

Comparative expression analysis of the renin–angiotensin system components between white and brown perivascular adipose tissue

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Abstract

Recent studies have demonstrated that the rat adipose tissue expresses some of the components necessary for the production of angiotensin II (Ang II) and the receptors mediating its actions. The aim of this work is to characterize the expression of the renin–angiotensin system (RAS) components in perivascular adipose tissue and to assess differences in the expression pattern depending on the vascular bed and type of adipose tissue. We analyzed Ang I and Ang II levels as well as mRNA levels of RAS components by a quantitative RT-PCR method in periaortic (PAT) and mesenteric adipose tissue (MAT) of 3-month-old male Wistar–Kyoto rats. PAT was identified as brown adipose tissue expressing uncoupling protein-1 (UCP-1). It had smaller adipocytes than those from MAT, which was identified as white adipose tissue. All RAS components,

except renin, were detected in both PAT and MAT. Levels of expression of angiotensinogen, Ang-converting enzyme (ACE), and ACE2 were similar between PAT and MAT. Renin receptor expression was five times higher, whereas expression of chymase, AT_{1a}, and AT₂ receptors were significantly lower in PAT compared with MAT respectively. In addition, three isoforms of the AT_{1a} receptor were found in perivascular adipose tissue. The AT_{1b} receptor was found at very a low expression level. Ang II levels were higher in MAT with no differences between tissues in Ang I. The results show that the RAS is differentially expressed in white and brown perivascular adipose tissues implicating a different role for the system depending on the vascular bed and the type of adipose tissue.

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Introduction

The renin–angiotensin system (RAS) plays a central role in blood pressure regulation by modulating vascular tone, vascular structure and renal function. The substrate of the system, angiotensinogen, is cleaved by renin, producing angiotensin I (Ang I), which is then converted to Ang II by either Ang-converting enzyme (ACE) and/or chymase. The decapeptide Ang I is also a substrate of ACE2, which catalyzes Ang 1–7 production. Ang II is considered to be the major effector peptide of the RAS by binding two different receptors, AT₁ and AT₂. The AT₁ receptors are widely distributed and mediate most of the biological responses that contribute to the known pressor, trophic, and pro-inflammatory effects of Ang II, whereas the AT₂ receptors antagonize several of the AT₁ receptor-mediated responses

(for review see Paul *et al.* (2006)). In rodents, the existence of two AT₁ receptor isoforms, AT_{1a} and AT_{1b}, has already been described (Iwai & Inagami 1992).

During the last decade, the existence of a local RAS in adipose tissue and its functional importance has attracted closer attention (Gorzelnik *et al.* 2002, Engeli *et al.* 2003, Paul *et al.* 2006). The white adipose tissue (WAT) is the most abundant source of angiotensinogen after the liver (Phillips *et al.* 1993). Moreover, almost all components of the system have been described in both white and brown adipose tissues (BAT; Cassis *et al.* 1988a, Engeli *et al.* 1999, Paul *et al.* 2006). From a functional point of view, adipose Ang II has been implicated in adipocyte homeostasis regulating growth, differentiation, and metabolism (Darimont *et al.* 1994, Harp & DiGirolamo 1995, Saint-Marc *et al.* 2001, Janke *et al.* 2002). Furthermore, a pathophysiological relevance of Ang II

production in the adipose tissue for blood pressure regulation cannot be excluded (Massiera *et al.* 2001, Engeli *et al.* 2003). Thus, an increased local formation of angiotensinogen and Ang II in rat adipocytes due to overfeeding might represent a link between increased adipose tissue mass and hypertension in rodents (Frederich *et al.* 1992, Boustany *et al.* 2004). Similarly, local Ang II formation in adipose tissue is increased in obese hypertensive subjects (Giacchetti *et al.* 2002, Gorzelniak *et al.* 2002).

While most of the studies investigating the RAS in adipose tissue have focused on subcutaneous and visceral adipose tissues, little attention has been paid to perivascular fat. The latter tissue surrounds almost all blood vessels in different amounts. We and others have recently demonstrated the paracrine role of perivascular adipose tissue (PVAT) in the regulation of vasomotor function (Soltis & Cassis 1991) due to the synthesis and release of several vasoactive factors, such as the adipocyte-derived relaxing factor (Löhn *et al.* 2002, Dubrovskaja *et al.* 2004, Verlohren *et al.* 2004, Galvez *et al.* 2006), leptin (Galvez *et al.* 2006), and superoxide anions (Gao *et al.* 2006). The aim of this work is to characterize the expression of the RAS in two types of PVAT, i.e. mesenteric (MAT) and periaortic adipose tissues (PAT), and to assess whether the system is differentially expressed depending on the vascular bed and type of adipose tissue.

Materials and Methods

Animals

Experiments were conducted in 3-month-old male Wistar-Kyoto (WKY, $n=6$, 250–300 g; Charles River, Barcelona, Spain) maintained under controlled light (12-h light cycles from 0800 to 2000 h) and temperature (22–24 °C) conditions. The rats were fed a normal rat chow (A.04, Panlab, Barcelona, Spain) and had free access to tap water. Animals were killed by decapitation and the MAT and PAT were rapidly removed. The Institutional Animal Care and Use Committee approved all experimental procedures according to the guidelines for ethical care of experimental animals of the European Community, as well as the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

Isolation of adipocytes

Adipocytes were prepared from MAT and PAT according to the method of Rodbell (1964) with minor modifications. Briefly, adipose tissue was cut into small pieces and digested with collagenase A (1 mg/ml) in Krebs-Ringer bicarbonate (KRB) buffer (pH 7.4) containing 4% (w/v) BSA (fatty acid free, fraction V) and 5.5 mmol/l glucose at 37 °C in a O₂/CO₂ (19:1) atmosphere with continuous vigorous shaking (60 cycles/min). Subsequently, fat cells were dispersed and

filtered through a silk screen, washed thrice with KRB buffer to eliminate collagenase, and resuspended in the same buffer. The size of the fat cells were measured by direct microscopic determination, and the mean adipocyte diameter was calculated from measurements of 100 cells.

Histological study by confocal microscopy

Sections of aortae and intact mesenteric resistance arteries with adherent PVAT were washed in PSS and mounted between a slide (provided with a well to avoid vessel compression) and a cover glass. The tissue was visualized with a Leica TCS SP2 confocal system (Leica Microsystems, Wetzlar, Germany) fitted with Argon and Helio-Neon laser sources and coupled to a Leica DMIRE 2 microscope, using the 488/515 nm line of the microscope as adipocytes exhibited autofluorescence at this wavelength. Adipocytes were localized and of serial optical sections (1 µm thick) were captured from several areas with 20× oil objective for MAT and with 20× objective zoom 3 for PAT. Quantification of adipocyte diameter was obtained with Metamorph image analysis software (Universal Imaging Co., West Chester, UK).

Western blot for UCP-1

UCP-1 was measured in PAT and MAT. BAT was used as positive control. Briefly, the tissues were homogenized in ice-cold buffer containing 0.42 M NaCl, 20 mM HEPES (pH 7.9), 1 mM Na₄P₂O₇, 1 mM EDTA, 1 mM EGTA, 1 mM dithiothreitol, 20% glycerol, 1 µg/ml aprotinin, 1 µg/ml leupeptin, 20 mM sodium fluoride, 1 mM trisodium orthovanadate, and 2 mM phenylmethylsulfonyl fluoride. After 20 min ice-cold incubation, homogenates were centrifuged for 10 min and the pellet was discarded. Equivalent amounts of proteins (50 µg) present in the supernatant were loaded in Laemmli buffer (50 mM Tris (pH 6.8), 10% SDS, 10% glycerol, 5% mercaptoethanol, and 2 mg/ml blue bromophenol) and size separated in 15% SDS-PAGE. Proteins were transferred to polyvinylidene difluoride (PVDF) membranes (Amersham Pharmacia) using a transblot apparatus (Bio-Rad). For immunoblotting, membranes were blocked with 5% nonfat dried milk in Tween-PBS (TPBS) for 1 h. Primary antibodies against UCP-1 (Affinity BioReagent, Golden, CO, USA) were applied at a dilution of 1:1000 overnight at 4 °C. After washing, appropriate secondary antibodies (anti-rabbit IgG-HRP conjugated, Amersham Bioscience) were applied for 1 h at a dilution of 1:2000. Blots were washed, incubated in commercial enhanced chemiluminescence reagents (Amersham Bioscience), and exposed to autoradiographic film. To prove equal loadings of samples, blots were re-incubated with β-actin antibody (Sigma) and secondary antibody (antimouse IgG-HRP conjugated, 1:4000, Amersham Bioscience). Films were scanned using a GS-800 Calibrated Densitometer (Bio-Rad) and blots were quantified using Quantity One software (Bio-Rad). The values were normalized by β-actin to account for variations in gel loading.

RNA isolation

Total RNA of adipose tissue was isolated using RNeasy lipid tissue mini kit (Qiagen) according to the manufacturer's protocol. An on-column DNase digestion step was included in the protocol with RNase-free DNase set (Qiagen) to minimize genomic contamination. Concentration and purity of isolated RNA was determined by measuring the extinction at 260 and 280 nm.

Reverse transcription (RT) and quantitative real-time PCR

One microgram of total RNA was reverse transcribed in 40 μ l final volume using First Strand cDNA synthesis kit (Fermentas GmbH, St Leon-Rot, Germany) according to the manufacturer's instructions. Transcribed cDNA was stored at -20°C until further use.

To quantify expression levels of genes of interest, we employed the real-time quantitative RT ('TaqMan') PCR as previously described (Bolbrinker *et al.* 2006). Briefly, the ABI PRISM 7000 SDS instrument in conjunction with the ABI TaqMan Universal Master Mix (Applied Biosystems, Darmstadt, Germany) was used to perform the assays with a final concentration of 300 nM for the primers and 100 nM for the probes. Fluorogenic probes and primers were synthesized by TIB Molbiol (Berlin, Germany). For details of primer and probe sequences, see Table 1. Where no sequence for the probe is given either a SYBR Green dye-based assay was used for quantification according to the manufacturer's protocol (TAQurate GREEN Real-Time PCR MasterMix, Epicentre Biotechnologies, Madison, WI, USA) or no quantification was performed. To avoid signals of genomic origin, intron-spanning primers were used and primers were checked for genomic signal before use. Specificity of the product was confirmed by sequencing.

Relative quantification was done using the standard curve method. Every sample was measured in triplicate. To normalize our expression data, we used 18S as a housekeeping gene as previously described (Wendt *et al.* 2007), as well as hypoxanthine-guanine phosphoribosyl-transferase (HPRT).

Determination of Ang I and Ang II levels by HPLC

Ten milligrams of adipose tissue were homogenized in 100 μ l Tris (50 mmol/l, pH 7.4), 0.1 mmol/l EDTA, and 1 mg/ml bacitracin. After centrifugation at 19 000 g for 10 min, 50 μ l supernatant were loaded on a Kromasil C8 column (0.4 \times 20 cm) and eluted by means of a linear gradient from 26 to 70% acetonitrile/water containing 0.05% trifluoroacetic acid. Fractions containing Ang I and Ang II were collected, then concentrated in a speed-vacuum system and resuspended in 50 μ l Tris (50 mmol/l, pH 7.4). Ang I and Ang II were quantified using specific EIA kits for rat Ang I and Ang II respectively (Phoenix, Europe, Karlsruhe, Germany).

Statistical analysis

All values are given as mean \pm s.e.m. Student's *t*-tests or ANOVA were used as appropriate. A value of $P < 0.05$ was considered statistically significant; *n* represents the number of samples.

Results

Characterization of perivascular adipocytes

Figure 1 shows adipose tissues surrounding the mesenteric artery (Fig. 1a–c) and the aorta (Fig. 1d–f). Perivascular adipocytes from MAT were larger in size (mean diameter = $40.2 \pm 3 \mu\text{m}$; Fig. 1b and c) compared with adipocytes obtained from PAT, which had smaller diameters (mean diameter = 9.4 ± 0.2 ; Fig. 1e and f). The complete distribution of the cell sizes, as well as the analysis and comparison of the distributions by curve fitting of mesenteric adipocytes has been previously published by our group (Gálvez *et al.* 2006).

In order to determine the type of adipose tissue (i.e. WAT, or BAT), expression of UCP-1, a specific marker for BAT, was determined. Figure 2 shows that PAT is positive for UCP-1. The level of UCP-1 expression was similar to that of BAT used as positive control, thus suggesting that PAT is brown. MAT only showed a low expression of UCP-1, suggesting that it is WAT.

mRNA expression analysis of RAS components

We first performed qualitative expression analysis of the RAS components. This analysis revealed that angiotensinogen, ACE, ACE2, and chymase, as well as (pro)renin, AT_{1a}, and AT₂ receptors are expressed in both PAT and MAT, while renin expression could not be detected. Expression levels of the AT_{1b} receptor in PAT and MAT were very low, and therefore no further quantification analysis was performed (Fig. 4a).

Angiotensinogen

Angiotensinogen expression was detected in PVAT and was similar between PAT and MAT (Fig. 3a; Angiotensinogen/HPRT: $100\% \pm 26\%$ vs $143\% \pm 22\%$, $P = 0.27$).

(Pro)renin receptor

(Pro)renin receptor levels were approximately fivefold lower in MAT than in PAT ($P = 0.0059$; Fig. 3b; (pro)renin receptor/HPRT: $100\% \pm 15\%$ vs $14\% \pm 0.4\%$, $P = 0.003$). To our knowledge, this is the first study reporting renin receptor expression in BAT.

Table 1 Real-time quantitative RT-PCR: primers and probes

Gene	Gene symbol/ensembl gene ID	Forward (F), reverse (R)	Probe
Angiotensinogen	Agt/ENSRNOG00000018445	F: CTACGTTCACTTCCAAGGGAAGA R: CACAGACTGAGGTGCTGTTG	CCAGAACTCATGGAGCCCAGTCAGCT
Renin receptor	Atp6ap2/ENSRNOG00000003858	F: CATCCCTGGTGAGGAAGTCAAG R: CGCAAGGTTGTAGGGACTTTG	CATCCTTGAGACGAAACA
Renin	Ren1/ENSRNOG00000002937	F: GGTGCCCTCCACCAAGTGT R: GCTAGAGGATTCCGAGGAGTCA	TCCCCTCTACACTGCCTGTGAGATTCACA
ACE	Ace/ENSRNOG00000007467	F: TTGTCTGTCACCTGGAGCCTGA R: CCACACCCAAAGCAATTCTTC	TGACAAATATAATGGCCACGTCCCGGT
ACE2	Ace2/ENSRNOG00000003911	F: GAGGAGAATGCCAAAAGATGA R: GAAATTTGGGCGATCTTGA	CTGCGGCCAAATGGTCTGCCTT
Chymase	Cma1/ENSRNOG00000020563	F: GGCAGAAGCTCGAGGTTGAA R: GCCTAGGGTTAGCTTGGCTTTC	CATTCATCCAAACTATG
AT _{1a} (exon 1+3)	Agtr1a/ENSRNOG00000018346	F: CACATTCCCTGTCAAGTGGATT R: CATTGTGTTTTCTGGTTGAGTTG	NA
AT _{1a} (exon 1+2+6 bp+3)		F: CCACATTCCCTGAGTTAACATATGA R: GAGTTGGTCTCAGACACTATTCGAAA	ATCACCAGGTAAAGGTC
AT _{1b}	Agtr1b/ENSRNOG00000010640	F: ACCAGACCAGACACACACCTT R: TCTTGGATTCTTTAATTCCATCTCA	NA
AT ₂	Agtr2/none	F: CCTTCTGTATTGTTTCGTTGGA R: TGGAGCCAAGTAATGGGAACCTC	CCGCTTCCAACAGAAGCTCCGTAGTG
18S	Rnr1/none	F: GGAGCCTGCGGCTTAATTT R: CAACTAAGAACGGCCATGCA	CAATCTGTCAATCCTGTCCGTGCCG
HPRT	Hprt/ENSRNOG000000031367	F: CTCATGGACTGATTATGGACAGGACT R: TCCAGCAGGTCAGCAAAGAAC	NA

Forward (F) and reverse primers (R) (5′–3′) for PCR amplification and fluorogenic probes (5′ FAM - 3′ TAMRA) are given for the analysis of quantitative real-time PCR; NA, not applicable as SYBR green-based assay was used for detection (AT_{1a} (exon 1+3), HPRT) or no quantification was performed (AT_{1b}).

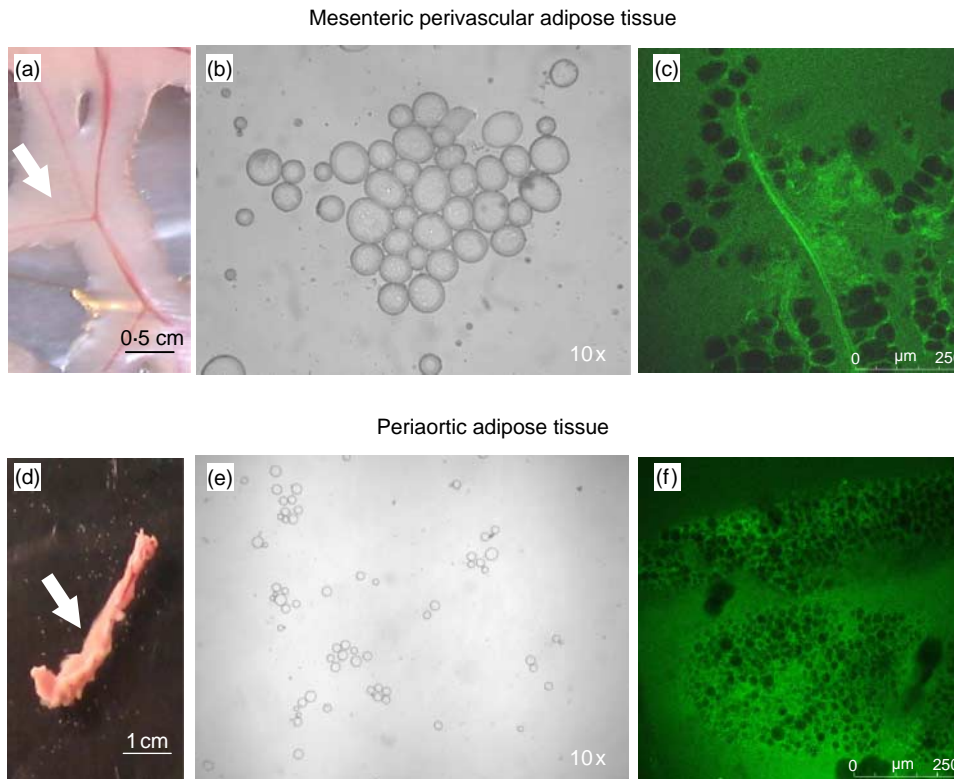


Figure 1 MAT and PAT of Wistar-Kyoto rats. Upper panel: (a) mesenteric artery surrounded by adipose tissue (arrow), (b) adipocytes isolated from fresh MAT (magnification 10 \times), and (c) adipocytes in a whole section of MAT observed by confocal microscopy. Lower panel: (d) aorta surrounded by adipose tissue (arrow), (e) adipocytes isolated from fresh PAT (magnification 10 \times), and (f) adipocytes in whole section of PAT observed by confocal microscopy. Full colour version of this figure available via <http://dx.doi.org/10.1677/JOE-07-0284>

ACE, ACE2, and chymase

Expression levels of ACE showed a trend for higher expression levels in MAT as observed by normalization with 18S (Fig. 3c), which was significant when normalizing data with HPRT ($100\% \pm 14\%$ vs $195\% \pm 25\%$, $P=0.018$). ACE2 levels (Fig. 3d) were similar between PAT and MAT (ACE2/HPRT: $100\% \pm 27\%$ vs $148\% \pm 19\%$, $P=0.220$). By contrast, chymase expression was almost sevenfold higher in MAT compared with PAT ($P=0.0013$; Fig. 3e; chymase/HPRT: $100\% \pm 15\%$ vs $627\% \pm 54\%$, $P<0.001$).

Angiotensin receptors

The AT₂ receptor expression was significantly higher in MAT than in PAT ($P<0.0001$; Fig. 3f; AT₂ receptor/HPRT: $100\% \pm 26\%$ vs $790\% \pm 111\%$, $P=0.006$).

While generating the standard curve for quantification of AT_{1a} receptor, we detected three different bands in RT-PCR indicating the existence of three splice variants of the receptor in adipose tissue (Fig. 4b). Sequencing revealed specificity of all three products. The lowest band representing the shortest amplicon comprises AT_{1a} lacking exon 2 (exon 1+3).

The band in the middle with a size of about 300 bp represents the wild-type form after normal splicing (exon 1+2+3). The analysis of the amplicon represented by the band above revealed an insertion of 6 bp derived from intronic sequence (exon 1+2+6 bp+3). For quantification of the shortest and the longest isoform, two specific assays were designed (Table 1). Expression of the AT_{1a} exon 1+3 was similar between PAT and MAT (Fig. 4c; AT_{1a} receptor (exon 1+3)/HPRT: $100\% \pm 39\%$ vs $142\% \pm 9\%$, $P=0.381$), whereas expression of the longest amplicon with exon 1+2+6 bp+3 was significantly higher in MAT compared with PAT ($P<0.0001$; Fig. 4d AT_{1a} receptor/HPRT: $100\% \pm 13\%$ vs $482\% \pm 40\%$, $P<0.001$).

Ang I and Ang II peptide levels

In order to assess the functionality of the RAS in PVAT and to validate the RT-PCR expression analysis, we determined levels of Ang I and Ang II peptides in these tissues. Ang I and Ang II were detected in both PAT and MAT. There were no significant differences in the amount of Ang I between the adipose tissues (Fig. 5a). However, Ang II protein level was significantly higher in MAT than in PAT ($P=0.043$; Fig. 5b).

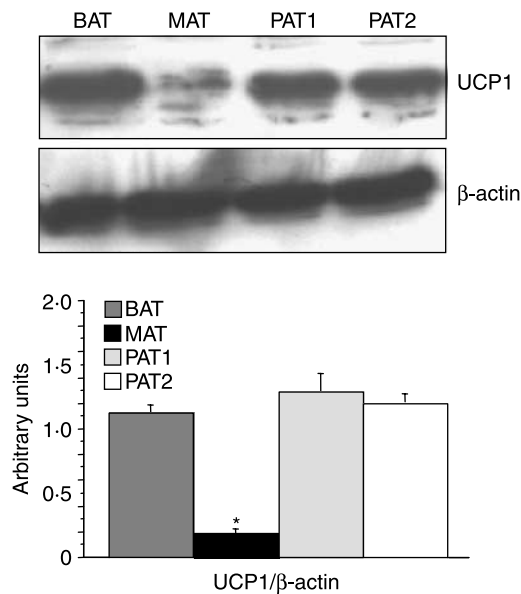


Figure 2 Immunoblots of UCP-1 obtained from interscapular brown adipose tissue (BAT), mesenteric adipose tissue (MAT), and periaortic adipose tissue (PAT). Bottom panel summarizes the level of UCP-1 expression normalized by β -actin as mean \pm S.E.M. Values correspond to three individual values. * $P < 0.05$ compared with values obtained from BAT.

Discussion

In this study, we show expression of all components of the RAS, except renin, in perivascular adipose tissue of the aorta and mesenteric arteries from WKY rats. An important new finding is the expression of the (pro)renin receptor, ACE2 and of three AT_{1a} receptor isoforms in perivascular adipose tissue. Gene expression levels of some RAS components are different between periaortic (BAT) and perimesenteric fat (WAT). The finding that Ang II levels and both AT_{1a} and AT_2 receptor expression levels are higher in mesenteric fat suggests that RAS-mediated effects might differ depending on the type of surrounding adipose tissue and the type of vascular bed.

Adipose tissue and adipocytes contain most of the components of the RAS giving rise to local Ang II synthesis at the tissue level (Saye *et al.* 1993, Harp & DiGirolamo 1995, Crandall *et al.* 1999, Engeli *et al.* 1999, 2000, Schling *et al.* 1999). Previously, the vast majority of studies investigating the expression of RAS components in adipose tissue have been performed in subcutaneous and visceral fat mainly obtained from epididymal and retroperitoneal tissues respectively. So far, no study has assessed the presence of the RAS in perivascular adipose tissue. Whether perivascular adipose tissue can be considered as visceral adipose tissue or not, is a matter of definition. According to Mårin *et al.* (1992), visceral (or intraperitoneal) fat is mainly composed of omental, mesenteric, and retroperitoneal fat masses. In view of this definition, adipose

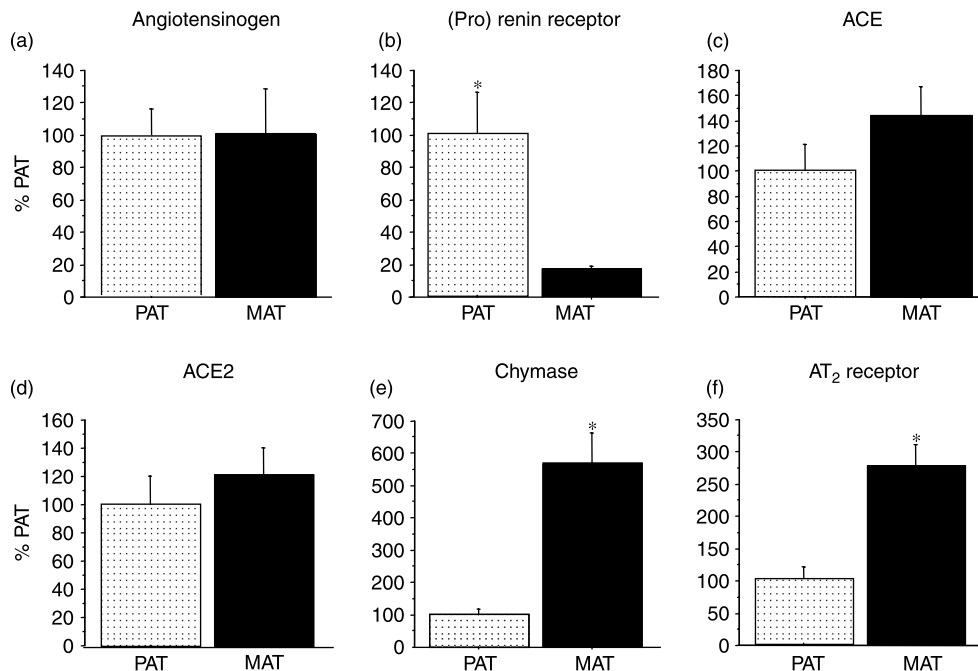


Figure 3 mRNA expression of RAS components in perivascular adipose tissues: periaortic adipose tissue (PAT) and mesenteric adipose tissue (MAT). (a) Angiotensinogen, (b) (pro)renin receptor, (c) ACE, (d) ACE2, (e) chymase, and (f) AT_2 receptor. All results are normalized to the housekeeping gene 18S and are expressed as percentage of the expression level in PAT. $n = 6$ for each group. * $P < 0.05$.

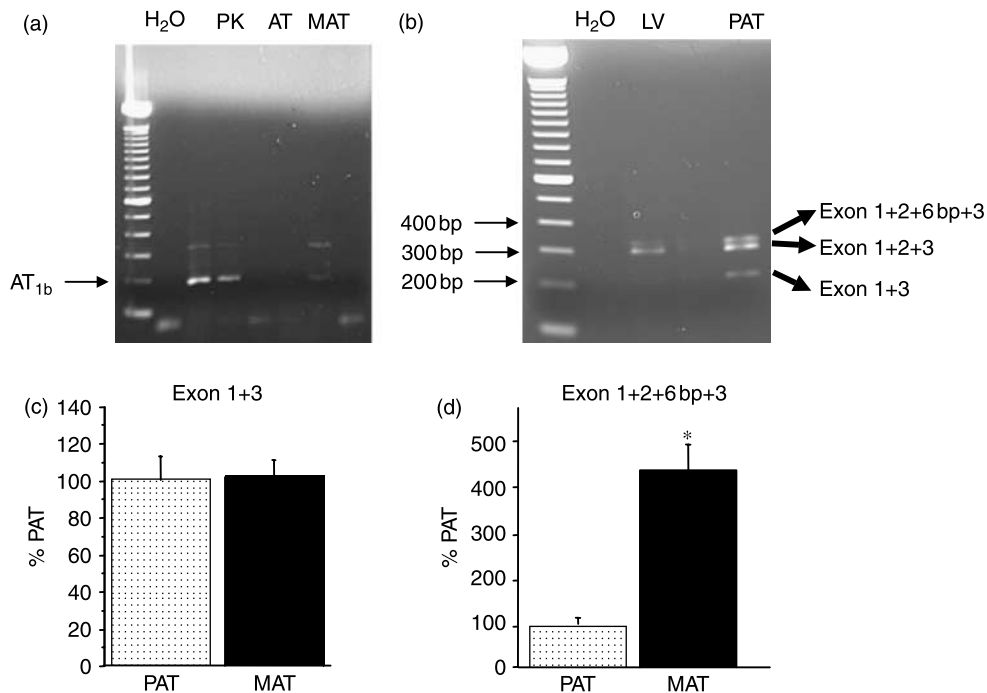


Figure 4 The AT_{1a} receptor isoforms in periaortic adipose tissue. (a) RT-PCR products of the AT_{1b} receptor in kidney (K), but absence in PAT and MAT. (b) RT-PCR products indicating the existence of three splice variants of the AT_{1a} receptor in adipose tissue. Expression levels in PAT and MAT of (c) Exon 1+3, and (d) Exon 1+2+6 bp+3. Results are normalized to the housekeeping gene 18S and are expressed as percentage of the expression level in PAT. * $P < 0.05$.

tissue surrounding mesenteric arteries can be considered visceral fat, whereas PAT and fat surrounding other blood vessels are not included in this definition. We have compared RAS expression in PAT and MAT. Our characterization of these adipose tissues shows that periaortic fat is brown (BAT) with smaller adipocytes, whereas mesenteric fat is WAT with larger adipocytes. This is in line with previous suggestions (Kortelainen *et al.* 1993, Matthias *et al.* 1994, Hausman *et al.* 2002), although it is the first demonstration of a differential expression of UCP-1 in PAT and MAT. Angiotensinogen expression and release from adipose tissue is well known. In fact, WAT is the most abundant source of angiotensinogen after the liver (Campbell & Habener 1987, Phillips *et al.* 1993). Visceral adipose tissue exhibits higher angiotensinogen levels than subcutaneous fat from rodents (Serazin-Leroy *et al.* 2000) and humans (Giacchetti *et al.* 2002). Moreover, high levels of angiotensinogen mRNA have been detected in PAT (Campbell & Habener 1987, Cassis *et al.* 1988a,b). In the present study, we can show that angiotensinogen expression is similar between white and brown PVATs.

Regarding renin mRNA expression in adipose tissue, there are conflicting results in the literature, which might be related to the species and type of adipose tissue (Engeli *et al.* 2003 and references therein). In our study, the renin mRNA expression was undetectable in either type of PVAT investigated, which is at variance with previously reported studies for other types of adipose tissues (Engeli *et al.* 1999, Giacchetti *et al.* 2002).

Our finding supports the concept that the functional role of RAS in PVAT does not depend on local renin synthesis but rather on renin or prorenin uptake from the circulation.

It is of interest that we detected the expression of the recently identified (pro)renin receptor (Nguyen *et al.* 2002, Danser *et al.* 2007) in both MAT and PAT. The presence of this receptor has been reported recently in human subcutaneous (Engeli *et al.* 2005, Achard *et al.* 2007) and visceral adipose tissue (Zorad *et al.* 2006, Achard *et al.* 2007). In our study, expression of the (pro)renin receptor could be detected in both white and brown PVATs, with higher levels of mRNA expression in periaortic fat.

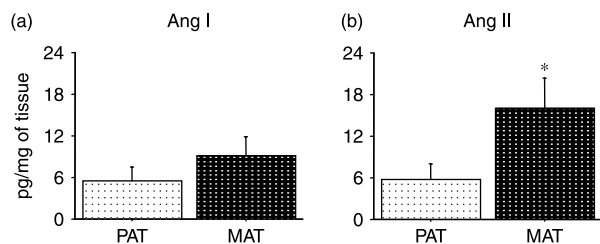


Figure 5 Ang I and Ang II peptide levels in perivascular adipose tissues: periaortic adipose tissue (PAT) and mesenteric adipose tissue (MAT). (a) Ang I and (b) Ang II levels. Results are expressed as pg/mg of tissue. $n = 6$ for each group. * $P < 0.05$.

The expression of angiotensinogen in PVAT highlights the potential role of local periadventitial synthesis of Ang I for the first time. This is confirmed by our detection of high levels of Ang I in both perimesenteric and periaortic fat. The Ang I levels were not different between both types of adipose tissue.

Regarding Ang II, we detected higher levels of this peptide in MAT. Other authors have found a fourfold higher amount of Ang II in rat BAT (Campbell *et al.* 1993, 1995, Shenoy & Cassis 1997) compared with WAT (Phillips *et al.* 1993). The Ang II synthesis in these tissues can be catalyzed by either ACE or chymase. ACE has been detected in WATs (Jonsson *et al.* 1994, Engeli *et al.* 1999, Schling *et al.* 1999, Giacchetti *et al.* 2002), whereas there are no data in the literature regarding ACE expression in BAT. ACE mRNA was found in subcutaneous and visceral adipose tissue in rodents (Crandall *et al.* 1992, Pinterova *et al.* 2000) and humans (Jonsson *et al.* 1994, Engeli *et al.* 1999, Giacchetti *et al.* 2002). Moreover, ACE activity was detected in human preadipocytes (Schling *et al.* 1999). Expression of α -chymase has been described to date only in human adipocytes (Engeli *et al.* 1999). The fact that expression levels of both ACE and chymase mRNA are higher in MAT than in PAT might explain the higher amount of Ang II levels in this tissue and might result in different treatment effects at the vascular tissue level during treatment with ACE inhibitors. Hence, local suppression of Ang II synthesis in response to ACE inhibition may be limited in PAT, but not in MAT due to its higher amounts of chymase expression.

The ACE homolog ACE2, which generates angiotensin 1–7 from Ang I (Tipnis *et al.* 2000), has not been previously described in adipose tissue. Here, we show equal expression of ACE2 in both white and brown PVATs.

The angiotensin receptor subtype that is most abundantly expressed in both WAT (Burson *et al.* 1994, Cassis *et al.* 1996, Crandall *et al.* 1999, Adams *et al.* 2002) and BAT (Cassis *et al.* 1996) is the AT₁ receptor subtype. Cassis *et al.* (1996) described that WAT contains a higher number of AT₁ receptors than BAT. Accordingly, we found that MAT exhibits a higher AT₁ receptor expression than PAT. Mice and rats possess two isoforms of the AT₁ receptor (AT_{1a} and AT_{1b}) encoded by two different genes (Sasamura *et al.* 1992). In PVAT, we detected AT_{1b} receptor expression only at a very low level. Interestingly, we found three isoforms of the AT_{1a} receptor in both types of adipose tissue. Existence of three variants of this receptor has been recently described in cardiac fibroblasts (Zhang *et al.* 2004, Cowling *et al.* 2005). Rat AT_{1a} receptors contain three exons of which exon 1 and exon 2 represent the 5'-untranslated region (Cowling *et al.* 2005). It was shown that translation of the AT_{1a} receptor is reduced in the presence of exon 2, so that the smallest splice variant appears to be translated more efficiently (Cowling *et al.* 2005). We observed higher levels of the variant containing exon 2 in MAT compared with PAT, whereas the short variant was expressed in similar amounts.

Expression of AT₂ receptors has recently been reported in epididymal adipose tissue from WKY rats (Zorad *et al.* 2006). Here, we also show AT₂ receptor expression in PVAT with a higher level of expression in mesenteric fat.

In conclusion, we show expression of all components of the RAS, except renin, in PVAT of the aorta and mesenteric arteries from WKY rats. Although Ang I peptide levels are similar between PAT and perimesenteric adipose tissues, both AT_{1a} and AT₂ receptor levels are higher in MAT. This suggests that, under certain circumstances, perivascular RAS might play a different role depending on the type of surrounding adipose tissue and the type of vascular bed. There are several issues that deserve future investigation. Since adipose tissue is a complex mixture of various cell types (adipocytes, stromal vascular cells, macrophages, etc.) and we have used whole-tissue homogenates, our study does not clarify the cell type where each RAS component is expressed. Another intriguing question relates to the possible physiological role of the perivascular adipose RAS. Due to the contractile, trophic, and pro-inflammatory effects of Ang II, the local production of this peptide in PVAT could have a substantial impact on vascular function and structure and might play an important role in the development of obesity-associated hypertension and vascular remodeling.

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