Physiology and Pathophysiology of the Adipose Tissue
Renin-Angiotensin System

Stefan Engeli, Raymond Negrel, Arya M. Sharma

Abstract—The renin-angiotensin system has long been recognized as an important regulator of systemic blood pressure and renal electrolyte homeostasis, and local renin-angiotensin systems have also been implicated in pathological changes of organ structure and function by modulation of gene expression, growth, fibrosis, and inflammatory response. Recently, substantial data have been accumulated in support of the notion that adipose tissue, besides other endocrine functions, also hosts a local renin-angiotensin system. In the first part of this review, we describe the components of the adipose tissue renin-angiotensin system in human and rodent animal models with respect to regulation of angiotensinogen expression and secretion, formation of angiotensin peptides, and the existence of angiotensin II receptors. In the second part, we describe the role of the adipose tissue renin-angiotensin system in the process of adipogenic differentiation and in the regulation of body weight. We also detail the differential regulation of the adipose tissue renin-angiotensin system in obesity and hypertension and thereby also speculate on its possible role in the development of obesity-associated hypertension. Although some findings on the adipose tissue renin-angiotensin system appear to be confusing, its involvement in the physiology and pathophysiology of adipose tissue has been confirmed by several functional studies. Nevertheless, future studies with more carefully described phenotypes are necessary to conclude whether obesity (by stimulation of adipogetic differentiation) and hypertension are associated with changes of renin-angiotensin system activity in adipose tissue. If so, the physiological relevance of this system in animal models and humans may warrant further interest. (Hypertension. 2000;35:1270-1277.)

Key Words: adipose tissue ■ angiotensin II ■ hypertension, obesity ■ obesity ■ prostacyclin ■ renin-angiotensin system

The renin-angiotensin system (RAS) has long been recognized as an important regulator of systemic blood pressure and renal electrolyte homeostasis. Over the last decade, several components of the RAS have been detected in a variety of tissues, for example, adrenal gland, kidney, brain, heart, and blood vessels. Consequently, the concept of local RAS as regulators of normal organ function has emerged.1–3 In addition, local RAS have also been implicated as major players in pathological changes of organ structure and function by modulation of gene expression, growth, fibrosis, and possibly inflammatory response.4–6 Indeed, the cardiac RAS plays a critical role in the hypertrophic response to pressure load as well as in tissue remodeling after myocardial infarction,7–10 and the renal RAS has been shown to be involved in fibrotic changes caused by inflammatory and metabolic diseases.11–14 Consistent with these observations, pharmacological blockade, either by angiotensin-converting enzyme (ACE) inhibitors or type 1 angiotensin-receptor (AT1) antagonists, is widely used in patients with hypertension, left ventricular hypertrophy, myocardial infarction, congestive heart failure, and diabetic nephropathy.15–19 Recently, substantial data have been accumulated in support of the notion that a local RAS is also present in adipose tissue. The occurrence of a local RAS in adipose tissue might appear intriguing, and its physiological meaning thus deserves to be discussed in more detail. In the first part of this article, we review current data on several components of the adipose tissue RAS in human and rodent animal models. In the second part, we describe the involvement of this local RAS in the regulation of adipose tissue physiology and speculate on its possible role in the pathophysiology of obesity and obesity-associated hypertension.

Angiotensinogen Expression and Secretion in Adipose Tissue

Investigation of angiotensinogen (AGT) in adipose tissue began in 1987, when AGT−mRNA was found in periaortic brown adipose tissue (BAT) and in cells found within the rat aorta wall.20 Subsequently, AGT secretion and AGT−mRNA were detected in several rat adipose tissue depots and in adipocytes isolated from rat arterial vessel walls, atria, and

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From the Department of Internal Medicine, Division of Endocrinology and Nephrology, Benjamin Franklin Clinic, Free University of Berlin (Germany) (S.E., A.M.S.), and the Institute of Signaling, Developmental Biology and Cancer Research, CNRS UMR 6543, Centre de Biochimie, Faculté des Sciences, Université de Nice-Sophia Antipolis, Parc Valrose, Nice Cedex, France (R.N.).

Correspondence to Prof Arya M. Sharma, Dept of Nephrology and Hypertension, Franz-Vohard-Clinic, University Clinic Charité, Humboldt University, Wiltenbergstr. 50, 13122 Berlin, Germany. E-mail sharma@zedat.fu-berlin.de

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mesenterium.\textsuperscript{21–23} In humans, AGT expression has been demonstrated in adipose tissue,\textsuperscript{24–26} in primary cultured adipocytes,\textsuperscript{24,25} and in differentiating preadipocytes.\textsuperscript{27} In fact, increasing AGT expression and secretion is a characteristic feature of preadipocyte differentiation and is therefore considered a late marker of adipocyte differentiation.\textsuperscript{25–32} Cis and trans regulators of AGT expression during adipogenic differentiation have been identified in mouse 3T3-L1 preadipocytes.\textsuperscript{33–36}

Fatty acids,\textsuperscript{31} glucocorticoids,\textsuperscript{32} and possibly tumor necrosis factor-\(\alpha\)\textsuperscript{37} have been shown to modulate AGT expression in Ob1771 and 3T3-L1 clonal preadipocyte cell lines. In contrast, well-known activators of liver AGT expression, such as estrogens, triiodothyronine, and angiotensin II (Ang II),\textsuperscript{21} were without effect in Ob1771 cells,\textsuperscript{32} and glucose likewise did not change AGT expression in 3T3-L1 cells.\textsuperscript{24} Insulin is another important stimulator of liver AGT expression, but conflicting results have been obtained on this hormone in adipose tissue: In vivo, streptozotocin-induced insulin deficiency in Sprague-Dawley rats resulted in a fall of adipose tissue AGT expression, which was restored by insulin treatment,\textsuperscript{38} but insulin did not change AGT secretion in primary cultured adipocytes of Obese Zucker rats.\textsuperscript{39} Furthermore, insulin stimulated AGT expression in 3T3-L1 cells\textsuperscript{24} but depressed it in Ob1771 and 3T3-F442A cells.\textsuperscript{77}

Recent studies with the central-acting sympatholytic agent \(\alpha\)-methyl-\(p\)-tyrosine resulted in decreased adipose tissue AGT expression in wild-type mice,\textsuperscript{40} implicating the sympathetic nervous system as a stimulator of AGT. On the other hand, sympathetic activators such as isoproterenol decreased AGT expression in 3T3-L1 cells,\textsuperscript{24} and fasting, usually accompanied by sympathetic activation, did not change adipose tissue AGT expression in wild-type mice.\textsuperscript{40}

In Sprague-Dawley rats, adipose tissue AGT expression increased in response to bilateral nephrectomy or treatment with the ACE inhibitor enalapril\textsuperscript{21} but was not affected by a sodium-restricted diet.\textsuperscript{22} Aging, usually associated with weight gain, resulted in decreased adipose tissue AGT expression in Wistar-Kyoto and Wistar Fatty rats but not in Sprague-Dawley and Obese Zucker rats.\textsuperscript{41–43} AGT expression was higher in visceral than in subcutaneous adipocytes in these rat strains,\textsuperscript{42,44} a finding recently also reported in humans.\textsuperscript{45,46} Gender differences in AGT expression in human adipose tissue are controversial\textsuperscript{25,45,46} but have been reported in Sprague-Dawley rats, in which testosterone is a strong activator of adipose tissue AGT expression.\textsuperscript{44}

**Generation of Angiotensin Peptides in Adipose Tissue**

Renin activity has been found in rat BAT even after bilateral nephrectomy,\textsuperscript{37} and detection of renin-mRNA in human adipocytes\textsuperscript{26} and its increase during human preadipocyte differentiation have recently been reported.\textsuperscript{27} However, other groups failed to confirm these results (see Table 1 for further details).\textsuperscript{25,41,48} Thus, the origin of adipose tissue renin activity remains unsolved. Further studies will be required to determine whether renin is produced by adipocytes, or, as has been reported for other tissues,\textsuperscript{49–51} the presence of renin and reninlike activity in adipose tissue is due to uptake of the circulating enzyme. Interestingly, the expression of the renin-binding protein gene has recently been reported in human adipocytes.\textsuperscript{25,27} This intracellular localized enzyme\textsuperscript{52} is identical to \(N\)-acyl-\(D\)-glucosamine 2-epimerase, usually involved in neuroamine acid metabolism, and apparently functions as a renin inhibitor,\textsuperscript{53} leading to a fall in blood pressure when given intravenously.\textsuperscript{54} Modulation of renin activity in adipose tissue by this protein therefore appears to be possible.\textsuperscript{52}

Consistent with the fact that AGT is a late marker of differentiation in mouse and human preadipocytes,\textsuperscript{27–32} the production of Ang II has been shown to increase during differentiation of human preadipocytes\textsuperscript{37} and can be blocked with the ACE inhibitor captopril in rat adipose tissue.\textsuperscript{41} This finding is in agreement with several reports of ACE expression and activity in human adipocytes.\textsuperscript{25–27,45,55} Stronger ACE expression was found in human visceral than in subcutaneous adipose tissue\textsuperscript{45}; obesity, on the other hand, was not shown to influence ACE expression in humans.\textsuperscript{45} Recent studies in human adipose tissue revealed the expression of the Ang I–forming enzyme cathepsin D\textsuperscript{26} as well as the Ang II–forming enzymes chymase\textsuperscript{25} and cathepsin G.\textsuperscript{26} The contribution of these enzymes to the generation of angiotensin peptides in adipose tissue remains to be clearly established, since inhibitors of ACE (ethylenediaminetetraacetic acid, EDTA), chymase (4,2-aminooethyl-benzenesulfonylfluoride, AEBSF), and cathepsin G (pepsatin) did not influence Ang II–forming activity in homogenates of 3T3-F442A preadipocytes\textsuperscript{48} (see Table 2 for further details).

**TABLE 1. Angiotensin I–Forming Enzymes in Adipose Tissue and Adipocytes**

<table>
<thead>
<tr>
<th>Models Investigated</th>
<th>REN mRNA</th>
<th>REN Protein</th>
<th>Ang I Formation</th>
<th>Other Enzymes</th>
<th>Inhibition of Ang I Formation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human (mature adipocytes)</td>
<td>–/+</td>
<td>nd</td>
<td>nd</td>
<td>cathepsin D-</td>
<td>nd</td>
</tr>
<tr>
<td>(differentiating preadipocytes)</td>
<td>[27]</td>
<td>[27]</td>
<td>[27]</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Rat adipose tissue</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>nd</td>
<td>+: kallikiren and REN-Ab</td>
</tr>
<tr>
<td>(Sprague-Dawley, Wistar)</td>
<td>[47]</td>
<td>[47]</td>
<td>[41, 47]</td>
<td>[47]</td>
<td></td>
</tr>
<tr>
<td>Mouse clonal cell lines (3T3-F442A)</td>
<td>–</td>
<td>nd</td>
<td>+</td>
<td>cathepsin D-</td>
<td>–: pepstatin</td>
</tr>
<tr>
<td></td>
<td>[48]</td>
<td>[48]</td>
<td>[48]</td>
<td>mRNA [48]</td>
<td></td>
</tr>
</tbody>
</table>

Ab indicates antibodies; nd, not determined; REN, renin; +, found; and –, not found.
Presence of Angiotensin Receptors in Adipose Tissue

AT₁ receptors were first identified in 1993 in adipocyte membranes prepared from rat epididymal fat tissue. Since then, evidence on mRNA, protein, and functional levels for AT₁ receptors as well as AT₂ receptors has been obtained in rodent models and in human adipocytes by several investigators, but the function of these receptors remains to be determined. In vivo, adipose tissue expression of the gene for AT₁ (AGTR1) appears to be age-dependent, since old, obese Sprague-Dawley rats had lower AT₁ receptor densities than younger, leaner controls. It has therefore been hypothesized that AT₁ receptor downregulation may be the result of increased adipose tissue formation of Ang II as a result of the development of obesity in older rats. In contrast, long-term treatment of these rats with the specific AT₁ antagonist losartan, which is usually accompanied by increased Ang II plasma and tissue levels, also resulted in downregulation of AT₁ receptors. Obesity did not change adipose tissue AT₁ density in obese Zucker rats but was associated with increased AGTR1 expression in both visceral and subcutaneous adipose tissue in human subjects, with stronger AGTR1 expression in visceral adipocytes at any time and any body weight.

Physiological Importance of Adipose Tissue RAS

Adipose tissue not only contains adipocytes but also fibroblast-like cells (eg, preadipocytes), smooth vascular muscle cells, endothelial cells, sympathetic nerve fibers, and mononuclear and lymphocytic cells. The Figure summarizes the possible effects of Ang II on these potential cellular targets in adipose tissue. In addition to the well-known subcutaneous and omental adipose tissue depots, adipocytes can be found in close association to nearly all organs, either as an adipose tissue capsule (eg, kidney, heart, epididymis) or as a fibrous cover containing adipocytes (eg, extima of blood vessels).
vessels). On the basis of the mechanisms proposed in the
Figure, it appears reasonable to speculate that Ang II,
released by adipocytes, is of potential importance for the
physiology and perhaps pathophysiology of adipose tissue
and organs in close communication with adipocytes.

Role of RAS in Growth and Differentiation of
Adipose Tissue
Ang II acts as a well-recognized growth factor in a variety of
tissues and cells,66–70 and recent data suggest that Ang II may
also play a role in adipocyte growth and differentiation.58,61,71
Stimulation of human preadipocytes with Ang II resulted in an
acceleration of the G1-phase of the cell cycle and increased
expression of the cell cycle regulator cyclin D1.58 Furthermore,
antisense oligonucleotides directed against the
differentiation-specific element binding protein resulted in a
dose-dependent inhibition of lipid accumulation in mouse
3T3-L1 preadipocytes.71 This protein acts as a transactivator
by binding to the differentiation-specific element in the AGT
promoter and thereby initiates AGT activation during adipo-
genic differentiation of the 3T3-L1 clonal cell line.36

With respect to preadipocyte differentiation, it is worth
noting that prostaglandin I2 (PGI2=prostacyclin), which is a
major metabolite of arachidonic acid in rodent and human
adipose tissue,72–74 is a potent and specific autocrine effector
of adipogenic differentiation.61,75–79 Interestingly, PGI2 secre-
tion by adipocytes is induced on exposure to Ang II, both in vitro61,86 and in the interstitial fluid of rat adipose tissue in vivo.61 Moreover, Ang II induces in a paracrine manner the
differentiation of preadipocytes into adipocytes, as has been
demonstrated in coculture experiments of matured Ob1771
preadipocytes with undifferentiated Ob1771 preadipocytes.61 In
this experimental setting, PGI2 was secreted exclusively from
matured Ob1771 cells and acted as a chemical relay for the
action of Ang II.61 Consistent with the involvement of PGI2 as
an Ang II–induced paracrine messenger, its adipogenic effect
was suppressed by inhibitors of prostaglandin synthesis such as acetyl salicylic acid as well as by neutralizing antibodies
against PGI2.61 It is of interest to note that evidence of the
ability of Ang II to induce rat adipose precursor cells to
differentiate ex vivo in adipose tissue explants has recently
also been obtained: Immunostaining of a differentiation
marker (glycerol-3-phosphate dehydrogenase, GPDH) re-
vealed a decrease of the proportion of undifferentiated
GPDH-negative cells on exposure to a stable analogue of PGI2 or to Ang II, whereas that of differentiating GPDH-
positive cells is increased. As expected for an involvement of PGI2 as a paracrine chemical relay of Ang II, this adipogenic
effect of Ang II again is abolished in the presence of acetyl
salicylic acid (P. Saint-Marc, C. Darimont, G. Ailhaud, L.
Kozak, R. Negrel, unpublished data, 1999).

In the mouse Ob1771 system, the AT2-receptor antagonist
PD123319 but not the AT1-receptor antagonist losartan was
able to counteract the indirect adipogenic effect of Ang II.61
Although in contrast, Ang II–induced secretion of prostaglan-
dins from rat adipocytes appears to be mediated by the AT1
receptor,43 it can be hypothesized that Ang II, cleaved from
AGT secreted by mature adipocytes, may act in a paracrine
manner on AT2 receptors to induce the production and release
of PGI2, thereby promoting adipogenic differentiation in the
Ob1771 model. Nevertheless, the exact profile of action of the
different Ang II receptors in the process of adipogenic
differentiation appears to depend on the species or models
investigated. Schling and Löffler60 reported upregulation of
AGTR2 expression and downregulation of AGTR1 expression
during in vitro differentiation of human preadipocytes.

Thus, a balance between AT1- and AT2-dependent mecha-
nisms, related to adipocyte hypertrophy and adipose tissue
hyperplasia in the various models studied, might be of
importance83 and may be explained by the actual Ang II
receptor status. The involvement of AT2 receptors in preadi-
pocyte differentiation coupled to PGI2 production61 and that
of AT1 receptors in the acceleration of the preadipocyte cell
cycle58 as well as the differential pattern of angiotensin-
receptor expression in mouse Ob1771 and 3T3-L1 and human
preadipocytes27,58,60,61,82 clearly indicate that additional ex-
periments are needed to clarify the involvement of the
different Ang II–receptor subtypes in these models of various
species.

Ang II, Body Weight Regulation, and Adipose
Tissue Metabolism
In Sprague-Dawley rats, Ang II infusions resulted in weight
loss84,85 and reduction of white adipose tissue mass.86 This
effect was independent of blood pressure changes and was
abolished by losartan.84 In pair-feeding experiments, 70% of
the weight loss was attributable to decreased food intake,84
whereas other investigators found no changes in food intake
but an increased body temperature.86 Ang II could thus appear
as anorexigenic and as an effector of energy expenditure.
In contrast, studies in rats and humans have reported weight loss
with the administration of ACE inhibitors87–89 and age-
related white adipose tissue hypertrophy in rats was pre-
vented by the long-term administration of the AT1 antagonist
losartan.43

Ang II–associated weight loss84–86 may be ascribed to an
AT1-dependent lipolytic effect. However, lipolytic activity of
Ang II has neither been reported in vitro61,70,72 nor in vivo.80
In contrast, in vitro studies demonstrated lipogenic effects of
Ang II in 3T3-L1 and human adipocytes, along with increased
activity and expression of GPDH and fatty acid synthase.63 In
this later study, receptor binding experiments detected only
AT2 receptors, but Ang II–associated lipogenesis was inhib-
ited by both the AT2 antagonist PD123319 and the AT1
antagonist losartan.62

Ang II–induced norepinephrine release from BAT in obese
Zucker rats is more pronounced in young, preobese rats as
compared with older, obese animals.80 This may result in
impaired thermogenesis in older animals and thus may be a
mechanism leading to age-associated obesity. Cold exposure
in Sprague-Dawley rats increased Ang II concentrations in plasma and BAT, increased AT₁-receptor density in BAT, and increased norepinephrine release as well as decreased its reuptake in BAT.91,92 These changes in norepinephrine turnover on cold exposure were completely prevented by treatment with losartan.91 Thus, cold exposure activates the systemic as well as the local BAT RAS, and this might be a possible mechanism leading to the well-known sympathetic activation in cold-exposed animals. In addition, cold-exposed, pair-fed animals did not show any increase of plasma Ang II levels, meaning that increased food intake, usually seen on cold exposure, appears to be important at least for the systemic activation of the RAS.92

Interestingly, 2 recent randomized trials have demonstrated that treatment with the ACE inhibitors captopril (Captopril Prevention Project, CAPPP)93 or ramipril (Heart Outcomes Prevention Evaluation, HOPE)94 may reduce the incidence of type 2 diabetes and of diabetes-related end points. Whether or not this effect is related to an effect of ACE inhibition on insulin sensitivity or is mediated by an effect on adipose tissue metabolism remains to be determined.

Regulation of Adipose Tissue RAS in Obesity and Hypertension

A positive relation between AGT plasma levels and blood pressure was first described in 1979 by Walker et al95 and has since been confirmed not only in humans,96,97 but also in rat models of hypertension.98,99 It is further interesting to note that some studies found positive correlations between plasma AGT levels and body mass index in different human populations100–103 and that linkage between obesity and an AGT polymorphism was demonstrated in a genetic isolated population.104 Not only plasma AGT but also plasma renin activity45,105,106 and plasma ACE activity100 were positively correlated to the body mass index in obese human subjects. These findings were not repeated in Obese Zucker rats; however, infusion of Ang II led to a stronger blood pressure increase in obese compared with lean animals.107

Besides a significant relation between blood pressure, body mass index, and plasma AGT levels in lean normotensive subjects,108 we reported that ∼20% of the plasma AGT variance could be explained by plasma leptin levels in this study. Taking plasma leptin as an indicator of adipose tissue hormone expression in adipose tissue and refeeding by an increase.111 Stimulation of adipose tissue AGT expression by food intake might be a possible explanation for the refeeding hypertension model. In this rat model of obesity-associated hypertension, high blood pressure usually develops as a result of fasting and refeeding cycles, but to date, sympathetic activation has been the only mechanism examined in this model.112–115

It is important to recall that AGT expression is positively regulated by fatty acids31 and carbaprostacyclin116 by means of a transcriptional mechanism, implicating the peroxisome proliferator–activated receptors PPARγ and/or PPARα.117–120 Such a mechanism might be a possible link between AGT regulation in adipose tissue, food intake, and the metabolic disturbances accompanying obesity. Nevertheless, no peroxisome proliferator–response element has so far been reported within the AGT promoter region. AGT expression in adipose tissue of animal models of obesity and hypertension as well as in obese and hypertensive subjects has been investigated with positive42,46,98,99,110,111 and negative results.24,41,45,121 In that respect, the AGT-deficient hypotensive mouse model, which has been generated by homologous recombination,122 appears as an interesting tool to study adipose tissue cellularity and blood pressure in response to low- or high-fat feeding, as compared with wild-type animals.123

Conclusions

Findings on AGT secretion, generation of Ang peptides, and activity of Ang II receptors confirm the existence of a local RAS in adipose tissue. However, available data reveal still unsolved problems. AGT gene expression in adipose tissue is subject to differential regulation, but the data are incomplete and sometimes controversial. Formation of Ang peptides has been demonstrated in adipose tissue, but the pathways involved have not been definitely characterized. The presence of both subtypes of Ang II receptors is supported not only by the finding of mRNA or protein but also by ligand-binding as well as functional and pharmacological studies. Nevertheless, the reported patterns of Ang II–receptor subtypes vary substantially between the different models. These inconsistencies may be due to the high number of model organisms and systems that have been investigated. If all species, strains, tissues, and clonal cell lines are considered, the number of models investigated reaches ∼20. In addition, these models not only belong to different species and strains but also represent various stages of adipose cell differentiation, starting with mouse preadipocyte cell lines, which have been investigated during their complete course of differentiation, ending with freshly isolated, mature human adipocytes investigated ex vivo.

Although some findings on the adipose tissue RAS appear to be confusing, its involvement in the physiology and
pathophysiology of adipose tissue has been confirmed by several functional studies. Especially, adipose tissue development and metabolism have been shown to be regulated by Ang II in vitro and in vivo. Nevertheless, the possible contribution of locally produced Ang II on blood pressure regulation still remains to be established. Future studies with carefully described phenotypes are necessary to conclude whether obesity and hypertension are associated with changes of RAS gene expression and activity in adipocytes and, if so, the physiological relevance must be tested in in vivo models. Future studies will also determine whether the local adipose tissue RAS is involved in the beneficial effects of ACE inhibitor treatment on the development of type 2 diabetes, as has been demonstrated by recent randomized cardiovascular prevention trials (CAPPP and HOPE).

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