The FAT Score, a Fibrosis Score of Adipose Tissue: Predicting Weight-Loss Outcome After Gastric Bypass

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Context: Bariatric surgery (BS) induces major and sustainable weight loss in many patients. Factors predicting poor weight-loss response (PR) need to be identified to improve patient care. Quantification of subcutaneous adipose tissue (scAT) fibrosis is negatively associated with post-BS weight loss, but whether it could constitute a predictor applicable in clinical routine remains to be demonstrated.

Objective: To create a semiquantitative score evaluating scAT fibrosis and test its predictive value on weight-loss response after Roux-en-Y gastric bypass (RYGB).

Methods: We created a fibrosis score of adipose tissue (FAT score) integrating perilobular and pericellular fibrosis. Using this score, we characterized 183 perioperative scAT biopsy specimens from severely obese patients who underwent RYGB (n = 85 from a training cohort; n = 98 from a confirmation cohort). PR to RYGB was defined as $<$28% of total weight loss at 1 year (lowest tertile). The link between FAT score and PR was tested in univariate and multivariate models.

Results: FAT score was directly associated with increasing scAT fibrosis measured by a standard quantification method (P for trend <0.001). FAT score interobserver agreement was good ($\kappa = 0.76$). FAT score $\geq$2 was significantly associated with PR. The association remained significant after adjustment for age, diabetes status, hypertension, percent fat mass, and interleukin-6 level (adjusted odds ratio, 3.6; 95% confidence interval, 1.8 to 7.2; $P = 0.003$).

Conclusion: The FAT score is a new, simple, semiquantitative evaluation of human scAT fibrosis that may help identify patients with a potential limited weight-loss response to RYGB. (***J Clin Endocrinol Metab* 102: 2443–2453, 2017**)

Compared with lifestyle interventions, bariatric surgery (BS) procedures have proven their efficacy with major sustainable weight loss, comorbidity remission, and overall mortality reduction in severely obese patients (1). Subsequently, the number of BS procedures has dramatically increased during the past 10 years (2) and

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Abbreviations: AT, adipose tissue; BS, bariatric surgery; CI, confidence interval; CLS, crownlike structure; FAT score, fibrosis score of adipose tissue; IL-6, interleukin-6; NAFLD, nonalcoholic fatty liver disease; OR, odds ratio; PCF, pericellular fibrosis; PLF, perilobular fibrosis; PR, poor weight-loss response; RYGB, Roux-en-Y gastric bypass; scAT, subcutaneous adipose tissue; T2D, type 2 diabetes.

may continue to rise because BS is now featured in the international guidelines for type 2 diabetes (T2D) treatment in obese patients, including those with a body mass index <35 kg/m^2 (3).

Although BS induces weight loss for most patients, there is substantial interindividual variability in the extent and profile of patient weight-loss trajectories and sometimes weight is regained (4, 5). Thus, identifying relevant baseline predictors of weight-loss response is essential to better inform BS candidates and to assist clinical staff in individualizing patient postoperative follow-up. Preoperative factors, such as age, baseline T2D, T2D severity (*i.e.*, hemoglobin A1c) (6–8), and psychological aspects (9), are linked with BS-induced weight loss. However, to date, these identified baseline variables exhibit limited predictive value for post-BS weight loss. In many patients (n = 1513) who underwent Roux-en-Y gastric bypass (RYGB), a wide selection of preoperative biological and psychological factors combined in a multivariate model only explained 14% of the weight-loss variability (7).

However, in these previous studies in which clinical variables were considered, the potential of adipose tissue (AT) structural alterations was not examined. During obesity development and progression, AT structure substantially changes and AT becomes dysfunctional (10). Immune cells, such as macrophages, infiltrate the AT. Some of these cells aggregate around adipocytes to form crownlike structures (CLSs), which are associated with markers of insulin resistance and low-grade inflammation (11, 12). Another important hallmark of AT alteration during obesity is the accumulation of extracellular matrix components, especially cross-linked collagens (13). Collagen accumulation, observed either around AT lobules [*i.e.*, perilobular fibrosis (PLF)] or surrounding adipocytes [*i.e.*, pericellular fibrosis (PCF)] (14) affects adipocyte biology (15). AT fibrosis in omental depot (and not subcutaneous depot) is also correlated with patient metabolic alterations (16). Moreover, we have shown that subcutaneous AT (scAT) fibrosis measured with picrosirius-red staining quantification is negatively associated with the 1-year post-BS weight-loss outcome (8, 14).

We hypothesized that the link between AT fibrosis and weight loss is explained by a dysfunctional scAT with reduced plasticity. Supporting this hypothesis, we previously observed a correlation between scAT fibrosis and tissue physical stiffness, measured with elastometry (8), and a negative correlation with fat mass (14). Furthermore, rodent studies show that mice that are genetically deficient in collagen 6 gain more weight than control mice when fed a high-fat diet (17). However, whether scAT fibrosis quantification could be used as a relevant predictive tool applicable in clinical routine remains unknown.

In most studies, AT fibrosis is measured by numeric quantification of picrosirius red-stained collagen, but this method has several limitations when considering the translation of results in routine clinical care. Measures depend tightly on both staining quality and slide preparation that can vary between samples. Likewise, fibrosis can be heterogeneous within a biopsy specimen, making the interpretation of a purely quantitative measure difficult. The technique is also time consuming (10 to 15 minutes per slide) and requires specific analytical software, which is not available in all histology laboratories. Finally, the reproducibility of results can be difficult to achieve because the method is based on color detection programs and, thus, relies on staining intensity and the scanned image quality.

Given these difficulties, our first objective was to create a simple, semiquantitative histological score to easily characterize scAT fibrosis, similar to what was done in the liver field for diagnostic scoring of nonalcoholic fatty liver disease (NAFLD) (18, 19). Second, we aimed to examine the clinical applicability of this scoring; in particular, its added value in predicting severely obese patients' post-BS weight-loss responses.

Materials and Methods

Study population

Perioperative histological samples were available for 868 patients from our ongoing BS cohort of 1748 patients enrolled in the Nutrition Department, Institute of Cardiometabolism and Nutrition, Pitié-Salpêtrière Hospital. Patients were operated on in the surgery departments of Hôtel-Dieu, Ambroise Paré, and Pitié-Salpêtrière hospitals in Paris between 1998 and April 2015. Among these patients, we retrospectively studied 564 obese individuals who underwent RYGB and for whom complete follow-up data 1 year postsurgery were available. Other surgeries, such as sleeve gastrectomy and longitudinal adjustable gastric banding, were excluded to eliminate any confounding effect on the amount of weight loss (20). Conversions of longitudinal adjustable gastric banding or sleeve to RYGB were also excluded because these patients display higher scAT fibrosis levels (8) and a poorer weight-loss response than patients undergoing RYGB for the first time (21).

Our semiquantitative score was created on a first random selection of 85 patients (training cohort). An independent validation cohort including another 98 RYGB patients was then constituted, which led to a total of 183 patients for the pooled cohort (Fig. 1). The sample size was chosen based on similar work in the liver field for the design of NAFLD histological score (n = 50) (18, 19) and was increased because of the high AT heterogeneity. Ethical approval was obtained from the Research Ethics Committee of Hôtel-Dieu Hospital (CPP Ile-de-France No.1). Informed written consent was obtained from all subjects, and the protocol was registered on www.clinicaltrials.gov (NCT01655017).

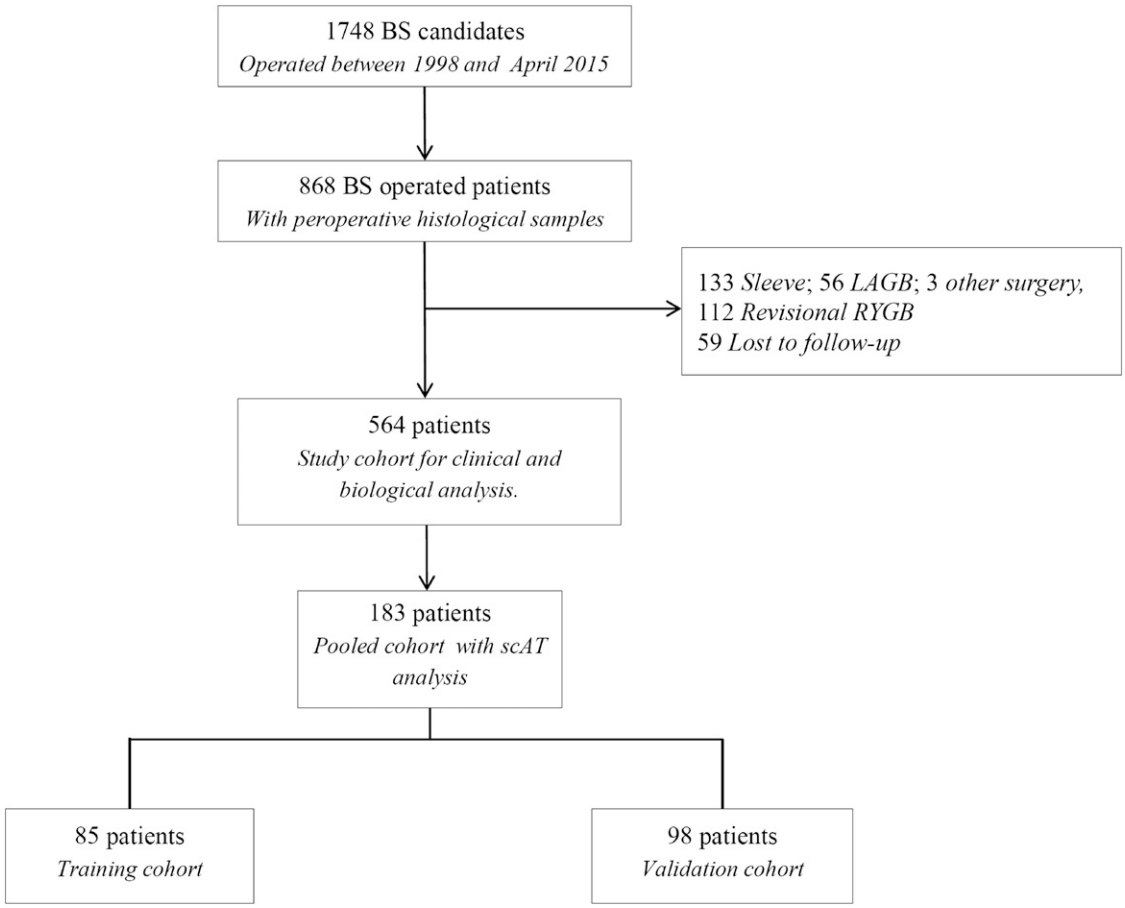

Figure 1. Study flow chart. LAGB, longitudinal adjustable gastric banding; revisional RYGB, conversion of longitudinal adjustable gastric banding or sleeve to RYGB.

Clinical, anthropological, and biological parameters

Patients' clinical and anthropometric characteristics were obtained at baseline (T0) and 1 year (T12) postsurgery. Body composition was evaluated by whole-body fan-beam dual energy X-ray absorptiometry scan (Hologic Discovery, Marlborough, MA), as described previously (8). Blood samples were collected after 12-hour overnight fast before BS (T0). Perioperative surgical scAT biopsy specimens were recovered from the periumbilical area by trained surgeons, as previously described (14). Weight-loss outcome was defined using the percent of total body weight loss between T0 and T12, as recently recommended (22). Poor responders were defined as the lowest tertile of percent of total body weight loss [*i.e.*, <28% in our cohort of RYGB obese patients (n = 564)].

Tissue preparation and histological scoring of scAT

Creation of the scAT fibrosis score

Surgical scAT biopsy samples were fixed and embedded in paraffin and sliced into 5 μm-thick sections. Collagen was stained with picrosirius-red (mainly collagen I and III) and the total area was scanned at ×20 magnification and resolution of 0.24 μm/pixel. Detection thresholds were adjusted with an image-analysis module using Calopix software (TRIBVN). Manual delimitation of red-stained AT, avoiding blood vessels and staining artifacts, was then performed (15 min/slide). Total fibrosis quantification was expressed as the ratio of picrosirius-red–stained fibrous tissue area to the total tissue surface, as described previously (8, 14, 23). The entire scAT sample was examined; fibrosis depots were considered for the scoring if they represented >5% of the slide and scAT fibrosis was separated into two categories: PLF and PCF, as described previously (14).

PLF (*i.e.*, the accumulation of collagen surrounding AT lobules) was categorized as follows: PLF 0: no or very limited PLF (thickness clearly less than the diameter of one average adipocyte); PLF 1 (moderate): thickness similar to the diameter of one average adipocyte; and PLF 2 (severe): thickness similar to the diameter of two average adipocytes. PCF (*i.e.*, the accumulation of collagen around adipocytes localized in the depth of the AT lobules) was categorized as follows: PCF 0: no PCF; PCF 1 (moderate): thin PCF or thick PCF but no "trapped adipocytes" (*i.e.*, adipocyte surrounded by PCF); and PCF 2 (severe): thick PCF and presence of trapped adipocytes.

A combined semiquantitative scAT fibrosis score [fibrosis score of adipose tissue (FAT score)] was then attributed to each patient according to the following four stages: stage 0: no PLF and no PCF; stage 1: moderate PLF and/or moderate PCF; stage 2: severe PLF or severe PCF; and stage 3: severe PLF and severe PCF.

The definitions of the different grades of PLF, PCF, and FAT score stages are detailed in Fig. 2.

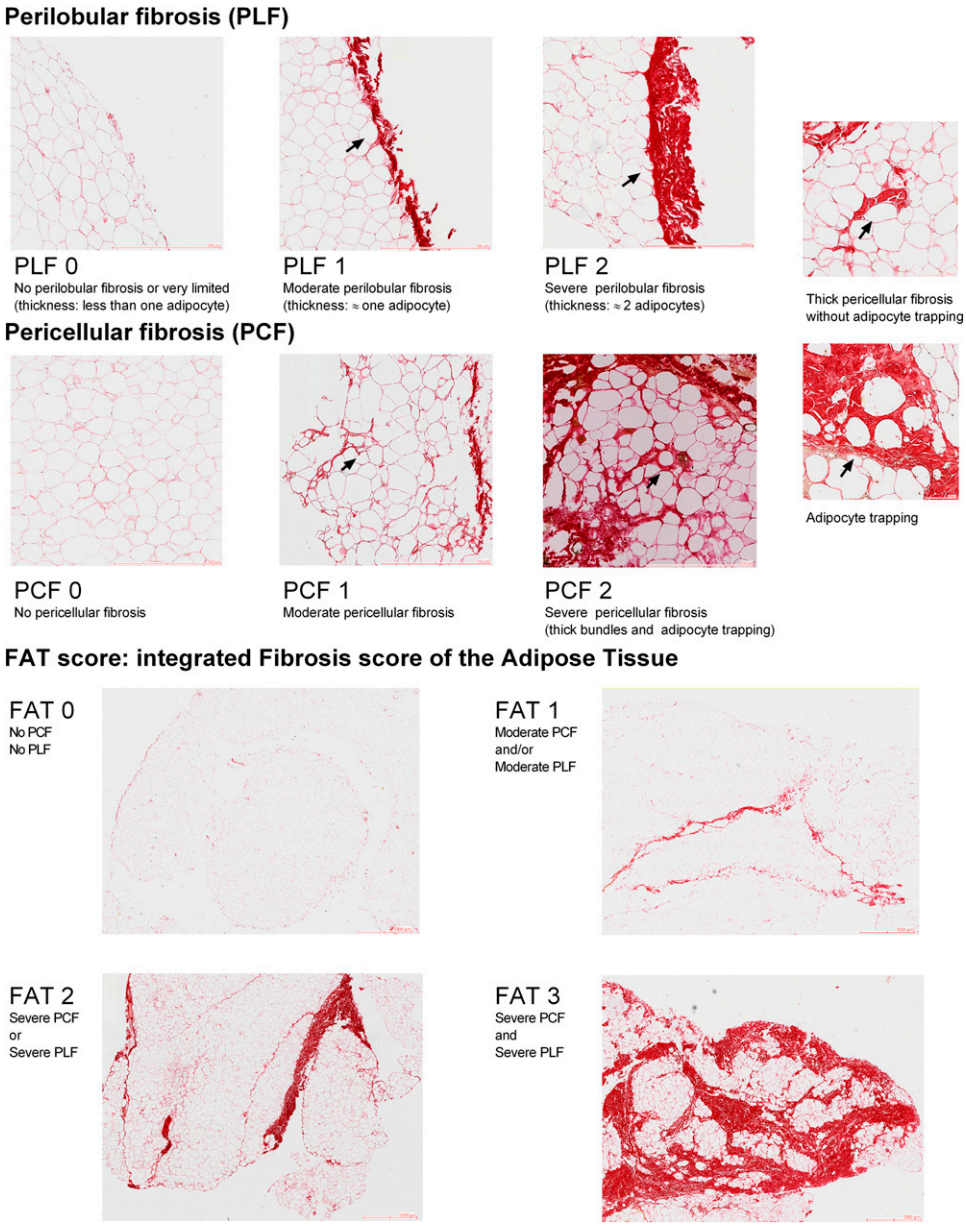

Figure 2. The FAT score. Images are perioperative scAT biopsy specimens stained with picrosirius red to reveal fibrosis (*i.e.*, collagen accumulation).

AT slides were analyzed and scored independently by four observers blind to clinical data (*i.e.*, P.B., J.T., F.C., and J.A.-W.). For slides without a unanimous agreement, a consensual score was obtained by subsequent analysis of the slide by all observers. Total fibrosis (determined by picrosirius-red quantification) was used as a reference to validate our semiquantitative scoring.

Accumulation of scAT macrophages

Total AT macrophages were detected by immunochemistry using an automated immunochemistry stainer BOND-MAX (Leica Biosystems, Newcastle, United Kingdom) with CD68 cell-specific staining (KP1 clone antibodies; Dako, Glostrup, Denmark) (24). CLSs were defined as three or more CD68-positive cells surrounding an adipocyte, as described previously (11), and CLS density was further defined as the number of CLSs per square centimeter of AT. M2 AT macrophage density was measured using CD163 (EDHu-1 clone; Biorad, Marnes-la-Coquette, France) cell-specific antibodies (25).

Adipocyte size

Mean adipocyte surface was measured on each slide on two randomly placed 1000-μm × 1000-μm squares, using a dedicated color-detection program, Histolabs (HEWEL, Paris, France) with a semiautomatic object detection and manual correction when necessary.

Statistical analysis and model construction

Data are expressed as mean ± standard deviation; categorical variables are given as numbers and percentages. Continuous data with no Gaussian distribution were log-transformed. Baseline characteristics of the two independent patient groups were compared using χ^2 test for categorical

data and Student *t* test for continuous data. Weighted κ scores were used to measure the degree of interobserver agreement and were estimated using intraclass correlation coefficient (26). Clinical and biological predictors of poor response were assessed by computing univariate odds ratios (ORs) in our cohort of 564 RYGB patients. ORs for a poor weight-loss response (PR) were calculated for each stage of the FAT score before and after adjustment for potential confounders. All analyses were conducted using R software version 3.0.3 (http://www.r-project.org) and GraphPad Prism 6.0. (GraphPad Software, La Jolla, CA). *P* values <0.05 were considered to denote statistically significant differences.

Predictive scoring models based on clinical and histological data were constructed using a machine-learning method that simultaneously learns which variable to select, its optimal binning, and its optimal weights to produce an optimal score (*i.e.*, the weight-loss response). To learn bins and weights, we minimized an empirical risk on the cohort. The classification algorithm we used is a sparse support vector machine. We add integrity constraints to our task so that the resulting weights are integers. The optimization problem is solved with linear integer programming. We use the IBM ILOG CPLEX Optimization Studio (IBM, Armonk, NY), which is a state-of-the-art solver for constrained optimization. We ran 10-fold cross validation to account for overfitting. The computations were done with R version 3.1.3 and the "Rcplex" package (interface to the IBM CPLEX Studio).

Results

FAT score: an easy semiquantitative measure of scAT fibrosis

Using our new semiquantitative FAT score, we staged scAT slides from 183 patients (pooled cohort). Patients' baseline clinical characteristics are shown in Table 1 and demonstrate no relevant differences between the training and validation cohorts. The interobserver agreement for the FAT score, before the consensual analysis, was good for the overall stages (κ = 0.76) but performed better for the PLF (κ = 0.75) than for the PCF (κ = 0.63). The FAT score stages distribution was similar between the training and validation groups, as shown in Fig. 3(a). FAT score stages were associated with increasing total collagen accumulation measured by picrosirius-red staining [*P* for linear trend < 0.001; Fig. 3(b)].

Table 1. Baseline Characteristics of the Scored Patients and 1-Year Weight Loss

Characteristic	Pooled Cohort (n = 183)	Training Cohort (n = 85)	Validation Cohort (n = 98)	*P* Value[a]
Age, y	42.9 ± 12.3	43.2 ± 11.9	42.6 ± 12.6	0.72
Female patients, %	75.8	83.5	74.5	0.20
Diabetes status, %				0.65
Nondiabetic	61.2	63.5	59.2	
Diabetic	38.8	36.5	40.8	
Hypertension, %	47.5	43.5	51.0	0.36
Sleep apnea syndrome, %	62.8	58.8	66.3	0.34
Body composition				
BMI, kg/m^2	46.6 ± 6.4	46.2 ± 6.6	47.0 ± 6.3	0.41
Total body fat mass, %	48.2 ± 5.8	48.9 ± 5.4	47.6 ± 6.1	0.13
Serum markers				
Fasting glycemia, mmol/L	6.4 ± 2.4	6.3 ± 2.4	6.5 ± 2.4	0.46
HbA1C, %	6.46 ± 1.34	6.36 ± 1.37	6.54 ± 1.32	0.37
Cholesterol, mmol/L	4.73 ± 0.94	4.86 ± 0.91	4.61 ± 0.96	0.08
HDL cholesterol, mmol/L	1.15 ± 0.34	1.19 ± 0.34	1.12 ± 0.35	0.14
LDL cholesterol, mmol/L	2.87 ± 0.88	2.98 ± 0.85	2.78 ± 0.91	0.14
Triglyceride level, mmol/L	1.56 ± 0.89	1.51 ± 0.69	1.6 ± 1.03	0.87
ASAT, IU/L	30.3 ± 12.5	30.4 ± 14.2	30.2 ± 10.7	0.80
ALAT, IU/L	35.4 ± 22.6	33.4 ± 21.9	37.2 ± 23.1	0.15
GGT, IU/L	44.1 ± 31.8	41.6 ± 28.6	46.3 ± 34.4	0.36
Leptin, ng/mL	51.9 ± 28.8	47.9 ± 23.2	55.5 ± 32.8	0.25
Adiponectin, μg/mL	4.8 ± 2.9	4.9 ± 3.2	4.7 ± 2.6	0.90
IL-6, pg/mL	4.0 ± 2.4	3.6 ± 1.7	4.4 ± 2.8	**0.04**
CRP, mg/L	8.5 ± 7.1	8.3 ± 7.6	8.6 ± 6.8	0.55
1-year weight loss				
Absolute, kg	40.0 ± 12.5	38.4 ± 11.1	41.5 ± 13.5	0.09
Relative, % from baseline	30.9 ± 7.7	30.6 ± 7.5	31.1 ± 8.0	0.63
Poor responders (weight loss <28%), %	36.1 (n = 66)	37.6 (n = 32)	34.7 (n = 34)	0.79

Results are given as mean ± standard deviation.

Abbreviations: ALAT, alanine transaminase; ASAT, aspartate aminotransferase; CRP, C-reactive protein; GGT, γ-glutamyl transferase; HbA1c, hemoglobin A1C; HDL, high-density lipoprotein; IL-6, interleukin-6; LDL, low-density lipoprotein.

[a]Comparison between training cohort and validation cohort, Student *t* test *P* value results for continuous data after log-transformation of skewed variables and χ^2 test *P* value results for categorical variables.

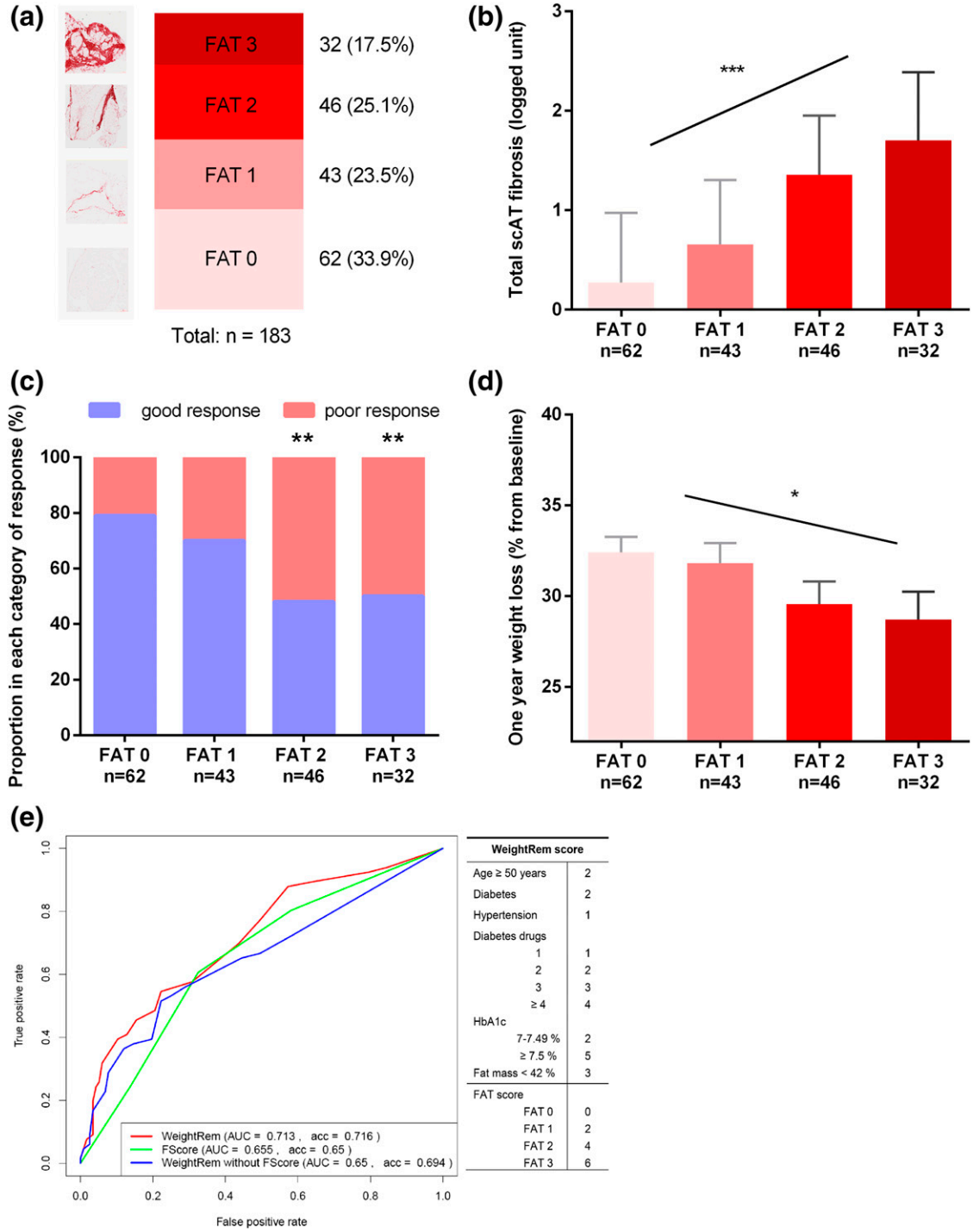

Figure 3. FAT score: association with scAT fibrosis and weight loss prediction. (a) Distribution of patients across FAT score stages. (b) Relationship between FAT score and scAT fibrosis picrosirius-red quantification. ***P for linear trend < 0.001. Total scAT fibrosis: quantification of picrosirius-red staining. (c) FAT score and post-RYGB weight-loss response. Poor response defined as 12-month total weight loss <28%. **OR for PR, >1 ($P < 0.01$). (d) FAT score and post-RYGB, 12-month percent weight loss. *P for linear trend < 0.05. Bars represent mean, lines represent standard error the mean. (e) Receiver operating characteristic curves of the clinical, histological, and combined scores for prediction of weight-loss response. acc, accuracy; AUC, area under the curve; HbA1c, hemoglobin A1C.

FAT score associates with increased M2 macrophage accumulation

We further examined whether the FAT score was linked with other features of AT alterations. The FAT score stages were associated with increasing CD163 stained-cell density ($P = 0.04$). Conversely, CLS density was negatively associated with the FAT score ($P = 0.02$), but no association was found between CD68 stained-cell density and FAT score ($P = 0.25$). These results suggest a link between higher scAT fibrosis and M2

macrophage accumulation in scAT. There was no association between FAT score and adipocyte size (Supplemental Fig. 1).

FAT score is associated with a poorer weight-loss response after RYGB

One year after RYGB, in the pooled cohorts (n = 183), there was an average difference of 17 kg in weight loss between the PRs and the good responders [respectively, 22.7% ± 3.6% weight loss (29.2 ± 6.0 kg) vs 35.6% ± 5.1% weight loss (46.2 ± 10.0 kg); Table 1]. We observed a significant negative relationship between the FAT score severe stages and the weight-loss response 1 year after RYBG [Fig. 3(c) and 3(d)]. This association between FAT score and PR was first observed in the training cohort [OR for PR, 4.1; 95% confidence interval (CI), 1.6 to 10.6; P = 0.003] and was subsequently confirmed in the independent validation cohort (OR for PR, 2.7; 95% CI, 1.2 to 6.5; P = 0.024). In the pooled cohort of 183 patients, a baseline FAT score ≥2 [FAT score of 2 or 3 (*i.e.*, severe stages)] was associated with a threefold increased risk of PR (OR for PR, 3.2; 95% CI, 1.7 to 6.1; $P < 0.001$; Table 2). We did not observe any difference between a FAT score of 2 and a FAT score of 3 in terms of association with PR [Fig. 3(c)].

Macrophage infiltration is not associated with weight-loss response

We then explored whether other markers of AT alteration could be used as biopredictors of weight loss. We first investigated CLS density because it can be quickly measured in the laboratory setting; however, it was not associated with PR in the training, validation, or pooled cohorts (pooled cohort OR, 0.96; 95% CI, 0.89 to 1.0; P = 0.13). Similarly, markers of total macrophage density, M2 macrophage density, and adipocyte size were not significantly associated with a PR [OR (95% CI), respectively, for CD68 density, CD163 density, and mean adipocyte surface: 1.29 (0.82 to 2.06), 1.23 (0.78 to 1.97), and 0.81 (0.5 to 1.27)].

FAT score associates with poorer weight loss independently of other predictive factors

To examine the specific contribution of the FAT score in weight-loss prediction, we assessed whether its association with PR was independent of other clinical predictors. In our large cohort of 564 RYGB candidates, several parameters were associated with PR in univariate analysis: older age; diabetes status; hypertension status; increased number of antidiabetic drugs; higher hemoglobin A1c, fasting glycemia, and interleukin-6 (IL-6) levels; lower fat mass; and leptin levels; (Table 3), in line with previous observations (7, 8).

Because some of these variables were highly intercorrelated, we built a model for multivariate analysis restricting to age, diabetes, hypertension status, percent fat mass, IL-6 level, and the FAT score. Interestingly, after adjustment for these variables, a FAT score ≥2 remained significantly and independently associated with PR [pooled cohort adjusted OR, 3.6; 95% CI, (1.8 to 7.2); P = 0.003; Table 2]. Consistently, we did not find any differences in terms of baseline clinical variables

Table 2. FAT Score and Weight-Loss Response to RYGB

	Univariate Model					
	Training Cohort (n = 85)		Validation Cohort (n = 98)		Pooled Cohort (n = 183)	
FAT Score	OR (95% CI)[a]	*P* Value	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value
0	ref	ref	ref	ref	ref	ref
1	1.4 (0.4–4.9)	0.63	1.9 (0.5–7.1)	0.30	1.6 (0.7–4.0)	0.28
2	4.6 (1.4–16.1)	0.013[b]	3.8 (1.2–13.4)	0.026[b]	4.1 (1.8–9.8)	<0.001[b]
3	4.8 (1.9–21.3)	0.031[b]	3.4 (1.0–12.5)	0.055	3.8 (1.5–9.7)	0.004[b]
<2	ref	ref	ref	ref	ref	Ref
≥2	4.1	<0.001[b]	2.7	0.023[b]	3.2 (1.7–6.1)	<0.001[b]
			Multivariate model[c]			
0	ref	ref	ref	ref	ref	ref
1	1.6 (0.4–7.4)	0.53	1.8 (0.4–8.1)	0.41	1.7 (0.6–4.7)	0.33
2	7.0 (1.4–16.1)	0.008[b]	3.9 (1.1–15.7)	0.046[b]	4.8 (1.9–12.9)	<0.001[b]
3	5.3 (1.1–29.5)	0.044[b]	3.9 (1.0–17.0)	0.060	4.0 (1.5–11.7)	0.008[b]
<2	ref	ref	ref	ref	ref	ref
≥2	5.1 (1.8–16.3)	0.003[b]	2.9 (1.1–7.7)	0.028[b]	3.6 (1.8–7.2)	0.003[b]

Abbreviation: ref, reference.

[a] ORs for predicting poor response to RYGB.

[b] $P < 0.05$.

[c] Adjusted for age, diabetes, hypertension, IL-6 level, and percent fat mass.

Table 3. Baseline Parameters Associated With Poorer Weight-Loss Response[a] to RYGB

Characteristics	RYGB Candidates (n = 564)	No.[b]	Standardized OR[b] (95% CI)	*P* Value	*P* Value[f]
Age, y	42.1 ± 11.9	564	1.34 (1.12–1.6)	0.002[d]	0.006[d]
Female patients, %	74.6	564	0.71 (0.48–1.07)	0.09	0.17
Diabetes status, %					
Nondiabetic	61.6		ref	ref	ref
Diabetic	38.4	564	2.07 (1.45–2.97)	<0.001[d]	0.001[d]
Treatment, %					
No treatment	13.9		ref	ref	ref
1 drug	66.1		3.3 (1.3–9.1)	0.015[d]	0.018[d]
≥3 drugs	20.0	217	5.8 (2.1–18.3)	0.001[d]	0.002[d]
Comorbidities, %					
Hypertension	50.0	557	1.81 (1.27–2.6)	0.001[d]	0.006[d]
Sleep apnea syndrome	63.6	555	1.16 (0.8–1.69)	0.43	0.50
Body composition					
Body mass index, kg/m^2	47.6 ± 6.9	564	0.93 (0.78–1.11)	0.44	0.51
Total body fat mass, %	48.3 ± 5.3	499	0.71 (0.59–0.86)	<0.001[d]	0.002[d]
Serum markers					
Fasting glycemia, mmol/L	6.3 ± 2.33	559	1.38 (1.16–1.64)	<0.001[d]	0.002[d]
HbA1C, %	6.48 ± 1.34	517	1.45 (1.21–1.75)	<0.001[d]	0.001[d]
Cholesterol, mmol/L	4.75 ± 0.94	551	0.9 (0.75–1.07)	0.23	0.33
LDL cholesterol, mmol/L	2.88 ± 0.85	552	0.84 (0.7–1)	0.06	0.12
HDL cholesterol, mmol/L	1.15 ± 0.33	551	0.99 (0.83–1.18)	0.90	0.90
Triglyceride levels, mmol/L	1.55 ± 0.79	552	1.11 (0.93–1.33)	0.25	0.34
ASAT, IU/L	29.6 ± 13.1	556	1.07 (0.89–1.27)	0.47	0.52
ALAT, IU/L	36.3 ± 23.5	556	0.98 (0.82–1.17)	0.85	0.89
GGT, IU/L	47.1 ± 40.2	557	1.12 (0.94–1.34)	0.19	0.29
Leptin, ng/mL	58.1 ± 29.9	551	0.79 (0.66–0.94)	0.009[d]	0.024[d]
Adiponectin, μg/mL	5.0 ± 3.0	537	0.85 (0.7–1.01)	0.072	0.14
IL-6, pg/mL	4.2 ± 2.9	520	1.23 (1.02–1.49)	0.031[d]	0.07
CRP, mg/L	9.3 ± 7.2	524	0.93 (0.77–1.12)	0.43	0.51

Results are given as mean ± standard deviation for continuous data and as percentage for categorical data. Skewed continuous data were log-transformed.

Abbreviations: ALAT, alanine transaminase; ASAT, aspartate aminotransferase; CRP, C-reactive protein; GGT, γ-glutamyl transferase; HbA1c, hemoglobin A1C; HDL, high-density lipoprotein; LDL, low-density lipoprotein; ref, reference.

[a]Poor response was defined as <28% weight loss at 12 months.

[b]No. for each variable represents the number of patients for whom data were available for the analysis.

[c]OR for poor response to RYBG.

[d]Benjamini-Hochberg false discovery rate adjusted *P* value of the OR.

[d]Adjusted $P < 0.05$.

(metabolic and morphologic) between the "low fibrosis" group of patients (FAT score, 0 or 1) and the "high fibrosis" group, in the scAT (FAT score, 2 or 3; Supplemental Table 1), which is in line with our previous findings (14).

Best prediction model of weight-loss response after RYGB: added value of the FAT score?

Our aim was to further build a scoring system to predict weight-loss response after BS and to assess the relevance of the FAT score in a multiparameter prediction score. For this purpose, we investigated the performance of three different scores built to predict PR: the FAT score; a weight loss prediction ("weight-rem") score including only a set of bioclinical predictors; and, finally, the weight-rem combined with the FAT score. The area under the curve for PR prediction was 0.65 for the weight-rem score, 0.66 for the FAT score, and 0.72 for the weight-rem plus the FAT score [Fig. 3(e)]. This indicates an added value of incorporating scAT pathology in scoring systems to predict weight-loss response 1 year after RYGB.

Discussion

Our current findings suggest the FAT score as a clinically applicable histological score to evaluate scAT fibrosis in a semiquantitative reproducible manner. Furthermore, we demonstrate its relevance in predicting patients' weight loss after BS: Severely obese subjects with a FAT score ≥2 before the intervention had a three- to fourfold increased risk of a poorer weight-loss response after RYGB surgery.

AT fibrosis has gained recognition as an important pathological alteration during obesity (10). Similar to

liver pathology (19), we hypothesized that defining stages of AT alterations would be important in examining large cohorts, for stratification of obese patients in research, and also in clinical care. We previously showed that the quantitative evaluation of picrosirius-red collagen accumulation at baseline is negatively associated with post-BS weight loss. However, this quantitative evaluation displays several pitfalls, as mentioned (*i.e.*, time for analysis, technical aspects, reproducibility, and sample heterogeneity). Conversely, our FAT score, based on a semiquantitative evaluation of PLF and PCF, is easily measured and reproducible, with good interobserver agreement [κ values were similar to those achieved in NAFLD scoring (19)].

Although BS induces major weight loss (27), the extent and trajectories of weight loss exhibit large interindividual variability (4). The weight-loss outcome depends on a combination of objective biological factors and psychological aspects (6–9), and the latter are sometimes difficult to evaluate and integrate into a predictive score. To date, obtaining an accurate weight-loss prediction remains arduous. Thus, to improve weight-loss prediction, we included a variable of scAT structural alterations into a predictive model. With this, we developed the FAT score, which is associated with a poorer weight-loss response independently of other known predictors (*i.e.*, age, diabetes, hypertension, IL-6 level, and percent fat mass). The predictive accuracy of our weight-rem plus FAT score is substantially higher than reported for previous models (7), which suggests that AT biology is an important element of an individual's weight-loss potential. Nevertheless, we are aware that this score successfully predicts the response for only 72% of the patients and, thus, still requires further improvement. This could be accomplished through inclusion of biological or psychological factors that are absent in our current model.

Our aim in creating a predictive score is not to replace the essential multidisciplinary discussions taking into account patients' medical and psychological information before deciding to proceed with BS. However, as already proposed for predicting T2D remission after BS (28), we believe that providing concrete biological evidence could help patients' information and, sometimes, decision making in complex cases. We believe that it would be preferable to have less-invasive diagnostic tools such as circulating biomarkers associated with scAT fibrosis, yet these systemic markers still need to be uncovered. On the other hand, perioperative scAT biopsy specimens do not add to the morbidity of the BS procedure; thus, measuring the FAT score to identify individuals at higher risk of PR could improve patient care. Indeed, in the context of precision medicine, this would allow intensifying the follow-up, nutritional advice, and/or psychological support. Whether these additional measures could be beneficial for these patients and improve weight loss outcomes requires further investigation.

We are aware that our study does not elucidate the mechanistic links between increased scAT fibrosis and reduced weight loss after BS. We hypothesize that AT structural alteration could directly impact AT plasticity, as suggested by (1) col VI KO mice gaining more weight than wild-type mice upon high-fat diet (17), (2) the negative association between scAT fibrosis and fat mass in obese patients (8), and (3) the positive association between scAT fibrosis and tissue physical stiffness measured with elastometry (8). Herein, we observed that our FAT score was associated with increasing CD163 cell infiltration [a marker associated with M2 macrophages (25)] but not CD68 cell infiltration [representing the overall AT macrophage infiltration (24)], thus showing a link between scAT fibrosis and a M2-like profile of immune cells, as previously described (29). Therefore, severe FAT score stages may be the signature of extensive tissue remodeling in obese patients who often have a history of repeated weight fluctuations, which could explain poorer weight-loss capacity.

Including immune cell infiltration (whether total macrophage, CLS, or M2 only) to the FAT score did not improve weight-loss prediction. It might be that distinct AT alterations are associated with different clinical outcomes. If scAT fibrosis is associated with limited fat-tissue expansion and weight-loss reduction, AT immune cell infiltration might play a more important role in the prediction of the post-BS metabolic improvement rather than weight loss. Indeed, omental macrophage infiltration is associated with NAFLD severity (30) and is improved after BS-induced weight loss (31). Likewise, CLSs are associated with insulin resistance and diabetes status independently of body mass index (11), and whether they could constitute predictors of diabetes remission has never been investigated (32) to our knowledge.

Our study presents some limitations. Inclusions were restricted to RYGB patients to avoid the potential confounding effect of surgery type on weight loss (20). Whether our results can be applied for other BS types, such as sleeve gastrectomy, will need further investigations. Weight-loss data were only available at 1 year after BS, which is not the weight nadir. We aim to further assess and confirm the predictive value of our score on longer term follow-up. Finally, our sample size was substantial for a designing stage of this histological score and comparable to previous work on other tissues (18), but it may have been too small to identify baseline clinical differences between high and low degrees of fibrosis, although none were found, using red-picrosirius quantification, in our previous studies (8, 14).

In conclusion, we designed a simple, semiquantitative evaluation of scAT fibrosis—the FAT score—which proved to be a good independent predictor of post-BS weight-loss outcomes. This score could constitute an AT pathology marker to improve patient characterization in obesity clinical research. In clinical care, the FAT score may contribute to the identification of BS poor responders and ultimately lead to more personalized patient follow-up.

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Author contributions: K.C., J.A.-W., and J.T. designed the study. K.C., J.A.-W., and C.P. contributed to patient recruitment and coordinated clinical investigation, patient phenotyping, and sample collection. L.G and J.-L.B. performed bariatric surgery procedures. P.B.L. and Y.L. conducted the experiments. P.B.L., Y.L., and G.L.N. performed numerical tissue analysis. P.B.L, F.C., J.A.-W., P.B., and J.T. contributed to adipose tissue scoring, and P.B.L., N.S., and J.-D.Z. analyzed data. P.B.L., Y.L., J.A.-W., and K.C. wrote the manuscript. All authors reviewed the manuscript. K.C. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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