Renin dynamics in adipose tissue: adipose tissue control of local renin concentrations

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Fowler JD, Krueth SB, Bernlohr DA, Katz SA. Renin dynamics in adipose tissue: adipose tissue control of local renin concentrations. Am J Physiol Endocrinol Metab 296: E343–E350, 2009. First published December 2, 2008; doi:10.1152/ajpendo.90693.2008.—The renin-angiotensin system (RAS) has been implicated in a variety of adipose tissue functions, including tissue growth, differentiation, metabolism, and inflammation. Although expression of all components necessary for a locally derived adipose tissue RAS has been demonstrated within adipose tissue, independence of local adipose RAS component concentrations from corresponding plasma RAS fluctuations has not been addressed. To analyze this, we varied in vivo rat plasma concentrations of two RAS components, renin and angiotensinogen (AGT), to determine the influence of their plasma concentrations on adipose and cardiac tissue levels in both perfused (plasma removed) and nonperfused samples. Variation of plasma RAS components was accomplished by four treatment groups: normal, DOCA salt, bilateral nephrectomy, and losartan. Adipose and cardiac tissue AGT concentrations correlated positively with plasma values. Perfusion of adipose tissue decreased AGT concentrations by 11.1%, indicating that adipose tissue AGT was in equilibrium with plasma. Cardiac tissue renin levels positively correlated with plasma renin concentration for all treatments. In contrast, adipose tissue renin levels did not correlate with plasma renin, with the exception of extremely high plasma renin concentrations achieved in the losartan-treated group. These results suggest that adipose tissue may control its own local renin concentration independently of plasma renin as a potential mechanism for maintaining a functional local adipose RAS.

renin-angiotensin system; angiotensinogen; myocardium; rat; plasma

THE RENIN-ANGIOTENSIN SYSTEM (RAS) has classically been considered a blood pressure and blood volume regulation system. Decreases in blood pressure, renal tubule sodium delivery, or angiotensin (Ang) II levels and increased sympathetic stimulation cause renal renin to be secreted into the plasma. Renin then acts on plasma angiotensinogen (AGT) to produce Ang I, which is further cleaved by Ang-converting enzyme to form the eight-amino acid peptide Ang II, the biologically active component of the RAS. Renin concentration is the primary rate-limiting step in this plasma cascade (7), although changes in plasma AGT levels can also influence the generation of angiotensins. Functional consequences are mediated primarily through Ang II binding to the Ang II type Ia receptor AT1R, which acts within the kidney, brain, adrenal glands, and vasculature to mediate increased blood volume and total peripheral resistance, thereby maintaining blood pressure (23).

Recently, increasing evidence has implicated a locally derived RAS within adipose tissue as an important regulator of adipose tissue growth, differentiation, metabolism, and inflammation (2, 4, 10, 11). Current literature indicates mRNA expression of all components necessary for a complete local RAS in adipocytes, including Ang II receptors, AGT, renin, and Ang-converting enzyme (13, 20, 22, 26). Ang II has been shown to induce lipolysis in 3T3-L1 adipocytes and human adipose cells (11). In addition, Ang II can increase expression of AGT in adipose tissue through its interaction with the Ang II type Ia receptor (19). Furthermore, Ang II type I receptor blockade in adipose tissue was shown to reduce the production of reactive oxygen species and improve some effects associated with adipokine dysregulation, including adiponectin expression and reduced TNFα and monocyte chemoattractant protein-I expression (18). Many reports implicate Ang II in the regulation of adipocyte differentiation, although any precise role remains unclear (4, 10, 22, 25).

Given recent research concerning the effects that locally produced Ang II is proposed to have within adipose tissue, the independence of adipose tissue RAS components from that of plasma RAS components has not been adequately addressed. Circulating RAS components change primarily with respect to salt and water balance as well as blood pressure and not with situations involving adipocyte metabolism or differentiation. Local generation of Ang II near adipose cell membranes should be, at least in part, a function of adipose interstitial fluid (ISF) renin and AGT concentrations. Plasma-derived renin and AGT are present in the ISF of many tissues, including the heart (9). Although adipose capillaries are not highly fenestrated, adipose tissue ISF albumin concentration has been shown to be 15% of plasma (5), and albumin permeability across adipose capillaries was found to increase with sympathetic stimulation and histamine release (24). Since renin and AGT are smaller than albumin, circulating renin and AGT could have access to adipose tissue. Therefore, we measured renin and AGT in adipose tissue and compared their levels with corresponding plasma levels. If plasma renin or AGT is able to move across adipose capillary walls, then plasma renin or AGT changes could result in concomitant adipose tissue RAS changes. If so, a local adipose RAS would not necessarily control local adipose growth, differentiation, metabolism, or inflammation independently of the plasma RAS.

Previous reports have measured the plasma contribution of RAS components in cardiac tissue (9, 15, 16). Analogous to that of adipose tissue, all components necessary for a local.
RAS within cardiac tissue have also been detected (30). Studies varying plasma concentrations of renin and AGT have generally shown that left ventricular cardiac renin and AGT concentrations are dependent on plasma concentrations of renin and AGT (9, 15, 16). Therefore, we also measured cardiac renin and AGT to compare the known changes of renin and AGT in cardiac tissue with the possible changes in adipose tissue during alterations of plasma renin and AGT.

In light of the many recent implications for the function of Ang II within adipose tissue, insight into the potential influence of plasma RAS components to that of adipose tissue will prove useful for elucidation of the control and functional significance of a local adipose RAS. To address this, rat plasma concentrations of renin and AGT were pharmacologically and surgically varied to examine how plasma fluctuations influence adipose tissue RAS component concentrations. The comparison was also extended to cardiac tissue. We tested the hypothesis that variations in the plasma RAS would be reflected in adipose tissue, as reported previously for cardiac tissue (9, 14, 16).

Manipulation of plasma renin and AGT was done with treatments previously shown to alter their plasma levels. Forty-eight-hour bilateral nephrectomy (BNX) removes the primary plasma renin source, resulting in decreased plasma renin and increased AGT (15). Long-term deoxycorticosterone acetate (DOCA) treatment causes plasma renin to fall. AGT plasma levels also fall (16) due to reduced chronic hepatic production of AGT secondary to reduced Ang II levels acting at hepatic AT1 receptors that normally stimulate AGT secretion (27). Losartan treatment inhibits negative feedback of renal renin secretion that normally stimulates AGT secretion (2). Losartan treatment (n = 11) was maintained for 3 wk prior to the rats being killed. Losartan animals gained 73.7 ± 4.7 g in 3 wk.

BNX. Both kidneys were removed from each animal by separate flank incisions under pentobarbital anesthesia (50 mg/kg ip injection). Ten milliliters of normal saline was added to the intra-abdominal cavity at the completion of surgery to maintain adequate hydration. Animals (n = 9) were maintained for 48 h with minimal food and free access to water, as published previously (14). Weight change was not measured over the course of 48 h.

Normal treatment. Rats (n = 12) were given no special treatment and were maintained with free access to food and water. Normal animals gained 82.6 ± 7.9 g over ~3 wk.

Tissue collection and perfusion. Animals were euthanized with 100% CO2 in a chamber able to accommodate the entire cage so as to reduce stress and subsequent sympathetically mediated renal renin secretion. When breathing had ceased and the animal was unresponsive, the chest cavity was quickly opened to expose the heart. A right or left visceral fat pad was randomly isolated, clamped, and removed from each animal (nonperfused visceral adipose tissue). Blood was drawn from the right ventricle into a syringe containing 5% EDTA, final 0.1%, immediately chilled, and centrifuged, and the plasma was frozen at −70°C for plasma analysis of RAS components. After blood collection, the left ventricular wall was pierced at its apex with a blunted 18-gauge needle, and a solution of 0.9% saline at 37°C was perfused at a pressure of 90 mmHg through the heart for ~10 s to flush the coronary circulation. The catheter was then advanced into the ascending aorta, and perfusion continued for an additional 45–60 s. Liver tissue and the testicular vein were monitored for blanching to ensure complete visceral adipose tissue perfusion. Upon completion of perfusion, the remaining visceral adipose fat pad, a retroperitoneal adipose tissue sample (perirenal fat), and left ventricular cardiac tissue (perfused tissues) were collected from each animal. Specifically, the visceral adipose tissue was a combination of omental and reproductive adipose tissue. Care was taken to remove both the testis and seminal vesicles from isolated white visceral adipose tissue. Tissues were diced into 3- to 4-mm sections, frozen, and stored at −70°C until extract preparation.

Extract preparation. Visceral and retroperitoneal adipose tissue homogenized at a ratio of 1 g of tissue to 1 ml of ice-cold protease inhibitor buffer (PIB) consisting of 0.15 M sodium phosphate buffer (pH 7.45), 1% BSA, 0.05% NaN3, and serine, metallo-, and thiol protease inhibitors [15 mM EDTA, 2.3 mM captopril, 2 mM 8-hydroxyquinoline, 10 mM sodium tetrathionate, 20 mM benzamidine, 10 mM N-ethylmaleimide, 3 mM Pefabloc SC [4-(2-aminomethyl)benzenesulfonyl fluoride], 20 μM leupeptin, and 450 μM aprotinin]. Cardiac extracts were homogenized at 1 g to 5 ml of PIB. Briefly, PIB was added to frozen tissue in a 1:5 ml dounce glass homogenizer. Samples were homogenized on ice to a uniform consistency. Homogenized samples were frozen at −70°C, thawed, and centrifuged at 100,000 g for 1 h. The aqueous phase, containing soluble RAS components, was removed and frozen at −70°C for subsequent assay of renin and AGT levels.

Renin concentration measurement. Adipose extract, plasma, or cardiac extracts were diluted in PIB to yield similar renin concentration measurements in each assay tube. One hundred-microliter assay tubes containing diluted samples, 50% PIB, 25% renin-free rat AGT [obtained from 48-h bilaterally nephrectomized rats, as described previously (16)], and 25% sodium phosphate buffer (0.15 M, pH 7.45, 2.5% BSA) were incubated at 37°C for 0, 3, and 6 h in duplicate. During the 37°C incubation, Ang I was generated by cleavage of AGT. Ang I initially present in the sample 0-h assay tube is subtracted from the 3- and 6-h time points to yield a linear generation of Ang I per milliliter plasma or gram tissue per hour. Ang I measurement is described below. As previously described, AGT levels in the assay are at saturating conditions such that Ang I generation over time is directly proportional to renin concentration (14–16).

Materials and Methods

Animals. Procedures involving animals were approved by the institution of animal care and use committee at the University of Minnesota. Male Sprague-Dawley rats were purchased from Charles River Laboratories at 251–275 g. After ~10 days of acclimation, rats were randomly divided into normal, DOCA, BNX, or losartan treatment groups. All animals had free access to standard rat chow and water unless noted otherwise.

DOCA treatment. One hundred milligrams of DOCA (Sigma) imbedded in a silastic pellet was subcutaneously implanted via a flank incision. In addition, unilateral nephrectomy was performed on the opposing flank for each animal according to a previously published laboratory protocol (16). Animals (n = 9) were maintained with 1% NaCl in their drinking water and normal chow for 3 wk. DOCA animals gained 46.4 ± 5.6 g in 3 wk.

Losartan treatment. Losartan (Merck Research Laboratories) was administered in the drinking water at a concentration of 0.4–0.5 mg/ml to result in a daily consumption of 30–40 mg·kg−1·day−1.
AGT measurement. Adipose extract, plasma, and cardiac extracts were diluted in PIB to yield similar AGT concentrations. One hundred-microliter assay tubes containing diluted samples and 50% PIB, 49% sodium phosphate buffer (0.15 M, pH 7.44, 2.5% BSA), and 1% semipurified rat renin source, obtained from perfused, homogenized, and ultracentrifuged rat renal cortex (14), were incubated at 37°C for 0-, 4-, and 6-h time points. During incubation at 37°C in the presence of excess renin, all AGT is cleaved to produce Ang I in a 1:1 molar ratio. Two time points are taken to ensure that the Ang I generation has been completed. AGT data are expressed as an average of both the 4- and 6-h time points corrected for any baseline measurement detected at time 0. Since renin is in significant excess and is able to convert ~100% of the AGT to Ang I within the first hour, 4- and 6-h time points are similar (Ang I degradation over 6 h is negligible). Ang I is measured by RIA as described below, and the AGT concentration was back calculated and expressed as nanomoles AGT per milliter plasma or gram tissue.

Measurement of Ang I. Ang I generated in both the renin and AGT assays was measured by RIA, as described previously (14). Briefly, addition of 125I-labeled Ang I and primary antibody directed against Ang I to each Ang I sample was followed by overnight incubation at 4°C. Secondary antibody (anti-rabbit IgG; Sigma) in a suspension of 4% polyethylene glycol (MW 8,000) with 0.5% rice starch was then added and incubated for 10 min at 4°C. Samples were centrifuged for 20 min at 2,000 g to precipitate antibody-bound Ang I, and the precipitates are counted in a γ-counter. Sample concentration of Ang I was calculated by comparing radioactive counts against control standards containing known Ang I concentrations.

Statistics. Differences in plasma renin and AGT were determined by one-way ANOVA. Renin values were log transformed to pass the normality test. Individual differences between groups were further assessed by the Holm-Sidak all-pairwise multiple comparison test after significance was obtained from the ANOVA procedure. Renin and AGT differences between visceral adipose, perfused visceral adipose, and perfused retroperitoneal adipose tissue across all four treatment groups were assessed by two-way ANOVA. Individual differences between groups were further assessed by the Holm-Sidak all-pairwise multiple comparison tests after a significant ANOVA result. Overall differences across all treatments for AGT in visceral and perfused visceral adipose tissue were assessed by the Wilcoxon signed-rank test. Cardiac extract renin and AGT differences between treatment groups, as well as weight gain differences, were assessed by one-way ANOVA. Cardiac extract data for renin and AGT analysis were natural log and arcsin square root transformed, respectively, to pass the normality test. Linear regression was performed between tissue and plasma renin or AGT. Statistical significance was set at P < 0.05.

RESULTS

Alteration of plasma renin concentration. To address the influence of plasma renin concentration on adipose and cardiac tissue renin levels, we surgically and pharmacologically altered the plasma levels of renin and measured their influence on adipose tissue renin concentrations. Plasma renin levels ranged from undetectable to >600 ng Ang I generated·ml⁻¹·h⁻¹. All treatment groups yielded significant changes in plasma renin concentration vs. normal animals (P < 0.05; Fig. 1A), and each treatment group had a significantly different mean plasma renin concentration than all other treatment groups except BNX vs. DOCA. Losartan treatment yielded average renin plasma concentrations that were >20-fold that of normal rats, whereas DOCA- and 24-h BNX-treated animals had nearly undetectably low plasma renin levels (P < 0.05; Fig. 1A).

Tissue renin concentrations. Adipose tissue renin concentrations were not statistically different between normal-, BNX-, and DOCA-treated animals (Fig. 1B). In addition, no statistical differences were observed between visceral adipose tissue [adipose extract (AE)], perfused visceral adipose tissue [perfused adipose extract (PAE)], or perfused retroperitoneal adipose tissue [retroperitoneal perfused adipose extract (PAEp)] within any treatment group (Fig. 1B). Normal-treatment adipose tissues contained renin levels that were not different than BNX or DOCA treatment despite ~100-fold greater plasma renin values in the normal-treatment group (Fig. 1, A and B). Losartan-treated animals had significantly higher adipose tissue renin concentrations,

Fig. 1. Plasma, adipose tissue, and cardiac renin concentrations. A: plasma renin concentrations among treatment groups. Normal (n = 9), deoxycorticosterone acetate (DOCA; n = 9), and losartan (n = 11). Renin activity [ng angiotensin (Ang) I·ml⁻¹·h⁻¹] is directly proportional to renin concentration. B: renin concentrations in adipose extract (AE), perfused adipose extract (PAE), and retroperitoneal perfused adipose extract (PAEp) among treatment groups. C: left ventricular cardiac extract renin concentrations among treatment groups. PAEp, n = 5; n is between 9 and 12 for all other groups. *P < 0.05 vs. all other treatment groups; +P < 0.05 vs. all other groups except DOCA; #P < 0.05 vs. all other groups except BNX.
~10–20 times that of normal, BNX, and DOCA animals \((P < 0.05; \text{Fig. } 1B)\). Left ventricular cardiac extract renin concentrations were statistically different between all treatment groups \((P < 0.05; \text{Fig. } 1C)\). Cardiac extract renin concentrations ranged from undetectable to \(>10 \text{ ng Ang } I \cdot \text{g}^{-1} \cdot \text{h}^{-1}\).

Adipose tissue renin vs. plasma renin. If plasma renin concentrations are reflected in proportional adipose tissue changes, then the significance of an independently controlled local RAS within adipose tissue is unclear. However, as shown in \text{Fig. } 2B, only the highest plasma renin values (losartan treatment group) were able to significantly change adipose renin values. Plasma renin concentrations did not correlate with visceral adipose renin among combined normal-, BNX-, and DOCA-treated animals \((R^2 = 0.015, P = 0.357, \text{combined perfused and nonperfused; Fig. } 2A)\) or within nonperfused and perfused adipose tissue when analyzed separately \((R^2 = 0.001, P = 0.877, \text{and } R^2 = 0.05, P = 0.224, \text{respectively})\). When analyzed on an individual treatment basis, only losartan treatment yielded a statistically significant positive correlation between visceral adipose tissue renin and plasma renin \((R^2 = 0.632, P < 0.001, \text{combined perfused and nonperfused data; Fig. } 2B)\). There was no apparent change in adipose tissue renin between plasma renin values of 0.01–100 \text{ ng Ang I} \cdot \text{ml}^{-1} \cdot \text{h}^{-1}\) (\text{Fig. } 2A). The 95% confidence interval for normal-treated animals was 6.5–45.9 \text{ ng Ang I} \cdot \text{ml}^{-1} \cdot \text{h}^{-1}\) (\text{Fig. } 2A, solid line). There was no consistent relationship between nonperfused (AE) and perfused (PAE) visceral adipose renin.

Cardiac tissue renin levels positively correlate with changes in plasma renin levels. In contrast to adipose tissue, left ventricular cardiac tissue renin concentrations were directly correlated to plasma renin. Cardiac tissue renin concentrations significantly correlated with changes in plasma renin across normal, BNX, DOCA \((R^2 = 0.537, P < 0.001; \text{Fig. } 2C)\), and losartan treatment \((R^2 = 0.849, P < 0.001; \text{Fig. } 2D)\). This is consistent with previously published cardiac and plasma renin associations \((9)\).

Alteration of plasma AGT concentration. Since adipose tissue was able to maintain a near-constant renin level in the

![Fig. 2. Plasma renin vs. visceral adipose tissue and cardiac tissue renin.](http://ajpendo.physiology.org/Downloaded from 10.220.33.6 on April 9, 2017)
face of large changes in plasma renin concentration, we extended this comparison to another RAS component, AGT. We assessed the levels of plasma, adipose, and cardiac tissue AGT to determine whether they were coordinately or independently regulated. Plasma AGT levels varied over a fivefold range for all treatment groups combined (Fig. 3A). BNX treatment plasma AGT was significantly different than DOCA and losartan treatments ($P < 0.05$). Losartan treatment was significantly different from normal treatment ($P < 0.05$; Fig. 3A).

**Tissue AGT concentrations.** Mean adipose tissue AGT levels ranged from 0.02 to 0.3 nmol/g tissue (Fig. 3B). Perfused retroperitoneal adipose tissue AGT levels were reduced compared with visceral adipose tissue and perfused visceral adipose tissue in normal- and losartan-treated animals (Fig. 3B). No statistical difference was seen between any adipose depot within the BNX- and DOCA-treated animals. Unlike renin, each treatment produced a significant difference in adipose tissue AGT for AE, PAE, and PAErp (Fig. 4B) except for normal vs. losartan treatment only within perfused retroperitoneal adipose tissue. Adipose tissue perfusion displayed a consistent trend of reduced AGT levels in the PAE compared with AE, although this lacked statistical significance within each treatment (Fig. 3B). However, combined data for AE vs. PAE across all treatment groups revealed a significant decrease in tissue AGT levels upon tissue perfusion of 11.1% ($P = 0.01$, $n = 41$; Fig. 3C). Left ventricular cardiac AGT concentrations ranged from 0.005 (losartan) to 0.05 nmol/g tissue (BNX) (Fig. 3D). Cardiac extract AGT concentrations differed between all treatment groups ($P < 0.05$; Fig. 3D).

**Adipose tissue AGT vs. plasma AGT.** Unlike renin, visceral adipose tissue AGT levels positively correlated with plasma AGT concentrations across all treatment groups for both perfused ($R^2 = 0.858$, $P < 0.001$) and nonperfused visceral adipose tissue ($R^2 = 0.732$, $P < 0.001$) (Fig. 4A). The 95% confidence interval for plasma AGT in normal-treated animals was 1.9–2.3 nmol AGT/ml (Fig. 4A, solid line). Plasma AGT levels (per ml) tended to be ~20-fold greater than adipose tissue AGT (per g) across all treatment groups.

**Cardiac tissue AGT levels positively correlate with changes in plasma AGT levels.** Cardiac tissue AGT levels positively correlated with plasma AGT concentrations across all treatment groups ($R^2 = 0.892$, $P < 0.001$; Fig. 4B). Hence, cardiac AGT (Fig. 4B) as well as adipose tissue AGT levels (Fig. 4A) varied directly with plasma levels over all four treatment

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**Fig. 3.** Plasma, adipose tissue, and cardiac angiotensinogen (AGT) concentrations. A: plasma AGT concentrations among treatment groups: normal ($n = 11$), BNX ($n = 9$), DOCA ($n = 9$), and losartan ($n = 11$). AGT concentration was back calculated from AGT conversion to Ang I (1:1 molar ratio) and expressed as nmol AGT/ml plasma or gram tissue. B: adipose tissue AGT concentrations. Adipose tissue, depot-specific, and extract AGT concentrations within treatment groups. Visceral (AE), PAE, and PAErp. PAErp, $n = 5$; $n$ is between 9 and 12 for all other groups. Data analyzed by 2-way ANOVA. C: PAE vs. AE visceral adipose extracts from all treatment groups combined ($n = 41$). Data assessed by Wilcoxon signed-rank test. D: left ventricular cardiac AGT concentrations among treatment groups. **$P < 0.05$ vs. DOCA and losartan; +$P < 0.05$ vs. normal; *$P < 0.05$ vs. all other AE treatment groups; +#$P < 0.05$ vs. all other PAE treatment groups; $\times P < 0.05$ vs. all other treatment groups except normal vs. losartan. #P < 0.05 vs. AE and PAE within treatment group; $\times P < 0.05$ vs. all other treatment groups.
Fig. 4. Plasma AGT vs. visceral adipose tissue and cardiac renin. A: normal- (n = 11), BNX- (n = 9), DOCA- (n = 9), and losartan-treated (n = 11) adipose AGT vs. plasma AGT. • Nonperfused visceral AE; ○ perfused visceral adipose tissue (PAE). Solid line represents the 95% confidence interval for plasma AGT concentration in normal animals. B: normal- (n = 5), BNX- (n = 6), DOCA- (n = 9), and losartan-treated (n = 11) cardiac AGT vs. plasma AGT.

DISCUSSION

The relationship between local adipose tissue RAS components and plasma RAS variation has not previously been addressed. The results of the present experiments demonstrated that adipose tissue renin was largely independent of circulating renin, whereas adipose tissue AGT was not independent of plasma AGT.

Since plasma renin can be highly variable to control blood pressure and Na\(^+\) homeostasis, adipose renin levels involved with local adipocyte biology would likely require independence from plasma renin fluctuations. To analyze this, we varied plasma renin (and AGT levels) using four treatment groups (Figs. 1A and 3A) and subsequently measured the associated changes in adipose tissue RAS components. This study does not address whether RAS components produced by adipose tissue can contribute to circulating RAS levels, as has been shown for AGT but not for Ang II (8). Instead, this study examines whether adipose tissue can maintain renin and AGT concentrations independently of plasma fluctuations.

Local generation of Ang II near adipose cell membrane Ang II receptors should be, at least in part, a function of adipose interstitial fluid renin and AGT concentrations. Since renin and AGT are receptors smaller than albumin, which has previously been shown to be present in adipose ISF (5), and since plasma-derived renin and AGT have been shown to be present in the ISF of many tissues, including the heart (9), we compared renin and AGT levels in plasma, adipose tissue, and cardiac tissue.

Adipose tissue was perfused to remove plasma renin and AGT circulating within the adipose tissue plasma compartment. Adipose plasma could have provided a false positive correlation between plasma and adipose RAS components. Therefore, perfused adipose tissue is composed of ISF and cells. Since the adipose plasma space is ~10% (by weight) of adipose tissue (24), if RAS components in adipose tissue are in equilibrium with plasma, a 10% decline in renin and AGT concentrations within the perfused visceral adipose tissue compared with nonperfused visceral adipose tissue should have been observed. This was true for AGT (Fig. 3, B and C) but not for renin (Fig. 1B).

Previous investigations have shown that, after BNX, plasma and cardiac renin levels decrease dramatically (14, 15). This has led to the suggestion that most cardiac renin is derived from plasma renin of renal origin (9, 14). In this report, we initially used a similar strategy to decrease plasma renin levels; however, visceral and retroperitoneal adipose renin did not fall after BNX compared with normal treatment (Figs. 1B and 2A) despite a large, highly significant decrease in plasma renin (Fig. 1A) and cardiac renin (Fig. 1C). Previous studies of interscapular brown adipose tissue homogenates from rats with BNX reported similar findings, noting that residual adipose vascular smooth muscle or vessel wall-associated plasma-derived renin could not be excluded as a potential explanation (28). However, this previous study employed an acidic renin enzymatic assay with AGT substrate. Our renin enzymatic assay using AGT substrate was performed at pH 7.45, and we have shown previously (14) that, when the assay is performed at pH 5.5, both renin and cathepsin D contribute to Ang I generation such that BNX artifactualy appears to cause no change in cardiac renin activity. Hence, an alkaline enzymatic incubation is required to help exclude cathepsin D activity from the renin assay. The rat renin assay in this study does not measure rat cathepsin D (14).

Since the initial BNX treatment was only 48 h long, and in an attempt to remove residual sources of bound plasma-derived renin activity, we also employed a 3-wk DOCA salt treatment group, where plasma renin was reduced to near zero for a much longer period of time. A possible study limitation was that DOCA animals gained significantly less weight than normals.
the RAS have previously been identified in adipose tissues (13, 20, 22, 26), it seems likely that a local adipose RAS can operate independently of the plasma RAS at least during situations involving normal and low plasma renin states.

Many reports have been published on the role of AGT levels in obesity, obesity-related hypertension, adipokine expression, and adipocyte metabolism (2, 3, 6, 21). Previously published reports have demonstrated that AGT protein expression restricted to adipose tissue is able to gain access to the plasma compartment and reconstitute a low level of plasma AGT (30%) compared with wild-type mice (21). In addition, AGT overexpression increased fat mass and adipocyte hypertrophy, whereas AGT-knockout mice displayed decreased fat mass and adipocyte size (17). Therefore, AGT levels are capable of ranging independently of circulating RAS regardless of AGT dynamics.

How adipose tissue is able to maintain renin concentration near normal levels in the face of chronically reduced plasma renin levels is unclear. It may be that renin cannot easily cross the adipose capillary wall, although larger molecules such as albumin and AGT apparently have adipose capillary permeability. In this case, adipose renin levels may be due to local adipocyte (or other cellular components) synthesis. Perhaps an adipocyte membrane renin-binding protein can maintain renin activity levels when plasma renin is low by activating prorenin. Renin-binding protein activation of prorenin has previously been hypothesized (12), and prorenin receptor has been detected in human adipose tissue (1). Alternatively, adipose renin that ultimately catalyzes Ang II formation may primarily be in the intracellular compartment and therefore not influenced by changes in a relatively small extracellular renin pool. An intracellular (intracrine) role of Ang II has recently been shown to be activated in cardiac fibroblasts by high glucose levels (29). Either of these two possibilities could also help explain why adipose tissue renin levels did not significantly decrease after saline perfusion. When losartan treatment significantly increases plasma renin, some plasma renin may then cross the capillary wall and contribute to the previously existing adipose-derived renin activity. Alternatively, adipose tissue Ang II receptor blockade may result in upregulation of adipose renin expression as in kidney tissue, although no upregulation was seen during BNX. Regardless, adipose tissue control of local renin concentrations may be a potential mechanism maintaining a functional local RAS independent of plasma fluctuations.

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GRANTS

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