Accumulation and Changes in Composition of Collagens in Subcutaneous Adipose Tissue Following Bariatric Surgery

Article in *The Journal of Clinical Endocrinology and Metabolism* · November 2015

DOI: 10.1210/jc.2015-3348

13 authors, including:

Adriana Torcivia
Assistance Publique – Hôpitaux de Paris
30 PUBLICATIONS 314 CITATIONS

Brigitte Bauvois
French Institute of Health and Medical Rese...
104 PUBLICATIONS 2,234 CITATIONS

Veronique Miette
65 PUBLICATIONS 599 CITATIONS

All content following this page was uploaded by Sandrine Bouchet on 23 November 2015.

The user has requested enhancement of the downloaded file. All in-text references underlined in blue are linked to publications on ResearchGate, letting you access and read them immediately.
Accumulation and Changes in Composition of Collagens in Subcutaneous Adipose Tissue Following Bariatric Surgery

Yuejun Liu¹,²,³,⁴, Judith Aron-Wisnewsky¹,²,³,⁵, Geneviève Marcelin¹,²,³, Laurent Genser¹,²,³,⁶, Gilles Le Naour⁷, Adriana Torcivia⁶, Brigitte Bauvois⁸, Sandrine Bouchet⁸, Véronique Pelloux¹,²,³, Magali Sasso⁴, Véronique Miette⁴, Joan Tordjman¹,²,³, Karine Clément¹,²,³,⁵

¹. Institute of Cardiometabolism and Nutrition, ICAN, F-75013, Paris, France; 2. INSERM, UMRS 1166, Nutriomic team 6, Paris, F-75013 France; 3. Sorbonne Universités, UPMC Université Paris 06, UMRS 1166, F-75005, Paris, France; 4. Echosens Research Department, Paris, France; 5. Assistance Publique-Hôpitaux de Paris (AP-HP), Pitié-Salpêtrière Hospital, Nutrition Department, Paris, F-75013 France; 6. Assistance Publique-Hôpitaux de Paris (AP-HP), Pitié-Salpêtrière Hospital, Department of Digestive and Hepato-Pancreato-Biliary Surgery, Paris, F-75013 France; 7. Assistance Publique-Hôpitaux de Paris (AP-HP), Pitié-Salpêtrière Hospital, Department of Pathology, UIMAP, UPMC Université Paris 06, Paris, F-75013 France; 8. Centre de Recherche des Cordeliers, INSERM UMRS 1138, Sorbonne Universités UPMC Paris 06, Université Paris Descartes Sorbonne Paris Cité, F-75006 Paris, France

Context: Extracellular matrix (ECM) in subcutaneous adipose tissue (scAT) undergoes pathological remodeling during obesity. However, its evolution during weight loss remains poorly explored.

Objective: To study histological, transcriptomic and physical characteristics of scAT ECM remodeling during the first year of bariatric surgery (BS)-induced weight loss and their relationships with metabolic and bioclinical improvements.

Design, Setting, Patients and Interventions: 118 morbidly obese candidates for BS were recruited and followed during one-year post-BS.

Main Outcome Measures: ScAT surgical biopsy and needle aspiration, as well as scAT stiffness measurement were performed in three sub-groups before and post-BS. 14 non-obese non-diabetic subjects served as controls.

Results: Significantly increased picrosirius-red stained collagen accumulation in scAT post-BS was observed along with fat mass loss, despite metabolic and inflammatory improvements and undetectable changes of scAT stiffness. Collagen accumulation positively associated with M2-macrophages (CD163⁺ cells) before BS but negatively after. Expression levels of genes encoding ECM components (e.g. COL3A1, COL6A1, COL6A2, ELN), cross-linking enzymes (e.g. LOX, LOXL4, TGM), metalloproteinases and their inhibitors were modified one-year post-BS. LOX expression and protein were significantly decreased, and associated with decreased fat mass, as well as other cross-linking enzymes. Although total collagen I and VI staining decreased one-year post-BS, we found increased degraded collagen I and III in scAT, suggesting increased degradation.

Conclusions: After BS-induced weight loss and related metabolic improvements, scAT displays major collagen remodeling with an increased picrosirius-red staining that relates to increased collagen degradation and importantly decreased cross-linking. These features are in agreement with adequate ECM adaptation during fat mass loss.
The extracellular matrix (ECM) in subcutaneous adipose tissue (scAT) undergoes substantial pathological remodeling during obesity. ECM accumulation, usually called fibrosis, is defined as an excessive deposition of ECM components (mainly cross-linked collagens) and impaired degradation (1). ECM accumulation is important in the regenerative step where it replaces damaged cells. However, if the damage persists, excessive ECM deposition harms tissue homeostasis and function (2). In obesity, scAT ECM accumulation reduces tissue plasticity and results in adipocyte dysfunction, ectopic fat storage, and metabolic disorders (1). Studies have shown the detrimental consequences of ECM accumulation in obesity and their associations with comorbidities. In mice, genetic ablation of MT1-MMP, a membrane anchored metalloproteinase degrading collagen I, leads to increased peri-adipocyte fibrosis and severe metabolic complications such as hepatic steatosis (3). Likewise, Collagen VI accumulation in obesity is associated with insulin resistance (4, 5). By contrast, the absence of collagen VI in high-fat diet or ob/ob mice results in uninhibited adipocyte expansion and associates with metabolic and inflammatory improvements (6). In obese subjects, scAT fibrosis is increased (7, 8). Moreover, higher scAT fibrosis at baseline is associated with lower weight loss one year postbariatric surgery (BS) (7, 9). In addition, scAT pericellular fibrosis is associated with liver fibrosis, suggesting that obesity is a profibrotic condition (9). Finally, the pericardial fat secreome was found to promote myocardial fibrosis (10). Overall, these studies underline the potential deleterious effects of obesity-induced scAT ECM accumulation.

Mechanistically, scAT fibrosis leads to adipocyte dysfunction and fibro-inflammation through a mechanotransduction pathway (11). Lysyl oxidase (LOX), an important matrix fibers’ cross-linking enzyme, contributes to tissue mechanical properties (12). In AT, LOX expression is up-regulated in high-fat diet or ob/ob mice. By contrast, inhibition of LOX activity leads to improved metabolism and inflammation (13). In obese subjects, scAT LOX expression is also increased (11). ScAT stiffness, measured noninvasively by transient elastography, associates with picrosirius-red stained scAT fibrosis and altered glucose homeostasis (9).

ECM turnover, a crucial process during excess ECM accumulation, is predominately regulated by the balance between matrix metalloproteinases (MMPs) and their endogenous tissue inhibitor of metalloproteinases (TIMPs). In obesity, a new relationship between MMPs and TIMPs is established and enables tissue remodeling. Enzymes (eg, MMP-3, -9, -11, -12, -13, -16, and -24) are expressed at low level in scAT, but are rapidly up-regulated during obesity, which eventually favors scAT expansion (1). Weight loss represents another condition that induces scAT remodeling, exhibiting by changes in expression of many ECM genes soon after BS (8, 14). Some studies have shown increased ECM deposition, eg, up-regulated collagens, particularly COL6A3, after major weight loss in a long term (14, 15). However, most of these studies focused on selected collagens at expression levels and did not explore the overall ECM characteristics. Furthermore, no study has yet evaluated the links between scAT ECM remodeling, stiffness, and modifications in cross-linking enzymes, and improved metabolic parameters after weight loss.

Herein, we examined fibrillar collagen accumulation, synthesis, and degradation as well as cross-linking enzymes, macrophage infiltration and scAT stiffness during the first year post-BS. We also analyzed relationships between ECM properties and metabolic and inflammatory parameters improvements observed post-BS.

Materials and Methods

Study Population

A total of 118 morbidly obese candidates for BS who met the recruitment criteria as previously described (7) were enrolled at the Institute of Cardiometabolism and Nutrition (ICAN), Nutrition Department and operated in the Department of Surgery, Pitié-Salpêtrière Hospital (Paris). Due to the difficulties to obtain large amount of scAT surgical biopsy sample in every subject during the follow-up and the number of experiments needed to perform on these samples, we divided our overall cohort into 3 groups according to different scAT measurements that were realized (study flowchart see Figure 1). However, subjects were part of the same prospective cohort and baseline (T0) characteristics were not significantly different (Table 1).

Group1 subjects (n = 52, age 40.1 ± 10.2yr, female n = 37 (71%), BS procedures: gastric banding (GB) n = 8 (15%), sleeve gastrectomy (SG) n = 16 (31%), Roux-en-Y gastric bypass (RYGB) n = 28 (54%)) accepted surgical scAT biopsy before (T0), 3 months (T3) and 12 months (T12) post-BS. Surgical biopsy was performed under local anesthesia in peri-umbilical area as described (15, 16). The collected scAT samples were used for explant experiments and histological analysis.

Group2 (n = 35, age 38.0 ± 10.0yr, female n = 24 (69%), BS procedures: GB n = 3 (8%), SG n = 16 (46%), RYGB n = 16 (46%)) underwent at T0, T3, and T12 scAT stiffness measurement (see below). A subgroup of 14 nondiabetic women from Group2 underwent scAT needle aspiration for RT-PCR analysis. Notably, 11 individuals with stiffness measurement were also part of Group1.

Group3 (n = 42, age 42.9 ± 10.5yr, female n = 42 (100%), BS procedures: RYGB n = 42 (100%)) underwent scAT needle aspiration at T0 and T12 for microarray analysis.

14 nonobese nondiabetic subjects (age = 41.6 ± 14.1yr, female 29%, BMI = 23.2 ± 3.3 kg/m²), who had elective abdominal programmed surgery without inflammatory diseases as described (7), were recruited as a control group. Perioperative scAT biopsy samples were collected in the same location as in obese
Table 1. Clinical and biological parameters of the study population

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group 1 (n = 52)</th>
<th>T0</th>
<th>T3</th>
<th>T12</th>
<th>Group 2 (n = 35)</th>
<th>T0</th>
<th>T3</th>
<th>T12</th>
<th>Group 3 (n = 42)</th>
<th>T0</th>
<th>T12</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (kg/m²)</td>
<td>45.8 ± 6.8</td>
<td>38.6 ± 6.0##</td>
<td>33.1 ± 6.0##</td>
<td>46.9 ± 7.7</td>
<td>39.2 ± 6.1##</td>
<td>33.1 ± 5.4##</td>
<td>47.1 ± 6.0</td>
<td>33.0 ± 5.0##</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type-2 Diabetes N (%)</td>
<td>15 (29)</td>
<td>7 (13)</td>
<td>8 (15)</td>
<td>12 (34)</td>
<td>4 (11)##</td>
<td>3 (10)##</td>
<td>14 (33)</td>
<td>4 (11)##</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension N (%)</td>
<td>16 (31)</td>
<td>16 (31)</td>
<td>12 (23)</td>
<td>13 (37)</td>
<td>10 (29)</td>
<td>12 (29)</td>
<td>12 (29)</td>
<td>8 (19)##</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sleep Apnea N (%)</td>
<td>31 (60)</td>
<td>24 (46)</td>
<td>15 (29)##</td>
<td>19 (54)</td>
<td>17 (48)</td>
<td>12 (29)</td>
<td>24 (57)</td>
<td>7 (17)##</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body composition</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>57.96 ± 12.98</td>
<td>48.73 ± 11.93##</td>
<td>36.40 ± 11.03##</td>
<td>61.75 ± 12.25</td>
<td>50.37 ± 11.00##</td>
<td>37.73 ± 9.29##</td>
<td>61.44 ± 11.67</td>
<td>36.01 ± 10.93##</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat-free mass (kg)</td>
<td>58.05 ± 6.03</td>
<td>51.12 ± 6.08##</td>
<td>57.34 ± 6.80##</td>
<td>49.09 ± 5.90</td>
<td>51.15 ± 5.57##</td>
<td>50.07 ± 6.67##</td>
<td>47.92 ± 3.44</td>
<td>57.32 ± 4.74##</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as means ± SD (standard deviation), unless otherwise stated. hsCRP, highly sensitive C-reactive protein; HOMA-IR, homeostatic model assessment-insulin resistance; HOMA-B (%), β cell function; HOMA-S (%): insulin sensitivity. There are 11 common subjects in Group 1 and Group 2. For continuous data, repeated ANOVA was used to compare three time points in Group 1 and Group 2, when p values of ANOVA were < 0.05. Holm-Sidak’s multiple comparison test was used to compare each of the two time points; student’s t test was used in Group 3. For categorical data, Fisher’s exact test was used. * P < 0.05 when compared to T0 in each group, # P < 0.05 when compared to T3 in Group 1 and Group 2. Subjects’ baseline characteristics (T0) in three groups were compared by ANOVA for quantitative data and by Fisher’s exact test for qualitative data. No significant differences were found except the BS type.

Clinical, Anthropological and Biological Parameters

Body composition was evaluated by whole body fan-beam dual energy X-ray absorptiometry (DXA) scan (Hollogic Discovery W, Bedford, MA) as described (9). Blood samples were collected after 12-hour overnight fast at T0, T3, and T12. Clinical variables were measured as described (7). Pancreatic β-cell function (insulin secretion), insulin sensitivity and resistance were estimated using Homeostatic Model Assessment – Continuous Infusion Glucose Model Assessment (HOMA-CIGMA) (17).

Measurement of scAT Shear Wave Speed by Transient Elastography

A new noninvasive medical device based on transient elastography (18), Adiposcan™ (Echosens, Paris, France), was developed to measure scAT shear wave speed (SWS) associated with scAT stiffness (9, 19). scAT stiffness was measured by the same operator in obese subjects (Group2) in the right peri-umbilical region at T0, T3 and T12.

Transcriptomic Experiments

ScAT samples obtained by needle aspiration at T0 and T12 (Group3) were stored at ~80°C for microarray analysis. Total RNA extraction, amplification, hybridization and raw data analysis were performed as described (20). The complete dataset is published in the NCBI Omnibus (http://www.ncbi.nlm.nih.gov/geo/) through the series accession number GSE72138.

RT-PCR for selected genes was performed as described (20), using total RNA extracted from scAT needle aspiration in 14 nonobese obese women (Group2) at T0, T3 and T12.
Tissue Preparation and Histological Analysis of scAT

A piece of surgical biopsy sample was fixed and embedded in paraffin and sliced into 5 μm-thick sections. Collagen was stained with picrosirius-red (mainly collagen I and III) and analyzed using Calopix software (Tribvn, Châtillon, France) in 36 subjects (Group1) at T0, T3 and T12 as described (9). Total collagen accumulation represents the ratio of the stained fibrous area to the total tissue surface. Pericellular collagen accumulation (ie, collagen surrounding adipocytes) represents the ratio of the stained area in 10 random fields avoiding fibrosis bundles. Adipocyte diameters were evaluated in the same 10 fields. Pericellular collagen accumulation was adjusted by adipocyte size to eliminate the effects of different adipocyte sizes in measure fields. Collagen I and VI, degraded collagen I, LOX and macrophages were detected by immunohistochemistry (IHC) using specific antibodies (Supplemental Table 1). Total macrophages were defined as CD68+ cells and M2-macrophages as CD163+ cells. Their results are expressed as the number of CD68+ or CD163+ cells related to 100 adipocytes (21). Collagen and elastin structures were analyzed using confocal microscopy and second-harmonic generation (SHG) microscopy on another piece of fixed scAT sample in 3 random obese subjects (Group1) as described (11).

ScAT Explant in vitro

Piece of surgical biopsy samples (Group1) was placed in a culture medium enriched in endothelial cell basal medium (Pro-mocell, Heidelberg, Germany), supplemented with 1% albumin free fatty acids (PAA Laboratoires, Velizy-Villacoublay, France) and antibiotics. After 24-hour incubation at 37°C, scAT explant secretion media were collected and frozen at –80°C for ELISA and zymography. The explant secretion was normalized to AT weight according to the ratio of 1 mL of culture medium for 0.1g scAT.

Protein Determination in scAT Explant

The concentrations of collagen III formation marker, N-proteases cleaved N-terminal propeptides of collagen III (PRO-C3), and degradation marker, MMP-9-generated neoepitope fragments of collagen III (C3M), in scAT explants were evaluated using two competitive ELISA kits developed by Nordic Biosciences A/S (Herlev, Denmark) (22). The protein profiles of proMMP-2 and proMMP-9 were analyzed by zymography as described (23).

Statistical Analyses

Data are expressed as mean ± SD, categorical variables as numbers and percentages, and values in graphs as mean ± SEM. Categorical data were analyzed using Fisher’s exact test. For continuous data, repeated one-way ANOVA was used to compare more than two groups and Holm-Sidak’s parametric multiple comparison for post hoc analysis; student’s t test was used to compare two groups. For small sample size (ie, n < 30), data were first transformed by natural logarithm if they did not follow a Gaussian distribution. Two-tailed p-values were considered significant below 0.05. All analyses were conducted using R software version 3.0.3 (http://www.r-project.org) and GraphPad Prism 6.0.

Results

Increased Collagen Deposition in scAT during BS-induced Weight Loss

Using picrosirius-red staining, scAT collagen was quantified in 36-paired obese subjects (Group1) at baseline (T0) and during the follow-up (T3 and T12). No significant difference in collagen accumulation was found among the different BS procedures at baseline (Supplemental Table 2). More abundant and thicker bundles of collagen fibers traversing the scAT were observed at T3 and T12 (Figure 2A, B, C). Several parenchymal areas were filled with less well-organized collagen in postoperative tissues (Figure 2C, enlarged image). A significant increase in the average of total and pericellular collagen was observed at T3 and T12 (Figure 2D). As expected, adipocyte size significantly decreased post-BS (Figure 2E), but this reduction was not correlated with collagen accumulation. Moreover, the increase in pericellular collagen remained significant after adjustment for adipocyte size reduction. Importantly, the fat mass reduction was negatively correlated with pericellular collagen accumulation (r=-0.40, P < .05). No other associations were observed between collagen accumulation and metabolic or inflammatory variables except for systemic HDL-cholesterol (Supplemental Table 3). Notably, the results held true in subgroup analysis of each BS procedure (data not shown).

Undetectable Changes in Tissue Stiffness despite Increased scAT Collagen Accumulation in scAT

Since we previously showed that collagen accumulation was associated with scAT rigidity and metabolic alterations in obesity (9), we next aimed to investigate scAT stiffness changes post-BS using Adiposcan™ at T0, T3 and T12 (Group2). To our surprise, despite increased collagen accumulation, no significant change in average SWS was detected at T3 and T12 compared to T0 (T0: 0.90 ± 0.29m/s, T3: 0.88 ± 0.28m/s, T12: 0.93 ± 0.43m/s, P = .58, Figure 2F). Even though we observed 2 major clusters of scAT stiffness individuals trajectories using K-means for longitudinal data (Kml) cluster (Supplemental Figure 1), we did not observe significant bioclinical differences at any time points that could possibly explain these trajectories (Supplemental Table 4). Overall, increased picrosirius-red stained collagen along with undetectable change in average scAT SWS could be considered as an adaptive phenomenon of ECM remodeling during weight loss, which requires further investigation.

M2-Macrophages Associate with Collagen Accumulation in scAT

M2 cells, alternatively activated macrophages, are implicated in the resolution phase of inflammation and tissue
remodeling (24). Using IHC, M2 cells (ie,CD163\(^+\) cells) and total macrophages (ie,CD68\(^+\) cells) in scAT were quantified in 15 obese subjects from Group1 at T0 and T12. The CD163\(^+\)/CD68\(^+\) ratio increased between T0 and T12 (0.38 ± 0.20 vs. 0.78 ± 0.58, \(P < .01\), Figure 3A), in agreement with a switch toward M2-macrophages during weight loss and their role in tissue remodeling. At T0, a strong positive association between CD163\(^+\) cells and pericellular collagen accumulation was observed (\(r = 0.76\), \(P < .01\), Figure 3D left panel). Although the number of CD163\(^+\) cells moderately increased at T12 (6 ± 3\% vs. 9 ± 4\%, \(P = .04\), Figure 3B), a negative association between CD163\(^+\) count and pericellular collagen accumulation was found (\(r = -0.65\), \(P = .02\), Figure 3D right panel). By contrast, the number of CD68\(^+\) cells decreased between T0 and T12 (17 ± 8\% vs. 14 ± 7\%, \(P = .04\), Figures 3C), but was not associated with collagen deposition at T0 or T12.

**Major ECM Remodeling at Transcriptomic Level after Weight Loss**

As BS-induced weight loss is accompanied by increased collagen deposition without detectable one-year change in SWS, we next characterized transcriptomic signatures of scAT at T0 and T12 in 42 women (Group3). Using a 5\% false-discovery rate, we detected 4236 up- and 2989 down-regulated genes (functional annotations see Supplemental Figure 2). We focused our analysis on genes encoding proteins involved in ECM structural components, profibrotic proteins, remodeling, and mechanotransduction. We found differential patterns of gene changes (Figure 4A). Particularly, genes encoding collagen III (COL3A1), collagen VI (COL6A1, COL6A2), and elastin (ELN) were significantly down-regulated, while collagen I (COL1A1) was unchanged. Collagen VI alpha 3 chain (COL6A3) was modestly up-regulated. Connective tissue growth factor (CTGF) and secreted phosphoprotein 1 (osteopontin, SPP1) were significantly down-regulated. MMP-9, TIMP1, TIMP2, and TIMP4 were also significantly modified. Importantly, some genes previously shown to be stimulated by mechanical stress (11), such as YAP, TEAD2, TEAD3, TEAD4, were not modified after weight loss (\(P > .05\)).

During collagen biosynthesis, major post-translational modifications take place and are mediated by important enzymes and chaperones. We found that the expression levels of most of these molecules were decreased at T12 (Figure 4B). Finally, we observed a significant down-regulation of genes encoding cross-linking enzymes such as LOX, lysyl oxidase-like 4 (LOXL4), transglutaminase1 (TGM1), procollagen-lysine 2-oxoglutarate 5-dioxygenase 2 and 3 (PLOD2 and PLOD3), suggesting that matrix fibers’ cross-linking was decreased post-BS (Figure 4A). These transcriptomic analyses confirm the strong remodeling of scAT following BS and show major transcriptomic modifications of enzymes involved in collagen biosynthesis, cross-linking, and degradation.

![Figure 2. ScAT evaluation. Collagen accumulation in scAT stained by picrosirius-red in one representative obese subject at baseline (T0) (A), 3 months (T3) (B) and 12 months (T12) (C) post-BS. Total and pericellular collagen accumulation (D) and adipocyte size (E) at T0, T3 and T12 in 36 obese subjects from Group1. Repeated ANOVA test and Holm-Sidak’s parametric multiple comparison test were used, * \(P < .01\). F, scAT stiffness, shear wave speed (SWS), was evaluated at T0, T3 and T12 post-BS measured by transient elastography in 35 subjects (Group2). Repeated ANOVA test, \(P > .05\).](https://press.endocrine.org/doi/10.1210/jc.2015-3348/fig-2)
Decreased Cross-linking of Matrix Fibers during Weight Loss Associates with Improved Metabolic Phenotype

We next explored cross-linking enzymes and their associations with metabolic phenotypes. We confirmed microarray data by RT-PCR and observed that LOX gene expression was significantly down-regulated at T3 and T12 (Group2) (Figure 5A). This was substantiated by decreased LOX protein staining surrounding adipocytes at T3 and T12 (Figure 5B) using IHC. By confocal microscopy and SHG in fixed scAT samples in 3 random obese subjects (Group1), we found a trend towards reduced collagen and elastin intensity at T3 (Supplemental Figure 3). Elastin protein at T3 had more twisted structures (Figure 5C), suggesting that scAT might become less rigid after weight loss.

We next examined the relationships between one-year changes in cross-linking enzyme expression and that of clinical variables (ie,T12-T0 variation) in Group3 (Figure 5D). The reduction of LOX gene expression was positively associated with the reduction of BMI, fat mass (kg), adipocyte volume, serum leptin and orosomucoid. Variation of LOXL1 was also associated with BMI, fat mass (kg), leptin, total- and HDL-cholesterol. Our gene expression results suggest that decreased post-BS cross-linked scAT matrix fibers link with improved weight loss.

Increased Collagen Degradation during BS-induced Weight Loss

Our team (7) and others (4) have shown that collagen I and III are more frequently observed in fibrous bundles, whereas collagen VI is surrounding adipocytes. Despite increased scAT collagen accumulation post-BS, we found decreased collagen I and VI staining at T12 (Supplemental Figure 4), suggesting that increased picrosirius-red staining may also indicate (at least partially) degraded collagen fragments. We tested this hypothesis by measuring collagen fragments with immunostaining, ELISA and zymography from scAT explants. We observed increased stained degraded collagen I surrounding adipocytes in scAT at T3 and T12 (Figure 5E). Accordingly, the concentration of degraded collagen III (C3M) in scAT at T12 was significantly increased compared to T0 (Figure 5F left panel). ProMMP-9 and proMMP-2 entities at 92 kDa and 72 kDa respectively were observed (Figure 5G). Despite individual variability, an increased trend of proMMP-2 at T3 in one nondiabetic obese subject and an increase of proMMP-9 at T3 followed by stabilization at T12 in two others were
observed. These changes in proMMPs were not detected in samples from type 2 diabetic obese subjects (Figure 5G). In parallel, newly synthesized collagen III (Pro-C3M) concentration was significantly decreased in obese compared to nonobese subjects and showed a nonsignificant increase at T3, T12 (Figure 5F right panel).

**Discussion**

Collagen accumulation in white AT is considered as an important pathological alteration associated with several comorbidities of obesity (1, 7, 9). Our results provide new insights into weight-loss induced AT remodeling in paired humans individuals before and one-year post-BS. Our results suggest that picrosirius-red stained collagen in scAT does not always refer to “pathological collagens”, but could be a signature of extensive tissue remodeling and collagen degradation following adipocyte shrinkage during weight loss along with improved clinical, metabolic and inflammatory outcomes.

During physiological tissue repair, ECM accumulation is a key regenerative step replacing tissue debris and dead cells (2). In pathological conditions, increased collagen deposition is not always synonymous with deleterious fibrosis. In myocardial injury, different types of fibrosis have been reported according to the progression and history of cardiomyopathies: a reactive “interstitial fibrosis” with ECM deposition in response to deleterious stimuli is considered pathological. Conversely, a “replacement fibrosis” that replaces myocytes after cell damage or necrosis may preserve the structural integrity of the myocardium (25, 26). As we did not find any association between adipocyte diameter reduction and pericellular collagen increase, we attribute this increased pericellular collagen to “replacement collagen” that occurs at adipocyte shrinkage sites. This is further supported by our observation of

![Figure 4](image-url)

**Figure 4. Transcriptomic signature of scAT ECM genes in obese subjects one year after BS** Gene expression levels from microarray data in scAT at baseline represented as dotted line (T0) and 12 months post-BS (T12) represented as bars in 42 women from Group3: A, genes involved in ECM remodeling (matrix fibers, cross linking, profibrotic protein, degradation proteins and adhesion protein) show important changes. B, most genes involved in post-transcriptional modifications of collagen are down regulated one year post-BS. They include i) enzymes involved in the hydroxylation of proline: prolyl 4-hydroxylase; prolyl-3 hydroxylase;ii) enzyme involved in glycosylation of hydroxylysine: GLT2D1;iii) chaperon molecules HSP47, GRP94, calexin (CANX) and disulphidesomerase (PDI) (HSPA5, DNAJC10, ERP29, PDIA4, PDIA6) and iv) enzymes involved in N- and C- propeptides of procollagens: ADAMTS1, ADAMTS2, ADAMTS5, ADAMTS14. By contrast, prolyl-3 hydroxylase (P3H2, P3H3) and ADAMTS9 genes were up-regulated. Data are presented as changes from baseline. *P < .05.
Figure 5. **Cross-linking of Matrix Fibers and collagen degradation and synthesis in scAT**. A, Lysyl oxidase (LOX) gene expression levels at baseline (T0), 3 months (T3) and 12 months (T12) post-BS in 14 obese nondiabetic women from Group2. B, LOX stained by immunohistochemistry in obese and nonobese subjects, X20. C, scAT elastin structure (magenta) was observed by second harmonic generation at T0 and T3 in one representative obese subject among the three, X20. D, correlation heatmap between changes of bioclinical parameters and changes of genes regulating cross-linking from T0 to T12 in 42 women from Group3. Correlations between gene expression and changes of...
some large parenchyma areas filled with less well-organized collagen. This replacement collagen, as part of the remodeling process, might be an adaptive and physiological phenomenon during weight loss.

Cross-linking is necessary for matrix fibers maturation and stabilization (27) and contributes to increased tissue stiffness. LOX is a major enzyme mediating collagen and elastin cross-linking. A relationship between LOX enzymatic activity and tissue stiffness was established in colorectal cancer and indicated a pivotal role of LOX-associated stiffness in driving colorectal cancer progression (12, 28). In obese subjects, scAT LOX gene expression is increased (11). Increased perioperative scAT pericellular collagen is associated with increased tissue stiffness measured by AdiposcanTM (9). Moreover, pericellular collagen leads to adipocyte constraints and stimulates genes encoding mechano-sensitive, inflammatory and profibrotic proteins such as CTGF in a 3D model (11). Herein, decreased LOX gene expression and protein, and increased elastin twist structure evaluated by SHG after weight loss clearly suggest decreased cross-linking and relaxed fibers.

ScAT stiffness measured by AdiposcanTM relates to adipose tissue rigidity in severe obesity before weight loss and is associated with picrosirius-red stained collagens and metabolic alterations (9). Surprisingly, we found increased post-BS collagen accumulation without significant change in average scAT stiffness measured by AdiposcanTM despite large interindividual variability. These results, associated with improved metabolic alterations after BS, suggest that the major ECM remodeling observed after weight loss might be adaptive. Profiles of stiffness changes were observed but without significant link with clinical parameters at this stage. In addition, our results also suggest that transient elastography AdiposcanTM might be more sensitive to severe cross-linked and dense fibrosis (ie, “pathological fibrosis”) as showed in liver stiffness measurements (29, 30), thus explaining why AdiposcanTM fails to detect small decreases in post-BS stiffness or alternatively to quantify adaptive ECM remodeling (ie, less cross-linked and more degraded collagens) not linked to pathological conditions. Therefore, AdiposcanTM might be more appropriate to better stratify obese individuals before any drastic weight intervention or to noninvasively predict weight loss outcomes (9), a feature which needs further study in extended cohorts. Furthermore, other scAT changes occurring after weight loss might also influence tissue stiffness, such as the amount and types of lipids in adipocyte or scAT vascularity. In addition, some genes involved in mechano-transduction pathway YAP/TEAD were unchanged while the downstream profibrotic gene CTGF was down-regulated, suggesting again that weight loss induced increased collagen deposition was not associated with pathological constraint.

The transcriptomic study performed before and one-year post-BS confirmed intense tissue remodeling. These results align with other observations of deceased major ECM gene and profibrotic proteins both after short-term BS-induced weight loss (14) or dietary intervention (31). We, herein, suggest that increased picrosirius-red staining is, at least partially, due to increased degraded collagens (collagen I, III) and eventually less newly synthesized collagens (collagen III) as shown by IHC and ELISA. Indeed, we found decreased staining of specific collagens such as collagen I and VI. Importantly, we went beyond the transcriptomic results obtained by McChulloc et al who observed only increased post-BS COL6A3 expression but not other collagen VI alpha chains or their protein content (14). Our microarray analysis displayed different expression changes of collagen VI alpha chains: COL6A1 and COL6A2 decreased whereas COL6A3 increased. It is well known that transcriptomic changes of subtype chains do not always relate to the same changes at the protein level. According to our immunostaining results, we found less collagen VI surrounding adipocytes post-BS.

Our zymography analysis in scAT revealed the presence of proMMP-2 and/or proMMP-9 proteins in obese non-diabetic subjects. ProMMPs are the inactive zymogen forms. There are growing evidences of the ability of proMMP-2 and proMMP-9 to directly activate classical signaling pathways involved in cell growth, survival, mi-

---

Legend to Figure 5 Continued...

HbA1c and glyceremia were analyzed separately in nondiabetic (nonDM, n = 28) and Type-2 diabetic (DM, n = 14) subjects. HOMA-IR, HOMA-B% and HOMA-S% were only analyzed in nondiabetic subjects. Pearson’s coefficients of each correlation are represented as blue (negative correlation) or red (positive correlation), * P < .05. E, degraded collagen I in scAT stained by immunohistochemistry in one representative obese subject at T0, T3 and T12 and one representative nonobese subject. F, degraded collagen III (left panel) and newly synthesized collagen III (right panel) in scAT explant measured by ELISA in 5 non obese (Non Ob) and 10 obese subjects (Group1). Diabetic subjects are in red, * P < .05. G, analysis of (pro)MMP-2 and (pro)MMP-9 presence in scAT by gelatin zymography in 3 obese nondiabetic (Ob), 3 obese diabetic (Ob Diab) and 2 nonobese subjects. Ob Diab 1 was under metformin at T0, treatment that was stopped after BS therefore absent at T3, T12, Ob Diab 2 was taking sitagliptin, glimepiride, metformin at T0, metformin alone at T3, and no more treatments at T12; Ob Diab 3 was taking basal insulin, liraglutide, glimepiride and metformin at T0, basal insulin and metformin at T3, glimepiride and metformin at T12. U937 cells (ATCC CRL-1593.2) stimulated with 100 U/ml recombinant TNF for 48 hours were used as positive control. ProMMP-2 (72 kDa) and proMMP-9 (92 kDa) were detected as transparent bands on the background of Eza-blue stained gelatin.
Adipose Tissue Remodeling during Weight Loss

J Clin Endocrinol Metab

Regeneration, and angiogenesis (32). In metabolically healthy obese individuals, scAT proMMP-9 zymographic activity is increased, suggesting that proMMP-9 might be linked with better metabolic profile (33). The fact that we did not observe the difference in obese diabetic individuals seems to be in accordance with this last point, or could also be due to the effect of antidiabetic drugs. Exploring the co-expression of proMMPs and TIMPs in the context of scAT remodeling and improved metabolism deserves further consideration.

The mechanisms leading to fibrosis synthesis and degradation at the cellular level need to be better delineated in AT. AT macrophages (ATM) are triggers of fibrosis (34). We previously showed that both diet and BS-induced weight loss improve inflammatory profiles despite non-negligible interindividual variations (14, 24, 35). Here, we observed increased CD163+/CD68+ ratio due to increased CD163+ cells and decreased CD68+ cells during weight loss, a profile of activated state of ATM shifted towards M2 relative to M1, as previously shown after 3 months post-BS (24). In addition, CD163+ cells before BS associated with pericellular collagen accumulation, indicating a role in the generation of fibrosis in obese scAT. M2 cells have a complex role in tissue repair and fibrosis: besides direct effects of M2 cells on promoting and suppressing collagen synthesis and fibrosis development, M2 cells are inducers of Treg cells, which are implicated in fibrosis suppression and can directly produce MMPs and TIMPs, thus controlling ECM turnover (36). The reason why we found a significant negative association between CD163+ cells and collagen accumulation at T12 is unknown, but may suggest a balanced involvement of several cell types during this remodeling process and warrants further exploration. Our previous studies that described changes of scAT immune cells before and after weight loss have used IHC method for cell quantification (14, 24, 37). However, due to the clinical difficulties in acquiring sufficient and repeated post-BS scAT surgical biopsy samples in obese subjects during the follow-up, it was hard to compare our IHC observation to other methods such as fluorescence-activated cell sorting (FACS) for quantifying immune cells infiltration.

Some questions remain unanswered. Our clinical study aimed at evaluating the changes in scAT ECM until one year, the nadir point of post-BS weight loss in many individuals (38). The kinetic changes (amount, type, cross-linking) of collagen fibers with longer duration of post-BS weight loss, stabilization, or weight regain remain to evaluate. Some studies showed interesting results. For example, after two years post-BS weight stabilization, ex-obese subjects still presented the same amount of picrosirius-red stained scAT fibrosis as morbidly obese subjects, despite improvements in adipocyte hypertrophy and inflammation infiltration (39). However, this ex-obese group was compared to an independent group of pre-BS obese individuals, which might induce bias in the results due to important intervariability in AT fibrosis. Therefore, these findings should be confirmed in samples from same individuals obtained before and after BS, as we herein assessed. Furthermore, the type of collagens and cross-linking enzymes were not investigated. In addition, obese subjects experience periods of weight fluctuations even post-BS (38) that could possibly subsequently modify their adipose tissue ECM characteristics. We previously showed that 59 subjects who underwent RYGB after an initial failure of gastric banding displayed significantly higher total collagen accumulation than primarily operated subjects (9), suggesting again that weight fluctuations impact on ECM remodeling. Therefore, it is of interest to pursue the follow-up of our obese subjects, who were already investigated at baseline and follow-up until one year, to evaluate longer-term scAT remodeling and potential relationships with BS outcomes. In addition, there is very few data concerning the change of visceral adipose tissue characteristics. In one human study, obese subjects displayed decreased fat diameter in visceral AT as measured by ultrasound (40). In rodent, mice which underwent BS demonstrated decreased infiltration of T-lymphocytes and macrophages in visceral AT (41). Further study in these post-BS features in humans would be of major interest. However, there are clinical and ethical limitations to such explorations, and the development of non-invasive measures (eg, imaging) is indispensable.

In conclusion, this study provides new insights into scAT adaptation during drastic weight-loss and shows that increased picrosirius-red staining is a signature of tissue remodeling with increased collagen degradation and less cross-linked fibers. It will be critical to follow patients during long-term weight loss and to determine the impact of scAT remodeling on metabolic improvements.

Acknowledgments

We are grateful to the patients who contributed to this work and especially those who accepted repeated surgical biopsies during the follow-up. We thank Valentine Lemoine for patients’ follow-up, Florence Marchelli for data management, and Rohia Allili for her contribution in bio-banking. We thank Frédéric Charlotte, Annette Lescot and Anne Gloaguen for scAT tissue preparation and picrosirius-red staining. We thank Victoria Dubar for helping in immunohistostaining. We thank Claire Lovo, Aurélien Dauphin, and Christophe Klein for performing SHG acquisition and for their help in analysis (Plate-forme d’Imagerie Cellulaire Pitié Salpêtrière). We thank Nataliya Sokolovska for her help in
KML analysis. We thank Brandon Kayser, Institute of Cardiometabolism and Nutrition (ICAN), for editorial/typing support.

Address all correspondence and requests for reprints to: Karine Clément, MD, PhD, Address: E3M building – sixth floor, 46–83 Boulevard de l’Hôpital, 75 013, Paris. Tel: 33(0) 1 4217 7928. E-mail: karine.clement@psl.aphp.fr.

Disclosure summary: Y.L. received support from Echosens for her PhD program, M.S. and V.M. are employees from Echosens. All other authors including J.A.W., G.M., L.G., G.L.N., A.T., B.B., S.B., J.T., K.C. declare no conflict of interest.

Clinical Trial Registration Number: ClinicalTrials.gov NCT01655017

This work was supported by several clinical research contracts (Assistance Publique-Hôpitaux de Paris CRC FIBROTA to JAW and KC and PHRC 0702 to KC) and funding from the Fondation pour la Recherche Médicale (ANR, Adipofib), the national program “Investissements d’Avenir” with the reference ANR-10-IAHU-05 and CIFRE N° 2012/1180.

References


This work was supported by several clinical research contracts (Assistance Publique-Hôpitaux de Paris CRC FIBROTA to JAW and KC and PHRC 0702 to KC) and funding from the Fondation pour la Recherche Médicale (ANR, Adipofib), the national program “Investissements d’Avenir” with the reference ANR-10-IAHU-05 and CIFRE N° 2012/1180.

References


