | 33.4 ± 2.5[a] |
| Waist (cm) | 126.8 ± 2.0 | 114.4 ± 2.0[b] | 100.0 ± 3.2[b] | 114.4 ± 4.5 | 100.5 ± 3.2[b] |
| WHR | 0.89 ± 0.02 | 0.89 ± 0.03 | 0.87 ± 0.03 | 0.96 ± 0.02 | 0.93 ± 0.02[c] |
| Fasting glucose (mg/dl) | 108.6 ± 8.0 | 88.7 ± 2.0[b] | 89.7 ± 3.0[b] | 96.0 ± 2.3 | 88.0 ± 1.9[c] |
| Fasting insulin (μU/ml) | 21.0 ± 3.1 | 15.2 ± 2.6[c] | 11.2 ± 2.9[c] | 14.5 ± 3.2 | 8.6 ± 1.3 |
| HOMA | 6.0 ± 1.6 | 3.4 ± 0.7[c] | 2.7 ± 0.9[b] | 3.4 ± 0.8 | 1.9 ± 0.3 |
| QUICKI | 0.31 ± 0.01 | 0.33 ± 0.01[b] | 0.35 ± 0.01[a] | 0.33 ± 0.01 | 0.36 ± 0.01[c] |
| Triglycerides (mg/dl) | 114 ± 12 | 100 ± 20 | 86 ± 7 | 117 ± 14 | 80 ± 8[a] |
| Cholesterol (mg/dl) | 210 ± 9 | 173 ± 9 | 165 ± 6[a] | 204 ± 9 | 177 ± 7[c] |
| LDL cholesterol (mg/dl) | 142 ± 8 | 110 ± 6 | 99 ± 5[a] | 127 ± 8 | 109 ± 5 |
| HDL cholesterol (mg/dl) | 44 ± 3 | 43 ± 4 | 48 ± 2[b] | 54 ± 3 | 52 ± 3 |
| Leptin (ng/ml) | 71.0 ± 6.1 | 31.8 ± 1.4[b] | 28.9 ± 4.2[b] | 35.0 ± 6.8 | 13.4 ± 2.9 |
| CRP (mg/liter) | 9.1 ± 1.2 | — | 4.9 ± 1.6 | 9.0 ± 3.0 | 4.9 ± 3.1 |
| Fibrinogen (mg/dl) | 378 ± 20 | 380 ± 38 | 338 ± 14 | 398 ± 26 | 384 ± 84 |
| ALT (UI/liter) | 26 ± 4 | — | 23 ± 2 | 30 ± 5 | 18 ± 3[b] |
| AST (UI/liter) | 18 ± 4 | — | 17 ± 2 | 17 ± 2 | 14 ± 1[c] |
| ALP (UI/liter) | 116 ± 9 | — | 104 ± 12[c] | 99 ± 5 | 83 ± 6[c] |
| γ-GT (UI/liter) | 28 ± 5 | — | 14 ± 2 | 24 ± 3 | 15 ± 3[c] |

Data are mean ± SEM. Differences between groups were analyzed by two-tailed paired *t* tests. CRP levels were logarithmically transformed for statistical analysis due to their nonnormal distribution. ALP, Alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; γ-GT, γ-glutamyltransferase; WL, weight loss.

[a] $P < 0.0001$ *vs.* before WL.

[b] $P < 0.01$ *vs.* before WL.

[c] $P < 0.05$ *vs.* before WL.

creased circulating concentrations of YKL-40, which is in agreement with previous studies (9, 11). Different cross-sectional studies concluded that WHR is a better predictor of cardiovascular and T2D risk factors than BMI (16). In this regard, in our study patients with higher WHR showed increased levels of circulating YKL-40 levels. Moreover, the positive correlation between gene expression levels and circulating concentrations of YKL-40 suggests that VAT contributes to the increased plasma YKL-40 levels in obesity-associated T2D. Visceral adipocytes are metabolically more active displaying huge differences in gene expression levels compared with sc adipocytes. The production and release of inflammatory cytokines is reportedly increased in VAT, suggesting that an excess visceral adiposity is related to a more proinflammatory state (2). Noteworthy, it has been shown that *YKL40* gene expression is higher in visceral than sc SVFCs (17), probably due to the greater infiltration of macrophages taking place in omental fat. Moreover, high mRNA and protein expression levels of YKL-40 analyzing separately subpopulations of macrophages in different tissues obtained from diverse inflammatory processes and extracellular matrix remodeling conditions have been described (4).

In our study, the presence of YKL-40 by immunohistochemistry in human VAT is clearly shown. YKL-40 labeling was detected in CD68-positive macrophages as might be expected but, contrarily to other studies (17), a marked staining in fully mature adipocytes was also observed. Moreover, no differences in mRNA expression of *YKL40* between adipocytes and SVFCs were detected. In this regard, gene expression levels of *YKL40* from preadipocytes had been reported to be remarkably low compared with those of macrophages (17). A plausible explanation may depend on a *YKL40* gene expression increase taking place during the differentiation process from preadipocytes to mature adipocytes. Our findings suggest that both macrophages and adipocytes are involved in the secretion of YKL-40. Because mRNA levels of *YKL40* and *CD68* were positively correlated, *YKL40* expression levels may reflect the degree of VAT macrophage infiltration. To gain more insight into the source of production, we further analyzed the gene expression levels of *YKL40* in isolated adipocytes, which revealed a higher expression in patients with T2D compared with obese NG volunteers, thereby suggesting a more pronounced contribution of visceral adipocytes to the elevated concentrations of YKL-40 observed in T2D.
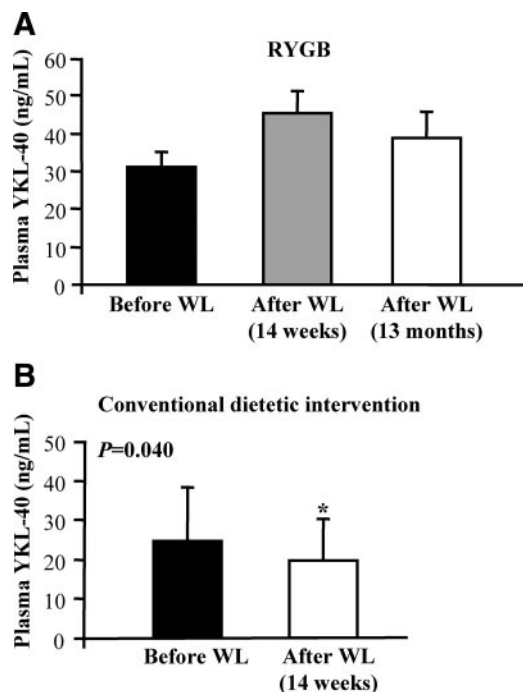
**FIG. 3.** Comparison of plasma YKL-40 concentrations determined in obese patients before and after weight loss (WL) achieved by RYGB or a conventional lifestyle intervention. Effect after RGYB (n = 26) (A) and after a conventional hypocaloric regimen (n = 20) (B) is shown. Values are means ± SEM. Statistical differences were assessed by two-tailed paired Student's t tests. *, $P < 0.05$ vs. prediet values.

In agreement with previous results (9–11), increased YKL-40 levels in patients with T2D were observed. These data together with the positive correlation of plasma YKL-40 with fasting glucose, insulin, and the HOMA index as well as the negative correlation with the QUICKI highlight a link between YKL-40 and hyperglycemia and, consequently, with the development of insulin resistance and T2D. In accordance with this, a correlation between YKL-40 and glycemic-related parameters such as hemoglobin A1c has been also shown (12). Patients with type 1 diabetes exhibit a positive association between increased YKL-40 levels and elevated albuminuria, indicating that YKL-40 may be a marker of renal vascular damage progression (12). In this sense, we found that increasing concentrations of YKL-40 were associated with a decline in GFR, pointing to a plausible implication of YKL-40 in the impaired kidney function in obese T2D patients, given the need for its renal excretion. Moreover, a positive correlation of YKL-40 with circulating creatinine concentrations together with a negative correlation with urinary creatinine levels was detected in the univariate analysis. No associations with obesity-related variables were identified in the study. In this regard, Nielsen et al. (9) did not find a correlation between YKL-40 and BMI.

Reduced YKL-40 levels in obese patients after bariatric surgery have been described indicating an association with adipose tissue and weight loss (10). In the present study,

the impact on YKL-40 levels of weight loss achieved by both RYGB and a conventional lifestyle intervention has been evaluated. Obese patients either after the surgical procedure or following a conventional hypocaloric diet experienced a similar decrease in body weight after a short-term period. Interestingly, diet-induced weight loss was followed by a reduction in YKL-40 concentrations, whereas no differences in surgically treated patients were observed. To gain more insight into the effects of RYGB, changes in YKL-40 concentrations after a long-term follow-up were also assessed. Oppositely to Hempen et al. (10), in our study, a similar surgery-induced weight loss did not induce a statistically significant reduction in circulating levels of YKL-40. The study by Hempen et al. (10) included only 17 patients with an unbalanced gender selection and the specific bariatric surgery intervention was not specified. In this sense, mean changes in risk factors reportedly vary according to the specific type of surgical procedure used (18). Noteworthy, a positive correlation between differences in YKL-40 levels and changes in insulin concentrations in RYGB was observed, which highlights the role of YKL-40 in relation with insulin resistance rather than with obesity development. Similarly, it has been reported that TNF-α concentrations remained unchanged after weight loss after bariatric surgery (19), whereas its levels decreased after weight loss induced by a hypocaloric diet (20). Plausible explanations for both the YKL-40 and TNF-α findings relate to the stressful effects caused by relative starvation or surgery itself (21). Alternatively, due to the fact that our patients remained obese and with higher BMI and BF compared with the patients on the hypocaloric diet, the potential existence of a threshold level for adiposity before any effect on the circulating concentrations of these adipokines becomes evident. In this sense, we showed that obese patients on the hypocaloric diet experienced a significant decrease in WHR, whereas patients who underwent bariatric surgery did not exhibit significant differences. Due to the fact that YKL-40 concentrations are closely correlated with WHR, the lack of a WHR reduction in patients after RYGB may be influencing the absence of statistically significant YKL-40 decrease. Nonetheless, this heterogeneous response and the differences in the inflammatory response after these two ways of weight loss still remain to be clarified. In line with this, an increase in osteopontin plasma concentrations concomitantly to weight loss after bariatric surgery and independent from the insulin sensitivity improvement and the decrease in inflammatory markers has been observed (22). In addition, the metabolic changes taking place in the gastrointestinal tract after RYGB may be influencing the lack of decrease in YKL-40 concentrations.

Because insulin resistance in obese patients is partially caused by a chronic low grade-inflammatory state, our results indicate that YKL-40 might play a role in this process. Our findings in relation to inflammation are in line with previous reports where an association with MCP-1 was detected (10). A positive association of YKL-40 with TNF-$\alpha$ and HGF, well-established markers of chronic inflammation, was observed in the present study. Moreover, *YKL40* gene expression levels were positively correlated with VAT *TNFA* mRNA. No association between YKL-40 and the acute phase reactants, CRP and serum amyloid A, was observed in the present study, implying that elevated YKL-40 levels are independent from the increased concentrations of both inflammatory markers. Our finding is in accordance with another study (11), although a strong association of YKL-40 with CRP has been described in patients with rheumatoid arthritis as well as in elderly women (23, 24). In this regard, we show that YKL-40 gene and protein expression levels in VAT are associated with IL-6 and CRP. Multivariate linear regression analysis identified MCP-1 and HOMA as independent factors predicting circulating YKL-40, suggesting a marked influence of glucose homeostasis and inflammation on YKL-40 regulation. This leads to a possible role of YKL-40 in insulin resistance in relation to its direct or indirect effects on inflammation. YKL-40 is a secreted protein, suggesting that its sites of actions are most likely to be extracellular. However, the activation of cytoplasmic signal transduction pathways suggests that YKL-40 interacts with one or several signaling components on the plasma membrane. Specific cell-surface, soluble receptors or potential ligands for YKL-40 have not been identified yet. In this regard, the study of the actual YKL-40 receptors together with the signaling pathways triggered by these receptors may help to better understand the role of YKL-40 in adipose tissue.

In summary, we have identified the up-regulation of gene and protein expression levels of YKL-40 in VAT as well as an association with its circulating concentrations, suggesting the contribution of visceral fat to the increased circulating YKL-40 levels in obesity-associated T2D. The decrease in insulin concentrations observed in RYGB-induced weight loss was correlated with the reduction in YKL-40 levels, reinforcing its involvement in glucose homeostasis. Only diet-induced weight loss was able to significantly reduce circulating YKL-40 concentrations in obese patients in our study. The association between YKL-40 with different proinflammatory markers highlights an involvement in the low-grade chronic inflammation accompanying obesity-associated T2D. The biological function of YKL-40 is not yet clear, but our results strengthen the view for an involvement of YKL-40 in the low-grade inflammation associated with the development of T2D.

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