ONLINE APPENDIX

EXPERIMENTAL PROCEDURES

Figure 1: Electron microscopic observation of WAT fibrosis.
Small fragments of tissue were fixed in 4% glutaraldehyde in 0.1M phosphate buffer, pH 7.4, for 4 h, post-fixed in 1% osmium tetroxide, and embedded in an Epon-Araldite mixture. Semithin sections (2µm) were stained with toluidine blue, and thin sections were obtained an ultratome, stained with lead citrate, and examined with transmission electron microscope.
We observed deposit of fibrin (F) **(a)** and well organized collagen I (Col) **(b)** between adipocyte. The panel **(c)** shows the presence of less organized fibbers, those evocate collagen VI fibbers (Col).
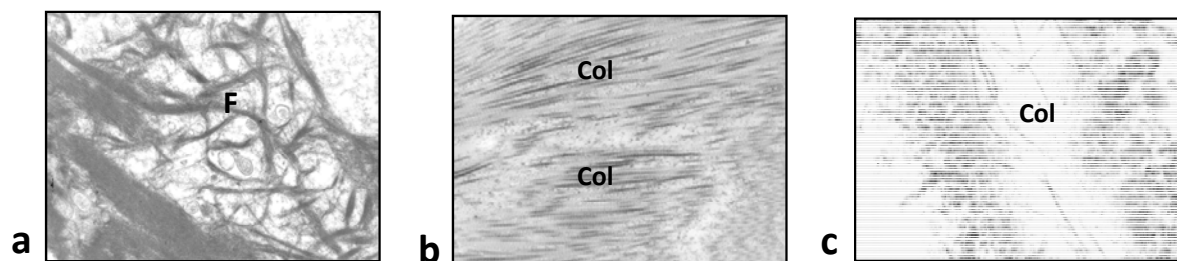

Figure 2: Immunohistochemistry of obese scWAT with a perilipin antibody.
scWAT biopsies were obtained during gastric in obese subject. Biopsies were fixed overnight at 4°C in 4 % paraformaldehyde and processed for standard paraffin embedding. Sections of 5 µm were stained as described below and observed under a Zeiss 20 Axiostar Plus microscope (Zeiss, Germany). We used a perilipin antibody (Lifespan Biosciences, Seattle, USA). Method specificity tests were performed by omission of primary antibodies.

Obese adipose tissue section was stained with a perilipin antibody. Positive staining appeared in brown. Black arrow indicates adipocyte perilipin positive and open arrow indicates adipocyte perilipin negative. This slide is representative of 5 independent experiments.
F: Fibrosis.

Adipocyte diameters measure in parenchyma and in fibrotic areas.
Digital photomicrographs of H&E slides were analyzed using PerfectImage Software (Claravision, France). We measured the diameter of all adjacent adipocytes from each of 4 separate photographs of the same slide in parenchyma, at distance from fibrosis area. The diameter of adipocytes close or within fibrosis was measured on some slides. These measures were performed at x 10 magnification, with 200-300 adipocyte diameters measured for each biopsy. The data were transferred to an Excel program to calculate the mean adipocyte diameter and standard deviation for each sample.

RESULTS

Table 1: Clinical parameters of lean and obese subjects

Clinical parameters	Obese subjects (n=65)	Lean subjects (n=9)	p value
Age (years)	39.9 ± 1.4	46.0 ± 4.2	NS
Gender (M/F)	15/50	2/7	NS
Adiposity markers			
BMI (kg/m²)	48.2 ± 0.8	22.8 ± 0.6	<0.0001
Body weight (kg)	135.0 ± 3.0	59.4 ± 1.6	<0.0001
Fat mass %	46.2 ± 0.69	ND	
Leptin (ng/ml)	63.0 ± 3.1		
Plasma Glucose homeostasis			
Glycaemia (mmol/l)	6.55 ± 0.37	5.10 ± 0.21	
Insulinemia (μU/mL)	19.02 ± 1.81		
QUICKI	0.31 ± 0.003		
Diabetic	32.3 %	0%	
Adiponectin (μg/ml)	6.08 ± 0.36		
Plasma lipid homeostasis			
Total cholesterol (mmol/l)	5.00 ± 0.12	4.80 ± 0.55	NS
Total triglycerides (mmol/l)	1.53 ± 0.1	1.10 ± 0.27	<0.0001
HDL cholesterol (mmol/l)	1.28 ± 0.05	1.3 ± 0.22	NS
Liver test			
AST (IU/L)	24.5 ± 1.4	24.2 ± 6.4	NS
ALT (IU/L)	35.4 ± 3.4	24.5 ± 6.8	NS
γGT (IU/L)	48.9 ± 5.2	16.7 ± 3.31	<0.0001
Inflammatory markers			
Plasma hsCRP (mg/dl)	0.91 ± 0.08		
Plasma IL-6 (pg/ml)	3.94 ±0.32		
Adipose tissue phenotype			
Sc adipocyte diameters (μm)	75.14 ± 1.16		
ATM scWAT (%)	14.70 ± 0.99		
Om adipocyte diameters (μm)	66.83 ± 1.25		
ATM oWAT (%)	28.78 ± 1.82		

NS: not significant; ND: not determined

Table 2: Multiple linear regression modeling

Triglycerides	F ratio	p value
Log total oWAT fibrosis	**7.51**	**0.008**
oWAT adipocyte size	**6.79**	**0.012**
Diabetic status	3.00	0.089
Log age	0.22	0.640
Log BMI	0.02	0.892
Gender	<0.01	0.981

Association between triglyceride level and total oWAT fibrosis remains significant after adjustment with gender, diabetic status, age, BMI and omental adipocyte size (least squares means analysis).

Table 3: Clinical parameters in cluster of fat mass loss

	Group B	Group A	Group C	Anova
Age (year)	37.6 ± 2.91	39.6 ± 2.43	45.2 ± 3.79	NS
Gender (F/M)	11/4	20/1	8/1	NS
Adiposity markers				
Body Weight(kg)	130 ± 4.17	121 ± 3.50	133 ± 5.30	NS
BMI (kg/m²)	44.5 ± 1.33	45.6 ± 1.13	50.0 ± 1.72[*†]	0.044
Fat mass (%weight)	43.8 ± 1.12	46.5 ± 0.95	48.5 ± 1.44[*]	0.040
Leptin (ng/ml)	53.2 ± 5.95	65.8 ± 5.03	71.2 ± 7.68	NS
Glucose homeostasis				
Glycaemia (mmol/l)	5.19 ± 0.39	5.90 ± 0.33	6.41 ± 0.50	NS
Insulinemia (µU/mL)	16.5 ± 4.19	24.1 ± 3.54	16.6 ± 5.41	NS
Adiponectin (µg/ml)	6.20 ± 0.56	5.07 ± 0.49	7.10 ± 0.73	NS
Diabetic (%)	27	33	44	NS
Lipids				
Cholesterol (mmol/l)	4.86 ± 0.25	5.17 ± 0.22	4.65 ± 0.33	NS
Triglycerides (mmol/l)	1.26 ± 0.10	1.35 ± 0.09	1.29 ± 0.13	NS
HDL cholesterol (mmol/l)	1.36 ± 0.19	1.56 ± 0.16	1.45 ± 0.24	NS
Liver test				
AST	22.0 ± 2.48	24.6 ± 2.13	22.7 ± 3.09	NS
ALT	31.6 ± 6.09	37.0 ± 5.23	27.2 ± 7.60	NS
GGT	51.6 ± 11.7	49.6 ± 10.3	41.3 ± 14.6	NS
Inflammatory markers				
Plasma hsCRP (mg/l)	0.79 ± 0.17	0.89 ± 0.13	0.72 ± 0.22	NS
Plasma IL-6 (pg/ml)	2.68 ± 0.66	3.81 ± 0.55	6.04 ± 0.84[*†]	0.013

[*] Significant difference between C and B;
[†] Significant difference between C and A.
NS: not significant
Group C corresponds to the group of subjects experiencing the lowest loss of fat mass.
Group B corresponds to the group of subjects experiencing the highest loss of fat mass.

Table 4: Multiple linear regression modeling.

		total fibrosis		pericellular fibrosis	
		t-value	*p-value*	*t-value*	*p-value*
% of fat mass loss at 3M	fibrosis	- 2.62	0.012	- 1.69	0.098
	IL-6	- 2.13	0.039	- 2.32	0.024
% of fat mass loss at 6M	fibrosis	- 2.10	0.041	- 1.97	0.055
	IL-6	- 2.88	0.006	- 3.12	0.003
% of fat mass loss at 12M	fibrosis	- 1.80	0.078	- 1.92	0.062
	IL-6	- 1.95	0.057	- 2.12	0.039

Association between the percentage of fat mass loss at 3M, 6M and 12M post-surgery and total scWAT fibrosis remains significant after adjustment with circulating IL-6 level. The same association appears weaker with pericellular scWAT fibrosis.
t-value: ratio of the parameter estimate to its standard error.