Renin receptor expression in human adipose tissue

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1Institut National de la Santé et de la Recherche Médicale UMR 626, Marseille; 2Faculté de Médecine, Université de la Méditerranée, Marseille; 3Centre d’Investigation Clinique, Assistance Publique-Hôpitaux de Marseille, Marseille; 4Service de Gynécologie-Obstétrique, CHU Nord, Assistance Publique–Hôpitaux de Marseille, Marseille; 5Institut National de la Santé et de la Recherche Médicale U36, Collège de France, Paris, France

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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 membranial 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 (omental) biopsies were obtained for each patient, regardless of the menstrual cycle phase, during surgery for gastrol-plasty, or for noninfecious, nontumoral gynecological disease. Biopsies were fixed for 3 h in 4% paraformaldehyde and stored in 70% ethanol at 4°C until paraffin embedding. Five-micron-thick sections were cut with a Leica microtome and apposed onto slides (Superfrost Plus; CML, Nemours, France).

Fasting glycemia, total and HDL cholesterol, and triglycerides were measured using automated enzymatic assays (Vitros; Ortho-Clinical Diagnostics, Rochester, NY); coefficients of variation were 0.60, 0.77, and 0.88%, respectively. Circulating insulin and plasminogen activator inhibitor type 1 (PAI-1) levels were measured using an immunoradiometric assay (Sanofi-Pasteur Diagnostics, Paris, France) or an in-house ELISA, respectively; coefficients of variation were 4.0 and 9.8%, respectively.

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 (154 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 isobutylmethylxanthine (IBMX), insulin transferrin selenium (1:100; Sigma-Aldrich), 10 mmol/l dexamethasone, and 1 mmol/l triiodothyronine. After 4 days of incubation, IBMX was withdrawn from the medium to induce differentiation (day 0 postinduction). The culture medium was then routinely changed every other day. Every 4 days, total RNA from cells grown in regular plates was isolated using Trizol reagent (Invitrogen, Cergy Pontoise, France), whereas cells grown in Lab-Tek chambers 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.

Immunohistochemistry

After dewaxing and rehydration, slides were heated in a microwave for 5 min in a solution containing 10 mmol/l sodium citrate and 1 mmol/l EDTA, pH 6.0 to maximize antigen retrieval, incubated in 1% H₂O₂ for 20 min to inactivate endogenous peroxidases and blocked with 3% normal goat serum (NGS) in PBS for 2 h. For detection of RenR alone or adiponectin, slides were incubated overnight in PBS containing 1% NGS and either a rabbit anti-RenR antiserum diluted 1:1000, as previously described (28), or in a monoclonal anti-adiponectin antibody (clone HADI 773; Alexis Biochemicals, Lausen Switzerland) diluted 1:10, washed three times, and subsequently incubated for 2 h with a biotinylated horse anti-mouse/anti-rabbit IgG (Vector Laboratories, Burlingame, CA) diluted 1:200, for 2 h with the avidin biotin complex. The signal was revealed by using DAB chromogen substrate in the presence of 0.01% H₂O₂ for 5 min. The slides were counterstained with orange acridine (0.5 μg/ml), and bright-field images were observed with a DM-RB microscope (Leica Microsystems, Wetlzer, Germany) and digitized using a Coolsnap charge-coupled device camera (Roper Scientific, Duluth, GA). Control sections included incubation with the preimmune rabbit antiserum. For double labeling, background autofluorescence was quenched before the assay by using an in-house-built photobleaching box (27). VAT sections were blocked with PBS containing 3% NGS and subsequently incubated overnight in PBS containing 1% NGS, the anti-RenR antiserum, and a monoclonal antibody either anti-CD 68 (clone 54H12; Serotec, Cergy Saint Christophe, France) diluted 1:20, or anti-CD 45 (clone 2B11; DAKO, Glostrup, Denmark) diluted 1:50, or anti-PAI-1 (kindly provided by Prof. P. Declerck, Faculty of Pharmaceutical Sciences, Leuven, Belgium) diluted 1:200, or antirenin (kindly provided by Pr. Bruneval, G. Pompidou European Hospital, Paris, France) diluted 1:2,000. Slides were subsequently rinsed three times in PBS, and incubated 2 h with fluorescein-conjugated goat anti-rabbit IgG and rhodamine-conjugated goat anti-mouse IgG (Jackson ImmunoResearch, West Grove, PA, both diluted 1:200). Preparations were then observed using a True Confocal Scanner microscope (×63 magnification) coupled to 4D software. Controls included incubation with rabbit preimmune serum and omission of the monoclonal antibody.

For adipose tissue biopsies, the number of nuclei and of RenR immunoreactive cells was manually counted, using fluorescence or bright-field light, respectively, at a ×20 magnification, in the interadipocytes stromal fraction (plain stromal areas being too few to allow for a good quality quantification) in four randomly chosen fields (each measuring 1.4 mm²) for each adipose compartment and each patient. For the cell culture experiments, the total number of cells and the number of RenR or adiponectin immunoreactive cells were manually counted at a ×20 magnification. Results are expressed as the percentage of labeled cells.

In Situ Hybridization

Paraffin sections were processed and hybridized as previously described (15). The probe was a 401-bp cDNA fragment corresponding to bases 357–758 of the human RenR cDNA (28) subcloned in pPCR script, linearized with SstI (antisense probe) or PsI (sense probe) and labeled with [35S]UTP (PerkinElmer, Courtaboeuf, France). After hybridization and washing, slides were dipped in nuclear emulsion (1:1 in water, K5; Ilford, Saint-Priest, France) and exposed for 2 mo. After development, sections were counterstained with neutral red.

Quantitative RT-PCR

Quantification and RNA purity were evaluated using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies) before RT that was performed as previously described (2). Amplification was performed in a final volume of 25 μl with an Abiprim 7700 (PerkinElmer). The PCR conditions were as follows: 2 min at 50°C, 10 min at 95°C followed by 40 cycles of two-step PCR reaction denaturation at 95°C for 15 s, and an annealing extension at 60°C for 1 min. Each sample contained 2 μl of cDNA, 1 μl SYBR Green PCR...
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'-GGT TAC GTC GTC TCC ACC G-3'; 18S rRNA: forward 5'-CTT CCA CAT CCA AGG AAG GCA-3', reverse 5'-TTT TTC GTC ACT ACC CCC CC-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 phentanthalone (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, Gif sur Yvette, 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. IA) or within stromal areas (Fig. 1E), and in vessel walls, near the intima (Fig. 1Q). No staining was found when the RenR antisera 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 adiponect-
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
short exposure to renin was followed by a long-lasting dose-
primary cultures of human stromal cells demonstrated that
treatment with endothelin (20), insulin (29), or acylation-
comparable to that previously described in 3T3-L1 cells after
cells induced a rapid and transient activation of ERK 1/2
alyzed within stromal cells and that renin addition to 3T3-L1
revealed that RenR and renin immunoreactivities were colo-
functional, i.e., is able to bind renin and is linked to second
ers that adipose RenR is specifically synthesized 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 cortisol) specifically in adipose tissue
have high blood pressure together with increased adipose AGT
mRNA and circulating AGT and renin concentrations dispro-
portionate to the large increase in plasma ANG II (26). Al-
though adipose AGT 1 could be cleaved from AGT by non-
pecific enzymes, such as other aspartyl proteases, it is recog-
nized 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 South-
ern blot analysis, showed that isolated human adipocytes, but
not stromal cells, contained renin mRNA. Schling et al. (32)
demonstrated that, after proliferation, human preadipocytes
expressed renin mRNA. However, using RT-PCR, Engeli et al.

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 expres-
sion 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, demon-
strating 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 colo-
ralized 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 immunohisto-
chemical 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-synthe-
sizing 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 produc-
tion 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 treat-
ment 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
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expressed renin mRNA. However, using RT-PCR, Engeli et al.

![Graph](image.png)

Fig. 4. Effect of renin on ERK 1/2 phosphorylation status of 3T3-L1. Cells
were incubated with 10 nM renin for 0, 10, and 30 min. Results are means ±
SE of 3 experiments. *P < 0.05.

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

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<th>Lean Age 40.5 ± 5.4</th>
<th>Obese Age 5.4 9.3</th>
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<td>BMI, kg/m²</td>
<td>Lean 21.0 ± 2.2</td>
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<td>Fat, %</td>
<td>Lean 23.3 ± 4.7</td>
<td>Obese 4.7 43.0</td>
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<td>WHR</td>
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<td>Obese 0.85 0.1</td>
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<td>Obese 9.94 4.36</td>
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<td>Obese 2.25 0.87</td>
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<td>Obese 4.72 0.93</td>
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<td>Obese 1.18 0.48</td>
</tr>
<tr>
<td>Triglycerides, mmol/l</td>
<td>Lean 0.79 ± 0.38</td>
<td>Obese 1.56 0.83</td>
</tr>
<tr>
<td>PAI-1 (ng/ml)</td>
<td>Lean 5.6 ± 5.1</td>
<td>Obese 35.2 27.3</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.
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, 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. Tomlinson 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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