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Adipose Tissue Collagen VI in Obesity

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Objectives: Basic science studies show that the extracellular matrix of adipose tissue, mainly represented by collagen VI, is dysfunctional in obesity and contributes to the development of the metabolic syndrome. We hypothesized in humans that increased collagen VI α3-subunit (COL6A3) mRNA is associated with adipose tissue macrophage chemotaxis and inflammation and that weight gain is accompanied by changes in the expression of COL6A3.

Research Design and Methods: Adipose tissue biopsies were obtained from a cross-sectional study (n = 109), an overfeeding study (n = 9), and a pioglitazone treatment study (n = 14). Adipose tissue gene expression was measured by quantitative RT-PCR, immunohistochemistry, and adipocyte sizing by fixation with osmium and Coulter counting. Body composition was measured by dual-energy x-ray absorptiometry and visceral adipose tissue by computed tomography. Patients with high or low COL6A3 mRNA were compared by one-way ANOVA.

Results: In humans, immunohistochemistry revealed that COL6 is present in adipose tissue extracellular matrix. COL6A3 mRNA is correlated with body mass index ($r = 0.60$, $P < 0.0001$) and fat mass ($r = 0.41$, $P < 0.0001$). COL6A3 expression was similar in obese *vs.* type 2 diabetes patients. Obese subjects with high COL6A3 mRNA had greater visceral adipose tissue mass ($P < 0.05$), lower size of small and medium adipocytes ($P < 0.05$), more CD68+ and CD163/MAC2+ macrophages, and increased macrophage inflammatory protein-1α and macrophage chemoattractant protein-1α mRNA ($P < 0.05$). Eight weeks of overfeeding increased body weight and COL6A3 mRNA ($P < 0.05$). Pioglitazone decreased COL6A3 mRNA, and the change was inversely proportional to baseline COL6A3 mRNA ($r = -0.95$, $P < 0.0001$).

Conclusion: These results are consistent with basic science data, suggesting that COL6A3 might contribute to adipose tissue inflammation. **(*J Clin Endocrinol Metab* 94: 5155–5162, 2009)**

Adipose tissue has been extensively studied for its role in the etiology of the metabolic syndrome. The main focus has been on the location, quantity, and molecules secreted from adipose tissue (1). Several basic science models support the view that the extracellular matrix in adipose tissue is dysfunctional in obesity and contributes to the metabolic syndrome (2). Adipose tissue extracellular matrix contains multiple types of collagen including I, IV, V, VI, VII, VIII, and IX; among these collagen VI is highly enriched in adipose tissue (3). Collagen VI α3-subunit (COL6A3) mRNA is increased in diabetic mice (2). Obese ob/ob mice lacking the COL6A3 gene (COL6KO) have a better metabolic profile compared with wild-type mice. They also gain less weight when fed a high-fat diet (2). COL6KO mice are also insulin sensitive, despite larger sc adipocytes (2), which are typically associated with decreased insulin sensitivity (4). This suggests that COL6A3 restricts the storage of lipids in sc tissue. COL6KO mice also have lower macrophage content in adipose tissue and decreased expression of inflammatory molecules including the macrophage chemokine, macrophage chemoattractant protein (MCP)-1, and macrophage inflammatory protein (MIP)-1 (2).

Collagen VI (COL6) might act directly through molecules that increase (lumican) or decrease (decorin) mac-

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Abbreviations: BMI, Body mass index; COL6, collagen VI; COL6A3, COL6 α3-subunit; COL6KO, lacking the COL6A3 gene; CT, computer tomography; MCP, macrophage chemoattractant protein; MIP, macrophage inflammatory protein; PPAR, peroxisomal proliferator-activated receptor.

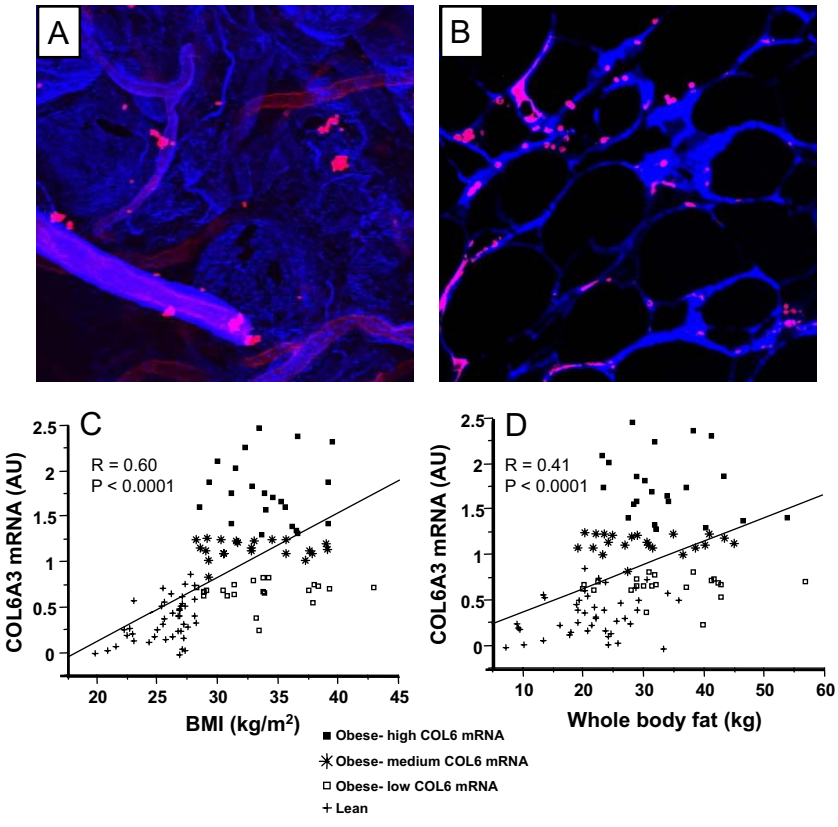

FIG. 1. COL6A3 is higher in obesity. Abdominal sc adipose tissue frozen tissue (A) and paraffin-embedded sections (B) labeled for capillaries (*red*) and COL6 (*blue*). Pearson correlation between COL6A3 mRNA and BMI (C) and COL6A3 mRNA and total fat mass in abdominal sc adipose tissue (D) of lean (*crosses*), overweight, and obese patients with low (*squares*), medium (*stars*), or high COL6A3 mRNA (*filled squares*).

rophage accumulation as shown by decreased lumican and increased decorin expression in adipose tissue of COL6KO mice (2). In human adipose tissue, we found that adipose tissue oxygenation and COL6A3 mRNA were significantly correlated ($R = -0.81, P < 0.05$), showing that patients with greater expression of COL6A3 have reduced adipose tissue oxygenation (5). In addition, reduced oxygenation was associated with adipose tissue inflammation (5–7). Taken together, this suggests that adipose tissue fibrosis might prevent angiogenesis and lie upstream of reduced oxygenation and inflammation in obese human adipose tissue.

Our aim was to explore the expression of COL6A3 in human adipose tissue. We hypothesized that in humans increased COL6A3expression is associated with adipose tissue macrophage chemotaxis and inflammation and weight gain is accompanied by changes in COL6A3 mRNA.

Subjects and Methods

Population and study design

There are three distinct populations investigated in this work: a cross-sectional study, an overfeeding study, and a pioglitazone treatment study. For all studies, subjects were excluded if they had significant renal, cardiac, liver, lung, or neurological disease, although controlled hypertension was acceptable if blood pressure was less than 140/90 mm Hg on medications. Subjects were excluded for alcohol or other drug abuse or smoking and were unwilling to abstain from caffeine and alcohol for 48 h before biopsy. Protocols were approved by the Institutional Review Boards of the Pennington Biomedical Research Center or the University of Maryland. All volunteers gave written informed consent after being informed of the nature of all procedures to be performed. The study population was a convenience sample in which we assayed existing biopsy material from our archives. As such, we assayed essentially all of the available samples without attempting to have an equal number of males and females.

Cross-sectional study

Baseline data were analyzed from patients participating in clinical studies for weight loss, diabetes treatment, metabolic/physiological studies, or gastric bypass surgery performed at Pennington Biomedical Research Center or the University of Maryland.

Overfeeding study

Six healthy young men and three women aged 18–28 yr with a body mass index (BMI) of 25.7 ± 0.99 kg/m^2 were admitted to the inpatient unit and consumed a weight-maintaining diet. Energy requirements were determined by whole room calorimetry and weight stability over 2 wk. Subjects then consumed a diet consisting of 44% fat, 15% protein, and 41% carbohydrate for 4 wk at +40% of energy requirements. Weight gain averaged 7.7 ± 2.13%.

Pioglitazone treatment study

Patients with type 2 diabetes were enrolled in a study to determine the effect of pioglitazone on adipose tissue (8). Diabetes was defined by a fasting plasma glucose of 126 mg/dl or greater at entry or fasting plasma glucose greater than 115 mg/dl and a 2-h glucose tolerance test of glucose 200 mg/dl or greater or current use of metformin or sulfonylureas. The treatment was given as a single daily dose of 30 mg/d each morning. The dose of pioglitazone was increased at 45 mg/d at wk 8 if fasting plasma glucose was greater than 100 mg/dl or the hemoglobin A1C was greater than 7.7%. Fourteen men and women changed their body weight on average by 1.3 ± 2.3 kg.

Body composition

Body fat mass was measured on a dual energy X-ray absorptiometer in the fan beam mode (QDR 4500; Hologic, Inc., Waltham, MA). Coefficient of variation for the measurement of percentage of body fat is 1.7%. Visceral fat and abdominal sc fat mass were measured by computer tomography (CT) scanning using a GE high-speed CT scanner. From the eight cross-sectional areas, visceral adipose tissue volume (liters) was cal-

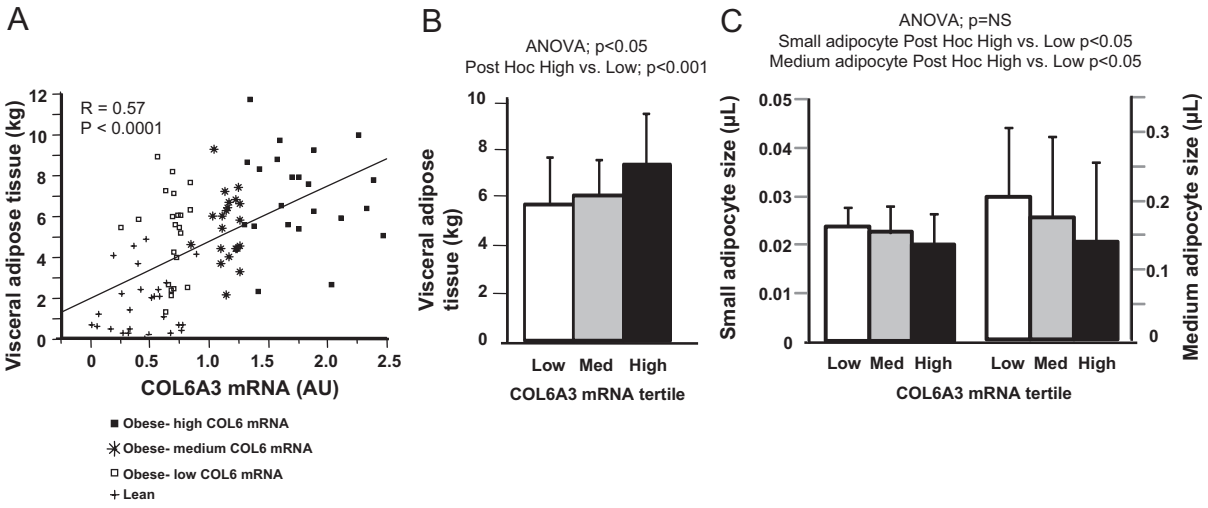

FIG. 2. COL6A3 expression and adipose tissue distribution. A, Pearson correlation between COL6A3 mRNA and abdominal visceral adipose tissue mass of lean (*crosses*), overweight and obese patients with low (*squares*), medium (*stars*), or high COL6A3 mRNA (*filled squares*). Both genders are included in this analysis. Visceral adipose tissue mass (B) and adipocyte size (C) were measured in the low (*white bars*), medium (*gray bars*), and high COL6A3 mRNA tertiles (*black bars*) and reported as mean ± SD.

culated and converted to kilograms visceral adipose tissue mass using the conversion factor 0.9193 kg/liter adipose tissue, as previously described (9).

Laboratory measures

The following assays were done on blood drawn after at least 10 h of fasting (overnight). Glucose was analyzed using a Beckman-Coulter Synchron CX7 (Brea, CA) and insulin via immunoassay on the DPC 2000 (Diagnostic Product Corp., Los Angeles, CA). The coefficient of variation for these assays is less than 2.0%. The homeostasis model assessment-insulin resistant index was calculated by multiplying the glucose (millimoles) by the insulin (microunits per milliliter) and dividing the product by 22.5 (10).

Adipose tissue biopsy

The biopsies were obtained after at least 10 h of fasting (overnight). Biopsy material was obtained from the following: 1) the abdominal sc adipose tissue only (Figs. 1–4); 2) paired samples from visceral and sc abdominal adipose tissue (supplemental Fig. 1SA, published as supplemental data on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org); and 3) paired samples from abdominal and gluteal sc adipose tissue (supplemental Fig. S1B). For the abdominal and gluteal sc adipose tissue biopsy, the skin was anesthetized with a mixture of lidocaine (2%) and bupivocaine (0.025%). Adipose tissue was obtained using a Bergstrom needle and processed at the bedside by washing in 37 C PBS. Visceral adipose tissue biopsy was obtained under anesthesia during a gastric bypass procedure (University of Maryland). Tissue was snap frozen for gene expression, preserved in 10% formalin for paraffin blocking or fixed in osmium solution for adipocyte sizing.

Quantitative real-time PCR

Human total RNA from about 100 mg AT was extracted in Trizol and purified by column purification (QIAGEN, Valencia, CA). All primers and probes were designed using Primer Express version 2.1 (Applied Biosystems, Foster City, CA). Sequences of primers and probes are shown in supplemental Table S1, published as supplemental data on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org. Quantitative real-time RT-PCR (11) were performed as one-step reactions in an ABI PRISM 7900 (Applied, Biosystems) using the following parameters: one cycle of 48 C for 30 min, then 95 C for 10 min,

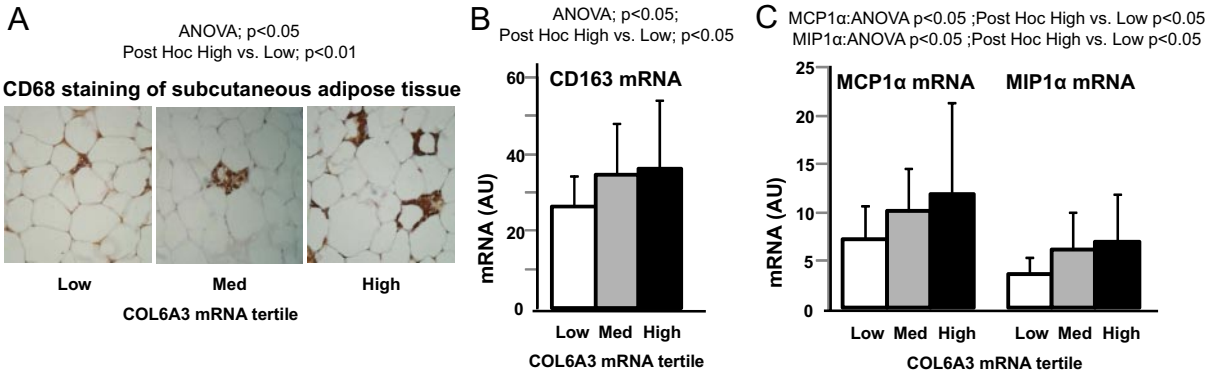

FIG. 3. COL6A3 expression and adipose tissue inflammation. A, Abdominal sc adipose tissue was stained for CD68+ macrophages. CD163/MAC2 mRNA (B) and MIP1α and MCP1α mRNA (C) were measured in the low (*white bars*), medium (*gray bars*), and high COL6A3 mRNA tertiles (*black bars*) and reported as mean ± SD.

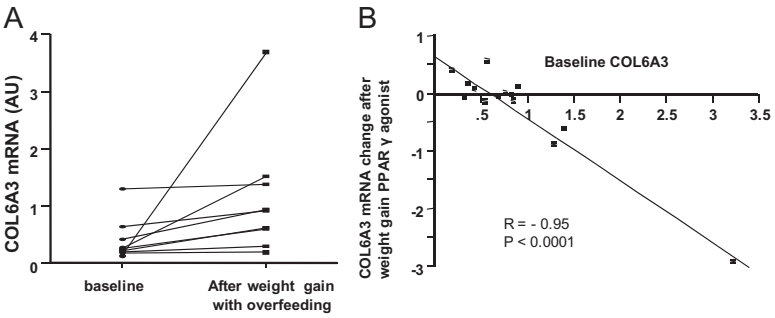

FIG. 4. COL6A3 expression changes with overfeeding and PPARγ agonist treatment. A, COL6A3 mRNA increased after weight gain by overfeeding. B, Obese patients with type 2 diabetes treated with a PPARγ agonist decrease COL6A3 mRNA proportional to COL6A3 baseline expression.

followed by 40 cycles at 95 C for 15 sec and 60 C for 1 min. The relative standard curve method was used to calculate the quantity of the target gene for each tissue extract with an internal control. The housekeeping gene cyclophilin B was previously demonstrated to be stable across lean and obese subjects (5, 12–14). Therefore, each sample value was divided by the quantity of cyclophilin B.

Immunomorphological analysis of adipose tissues

Frozen sc adipose tissue was incubated in blocking buffer (2 h), followed by rabbit polyclonal COL6 antibody 1:50 dilution (catalog no. ab6588; Abcam, Cambridge, MA) overnight. Next, sections were incubated with AlexaFlour 680 goat antirabbit antibody (catalog no. A21076; Invitrogen, Carlsbad, CA) and lectin-tetramethylrhodamine isothiocyanate conjugated from 10 μg/ml *Ulex europaeus* (catalog no. L4889; Sigma-Aldrich, St. Louis, MO) for 2 h. Images were collected using a 510 META confocal microscope (Zeiss, New York, NY) equipped with a ×20 (0.8 NA) objective and appropriate excitation lasers and bandpass filters.

Human abdominal sc adipose tissue was fixed in Bouin's solution, dehydrated, paraffin embedded, and serially sectioned (5 μm thicknesses). Sections were processed for immunohistochemical detection of the COL6 and CD68. The negative control was obtained by omitting the primary antibody during the immunostaining procedure. Adipose tissue sections were prepared for staining by being deparaffinized and dehydrated with Xylene for 20 min and incubation with 100, 90, 80, 70, and 60% ethanol. Slides were rehydrated with PBS (20 min).

COL6 staining

The slides were heated at 60 C for 30 min. Antigen retrieval was performed in 1 mM EDTA (pH 8.0) for 20 min. Bond Max stainer (Leica Microsystems, Bannockburn, IL) was used for immunohistochemistry. Incubation in blocking buffer was performed for 1 h followed by rabbit polyclonal COL6 antibody 1:50 dilution (catalog no. ab6588; Abcam) incubation for 1 h and rinsed with PBS four times for 10 min. AlexaFlour 680 goat antirabbit antibody (catalog no. A21076; Invitrogen) was applied for 1 h and rinsed five times and mounted using Aqua-Mount medium (catalog no. M7644–1; Cardinal Health, Elk Grove, CA).

CD68 staining

Sections were treated with 3% H_2O_2 in methanol for 30 min to block endogenous peroxidases followed by normal horse serum to reduce nonspecific staining. Consecutive sections were incubated overnight (4 C) with the monoclonal primary antibody antihuman CD68 (1:100; Santa Cruz Biotechnologies, Santa Cruz, CA). Antibody binding was detected with tyramide signal amplification kit (PerkinElmer Las Inc., Waltham, MA). Peroxidase activity was revealed using 3,3′-diaminobenzidine as a substrate. Quantitative analyses were performed according to the methods outlined elsewhere (15). Quantitative analyses of immunopositive cells were made with the assistance of the Metamorph/Metavue software (Molecular Devices, Dovington, PA). For macrophage quantization, adipocytes and macrophages were counted from five fields and the macrophages expressed as a percentage of total adipocytes counted. CD-68-positive cell number was assessed based on staining intensity in all samples by two independent observers.

Images of the stained sections were taken with a Zeiss Axioplan 2 upright microscope (Carl Zeiss) equipped with a Photometrics CoolSnap HQ CCD camera and a plan Apochromat ×20/0.75 objective lens.

Adipocyte size and number

Adipose tissue was fixed in osmium tetroxide and counted with a Coulter counter as previously described (16, 17). Adipocyte number in the abdominal sc adipose tissue was determined by dividing the sc abdominal fat mass (multislice CT) by the abdominal sc adipocyte mean size (18).

Statistical methods

After confirming the normal distribution using a Shapiro-Wilk test, COL6A3 mRNA was correlated with BMI, body fat mass, and visceral fat mass using the Pearson correlation coefficient. Within-subject comparison before *vs.* after intervention and between depots was done using a paired *t* test. Comparison between the obese without diabetes and obese with diabetes and between baseline and after weight gain was performed using an unpaired *t* test. Comparison between low, medium, and high COL6A3 expression tertiles was performed using a one-way ANOVA, followed by comparisons between the low and high COL6A3 expression tertiles using a *post hoc* contrast comparison with *P* set as significant if less than 0.05. Values are presented as mean ± SD, unless otherwise noted.

Results

COL6A3 mRNA increases with BMI

Clinical characteristics of the patients are presented in Table 1. Subjects were men (n = 84) and women (n = 25), aged 18–54 yr, with a broad range of BMI (19.7–42.9 kg/m^2), weight (52.8–128.7 kg), and body fat (7.2–57.0 kg). Abdominal sc adipose tissue mass ranged from 5.7 to 21.9 kg and abdominal visceral adipose tissue mass ranged from 1.2 to 11.6 kg. We showed for the first time that COL6 is present in humans in adipose tissue extracellular matrix by immunohistochemistry (Fig. 1, A and B). COL6A3 expression in abdominal sc adipose tissue was

TABLE 1. Clinical characteristics of the lean, overweight/obese, and patients with type 2 diabetes

	Lean	Overweight/ obese	Type 2 diabetes
Sex (female/male)	(25/18)	(0/66)	(8/7)
BMI (kg/m^2)	25.6 ± 2.1	33.5 ± 3.6	35.7 ± 4.7
Weight (kg)	74.1 ± 9.5	104.4 ± 12.0	101.7 ± 19.6
Total body fat (kg)	21.4 ± 6.9	32.6 ± 8.3	38.0 ± 10.1
VAT (kg)	2.6 ± 1.4	6.3 ± 2.0	25.6 ± 8.7
SAT (kg)	7.3 ± 2.0	10.2 ± 4.4	39.8 ± 13.2
COL6A3 mRNA (AU)	0.37 ± 0.22	1.20 ± 0.50	1.27 ± 0.95

Total body fat was measured by dual-energy x-ray absorptiometry. Abdominal visceral adipose tissue mass (VAT) and abdominal sc adipose tissue mass (SAT) were measured by multislice CT. COL6A3 mRNA was measured by RT-PCR and normalized to the housekeeping gene.

positively correlated with BMI (R = 0.60, $P < 0.0001$; Fig. 1C) and total body fat mass (R = 0.41, $P < 0.0001$; Fig. 1D). Independent of BMI, sex was a significant contributor to COL6A3 mRNA expression ($P < 0.001$) with women having lower COL6A3 expression compared with men (least square mean and SD: 0.63 ± 0.1 *vs.* 0.95 ± 0.1, respectively).

Not only adipose tissue mass but also adipose tissue distribution influences the development of the metabolic syndrome. COL6A3 mRNA was strongly correlated with abdominal visceral adipose tissue mass (R = 0.57, $P < 0.0001$; Fig. 2A) but not with sc abdominal adipose tissue mass (R = 0.19, P =NS). In addition, COL6A3 mRNA expression was lower in sc compared with visceral adipose tissue in men and women together (2.6 ± 1.7 *vs.* 10.8 ± 12.8 AU, respectively, Mean ± SD, $P < 0.05$; supplemental Fig. 1A), in men only (1.6 ± 0.1 *vs.* 4.0 ± 0.9 AU, respectively, $P < 0.05$) or in women only (3.0 ± 1.9 *vs.* 13.3 ± 14.4 AU, respectively, $P < 0.05$). Within the sc adipose tissue, we found that COL6A3 mRNA was lower in abdominal *vs.* gluteal adipose tissue in men and women (1.5 ± 1.1 *vs.* 2.4 ± 1.5 AU, respectively, $P < 0.05$, supplemental Fig. S1B), in women only (1.6 ± 1.6 *vs.* 2.5 ± 1.8 AU, respectively, $P < 0.05$) but not in men only (1.5 ± 0.6 *vs.* 2.4 ± 1.4 AU, respectively, $P = 0.06$).

Preclinical data showed that COL6KO mice are insulin sensitive (2). However, in BMI-matched obese patients without or with type 2 diabetes (BMI: 34.7 ± 3.0 *vs.* 35.7 ± 4.7 kg/m^2, respectively, P = NS) COL6A3 mRNA was similar (1.22 ± 0.5 *vs.* 1.27 ± 0.9 AU, respectively, P = NS).

Obese with high COL6A3mRNA have increased visceral adipose tissue mass and adipose tissue inflammation independent of BMI

We observed that at BMIs higher than 28 kg/m^2, COL6A3 mRNA varied greatly (Fig. 1A). Therefore, we split the subjects into two groups: lean (BMI < 28 kg/m^2) and overweight/obese (BMI ≥ 28 kg/m^2). COL6A3 mRNA was correlated with BMI in the lean group (R = 0.46, $P < 0.001$) but not the overweight/obese group (P = NS). The overweight/obese were split into tertiles of COL6A3 mRNA (clinical characteristics are presented in Table 2), which had similar BMI and similar body fat mass

TABLE 2. Clinical characteristics of overweight/obese subjects split by COL6A3 mRNA tertiles

	Low COL6A3	Medium COL6A3	High COL6A3	ANOVA	*Post hoc* high *vs.* low
COL6A3 mRNA (AU)	0.68 ± 0.13	1.16 ± 0.10	1.77 ± 0.36	[a]	[a]
Sex (female/male)	(0/22)	(0/22)	(0/22)		
Age (yr)	39 ± 10	44 ± 7	43 ± 8		
BMI (kg/m^2)	33.4 ± 4.0	32.9 ± 3.6	34.1 ± 3.1		
Weight (kg)	106 ± 12	100 ± 13	107 ± 10		
Total body fat (kg)	15.2 ± 7.0	16.5 ± 3.9	17.0 ± 5.8		
VAT (kg)	5.6 ± 2.0	6.0 ± 1.5	7.3 ± 2.1	[a]	[a]
SAT (kg)	10.2 ± 5.2	10.5 ± 3.7	9.9 ± 4.2		
Mean adipocyte size (μl)	0.76 ± 0.19	0.75 ± 0.13	0.77 ± 0.14		
Small adipocyte size (μl)	0.024 ± 0.004	0.022 ± 0.006	0.020 ± 0.006		[a]
Medium adipocyte size (μl)	0.21 ± 0.10	0.18 ± 0.12	0.14 ± 0.11		[a]
Large adipocyte size (μl)	0.44 ± 0.08	0.41 ± 0.11	0.39 ± 0.19		
Very large adipocyte size (μl)	1.43 ± 0.52	1.36 ± 0.40	1.60 ± 0.47		
Adipocyte number × 10^9	13.9 ± 6.1	13.9 ± 4.4	13.2 ± 5.7		
Fasting glucose (mg/dl)	95 ± 9	97 ± 11	98 ± 7		
HOMA-IR	4.0 ± 2.1	3.4 ± 1.5	3.4 ± 1.5		

COL6A3 mRNA was measured by RT-PCR and normalized to the housekeeping gene. Total body fat was measured by dual-energy x-ray absorptiometry. Abdominal visceral adipose tissue mass (VAT) and abdominal sc adipose tissue mass (SAT) were measured by multislice CT. Subcutaneous abdominal adipose tissue samples were fixed, digested, and analyzed on a Coulter counter. The diameter of each osmium-fixed TG droplet was used to calculate cell volume. For each participant, four subdistributions were determined: small, medium, large, and very large adipocyte. Adipocyte number was determined by dividing the sc abdominal fat mass by the abdominal sc mean size. HOMA-IR, Homeostatic model of assessment-insulin resistance index.

[a] $P < 0.05$.

(ANOVA, P = NS) but different body fat partitioning. Subjects in the high COL6A3 mRNA tertile had significantly more visceral adipose tissue (ANOVA, $P < 0.05$; *post hoc* contrast low *vs.* high, $P < 0.05$; Fig. 2B). The mean adipocyte size was not different between the low, medium, and high COL6A3 mRNA tertiles (0.76 ± 0.19 *vs.* 0.75 ± 0.13 *vs.* 0.77 ± 0.14 μl, respectively, ANOVA, P = NS; *post hoc* contrast low *vs.* high P = NS). sc adipocyte number was also similar ($13.9 \pm 6.1 \times 10^9$ *vs.* $13.9 \pm 4.4 \times 10^9$ *vs.* $13.2 \pm 5.7 \times 10^9$, respectively, ANOVA, P = NS). However, the average size of the small and medium adipocyte fractions was smaller in the high COL6A3 expression tertile compared with the low COL6A3 expression tertile subjects (ANOVA, P = NS, *post hoc* contrast low *vs.* high $P < 0.05$, Fig. 2C) with similar size of the large and very large adipocytes.

CD68 and CD163/MAC2 are robust macrophage markers. Subjects in the high COL6A3 tertile had increased macrophage content in adipose tissue as shown by higher CD68-positive macrophages per number of adipocytes (0.35 ± 0.21 *vs.* 0.28 ± 0.16 *vs.* 0.21 ± 0.12, respectively, ANOVA, $P < 0.05$; *post hoc* contrast low *vs.* high $P < 0.05$, Fig. 3A). Similarly, CD163/MAC2 mRNA expression was higher (36.1 ± 17.9 *vs.* 34.17 ± 13.2 *vs.* 26.2 ± 8.1 AU respectively, ANOVA, $P < 0.05$; *post hoc* contrast low *vs.* high $P < 0.05$; Fig. 3B). This is consistent with the increased expression of MCP1α and MIP1α observed in the high COL6A3 tertile subjects (ANOVA, $P < 0.05$; *post hoc* contrast low *vs.* high $P < 0.05$, Fig. 3C): MCP1α (11.6 ± 9.4 *vs.* 9.9 ± 4.3 *vs.* 6.9 ± 3.5 AU, respectively) and MIP1α (6.7 ± 4.7 *vs.* 5.9 ± 3.8 *vs.* 3.3 ± 1.7 AU, respectively).

COL6A3mRNA increases with overfeeding but not peroxisomal proliferator-activated receptor (PPAR)-γ agonist treatment

Given that COL6A3 mRNA correlates with adipose tissue inflammation, interventions that change adipose tissue inflammation might act through COL6A3. Obesity is associated with increased adipose tissue inflammation (19). Nine patients that participated in an overfeeding study gained weight (from 77.7 ± 13.0 kg average at baseline to 83.7 ± 14.6 kg; $P < 0.001$) and increased COL6A3 mRNA in adipose tissue from 0.4 ± 0.4 AU at baseline to 1.1 ± 1.1 AU ($P < 0.05$, Fig. 4A). COL6A3 mRNA increase after weight gain was bigger in subjects with low COL6A3 expression at baseline ($R = -0.33$, P = NS).

PPARγ agonist agents remodel adipose tissue and cause weight gain (8) along with decreasing adipose tissue inflammation (19, 20). Obese patients with type 2 diabetes treated with pioglitazone, a PPARγ agonist, gained weight (from 94.6 ± 21.1 kg at baseline to 95.9 ± 21.0 kg) but on average did not decrease COL6A3 mRNA (from 0.86 ± 0.76 at baseline to 0.57 ± 0.26 AU; P = NS). There was a greater fall in COL6A3 mRNA in those patients with high COL6A3 mRNA at baseline ($R = -0.95$, $P < 0.0001$; Fig. 4B). When the subject with the highest COL6A3 mRNA change was removed, the correlation remained significant ($R = -0.76$, $P < 0.01$).

Discussion

COL6A3 mRNA is increased in murine obesity. Based on compelling data from the COL6KO mouse, Scherer and colleagues (2) proposed that COL6 acts to constrain adipocyte expansion, leading to inflammation and insulin resistance. We found that COL6A3expression is increased in human obesity independent of diabetes. Obesity with high expression of COL6A3 have increased adipose tissue inflammation and visceral adipose tissue mass. Overfeeding, which increases inflammation, leads to an increase in COL6A3 expression. PPARγ agonist treatment, which decreases inflammation, decreases COL6A3 expression in proportion with the baseline COL6A3. Together with the basic science data, our results suggest that there are at least three consequences of increased COL6 in human obesity: first, to restrict the storage of lipid in sc adipose tissue, leading to storage in visceral adipose tissue; second, to reduce oxygenation of adipose tissue, which leads to inflammation; and third, to directly increase adipose tissue inflammation.

We studied the expression of COL6A3 mRNA in the sc adipose tissue of a large group of lean, overweight, and obese men and women. COL6 is present in human adipose tissue extracellular matrix, as shown by immunohistochemistry. COL6A3 mRNA was significantly increased at greater BMIs and greater fat mass. Because COL6A3 is a major component of adipose tissue extracellular matrix (3), this suggests that adipose tissue fibrosis is present in human obesity. Interestingly, we found that women have lower COL6A3 mRNA compared with men independent of BMI, possibly contributing to a better metabolic profile in women (21).

COL6 KO mice have higher insulin sensitivity compared with the ob/ob (2). We found no difference in COL6A3 expression between obese and obese with type 2 diabetes, suggesting that COL6A3 does not contribute to the development of diabetes. This is perhaps because both obese groups have reduced adipose tissue insulin sensitivity, but only the obese patients with type 2 diabetes had β-cell failure, which might not relate to COL6A3.

Most importantly, only the obese subjects had a large variation in COL6A3 mRNA measured in sc adipose tissue; lean subjects always had low COL6A3 mRNA. In-

creased visceral adipose tissue mass is associated with greater circulating inflammatory molecules, therefore greater risk for diabetes and cardiovascular disease. Interestingly, despite similar BMI and body fat, patients with high COL6A3 mRNA had significantly more visceral adipose tissue. In addition, in the cross-sectional study, we found that greater COL6A3 expression in sc adipose tissue is associated with greater abdominal visceral adipose tissue mass, suggesting that greater COL6A3 contributes to the metabolic disease risk. Consistent with the COL6KO mice (2), the average size of small and medium adipocytes was larger in the low COL6A3 mRNA tertile subjects.

Increased abdominal *vs.* gluteal and visceral *vs.* sc distribution of adipose tissue is a risk factor for cardiovascular disease and diabetes (21). We found that COL6A3 mRNA expression is lower in sc compared with visceral and in abdominal *vs.* gluteal adipose tissue. The significance is unclear but of general interest.

In parallel with the increase in COL6A3, adipose tissue macrophage content is increased, probably due to expression of the potent proinflammatory cytokines MCP1α and MIP1α. This suggests that high COL6A3 contributes to increased obese adipose tissue inflammation, perhaps through reduced oxygenation of adipose tissue, which induces inflammation *in vitro* (6, 7) and is associated with inflammation *in vivo* (5).

Given that higher COL6A3 mRNA is accompanied by adipose tissue inflammation, we hypothesized that interventions changing adipose tissue inflammation might act through COL6A3. Increased body weight is associated with adipose tissue inflammation (19), and we showed that it also increases COL6A3 expression. PPARγ agonist agents cause weight gain (8) but decrease adipose tissue inflammation and increases insulin sensitivity (19, 20). One mechanism by which PPARγ agonist treatment improves adipose tissue function may be to increase vascularization of adipose tissue (22). Animal studies suggest that remodeling angiogenesis leads to a decrease in fibrosis (23). Therefore, decreasing adipose tissue fibrosis might lay upstream increased vascularization, increased oxygenation, and decreased inflammation of adipose tissue. Alternately, PPARγ ligands might directly suppress the expression of COL6A3. Supporting these hypotheses, we showed in a pilot study that obese type 2 diabetes patients with high COL6A3 decreased COL6A3 mRNA when treated with PPARγ agonist.

In conclusion, COL6A3 expression is increased in human obesity independent of diabetes. Obese subjects with high COL6A3 have increased adipose tissue inflammation and increased visceral adipose tissue mass. Overfeeding increases COL6A3 expression, whereas PPARγ agonist treatment decreases COL6A3 expression; this change is proportional to the baseline expression of COL6A3. Together these data are consistent with a model in which fibrosis leads to local hypoxia, inflammation, and increased visceral adipose tissue. Alternately, hypoxia could increase the expression of COL6 and chemokine secretion, leading to macrophage infiltration and inflammation. Future studies are needed to study the direct effects of increased COL6A3 expression on inflammation and the distribution of adipose tissue.

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