Association of Adipose Tissue and Liver Fibrosis With Tissue Stiffness in Morbid Obesity: Links With Diabetes and BMI Loss After Gastric Bypass

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Context: Liver and white adipose tissue (WAT) develop inflammation and fibrosis.

Objective: The aim of the study was to evaluate the bioclinical relevance of WAT fibrosis in morbid obesity and diabetes and the relationships with tissue stiffness measured using a novel device.

Design and Setting: Observational and longitudinal studies were conducted in a hospital nutrition department.

Patients: Biopsies of liver and subcutaneous WAT (scWAT) and omental adipose tissue were collected from 404 obese bariatric surgery candidates, of whom 243 were clinically characterized before surgery and 3, 6, and 12 months after surgery. In 123 subjects, liver and scWAT stiffness was assessed noninvasively using vibration-controlled transient elastography (VCTE).

Interventions: Bariatric surgery was performed for some patients.

Main Outcome Measure: Adipose tissue fibrosis and stiffness and their link to obesity phenotypes were measured.

Results: scWAT fibrosis was positively associated with liver fibrosis (fibrosis score ≥2) ($\varrho = 0.14$; $P = .01$). VCTE-evaluated liver and scWAT stiffness was positively correlated with immunohistochemistry-determined liver ($\varrho = 0.46$; $P = .0009$) and scWAT fibrosis ($\varrho = 0.48$; $P = .0001$). VCTE-evaluated scWAT stiffness measures negatively associated with dual-energy x-ray absorptiometry-evaluated body fat mass ($R = -0.25$; $P = .009$) and were correlated with metabolic variables. Diabetic subjects showed increased scWAT stiffness. Participants less responsive to gastric bypass were older and more frequently diabetic, and they had increased body mass index, serum IL-6, and scWAT and liver fibrosis. Subjects with no diabetes and normal liver had higher fat mass and lower tissue fibrosis and stiffness.

Conclusion: scWAT stiffness was associated with tissue fibrosis, obesity, and diabetes-related traits. Noninvasive evaluation of scWAT stiffness might be useful in clinical practice. **(*J Clin Endocrinol Metab* 99: 898–907, 2014)**

One hallmark of obesity development and progression is tissue remodeling, particularly inflammation and increased fibrosis of liver and white adipose tissue (WAT). Liver fibrosis results from the gradual progression of liver injuries over time and can be a component of nonalcoholic fatty liver diseases (1). Although causal determinants are not well understood, liver fibrosis in obesity is associated with clinical and biological features, including age, insulin resistance, diabetes (2, 3), low-grade inflammation (elevated IL-6 and TNFα) (4, 5), gender (6), and ethnicity (7).

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Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CI, confidence interval; DXA, dual-energy x-ray absorptiometry; F, liver fibrosis score; GR, good responder; γGT, γ-glutamyl transpeptidase; HbA1C, glycosylated hemoglobin; HDL, high-density lipoprotein; IHC, immunohistochemistry; LR, less responsive; OR, odds ratio; oWAT, omental WAT; scWAT, subcutaneous WAT; VCTE, vibration-controlled transient elastography; WAT, white adipose tissue.

Liver fibrosis is also correlated with changes in tissue stiffness, which can be detected by noninvasive elastography (8). Macrophage accumulation in visceral WAT deposits is associated with aggravated liver inflammation and fibrosis in morbid obesity (9).

Recent data have shown associations between human obesity and major changes in the expressions of extracellular matrix components in WAT (10–13), accompanied by increased collagen depots and more collagen types, such as collagen I, III, and VI (11). Although excess collagen is linked with insulin resistance (13, 14), no study has reported details of the relationship between WAT collagen accumulation and potential changes in WAT stiffness, as observed in the liver. Moreover, a putative link between liver and WAT fibrosis has never been described in the context of obesity and diabetes (13). Thus, it remains unclear whether adipose tissue stiffness is associated with obesity-related variables and metabolic risks.

Reducing body weight leads to improvements in metabolic, liver, and cardiovascular complications (15), as well as ameliorations in WAT alterations, such as inflammation (10, 16). Diabetes improves after bariatric surgery-induced weight loss, which is currently the most efficient procedure for morbid obesity, but which has its own associated risks (17–19). Variability among individuals and weight regain remain major challenges (20, 21), with weight outcome failures occurring in about 30% of subjects after surgery (22). Putative predictors of weight loss include clinical and individual variables, such as gender, age, history of diabetes, and genetic and psychological traits (23, 24).

Little is known about the relevance of tissue structure alterations in the context of weight loss after gastric bypass. In a preliminary study with a small subject group (11), we showed that higher fibrosis around adipocytes (ie, periadipocyte fibrosis) is associated with reduced fat mass loss after gastric bypass. However, the predictive capacity of this putative tissue marker was not evaluated in combination with bioclinical variables and tissue alterations (11).

The present study examined morbidly obese subjects to determine the bioclinical relevance of WAT and liver fibrosis as evaluated by immunohistochemistry (IHC), as well as the relationships between IHC-evaluated fibrosis, tissue stiffness measured using a novel device, and bioclinical parameters.

Patients and Methods

Study samples

This study enrolled 404 obese subjects who had been prospectively included in a gastric surgery program at the nutrition departments of the Hôtel-Dieu and Pitié-Salpétrière hospitals (Paris, France), as described elsewhere (25, 26). During surgery, paired surgical sc and omental WAT (scWAT and oWAT, respectively) and liver biopsies were collected. A total of 139 subjects (34.4%) had type 2 diabetes, indicated by fasting glycemia of >7 mM or use of antidiabetic treatment. Of these diabetic subjects, 33 (23.7%) were untreated, 42 (30.2%) took metformin, 39 (28.1%) used insulin, and 25 (18%) used a combination of different treatments. Of the 404 study participants, 91 (22.5%) were taking hypolipidemic drugs (fibrates or statins).

Additionally, 59 subjects had undergone gastric banding ranging from a few months to 5 years before undergoing gastric bypass; these patients were referred to as the conversion group. The 345 primarily operated subjects were weight-stable for at least 3 months before gastric bypass. This group included 49 women (14.2%) who were considered metabolically healthy, having no diabetes or liver alterations (ie, no steatosis, inflammation, or fibrosis). The cohort did not include any healthy nondiabetic men. Table 1 shows the clinical and biological parameters of the participants. Among the 345 subjects evaluated at baseline, 243 were followed up at 3, 6, and 12 months after bypass surgery (Table 1). The Ethics Committee at Hôtel-Dieu Hospital approved the present clinical investigations. All subjects gave their informed, written consent before inclusion in the study.

Liver histopathology

Liver biopsies were formalin-fixed and paraffin-embedded, and serial sections were stained. The minimum set of stained sections included hematoxylin-eosin, picrosirius red, and Perls' staining. Biopsies were reviewed by a single liver pathologist (B.P.). Among the 345 patient samples, liver scoring for one patient could not be performed due to the poor quality of the liver biopsy; for the remaining 344 patients, fibrosis was scored at baseline according to Kleiner criteria (27).

WAT histopathology

scWAT and oWAT biopsies were processed, embedded in paraffin, and sliced into 5-μm-thick sections. Adipocyte diameters were evaluated using hematoxylin-eosin staining and Perfect Image software (Claravision). WAT biopsy slides were stained with picrosirius red, and the total area was scanned at $20\times$ magnification and resolution of 0.24 μm/pixel using a Na-

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Table 1. Characteristics of the Study Population

Variables	At Baseline (Before Bypass, n = 345)	Kinetic Studies (n = 243)			
		Before Bypass	3 mo After Bypass	6 mo After Bypass	1 y After Bypass
Age, y	42 (12)	42 (12)	42 (12)	42 (12)	43 (12)
BMI, kg/m^2	47.9 (7.4)	48.2 (7.0)	40.0 (6.5)	36.4 (6.4)	33.2 (6.4)
Body weight, kg	132.5 (25.34)	133.8 (23.2)	110.9 (20.5)	100.8 (20.0)	92.1 (19.8)
Total body fat mass, %	46.79 (5.69)	46.46 (5.55)	43.57 (5.92)	40.15 (6.39)	36.14 (7.12)
Diabetes status, n (%)	123 (35.7)	86 (35.4)	43 (17.6)	37 (15.2)	32 (13.2)
Fasting glycemia, mM	6.34 (2.36)	6.40 (2.50)	5.17 (1.28)	4.89 (0.95)	4.82 (0.77)
Fasting insulin, μU/mL	23.31 (37.54)	24.71 (42.98)	10.71 (6.3)	8.15 (4.85)	7.56 (6.55)
HbA1C, %	6.51 (1.3)	6.52 (1.36)	5.82 (1.35)	5.63 (0.57)	5.59 (0.56)
HOMA-IR	7.62 (22.07)	8.35 (25.89)	2.5 (1.85)	1.81 (1.3)	1.68 (1.84)
Triglycerides, mM	1.64 (1.3)	1.62 (1.41)	1.24 (0.56)	1.1 (0.48)	0.95 (0.42)
Cholesterol, mM	4.85 (1.03)	4.80 (1.03)	4.32 (0.88)	4.30 (0.85)	4.36 (0.82)
HDL cholesterol, mM	1.21 (0.34)	1.21 (0.35)	1.15 (0.33)	1.28 (0.39)	1.51 (0.4)
AST, IU/L	28.46 (12.44)	28.86 (13.0)	29.95 (15.48)	24.59 (7.12)	25.46 (8.79)
ALT, IU/L	36.24 (30.4)	37.32 (33.48)	36.46 (26.64)	26.17 (18.84)	27.44 (23.69)
γGT, mg/dL	50.15 (51.02)	50.65 (50.24)	29.81 (44.67)	27.08 (37.82)	26.17 (32.49)
Leptin, ng/mL	53.02 (24.88)	52.08 (23.44)	27.2 (15.57)	21.68 (13.32)	18.55 (14.08)
Adiponectin, μg/mL	5.71 (3.41)	5.61 (3.38)	6.65 (4.06)	7.79 (5.33)	9.01 (5.86)
IL-6, pg/mL	4.23 (3.44)	4.35 (3.45)	5.97 (15.45)	6.48 (18.77)	4.34 (14.12)
hsCRP, mg/dL	0.97 (0.85)	0.97 (0.82)	0.56 (0.63)	0.43 (0.52)	0.23 (0.56)

Abbreviations: hsCRP, highly sensitive C-reactive protein; HOMA-IR, homeostasis model of assessment-insulin resistance. Data are expressed as mean (SD) unless otherwise stated; n = 243 is a subset of the larger group; n = 345 at baseline before bypass.

noZoomer Hamamatsu scanner (Hamamatsu Photonics KK, Systems Division). Digital slides were visualized on a high-definition display (Barco Coronis Fusion; Barco) to pinpoint pathological fibrosis quantification (termed "IHC-fibrosis" below). Detection thresholds were adjusted with an image-analysis module using Calopix software (Tribvn). Total IHC-fibrosis quantification was expressed as the ratio of fibrous tissue area stained with picrosirius red to the total tissue surface, as previously described (10). Periadipocyte IHC-fibrosis was quantified by measuring the area of IHC-fibrosis in 10 random fields examined at 10× magnification (11). IHC-fibrosis quantification was performed on one section, and we verified that this slide was representative of the whole biopsy by quantifying 10 slides from the start to the end of the biopsy specimen. Results indicated that this quantification was homogeneous. This technique is routinely used to quantify liver fibrosis.

Bioclinical tests

Body composition was estimated by whole-body fan-beam dual-energy x-ray absorptiometry (DXA) scanning (Hologic Discovery W software, version 12.6; Hologic Inc) (28). Body fat and lean mass distribution were determined as described elsewhere (29). Thirty-four subjects were excluded because their preoperative weight exceeded the limit of the DXA (160 kg) or they did not fit entirely within the DXA field of view. Blood samples were taken after 12 hours of overnight fasting. Clinical variables were measured 1 month before the day of bariatric surgery as described elsewhere (9–11, 16). For 243 subjects, DXA measurements were taken before bypass and at 3, 6, and 12 months after bypass.

Measurement of tissue stiffness by transient elastography

In 123 subjects, liver stiffness was noninvasively assessed using the vibration-controlled transient elastography (VCTE) device (Fibroscan; Echosens) (30). Using the same principle that the VCTE uses for liver assessment, Echosens customized a novel prototype device called AdipoScan to measure scWAT stiffness in the same 123 obese subjects. The VCTE technology is based on the generation of a mechanical vibration, which induces the propagation of a shear wave in the tissue. The shear wave velocity is evaluated in the scWAT region of interest, which is anisotropic and heterogeneous and is related to tissue viscoelastic characteristics. The velocity increases with tissue stiffness. Using this new prototype, measurements were performed, localized near the umbilicus on the subject because abdominal scWAT was at its maximum thickness at this position. In that case, it is based on the use of a mini electromechanical transducer for generating the mechanical vibration, associated to a piezoelectric transducer for following the shear wave propagation (see Figure 2B). Importantly, this prototype is light to minimize the initial static force (pressure), and then to avoid compressing the tissue and modifying the viscoelastic properties of the WAT (see Figure 2B).

The intraoperator reproducibility was evaluated in five patients using the standardized coefficient of variation (SCV). AdipoScan measurements were reproducible (SCV = 4%).

Tissue stiffness by transient elastography was measured 1 month before the day of bariatric surgery.

Statistical analyses

Data are expressed as mean ± SD. Categorical variables are expressed as numbers and percentages. The Shapiro-Wilk test was used to test the Gaussian distribution of the biological parameters. Skewed variables were log-transformed to normalize their distribution before statistical analyses. Categorical data were analyzed using the χ^2 test or Fisher's exact test, as appropriate. Continuous data were analyzed with Student's t test or Wilcoxon's test, as appropriate. Correlation analyses were per-

Table 2. Comparison of Clinical and Biological Parameters at Baseline Between Patients With and Without Significant Liver Fibrosis

	Patients Without Significant Liver Fibrosis (F < 2)	Patients With Significant Liver Fibrosis (F ≥ 2)	*P* Value
n (%)	259 (75.3)	85 (24.7)	
Variables			
Female, n (%)	218 (84.1)	55 (64.7)	<.001
Age, y	42 (12)	43 (12)	.48
BMI, kg/m^2	47.43 (7.12)	49.28 (8.14)	.063
Total body fat mass, %	49.30 (5.21)	48.36 (5.04)	.25
Diabetes status, n (%)	75 (28.9)	47 (55.3)	<.001
Glycemic parameters			
Fasting glycemia, mM	6.01 (2.09)	7.34 (2.84)	<.001
Fasting insulin, μU/mL	21.69 (39.08)	28.49 (32.14)	.001
HbA1C, %	6.33 (1.21)	7.12 (1.40)	<.001
Lipid parameters			
Triglycerides, mM	1.56 (1.37)	1.87 (1.01)	.003
HDL cholesterol, mM	1.23 (0.36)	1.14 (0.30)	.026
Cholesterol, mM	4.90 (1.04)	4.69 (1.00)	.101
ApoA1, mM	1.38 (0.28)	1.29 (0.22)	.005
ApoB, mM	0.98 (0.28)	0.93 (0.28)	.28
Hepatic factors			
AST, IU/L	26.97 (10.10)	33.01 (17.25)	.003
ALT, IU/L	32.22 (19.28)	48.79 (49.74)	<.001
γGT, IU/L	42.87 (41.33)	73.23 (69.24)	<.001
Adipokines			
Leptin, ng/mL	52.79 (24.89)	53.73 (24.97)	.77
Adiponectin, μg/mL	5.77 (3.5)	5.53 (3.13)	.55
IL-6, pg/mL	4.02 (3.25)	4.89 (3.92)	.003
hsCRP, mg/dL	0.95 (0.93)	1.06 (0.81)	.050
Adipose-tissue fibrosis			
scWAT fibrosis, %	4.20 (4.77)	5.25 (4.83)	.018
Pericellular scWAT fibrosis, %	0.29 (0.41)	0.38 (0.49)	.052
oWAT fibrosis, %	4.16 (3.08)	4.55 (3.35)	.36

Abbreviations: hsCRP, highly sensitive C-reactive protein; Apo, apolipoprotein. Values are expressed as means (SD), unless otherwise stated. Parametric *P* values were obtained from Student's *t* test on log-transformed data or the χ^2 test for qualitative data. All parameters are at T0.

formed using Spearman's or Pearson's correlation, as appropriate. Univariate/multivariate logistic regression analyses were performed, and each odds ratio (OR) was calculated with a 95% confidence interval (CI).

For clustering analysis of patients' body mass index (BMI) losses after bariatric surgery, we used the K-means algorithm, which is specifically designed to deal with longitudinal data (KmL) (31). The KmL method is the implementation of "k-means" specifically designed to cluster trajectories. This method proposes a graphical interface for choosing the "best" number of clusters. Thus, the classification (low vs good responders to bariatric surgery) was based on the resulting observation. All *P* values are two-sided, and *P* values <.05 were considered to be statistically significant. All analyses were performed using R software, version 2.15.1.

Results

Association between WAT and liver IHC-determined fibrosis

Among the 404 initially recruited subjects, 59 underwent a bypass conversion that was prescribed due to weight regain after the first gastric banding. scWAT IHC-fibrosis accumulation significantly differed in primarily operated subjects (4.5%) compared to those with bypass conversion (8.1%; $P = .004$); therefore, the bypass conversion group was excluded from subsequent analysis.

Our findings confirmed that, among the investigated obese subjects for whom liver fibrosis score (F) was available, those with significant liver fibrosis (n = 85; F ≥2) were more frequently men, had higher rates of type 2 diabetes and dyslipidemia, and had elevated circulating IL-6 levels compared to the subjects with F < 2. Additionally, subjects with significant liver fibrosis exhibited increased total scWAT IHC-fibrosis (Table 2). Significant liver fibrosis (F ≥ 2) was positively associated with both scWAT total ($\varrho = 0.14$; $P = .012$) and periadipocyte IHC-fibrosis ($\varrho = 0.11$; $P = .044$). The amount of total IHC-fibrosis in scWAT was associated with oWAT IHC-fibrosis ($\varrho = 0.34$; $P < .001$), but no correlation was found between oWAT and liver fibrosis. When we excluded diabetic participants (35.6% of the population at baseline), we ob-

Table 3. Clinical and Biological Parameters at Baseline Between Nondiabetic Women Without Liver Disease (Healthy Liver) and With Significant Liver Fibrosis (F ≥ 2)

	Healthy Liver (n = 49)	F ≥ 2 (n = 27)	*P* Value
Variables			
Age, y	36 (11)	36 (9)	.041
Body weight, kg	128 (19)	131 (22)	.28
BMI, kg/m^2	46.6 (5.7)	49.5 (8.4)	.57
Total body fat mass, %	49.38 (4.87)	47.27 (3.96)	.024
Glycemic parameters			
Fasting glycemia, mM	5.07 (0.56)	5.32 (0.62)	.089
Fasting insulin, μU/mL	13.35 (7.03)	20.5 (11.99)	.005
HbA1C, %	5.73 (0.38)	5.81 (0.51)	.68
HOMA-IR	3.03 (1.66)	4.96 (3.28)	.004
Lipid parameters			
Triglycerides, mM	1.24 (0.68)	1.34 (0.83)	.66
Cholesterol, mM	5.04 (0.98)	4.69 (0.78)	.122
HDL cholesterol, mM	1.33 (0.39)	1.3 (0.31)	.99
Hepatic factors			
AST, IU/L	24.12 (7.43)	26.28 (7.99)	.157
ALT, IU/L	25.49 (15.7)	33.58 (17.52)	.049
γGT, mg/dL	36.47 (42.58)	41 (33.87)	.25
Adipokines			
Leptin, ng/mL	66.18 (28.69)	66.12 (25.23)	.89
Adiponectin, μg/mL	6.95 (4.57)	6.61 (3.17)	.93
IL-6, pg/mL	3.19 (1.77)	4.11 (2.36)	.052
hsCRP, mg/dL	0.98 (0.93)	1.1 (0.78)	.157
Adipose-tissue fibrosis			
scWAT fibrosis, %	3.96 (3.62)	6.01 (4.85)	.034
oWAT fibrosis, %	4.65 (4.61)	5.78 (2.99)	.026
Pericellular scWAT fibrosis, %	0.29 (0.39)	0.27 (0.33)	.62
Pericellular oWAT fibrosis, %	0.36 (0.5)	0.46 (0.51)	.31

Abbreviations: hsCRP, highly sensitive C-reactive protein; HOMA-IR, Homeostasis Model of Assessment–insulin resistance. Values are expressed as means (SD). *P* values obtained from Wilcoxon's test or Fisher's tests for qualitative data. All parameters are at T0.

served a positive correlation between significant liver fibrosis (F ≥ 2) and both total scWAT (ϱ= 0.15; P = .029) and oWAT IHC-fibrosis (ϱ= 0.14; P = .039).

In these 344 subjects, we further examined circulating metabolic and inflammatory variables that were potentially associated with WAT IHC-fibrosis at baseline. In contrast to our observations relating to liver fibrosis, we found no significant associations between WAT IHC-fibrosis and glucose or lipidic parameters. We only observed a significant association between scWAT IHC-fibrosis and circulating concentrations of IL-6 (ϱ= 0.12; P = .033).

WAT IHC-fibrosis negatively associated with body fat mass

At baseline, scWAT IHC-fibrosis was negatively associated with the percentage of fat mass (ϱ= −0.17; P = .003), and oWAT IHC-fibrosis was negatively correlated with weight (ϱ= −0.14; P = .010) and BMI (ϱ= −0.12; P = .022). Compared to women (n = 27) with significant liver fibrosis (F ≥ 2), the subgroup of 49 women with no diabetes and no liver alterations showed higher body fat mass (P = .024) but less IHC-fibrosis in both scWAT (P = .034) and oWAT (P = .026) (Table 3).

Tissue IHC-fibrosis associated with BMI reduction after gastric bypass

The group of 243 subjects prospectively followed after bariatric surgery showed major improvements in mean BMI, DXA-evaluated fat mass, and metabolic and inflammatory variables (Table 1). Use of the KmL method (31) to cluster the BMI trajectories after gastric bypass revealed three main groups of BMI loss: a less responsive (LR) group with a BMI loss of <25% (n = 70), and those with good (n = 119) or very good (n = 54) weight loss (Figure 1A). These latter two groups had a combined mean BMI reduction of 34.8% and were collectively termed good responders (GR). At 1 year after surgery, the LR group showed less improvement in body fat mass, diabetes status, and high-density lipoprotein (HDL) cholesterol compared to the GR group (Supplemental Table 1, published

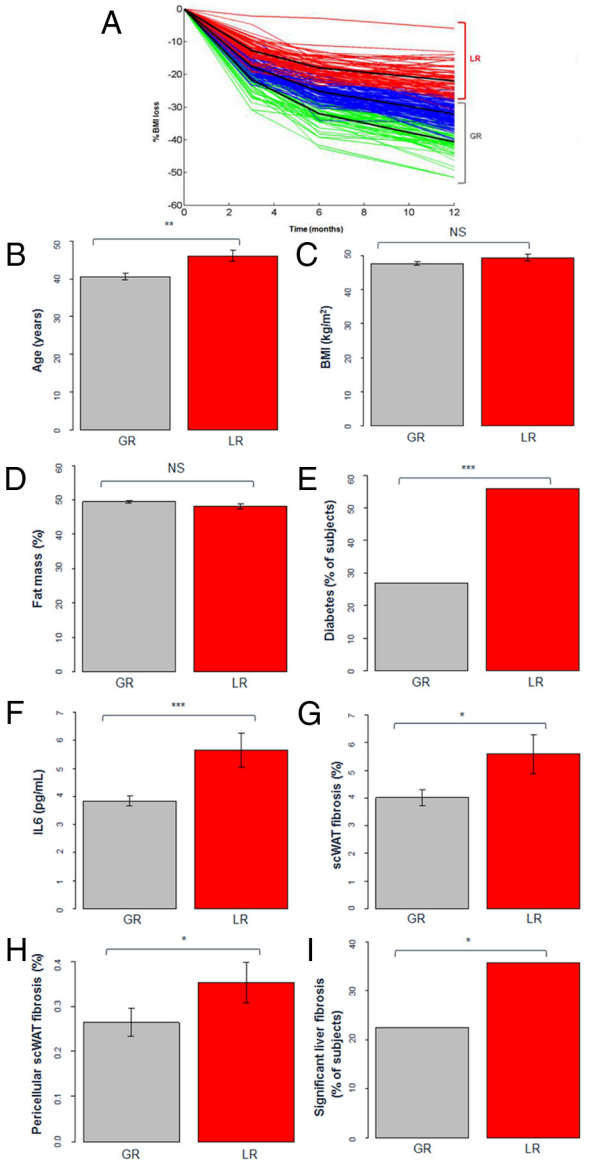


Figure 1. Baseline comparison between low and good responders to bariatric surgery-induced weight loss. A, Clustering of weight-loss profiles in 243 obese subjects: red, low responders to weight loss (LR); blue, good responders; green, very good responders. For further analyses, the good responders and very good responders are combined and referred to as good responders (GR). B–I, Baseline comparison between LR (n = 70) and GR (n = 173) groups in regards to age (B), BMI (C), DXA-evaluated fat mass (D), diabetes state (E), IL-6 circulating concentration (F), percentage scWAT IHC-fibrosis (G), percentage pericellular scWAT IHC-fibrosis (H), and liver fibrosis (F ≥ 2) (I). Data are expressed as mean ± SEM, or as percentage for diabetes and liver fibrosis. The *P* values were obtained using Student's *t* test on log-transformed data or the χ^2 test for qualitative data. NS, $P > .05$; *, $P < .05$; **, $P < .01$; ***, $P < .001$.

on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org).

Examination of the clinical and biological characteristics of the LR vs GR groups at baseline showed that the LR group was older, had a higher frequency of diabetes, and had increased circulating IL-6 concentrations, whereas mean baseline BMI and fat mass were similar in both groups (Figure 1, B–F, and Supplemental Table 2). Compared to the GR group, the LR group also had increased scWAT IHC-fibrosis (both total and periadipocyte fibrosis; Figure 1, G and H), and exhibited more significant liver fibrosis (Figure 1I and Supplemental Table 2). Interestingly, the 49 women with no liver alteration were more likely to be good responders after surgery (Table 3) with only four (13.7%) in the LR group, whereas 45 (86.3%) were in the GR group. This comparison could not be made in men, in whom no healthy livers were found.

Overall, the LR group was associated with diabetes status, age, fasting glycemia, glycosylated hemoglobin (HbA1C), IL-6, and binarized baseline BMI (1 for BMI ≥ 55, 0 for BMI < 55), as well as with total and pericellular IHC-fibrosis in scWAT (Table 4). Adjusting for age, diabetes, and IL-6 did not alter these associations. Regarding liver injury, the ORs increased with the severity of fibrosis score; OR values were 2.24 [95% CI, 1.09–4.60] for minimal fibrosis (F1), 2.50 [95% CI, 1.10–5.68] for significant fibrosis (F ≥ 2), and 6.48 [95% CI, 1.94–21.6] for severe fibrosis (F ≥ 3).

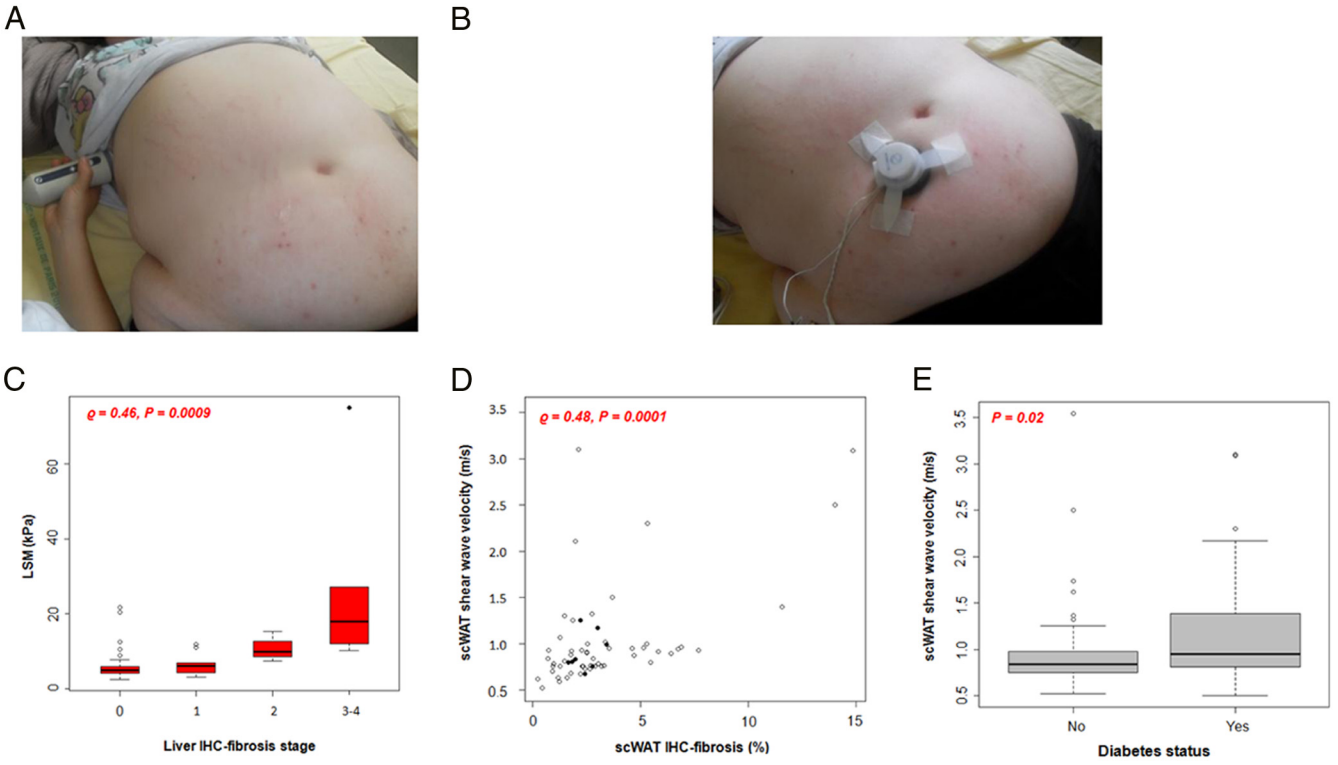
Physical measures of WAT stiffness associated with WAT IHC-fibrosis and dysregulation of glucose homeostasis

Liver fibrosis is associated with changes in tissue stiffness, which can be physically measured (29). To explore the possibility of a similar association with WAT IHC-fibrosis, we developed a new ultrasonic tool to measure shear-wave velocity in scWAT. Using this tool, we assessed tissue stiffness in 123 subjects (Figure 2, A and B), of whom 49 and 61 subjects, respectively, were also subjected to liver staging and adipose tissue IHC-fibrosis quantification.

First, we found that the shear-wave velocity of scWAT was positively correlated with liver stiffness (R = 0.3; *P* =

Table 4. Parameters Associated With the LR Group

Parameters	OR [95% CI]
Diabetes status	3.37 [1.90–6.00]
Age	1.05 [1.01–1.07]
Fasting glycemia	1.18 [1.06–1.33]
HbA1C	1.58 [1.25–1.99]
IL-6	1.18 [1.06–1.31]
BMI binarized at baseline (1 for BMI ≥ 55, 0 for BMI < 55)	1.45 [1.06–1.98]
Total scWAT fibrosis	1.45 [1.06–1.98]
Adjusted for age, diabetes, and IL-6	1.58 [1.10–2.28]
Pericellular scWAT fibrosis	1.29 [1.04–1.62]
Adjusted for age, diabetes, and IL-6	1.38 [1.07–1.79]



F

	scWAT shear wave velocity (m/s)	Liver stiffness measurements (LSM) (kPa)
Liver stiffness measurements in kPa	0.3 (P = .0003)	-
scWAT shear wave velocity in ms^{-1}	-	0.3 (P = .0003)
% Total body fat mass	−0.25 (P = .009)	−0.17 (P = .09)
% Total body lean mass	0.25 (P = .008)	0.19 (P = .06)
Fasting glycemia in mM	0.25 (P = .006)	0.30 (P = .002)
Fasting insulin in µU/mL	0.19 (P = .03)	0.64 (P < .0001)
% HbA1C	0.21 (P = .02)	0.28 (P = .005)
HDL cholesterol in mM	−0.18 (P = .04)	−0.20 (P = .04)
ApoA1 in mM	−0.14 (P = .14)	−0.13 (P = .23)
γGT in mg/dL	0.17 (P = .07)	0.50 (P < .0001)
AST in IU/L	0.11 (P = .21)	0.33 (P = .0009)
ALT in IU/L	0.06 (P = .53)	0.26 (P = .008)
Creatinine in mg/L	0.04 (P = .68)	0.25 (P = .02)
Uricemia in mg/L	0.01 (P = .95)	0.21 (P = .07)

Figure 2. Physical measures of scWAT and liver stiffness. A, Evaluation of liver stiffness measurements (LSM) using a Fibroscan XL probe. B, Area of measurement of shear-wave velocity in scWAT. C, Box-plot of LSM (kPa) according to stage of liver fibrosis in 49 subjects; 63.3% (n = 31) had a liver score of F0, 20.4% (n = 10) had F1, 6.1% (n = 3) had F2, 8.2% (n = 4) had F3, and 2% (n = 1) had F4 (solid dot). The only patient with the F4 stage of liver fibrosis also had the higher level of liver stiffness (75 KPa). D, Correlation analysis between shear-wave velocity in scWAT and percentage of scWAT IHC-fibrosis (n = 61 subjects). Filled circles represent eight women with no liver alterations and no diabetes. E, Box-plot of shear-wave velocity in scWAT (m/s) according to the diabetic status of 123 subjects; 42.3% (n = 52) had type 2 diabetes. F, Correlation analysis between shear-wave velocity in scWAT and liver stiffness measurements and clinical characteristics in morbidly obese subjects. Spearman's correlations were used in panels C and D, Student's *t* test was used in panel E, and Pearson's correlations were used in panel F.

.0003), which itself was positively associated with the fibrosis liver stage ($\varrho = 0.46$; $P = .0009$; n = 49; Figure 2C).

Second, we observed that scWAT shear-wave velocity was positively associated with scWAT IHC-fibrosis ($\varrho = 0.48$; $P = .0001$; n = 61; Figure 2D). In this group, nondiabetic obese women with no liver alterations also showed lower scWAT shear-wave velocity ($P = .02$), in agreement with their decreased adipose tissue IHC-fibrosis (Figure 2D, solid dots).

Third, in the 123 examined subjects, the shear-wave velocity of scWAT was correlated positively with several bioclinical parameters, including fasting glycemia and insulin, HbA1C, and fat-free mass, and negatively with body fat (%) and HDL cholesterol (Figure 2F). Diabetes status (n = 52, 42.3%) was also significantly associated with increased shear-wave velocity ($P = .022$) (Figure 2E). Similarly, liver stiffness was found to correlate positively with circulating creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyl transpeptidase (γGT), fasting glycemia, insulin, and HbA1C, and to negatively correlate with body fat (%) and HDL (Figure 2F).

Discussion

The present clinical study measured scWAT stiffness by shear-wave velocity using a prototype VCTE. Our results showed, for the first time, an association between scWAT IHC-fibrosis and physical measures of tissue stiffness and that this measure was associated with diabetes and metabolic variables. These findings demonstrated that WAT IHC-fibrosis shares common features with liver fibrosis in morbid obesity. However, because of the correlative nature of the relationship between liver and WAT IHC-fibrosis, we cannot speculate on the potential causal and kinetic relationships between these two phenomena. The observed negative association between body fat mass and VCTE, as well as the immunohistochemical quantification of collagens, suggests that diminished WAT stiffness (ie, with reduced collagen accumulation) enables fat mass enlargement, which may at least transiently protect the liver from severe injury. In contrast, increased WAT stiffness may represent a mechanical limitation to WAT expansion. This concept, related to the "adipose-tissue expandability hypothesis" (32), proposes that when the storage capacity of scWAT is reached, a flux of excess lipids is sent to ectopic sites, such as the liver, thus promoting metabolic complications. This concept is supported by the finding of morbidly obese subjects with healthy liver, no diabetes (ie, considered as "metabolically healthy"), increased total fat mass, and decreased scWAT IHC-fibrosis, as well as by the identification of increased WAT stiffness in diabetic obese subjects. However, it cannot be excluded that the diabetic milieu (ie, high glucose and high insulin) could also promote the maintenance and worsening of tissue alteration.

It is intriguing that the physical measures of WAT stiffness were associated with metabolic variables (glucose, insulin, and lipid values), whereas the immunohistochemical quantification of collagens (IHC-fibrosis) was not. We found that collagen amount explained 25.4% of VCTE signal variation, suggesting that factors other than collagen accumulation contribute to the modified stiffness of scWAT. Focused analysis of adipose deposits shows the presence of collagen types I, III, and VI (11). The fibrillar collagens I and III play a role in liver fibrogenesis (11, 33); they bind together, and subsequent tissue stiffness depends on the number of these links (34). A future goal will be to identify which specific collagen types predominantly associate with shear-wave velocity in WAT and whether collagen cross-linking contributes to tissue stiffness. Tissue stiffness may also be determined by other components, such as elastin, laminin, and fibronectin, as well as cellular components of adipose tissue (such as inflammatory cells) that are modified in obesity.

Our results also confirmed, in a larger group, our previous observations that collagen accumulation in WAT seems to be associated with low response to gastric bypass-induced weight loss, even when combined with predictive variables such as age, diabetes, and IL-6. Gradually increased liver fibrosis was also found to be a factor that could associate with low responsive weight loss. Although the reason for this association is unknown, it suggests a relationship between fibrosis-associated functional alterations of tissue function and a response to weight loss, which needs to be explored further. Further studies are needed to investigate the ability of new noninvasive physical methods to predict clinical outcomes following weight loss after bariatric surgery, as well as after dietary interventions.

It also remains unknown why fibrosis develops in human WAT. It has been suggested that persistent inflammatory stimulus causes excessive synthesis of extracellular matrix components and subsequent deposition of interstitial fibrotic materials (11). This phenomenon may particularly occur in the event of rapid and significant weight loss, and it is unknown whether it is reversible with time. An elegant study in mice demonstrated that weight cycling induced increased accumulation of proinflammatory T-cell populations, which could contribute to the negative metabolic consequences of repeated weight variations (35). Altogether, these data suggest that aggravation of adipose tissue inflammation and remodeling during

weight cycling may play a role in causing the metabolic abnormalities occurring in obesity.

In conclusion, the results of this clinical study suggest that human obesity, characterized by gradual enlargement of WAT deposits, causes a fibrotic condition that affects tissue remodeling, functioning, and stiffness. This condition has been described in the liver, as well as in lung, kidney, and heart diseases, for which obesity can be a risk factor (36, 37). Herein, we propose a noninvasive method based on Fibroscan technology to evaluate scWAT IHC-fibrosis, and to thus assess the phenotype of tissue stiffness and evaluate its clinical relevance in obesity and diabetes and during interventional follow-up.

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