Renin receptor expression in human adipose tissue

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Achard V, Boullu-Ciocca S, Desbriere R, Nguyen G, Grino M. Renin receptor expression in human adipose tissue. Am J Physiol Regul Integr Comp Physiol 292: R274–R282, 2007. doi:10.1152/ajpregu.00439.2005.—Adipose tissue synthesizes all components of the renin-angiotensin system. The renin receptor (RenR) is able, on renin binding, to increase its efficiency to generate angiotensin I from angiotensinogen. We demonstrate that RenR is specifically synthesized in the stromal portion of human adipose tissue in both isolated interadipocyte stromal cells and in stromal areas. RenR is expressed at the periphery of cells, strongly suggesting a membranal localization. RenR protein expression in primary cultures of human stromal cells decreased significantly during differentiation, whereas RenR mRNA levels did not change, demonstrating that RenR was expressed in both preadipocyte and nonpreadipocyte cells, and was regulated at a posttranscriptional level. Double-labeling immunohistochemistry of human adipose tissue sections revealed that RenR was colocalized with renin, whereas incubation of 3T3-L1, a preadipocyte cell line, with renin stimulated the phosphorylation state of the intracellular signaling pathway ERK 1/2, and short exposure of human adipose stromal cells in primary culture to renin was followed by a long-lasting dose-dependent increase of angiotensin I generation, indicating that adipose RenR is functional. We show, using a large set of human adipose tissue biopsies, that RenR expression was increased in visceral compared with subcutaneous adipose tissue of lean and obese patients. Taken together with our finding that RenR was colocalized with plasminogen activator inhibitor type 1, the main inhibitor of the fibrinolytic system in visceral adipose tissue, the above-mentioned data suggest that RenR plays a role in obesity-induced visceral adipose tissue accumulation and its accompanying cardiovascular complications.

immunohistochemistry; adipocyte differentiation; reverse transcriptase-polymerase chain reaction

CENTRAL OBESITY, WITH RESPECT to its continuously growing frequency and the severity of its complications, is now becoming a major public health concern (33). Central obesity is part of the metabolic syndrome, which associates insulin resistance, high blood pressure, and dyslipidemia, leading to increased prevalence of coronary artery disease and diabetes mellitus. The renin-angiotensin system (RAS), with its active peptide angiotensin II (ANG II) produced from angiotensinogen (AGT) via the action of renin and AGT-converting enzyme, has been implicated in the development of hypertension and insulin resistance in obesity, mainly via the ANG II/ANG type I receptor interaction. Indeed, circulating AGT is increased in obese patients (35) and in diet-induced obese rats (6), and pharmacological blockade of the RAS in obese subjects, in addition to lower blood pressure, delays or prevents the development of insulin resistance and diabetes (17). It has been recently demonstrated that AGT is produced in sites other than the liver, particularly in adipose tissue (13) and could participate in the circulating pool of AGT and ANG II (24). Interestingly, adipose tissue is able to process AGT to ANG II and, as a consequence, to participate in circulating ANG II levels, as recently demonstrated in humans (16). Because adipose tissue synthesizes the two ANG II receptors subtypes, adipose tissue RAS could influence, in a local paracrine/autocrine manner, adipose tissue growth and differentiation and the secretion of adipokines. Adipose tissue RAS is involved in multiple phenomena involving adipose tissue cell differentiation, recruitment, and function. In vitro in cells from rodent origin, ANG II has been shown to induce adipocyte differentiation via prostacyclin synthesis (1, 10, 30) and to positively regulate cycle cell progression of preadipocytes (9) and adipocyte physiology. AGT −/− mice showed reduced total fat mass compared with wild-type mice and selective AGT reexpression in adipose tissue led to elevated total fat mass (25). ANG II receptor type 2 (AT2R) −/− mice are protected against high-fat, diet-induced obesity and obesity-associated disorders like insulin resistance (36). ANG II binding to differentiated 3T3-L1 adipocytes increased GAPDH and fatty acid synthase activity (19). Conversely, it has been demonstrated that ANG II inhibits, through a paracrine negative feedback loop, further recruitment of preadipocytes by maturing adipocytes (18).

Recently, the identification and cloning of a functional renin receptor (RenR) opened new perspectives on the role of tissue RAS. This receptor is membrane associated, and high levels of its mRNA are detected in the heart, brain, placenta, and lower levels in the kidney and liver. Immunohistochemical studies allowed identification of RenR expression in the mesangium of glomeruli and in the subendothelium of coronary and kidney arteries. The binding of renin to its receptor induces a fourfold increase in the catalytic efficiency of AGT conversion to ANG I and an intracellular signal with phosphorylation of serine and tyrosine residues associated with an activation of MAP kinases ERK1 and ERK2 (28). Using RT-PCR, Engeli et al. (11) have recently reported that the RenR mRNA was present in adipose tissue extracts. However, because of the high expression of RenR mRNA in vascular structures, it was not clear whether the RenR was specifically synthesized in adipose tissue. As a consequence, we searched, using immunohistochemistry and in situ hybridization, for RenR expression in human adipose tissue. Primary cultures of human adipose stromal cells enabled us to study RenR protein and mRNA expression during

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preadipocyte differentiation and the effects of renin binding on ANG I generation, whereas 3T3L1, a preadipocyte cell line, was used to investigate the intracellular signal evoked by renin binding. Finally, we examined immunoreactive RenR expression in a large set of subcutaneous and visceral adipose tissue (SAT and VAT, respectively) samples obtained from lean or obese patients.

MATERIAL AND METHODS

Patients

This study was conducted in accordance with the guidelines proposed in The Declaration of Helsinki and approved by the local hospital ethics committee. All patients gave their informed consent. Thirty-one women, between 18 and 55 years of age with no endocrine, cardiovascular, hepatic, or systemic disease were investigated: 14 normal weight (BMI = 21.0 ± 2.2 kg/m²), and 17 obese (BMI = 37.9 ± 5.2 kg/m²). Patients who had followed a diet during the 3 mo preceding surgery or were taking corticoids, oral contraceptive, or antihypertensive drugs were excluded. Percentage body fat was assessed by impedancemetry. Abdominal superficial SAT and VAT (omentumal) biopsies were obtained for each patient, regardless of the menstrual cycle phase, during surgery for gastroplasty, or for noninfectious, nontumoral gynecological disease. Biopsies were fixed in 4% paraformaldehyde for 10 min and stored at 4°C in PBS until immunohistochemistry. The cell culture experiments were repeated three times with adipose tissue obtained from different patients.

Cell Culture

Stromal cell fractions were isolated from abdominal SAT obtained during plastic surgery in healthy women by collagenase digestion at 37°C for 45 min, as previously described (3). The stromal cell fraction was incubated in erythrocyte-lysing buffer (15 mmol/l NH₄Cl, 10 mmol/l KHCO₃, 0.1 mmol/l EDTA, pH 7.0) for 20 min. Cells were repeatedly washed, resuspended in DMEM/F-12 Ham medium (Sigma-Aldrich, Saint Quentin Fallavier, France), and incubated in CELLSTAR plates (Greiner Bio-One, Kremsmuenster, Austria) or Lab-Tek Chamber Slide (Nunc, Naperville, CA), in a humidified atmosphere of 5% CO₂ in air. Twenty-four hours after cell attachment, the medium was switched to a differentiation mixture consisting of the same medium enriched with 450 μmol/l NH₄Cl, 10 mmol/l KHCO₃, 0.1 mmol/l EDTA, pH 7.0) for 20 min. 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Master Mix (Applied Biosystems, Courtaboeuf, France), and 400 nmol/l of primers (Life Technologies, Cergy Pontoise, France). Primers were as follows: RenR: forward 5'-GCT CCC AGT GAG GAA AGA GTG TAT AT-3', reverse 5'-GGG TAC TCC GGT TTC ACC G-3'; adiponectin: forward 5'-AGA TGG CAC CCC TGG TGA G-3', reverse 5'-AGA TGG CAC CCC TGG TGA G-3'; adiponectin: forward 5'-GGG TAC TCC GGT TTC ACC G-3'; adiponectin: forward 5'-AGA TGG CAC CCC TGG TGA G-3'. The number of cycles required to generate a threshold of 0.03 fluorescence units was determined in triplicate for each sample. Results were accepted if the coefficient of variation was < 5% for the number of cycles required. Expression of adiponectin and RenR was normalized to 18S expression, and the result is given in arbitrary units (AU).

In Vitro Renin-Induced ANG I Generation

Human adipose tissue stromal cells were isolated and cultured as described above. At postinduction day (PID) 0 the culture medium was withdrawn and replaced by 1 ml DMEM/F-12 Ham medium containing increasing amounts of human recombinant renin (Cayman, Ann Arbor, MI) for 1 h at 37°C. Cells were then washed three times with PBS and incubated for 2 h at 37°C with 1 μM AGT (Sigma-Aldrich). The medium was collected in the presence of 1:10 vol of a 10× concentrated precooled inhibitor cocktail containing 4 μg/ml enalaprilat and 400 μg/ml 1,10 phenantroline (Sigma-Aldrich) to prevent AGT and ANG I conversion and centrifuged for 2 min at 4,000 rpm. Samples were stored at −20°C until ANG I assay. Tissue-bound renin was eluted by glycine treatment as previously described (28). The supernatant was centrifuged, concentrated, and desalted using Centricon columns (Millipore, Tullagreen, Ireland). ANG I was assayed using the GammaCoat Plasma Renin Activity kit (Diasorin, Stillwater, MN) with a sensitivity of 0.18 ng/ml. Renin was assayed using the Renin III generation radioimmunoassay kit (Cisbio, France) with a sensitivity of 2.5 pg/ml. Each measure was made in triplicate, and the experiment was repeated four times. To study the generation of ANG I by renin in solution, increasing amounts of renin diluted in DMEM/F-12 Ham medium were incubated in duplicate in cultures plates for 2 h at 37°C in the cell culture incubator in the presence of 1 μM AGT, and ANG I generation was measured as above described.

ERK 1/2 Phosphorylation Study

3T3-L1 (kindly provided by Dr J. F. Tanti) were grown in 96-well Falcon plates (Becton Dickinson, Franklin Lakes, NJ) in DMEM/F-12 Ham containing 10% fetal calf serum, 5 μg/ml insulin (Sigma-Aldrich) until they reached 80% confluence, and were then serum deprived for 18 h before the assay. The phosphorylation status of ERK 1/2 was studied by incubating cells in DMEM/F-12 Ham containing 10 nM human recombinant renin (Cayman) and 1 mg/ml saralasin (Sigma-Aldrich). After various amounts of time, cells were fixed with 4% formaldehyde for 20 min. The amounts of phosphorylated and total ERK 1/2 were measured using the fast-activated cell-based ELISA ERK 1/2 ELISA kit (Active Motif, Carlsbad, CA). Chemiluminescence was detected using the Chameleon device (HideX, Turku, Finland). Results are given as the ratio of phosphorylated ERK to the mean of total amount of ERK measured in the culture. The experiment was repeated three times.

Statistical Analysis

Results are expressed as means ± SE. Adiponectin and RenR gene expression in primary culture were analyzed by repeated-measures ANOVA followed by Fisher’s test. The Kolmogorov-Smirnov Z test was used to test differences in RenR expression in obese or lean SAT or VAT. The Mann-Whitney U-test was used to test for significance for renin activity. Differences over time course in the ERK phosphorylation status study were studied using ANOVA for repeated measures. Significance was defined by P < 0.05.

RESULTS

Localization of RenR Immunoreactivity and mRNA

SAT and VAT from lean and obese individuals collected during surgery were studied for RenR expression using immunohistochemistry. A strong signal was found in stromal cells, either between adipocytes (i.e., stromal-vascular cells, Fig. 1A) or within stromal areas (Fig. 1E), and in vessel walls, near the intima (Fig. 1Q). No staining was found when the RenR antiserum was replaced by the preimmune serum, demonstrating the specificity of the procedure (Fig. 1, B and F). RenR transcripts were revealed by in situ hybridization. Microautoradiographic signals followed the same pattern as the immunohistochemical signal (Fig. 1, C and G). Hybridization with the sense probe resulted in no signal above background (Fig. 1, D and H). Immunofluorescence revealed that adipose tissue RenR was localized in the cell periphery, strongly suggesting a membranal localization (Fig. 1M). Some of the interadipocyte stromal cells labeled for RenR showed a central round vacuole, reminiscent of a lipidic droplet (Fig. 1I), thus strongly suggesting a preadipocyte phenotype. The expression pattern of RenR immunoreactivity was similar in SAT and VAT in lean individuals and in one biopsy of epicardic adipose tissue (not shown).

Double staining of RenR and renin and analysis by immunofluorescence showed that RenR and renin were colocalized in adipose tissue in stromal cells both in SAT or VAT either between adipocytes (Fig. 1, J, K, and L) or in stromal areas. Macrophage specific antigen (CD 68) signal did not colocalize with RenR (Fig. 1, R, S, and T) nor did the leukocyte common antigen (CD 45, not shown). RenR and PAI-1 immunoreactivities were colocalized within isolated stromal cells (Fig. 1, N, O, and P).

RenR Expression During In Vitro Adipocyte Differentiation

Protein expression. Adipose tissue stromal cells obtained during surgery were cultured in vitro for 4 days and subsequently were differentiated into adipocytes. The experiment was repeated three times. The expression of RenR and adiponectin (used as an adipocyte-specific marker) were quantified by immunohistochemistry every 4 days from PIDs 0–16. At the onset of the culture, RenR immunoreactivity was localized in small round cells, whereas adiponectin labeling was not detectable (Fig. 2A). By PIDs 12–16, RenR immunoreactivity could still be detected in small rounded cells, whereas larger cells filled with a number of round vacuoles, which represent adipocytes, were not labeled for RenR and showed an intense labeling for adiponectin. The percentage of RenR-labeled cells decreased significantly during differentiation between PIDs 0 and 12 (71.4 ± 3.1 and 28.3 ± 7.3%, respectively, P = 0.02, Fig. 2B) and plateaued thereafter, whereas the percentage of adiponectin expressing cells increased steadily between PIDs 0 and 16 (0.2 ± 0.2 and 73.4 ± 11.5%, respectively, P < 0.01, Fig. 2B).

RNA

Total RNA was extracted at the same time points as for immunohistochemistry to measure RenR and adiponectin mRNA levels by quantitative RT-PCR. Results are expressed in AU as a ratio of 18S mRNA expression. Whereas adiponec-
Fig. 1. Expression and colocalization of the renin receptor (RenR) in human adipose tissue. RenR immunoreactivity (brown staining) was found in stromal cells either between adipocytes (A, arrows) or in stromal areas (E). Incubation with the preimmune serum (B and F) resulted in a lack of signal, demonstrating the validity of the procedure. Some stained cells had a small round inclusion evoking a lipidic droplet (I, arrow), suggesting that the RenR was expressed in differentiating preadipocytes. Fluorescence analysis indicated that RenR immunoreactivity was present in the periphery of stromal cells (M, arrows), suggesting a membranal localization, and near blood vessels endothelium (Q, arrow). In situ hybridization revealed that RenR mRNA expression (silver grains) followed a similar pattern as RenR immunoreactivity (C, arrow and G). No signal above background could be detected after hybridization with the sense probe (D and H), demonstrating the specificity of the antisense probe. Confocal microscopy analysis of double-labeling immunohistochemistry experiments showed that RenR was colocalized with renin (J: RenR; K: renin; L: RenR and renin, arrow), plasminogen activator inhibitor type 1 (PAI-1) (N: RenR; O: PAI-1; P: RenR and PAI-1, arrows), but not with CD 68 [R: RenR; S: CD 68; T: RenR and CD 68; note the cell bodies singly labeled for RenR (arrow) or for CD 68 (crossed arrows)].
tin mRNA level rose from 1 to 5.6 AU between PIDs 0 and 16, RenR mRNA levels were stable with time (0.3 and 0.5 AU at PIDs 0 and 16, respectively, Fig. 2C).

**ANG I Generation by Adipose Tissue Stromal Cells in Culture**

To investigate the catalytic activity of renin bound to adipose tissue, ANG I generation was measured in primary cultures of stromal cells. Cells were incubated with 0, 1, 5, or 10 nM renin, washed three times to remove unbound renin, and subsequently incubated with AGT. Figure 3 shows that preincubation with renin was followed by a dose-dependent increase of ANG I generation, with a maximal effect (i.e., generation of 1 ng·mL⁻¹·h⁻¹) obtained with 10 nM renin. We failed to estimate the amount of renin bound to stromal cells because, despite the fact that the samples were concentrated, renin levels eluted from wells incubated with 10 nM renin were below the detection limit of the renin radioimmunoassay (2.5 pg/ml). However, it should be stressed that 2.5 pg/ml of renin in solution could not, under our experimental conditions, catalyze the generation of more than 0.18 ng·mL⁻¹·h⁻¹ ANG I, demonstrating that the binding of renin to stromal cells is able to increase its catalytic activity.

**Effect of Renin on ERK 1/2 Phosphorylation Status of Cultured Adipose Cells**

ERK 1/2 phosphorylation was studied in the preadipocyte cell line 3T3-L1 that expressed both RenR mRNA and protein (not shown). Renin addition to 3T3-L1 cells grown to confluence induced a rapid, within 10 min, and transient (i.e., returning to basal values at 30 min) activation of ERK 1/2, resulting in a twofold increase in the ratio of phosphorylated/total ERK 1/2 (Fig. 4).

**RenR Expression in Interadipose Stromal Cells of SAT and VAT in Obese and Lean Patients**

SAT and VAT tissue biopsies were collected during surgery from 14 lean and 17 obese patients. Table 1 shows the anthropometric and biological parameters of the population studied. Fasting glycemia and total cholesterol were not statistically different between groups. Fat mass, waist-to-hip ratio, abdominal sagittal diameter, blood pressure, basal insulinemia, circulating concentrations of triglycerides, and PAI-1 were higher in obese than in control subjects, whereas circulating HDL concentrations were lower.

The expression pattern of RenR immunoreactivity did not differ in SAT and VAT between lean or obese individuals (not shown). Figure 5 depicts the frequency distribution of the percentage of RenR immunoreactive cells. The percentage of

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**Fig. 2.** In vitro expression levels of RenR and adiponectin protein and mRNA during preadipocyte differentiation. Adipose stromal cells were isolated and cultured in vitro for 16 days postinduction. A: photomicrographs of stromal cells immunostained for RenR or adiponectin at an early (day 0) or late stage (day 12) of differentiation. B: quantification of the immunocytochemical staining for RenR or adiponectin of differentiating stromal cells. Results are means ± SE of 3 independent experiments. C: quantification obtained by RT-PCR of RenR and adiponectin RNA in differentiating stromal cells. 18S rRNA was used as control. Results are the means ± SE of 3 independent experiments.

**Fig. 3.** ANG I generation by adipose tissue stromal cells in culture. Adipose tissue stromal cells were incubated for 1 h with increasing amounts of renin, washed 3 times, and then incubated with renin substrate for 2 h. Results are given as the amount of ANG I generated relative to controls. Experiments were performed in triplicate, and the results are the means ± SE of 4 independent experiments. *P < 0.05.

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stained stromal cells was comparable between subcutaneous fat depots of lean and obese individuals (18.2 ± 2.7 vs. 15 ± 2.8%, respectively (P = 0.7), and significantly increased in visceral compared with subcutaneous depot in lean or obese patients (22.0 ± 3.4 and 22.5 ± 3.9%, P = 0.044 and 0.041, respectively).

DISCUSSION

Our results demonstrate that both RenR mRNA and protein are present in human SAT and VAT. Engeli et al. (11) have recently shown, using RT-PCR, that human SAT extracts contain RenR mRNA. However, because of the high expression of RenR mRNA in vascular structures, it was not clear whether the RenR was specifically synthesized in adipose tissue. We show that RenR was expressed in vascular walls, but also in stromal areas and isolated stromal cells, demonstrating that human adipose tissue specifically synthesizes RenR. Our data also suggest that adipose tissue RenR is functional, i.e., is able to bind renin and is linked to second messengers. Indeed, double-labeling immunohistochemistry revealed that RenR and renin immunoreactivities were colocalized within stromal cells and that renin addition to 3T3-L1 cells induced a rapid and transient activation of ERK 1/2 comparable to that previously described in 3T3-L1 cells after treatment with endothelin (20), insulin (29), or acylation-stimulating protein (23). In addition, in vitro experiments using primary cultures of human stromal cells demonstrated that short exposure to renin was followed by a long-lasting dose-dependent increase of ANG I synthesis. Although the amount of renin bound to stromal cells in primary culture was below the detection level of our assay, our data strongly suggest that the catalytic activity of bound renin was more efficient than that of renin in solution, as previously described using RenR-transfected human mesangial cells (28). Both immunohistochemical analysis and primary cultures of human adipose tissue demonstrated that RenR was expressed by the stromal portion and strongly suggested that part of the RenR-synthesizing cells were preadipocytes. Our finding that RenR and PAI-1 immunoreactivities were colocalized further supports the above-mentioned assessment, because PAI-1, the main inhibitor of the fibrinolytic system, is specifically synthesized within adipose stromal cells (4).

Adipose RAS plays an important role in regulating systemic blood pressure. Indeed, adipose tissue is a major AGT production site (14). Rats exhibiting obesity in response to high-fat diet developed activation of the systemic and adipose RAS (6), and hypertension, which was significantly reversed by treatment with AT1 receptor antagonist (7). AGT −/− mice show decreased blood pressure, whereas AGT rescue driven by the AP2 promoter, which induces a specific expression in adipose tissue, normalizes their blood pressure (25). Animals with overexpression of AGT specifically in adipose tissue have high blood pressure and increased plasma levels of AGT (25). Mice overexpressing 11β-hydroxysteroid dehydrogenase type 1 (the enzyme that catalyzes the reactivation of inert dehydrocortico-sterone to active corticosterone) specifically in adipose tissue have high blood pressure together with increased adipose AGT mRNA and circulating AGT and renin concentrations disproportionate to the large increase in plasma ANG II (26). Although adipose ANG I could be cleaved from AGT by non-specific enzymes, such as other aspartyl proteases, it is recognized that renin is the major enzyme involved in AGT cleavage (8). Renin production in adipose tissue is still controversial. Indeed, Karlsson et al. (21), using RT-PCR followed by Southern blot analysis, showed that isolated human adipocytes, but not stromal cells, contained renin mRNA. Schling et al. (32) demonstrated that, after proliferation, human preadipocytes expressing renin mRNA. However, using RT-PCR, Engeli et al.

Table 1. Anthropometric and biochemical characteristics of the subjects enrolled in the study

<table>
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<tr>
<th></th>
<th>Lean</th>
<th>Obese</th>
<th>P</th>
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<tbody>
<tr>
<td>Age (years)</td>
<td>40.5±5.4</td>
<td>32.6±9.3</td>
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<tr>
<td>BMI, kg/m²</td>
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<td>Fat, %</td>
<td>23.3±4.7</td>
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<td>WHR</td>
<td>0.72±0.03</td>
<td>0.85±0.1</td>
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<td>Abdominal sagittal diameter, cm</td>
<td>19.2±1.6</td>
<td>31.6±3.1</td>
<td>&lt;0.0001</td>
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<td>Waist circumference, cm</td>
<td>70.2±5.0</td>
<td>105.1±8.3</td>
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<tr>
<td>Systolic BP, mmHg</td>
<td>110±13</td>
<td>134±17</td>
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<tr>
<td>Diastolic BP</td>
<td>61±16</td>
<td>75±10</td>
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<td>Fasting glucose, mmol/l</td>
<td>4.47±0.41</td>
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<td>Fasting insulin, μU/ml</td>
<td>6.72±1.55</td>
<td>9.94±4.36</td>
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<tr>
<td>Insulin/glucose</td>
<td>1.51±0.41</td>
<td>2.25±0.87</td>
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<td>Total cholesterol, mmol/l</td>
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<td>4.72±0.93</td>
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<td>HDL cholesterol, mmol/l</td>
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<td>Triglycerides, mmol/l</td>
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<td>1.56±0.83</td>
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<tr>
<td>PAI-1 (ng/ml)</td>
<td>5.6±5.1</td>
<td>35.2±27.3</td>
<td>0.0038</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = 14 lean group and 17, obese group. BMI, body mass index; WHR, waist-to-hip ratio; BP, blood pressure; PAI-1, plasminogen activator inhibitor-1.
(12) were unable to detect renin mRNA in human adipose tissue biopsies, whereas 3T3-F442A cell extracts were devoid of renin mRNA and contained an enzymatic activity that was not blocked by pepstatin and thus was different from renin, capable of processing AGT into ANG I (31). Using in situ hybridization, we failed to detect renin mRNA in the adipose tissue biopsies used for the present study (not shown). Interestingly, we demonstrated in the present study that immunoreactive renin and RenR were colocalized in freshly fixed adipose tissue biopsies, suggesting that, in vivo, adipose RenR was able to bind circulating renin. In addition, we show that in vitro renin binding to adipose cells induced a dose-dependent increase of the catalytic efficiency of renin, strongly suggesting that RenR plays an important role in adipose generation of ANG II. The above-mentioned hypothesis is consistent with the recent report of Harte et al. (16) who demonstrated, using sera obtained from the arterial circulation and from venous blood originating from subcutaneous abdominal adipose tissue, that human adipose tissue is a significant source of circulating ANG II.

We demonstrated that RenR expression was increased in VAT compared with SAT, probably through a posttranscriptional mechanism, as suggested by our in vitro experiments. The factors responsible for the upregulation of VAT RenR are not known. VAT is known to exhibit a low-grade inflammatory state, with increased macrophages infiltration, compared with SAT. However, we found that RenR was most probably not colocalized with CD 45 or with CD 68, suggesting that increased VAT RenR expression was not associated with macrophage or leucocyte invasion. Tomlison et al. (34) have demonstrated that the proliferation of stromal cells is higher in VAT than in SAT obtained either from lean or obese patients. Because our morphological analysis and our in vitro experiment showed that RenR was expressed in stromal cells in both preadipocytes and nonpreadipocytes, one can suggest that the above-mentioned phenomenon accounts for the increased expression of RenR in VAT. Such variation may be of importance with respect to the development of adipose tissue mass in obesity and its associated complications. We found that renin exposure of 3T3-L1 preadipocytes activated ERK1/2. Because it has been demonstrated in vitro that activation of ERK signaling regulates adipogenic transcription factors (29) and in vivo in mice that ERK1 is linked to the regulation of adipocyte differentiation, adiposity, and high-fat diet-induced obesity (5), the above-mentioned observation suggests that adipose RenR plays a role in VAT development in obesity. In addition,
because it is established both in clinical and in experimental studies that ANG II stimulates the expression of PAI-1 (22), our finding that RenR and PAI-1 immunoreactivities were colocalized indicates that RenR may also be involved in the thrombotic complications of increased central fat deposition.

In conclusion, we demonstrated that functional RenR is synthesized in the stromal portion of human adipose tissue, in both preadipocytes and nonpreadipocyte cells. Renin binding to preadipocyte cells in vitro stimulated the intracellular signaling pathways ERK 1/2 and potentiated ANG I generation. RenR expression was increased in VAT of both lean and obese patients, suggesting that RenR may play a role in central obesity-associated hypertension. Specific inhibitors of RenR may be useful for the treatment of patients with complicated visceral obesity.

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