Perivascular Adipose Tissue and Mesenteric Vascular Function in Spontaneously Hypertensive Rats

Beatriz Gálvez, Javier de Castro, Diana Herold, Galyna Dubrovskia, Silvia Arribas, M. Carmen González, Isabel Aranguez, Friedrich C. Luft, M. Pilar Ramos, Maik Gollasch, Maria S. Fernández Alfonso

Objective—Perivascular adipose tissue of normotensive rats releases a transferable factor that induces relaxation by opening voltage-dependent K⁺ (Kᵥ) channels. The relevance of these observations to hypertension is unknown.

Methods and Results—We characterized mesenteric perivascular adipose tissue from 3-month-old Wistar Kyoto rats (WKY) and aged-matched spontaneously hypertensive rats (SHR). Mesenteric bed (MB) weight and MB total lipid content were lower in SHR than in WKY. Freshly isolated MB adipocytes were smaller in SHR. Plasma triglycerides, glycerol, nonesterified free-fatty acids, and cholesterol were also lower in SHR. Plasma and mesenteric leptin were correlated with the quantity of mesenteric fat. To study vascular function, the MB was cannulated and perfused at a constant 2 ml/min flow. The Kᵥ channel blocker 4-aminopyridine (4-AP; 2 mmol/L) increased perfusion pressure less in SHR MB than WKY and was directly correlated with the mesenteric fat amount. In isolated mesenteric artery rings, 4-AP (2 mmol/L) induced a contractile effect that was attenuated in SHR compared with WKY. The anticontractile effects of perivascular fat were reduced in SHR mesenteric artery rings compared with WKY.

Conclusions—Differences in visceral perivascular adipose tissue mass and function may contribute to the increased vascular resistance observed in SHR. (Arterioscler Thromb Vasc Biol. 2006;26:1297-1302.)

Key Words: perivascular adipose tissue ■ ADRF ■ mesenteric arteries ■ SHR

Earlier studies uncovered a paracrine role for adipose tissue in the regulation of vascular function. Soltis and Cassis1 demonstrated that perivascular fat significantly attenuated vascular responsiveness of aortic rings to norepinephrine. More recent reports confirmed the inhibitory action of perivascular fat on aortic2 and mesenteric3 contractile response to a variety of vasoconstrictors. The anticontractile action is induced by an undefined transferable factor released by perivascular adipocytes, called adipocyte-derived relaxing factor (ADRF).2 ADRF is released from rat aortic perivascular tissue by a calcium- and cAMP-dependent mechanism.4 The anticontractile effect is mediated by opening of ATP-dependent K⁺ channels in the aorta5 and by activation of vascular smooth muscle voltage-dependent K⁺ (Kᵥ) channels in mesenteric arteries.6 The mesenteric vascular smooth muscle cell (VSMC) resting membrane potential is more hyperpolarized in arterial rings surrounded by fat than in rings without fat. Furthermore, the anticontractile effect is directly dependent on the amount of fat.7 These findings support the notion that perivascular fat contributes to the maintenance of basal tone in mesenteric arteries and to the regulation of mesenteric artery contractility by Kᵥ channel activation.3 Adipocyte-derived leptin also influences vascular tone.5–7 Pharmacological experiments showed that leptin induces a direct vasodilatation in aorta and mesenteric arteries.6 Interestingly, the vasodilatory effect of leptin on mesenteric arteries is attributable to VSMC membrane hyperpolarization,8 which is likely caused by activation of Kᵥ channels and release of endothelium-derived hyperpolarizing factor. Because the mesenteric bed (MB) is surrounded by high but variable amounts of perivascular white adipose tissue, changes in the amount of fat may have consequences for the regulation of mesenteric artery tone and systemic arterial blood pressure. We tested the hypothesis that visceral perivascular adipose tissue contributes to differences in vascular resistance in hypertension and analyzed the possible relationship between the amount and function of perivascular fat and hypertension.

Methods

Animals
Experiments were conducted in 3-month-old male Wistar Kyoto rats (WKY) and spontaneously hypertensive rats (SHR; 300 to 350 g;
Charles River; Barcelona, Spain) maintained under controlled light (12-hour light/dark cycles from 8:00 AM to 8:00 PM) and temperature (22°C to 24°C) conditions. The rats were fed a normal rat chow (A.04; Panlab) and had free access to tap water. The institutional animal care and use committee approved all experimental procedures according to European Community guidelines. Systolic and diastolic blood pressure was recorded directly in a group of animals under anesthesia (sodium pentobarbital 50 mg/kg). Because anesthesia might influence adipose tissue metabolism and lipid parameters, another group of animals was killed by decapitation and blood was collected in EDTA. Plasma samples were frozen in aliquots for biochemical determinations. The mesentry was rapidly separated from the intestine, weighed, and its volume was determined plethysmographically. Wet weight and volume were referred to rat body weight. The mesentry was then placed in a warm (37°C) solution of Krebs-Ringer bicarbonate buffer, pH 7.4, for adipocyte isolation or in cold (4°C) oxygenated (95% O₂/5% CO₂) physiological salt solution (PSS) for functional studies.

**Isolation of Adipocytes**

Adipocytes were prepared from mesenteric white adipose tissue according to the method of Rodbell with minor modifications. Briefly, mesenteric adipose tissue was cut into small pieces and digested with collagenase A (1 mg/mL) in Krebs-Ringer bicarbonate buffer (KRB), pH 7.4, containing 4% (wt/vol) BSA (fatty acid free, fraction V) and 5.5 mmol/L glucose at 37°C in a O₂/CO₂ (19:1) atmosphere with a continuous vigorous shaking (60 cycles/min). Subsequently, fat cells were dispersed and filtered through a silk gauze. The size of the fat cell was measured by direct microscopic determination, and the mean adipocyte diameter was calculated from measurements of 100 cells per animal. Because adipocytes have 95% lipid content and are spherical in shape, their volume and weight can be estimated from their diameter. Total cell lipid content was determined gravimetrically after organic extraction, and the number of fat cells was calculated by dividing the total lipid weight by the mean cell weight.

**Leptin Determination and Plasma Analysis**

For leptin determination and plasma analysis, please see the online supplement, available at http://atvb.ahajournals.org.

**Vascular Reactivity in the MB**

The mesenteric vascular bed was perfused as described previously. Briefly, the superior mesenteric artery was cannulated at its junction with the abdominal aorta, put on a platinum grid in an organ chamber, and perfused using a peristaltic pump (Ismatec) at constant flow (2 mL/min). At any given time, flow was maintained at a constant rate, changes in perfusion pressure were used as an index of changes artery resistance, and an increase or decrease in the perfusion pressure indicated either vasoconstriction or vasodilatation, respectively. After an equilibration period of 60 minutes and before starting the experiment, the vascular MB was contracted with 75 mmol/L KCl to assess contractility. Subsequently, the MB was contracted with noradrenaline (10⁻⁷ μmol/L), and acetylcholine (10⁻⁶ to 10⁻³ mmol/L) was added to functionally confirm the presence of the endothelium. In control experiments, contractility to 75 mmol/L of KCl increased with perfusion pressure but reached a plateau at ~2 mL/min. At any given flow rate, contractility was similar in WKY and SHRs.

**TABLE 1. Blood Pressure, Body Weight, and Adipose Tissue Characteristics in 3-Month-Old Male WKY and SHR (90± 7 days)**

<table>
<thead>
<tr>
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<th>WKY</th>
<th>SHR</th>
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<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>131±4</td>
<td>179±8*</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>95±6</td>
<td>136±6*</td>
</tr>
<tr>
<td>Rat body weight, g</td>
<td>315±4</td>
<td>320±9</td>
</tr>
<tr>
<td>Mesenteric weight, mg/g</td>
<td>12.0±0.4</td>
<td>10.0±0.4*</td>
</tr>
<tr>
<td>Mesenteric volume, L/g</td>
<td>12.0±0.1</td>
<td>11.0±0.3*</td>
</tr>
<tr>
<td>Mesenteric density, g/L</td>
<td>0.97±0.1</td>
<td>0.96±0.03</td>
</tr>
<tr>
<td>Lumbar adipose tissue, mg/g</td>
<td>8.0±0.3</td>
<td>5.0±0.4*</td>
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</table>

Data are expressed as mean±SEM of 12 determinations per strain. *P<0.05 between groups.

**Vascular Reactivity in Mesenteric Arteries**

Superior mesenteric artery or their first-order branches were cut in 2-mm segments. Periadventitial fat and connective tissue was either left intact, (+) fat rings, or removed, (−) fat rings with scissors. Rings were mounted in PSS buffer at 37°C in a small vessel myograph (Danish Myotechnology) at standard pretension of 1.12 mN/mm (1.98 mN/1.76 mm) or at a higher tension equivalent to that generated at 0.20× the diameter of the vessel at 100 mm Hg (ie, ~2 mN/mm in WKY and 1.7 mN/mm in SHR). Segments were threaded onto 2 stainless wires and suspended in 5-mL microvascular myograph baths (Danish Myotechnology) according to Mulpavy and Halpern. The arteries were equilibrated for 60 minutes, and then they were exposed to isomolar 60 mmol/L KCl-containing solution (KPSS) to ensure their viability and contractility. KPSS was similar to PSS except that NaCl was exchanged with equimolar KCl. After PSS washing, the vessels were subjected to the following protocols. In the first series of experiments, a cumulative concentration–effect curve to serotonin (10⁻⁴ to 7×10⁻⁶ mol/L) was generated for each vessel. In the second series of experiments, the effect of serotonin was investigated in rings pretreated with the K⁺ channel blocker 4-aminopyridine (4-AP). The effects were compared with contractions to 2 μmol/L serotonin 10 minutes before addition of 4-AP. In addition, cumulative concentration–effect curves to leptin, the K⁺ channel opener cromakalim, and the thromboxane receptor agonist U46619 were generated for each vessel. In all experiments, a separate vessel segment from each individual was simultaneously monitored as control vessel. Tension was expressed as a percentage of the steady-state tension (100%) obtained with isometric external KCl (60 mmol/L).

**Statistical Analysis**

All values are given as mean±SEM. Student t tests or ANOVA were used as appropriate. A value of P<0.05 was considered statistically significant; n represents the number of data.

**Results**

**Mesenteric Fat Depot Characteristics**

As shown in Table 1, SHR had higher systolic and diastolic blood pressures compared with WKY. MB wet weight and volume were significantly lower in SHR than in WKY, without differences in body weight or MB density. The lumbar fat pad weight was also significantly lower in SHR, indicating that changes in adipose tissue were not specific for mesenteric fat. The mean diameter of freshly isolated adipocytes (supplemental Figure Ia and Ib, available online at http://atvb.ahajournals.org) was significantly greater in WKY (36.9±3 μm) compared with SHR (26.9±4.3 μm), whereas the cell number was not different between strains (WKY=80±39 million cells/g tissue; SHR=107±31 million)
cells/g tissue). Because the population of mature adipocytes includes cells of variable size, we also analyzed the size distribution, which was shifted to the left in SHR, confirming that adipocytes were smaller in this strain (supplemental Figure Ic and Id). Furthermore, mesenteric total lipid content was significantly lower in SHR (43/10068 g lipid/100 g tissue) than in WKY (69/10068 g lipid/100 g tissue; P<0.05), indicating a reduced amount of SHR visceral fat.

Biochemical Analysis

Table 2 summarizes the metabolic and hormonal parameters. No glucose differences were observed between strains. However, the insulin concentration was significantly lower in SHR compared with WKY. SHR had lower circulating triglyceride, glycerol, nonesterified free-fatty acid, and cholesterol concentrations than WKY. Moreover, both plasma and mesenteric leptin levels were significantly reduced in SHR and were correlated with the quantity of mesenteric fat (P<0.05).

Vascular Reactivity in the Perfused MB

We used whole perfused MB to assess whether or not differences in perivascular fat between WKY and SHR influence vascular function. Basal perfusion pressure at 2 ml/min was similar between strains (WKY=6.9±0.6 mm Hg; SHR=7.1±0.6 mm Hg). Because mesenteric fat controls arterial function through ADRF by K<sub>v</sub> channel activation, we analyzed the effect of the blocker 4-AP. The increase in perfusion pressure induced by 2 mmol/L 4-AP was greater in WKY than SHR (Figure 1a). A significant correlation was observed between the amount of mesenteric fat and the contraction elicited by 4-AP (2 mmol/L; Figure 1b). KCl (75 mmol/L) induced a similar increase in perfusion pressure between strains (Figure 1c).

Although functional effects of mesenteric perivascular fat in normotensive rats were reported to be not dependent on NO, we analyzed the effect of basal NO production on vascular tone by perfusion with the nonspecific NO synthase inhibitor N<sub>2</sub>-nitro-arginine methyl ester. N<sub>2</sub>-nitro-arginine methyl (0.1 mmol/L) induced an increase in perfusion pressure that was not different between strains (WKY=1.6±0.3 mm Hg; SHR=2±0.5 mm Hg). In addition, acetylcholine produced a similar decrease in MB perfusion pressure in WKY and SHR (results not shown), indicating no differences in endothelium-dependent relaxation.

Vascular Reactivity in Mesenteric Arteries

Contractile responses were analyzed in isolated mesenteric artery rings with (+) fat and without (−) fat (supplemental Figure II). We first used standard vessel wall pretensions of 1.12 mN/mm, which give maximal force development in mesenteric rings of normotensive rats. At this pretension, the response to 60 mmol/L KCl was similar in rings from both strains, independent of fat (supplemental Figure IIIa). To analyze the ADRF contribution to mesenteric tone, rings were incubated with 4-AP. The contractile effect of 4-AP was greater in WKY than SHR (4-AP 2 mM; Figure 1b). KCl (75 mmol/L) induced a similar increase in perfusion pressure between strains (Figure 1c).

![Figure 1](http://atvb.ahajournals.org/)

**Figure 1.** a, Response to 2 mmol/L 4-AP in the perfused MB from WKY and SHR. b, Correlation between contraction to 4-AP in perfused MB from WKY and SHR and perivascular fat. c, Response to KCl 75 mmol/L in perfused MB from WKY and SHR. Contraction is expressed as increase in perfusion pressure in mm Hg. Data are shown as mean±SEM of 6 to 10 determinations per strain. *P<0.05 between groups.

<table>
<thead>
<tr>
<th>Metabolic and Hormonal Parameters of WKY and SHR Rats</th>
<th>WKY</th>
<th>SHR</th>
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<tbody>
<tr>
<td>Plasma triglycerides, mg/dL</td>
<td>145.1±12</td>
<td>94±5**</td>
</tr>
<tr>
<td>Plasma glycerol, μmol/L</td>
<td>264±17</td>
<td>203±19*</td>
</tr>
<tr>
<td>Plasma NEFA, μmol/L</td>
<td>621.7±91.2</td>
<td>309.2±27.2**</td>
</tr>
<tr>
<td>Plasma total cholesterol, mg/dL</td>
<td>65.3±3.1</td>
<td>53±0.8**</td>
</tr>
<tr>
<td>Plasma insulin, μg/L</td>
<td>2.6±0.3</td>
<td>1.6±0.4*</td>
</tr>
<tr>
<td>Plasma glucose, mg/dL</td>
<td>136±4.7</td>
<td>130±5.8</td>
</tr>
<tr>
<td>Plasma leptin, ng/mL</td>
<td>11.5±2.2</td>
<td>7.6±1.0*</td>
</tr>
<tr>
<td>Mesenteric leptin, pg/mg protein</td>
<td>324±48</td>
<td>80±12*</td>
</tr>
</tbody>
</table>

Data are expressed as mean±SEM of 6 determinations per strain. *P<0.05; **P<0.001 between groups.

NEFA indicates nonesterified free-fatty acids.
(2 mmol/L) was similar in (−) fat rings of both WKY and SHR rats (supplemental Figure IIIb). In contrast, 4-AP (2 mmol/L) significantly increased resting tension only in (+) fat rings from WKY rats (supplemental Figure IIIb). Moreover, concentration–response curves to serotonin were performed in absence and presence of 4-AP. The force generated by serotonin alone was less in (+) fat compared with (−) fat rings in both WKY and SHR (Figure 2a and 2b). This difference disappeared after preincubation with 4-AP (2 mmol/L; Figure 2c and 2d).

We next performed isometric contraction experiments at a vessel pretension equivalent to that generated at 0.9× the diameter of the vessel at 100 mm Hg (in the range of 2 mN/mm in WKY and 1.7 mN/mm in SHR). In mesenteric rings from WKY, the response to 60 mmol/L KCl was similar to the KCl response at a 1.12 mN/mm vessel pretension. However, in mesenteric rings from SHR, contraction to 60 mmol/L KCl was ~3-fold stronger compared with that induced at 1.12 mN/mm pretensions. In both strains, contractions to KCl at this pretension were independent of the fat presence. Under these conditions, serotonin induced lower contractions in (+) fat rings compared with (−) fat rings from WKY but not from SHR (Figure 3a and 3b).

We next analyzed a possible difference in the arterial smooth muscle response to a putative hyperpolarizing factor released from adipose tissue. For this purpose, we used the synthetic K⁺ channel opener cromakalim. Cromakalim produced dose-dependent relaxations of (+) fat mesenteric arterial rings of both WKY and SHR rats. The EC₅₀ values were not different between the 2 groups (WKY = 44.6 ± 11.7 nmol/L; SHR = 41.3 ± 11.3 nmol/L; n = 13), indicating that the WKY and SHR arterial smooth muscle sensitivity to membrane hyperpolarization was similar. This observation was confirmed using a vasoconstrictor that acts independently on smooth muscle potential changes in mesenteric artery rings, such as U46619.³ U46619 induced contractions in rings of

![Figure 2](http://atvb.ahajournals.org/)

**Figure 2.** a and b, Cumulative concentration–response curves to (10⁻⁸ to 7×10⁻⁶ M) serotonin (5-HT) in intact (+) fat mesenteric arteries (○) and (−) fat mesenteric arteries without periadventitial fat (●) of WKY (a) and SHR (b; n=4 each). c and d, Cumulative concentration–response curves to (10⁻⁸ to 7×10⁻⁶ M) 5-HT in intact (+) fat mesenteric arteries (○) and (−) fat mesenteric arteries without periadventitial fat (●) of WKY (c) and SHR (d) in the presence of 2 mmol/L 4-AP (n=4 each). Rings were mounted at resting tension of 1.12 mN/mm. *Significant with P<0.05.

![Figure 3](http://atvb.ahajournals.org/)

**Figure 3.** Cumulative concentration–response curves to (10⁻⁸ to 2×10⁻⁶ M) serotonin (5-HT) in intact (+) fat mesenteric arteries (○) and (−) fat mesenteric arteries without periadventitial fat (●) of WKY (a) and SHR (b; n=4 each). Rings were mounted at a tension equivalent to that generated at 0.9× the diameter of the vessel at 100 mm Hg. *Significant with P<0.05.
WKY and SHR rats, independent of perivascular fat (supplementary Figure IV).

We also analyzed the vasodilatory response to leptin in (+) fat and (−) fat WKY and SHR mesenteric arterial rings. Leptin produced dose-dependent relaxations in rings of both strains, irrespective of periadventitial fat (supplemental Figure V). However, the sensitivity to leptin was higher in mesenteric arteries from SHR (EC₅₀: [−] fat = 2.7 ± 1.0 pmol/L; [+]) fat = 2.9 ± 1.1 pmol/L) compared with WKY (EC₅₀: [−] fat = 11.7 ± 5.8 pmol/L; [+]) fat = 11.2 ± 4.3 pmol/L).

Discussion

The main finding in our study is that alterations in visceral perivascular fat mass and function may contribute to the increased vascular resistance in a hypertensive model. We found that SHR have less mesenteric perivascular adipose tissue with smaller adipocytes and a lower total lipid and leptin content compared with WKY. The lower amount of adipose tissue facilitates contraction in mesenteric arteries, likely attributable to a diminished paracrine regulation of perivascular fat on mesenteric arterial tone via KCa channels.

White adipose tissue is an endocrine organ that produces and releases numerous factors with a broad biological activity, including vasoactive factors such as ADRF²⁻⁴ and leptin.⁶ The mesenteric vascular bed, which significantly contributes to total peripheral vascular resistance, is surrounded by a considerable and variable amount of fat. Because fat inhibits vascular tone of small mesenteric arteries via KCa channels,⁴ and because abdominal visceral fat mass is directly correlated with the prevalence of hypertension,¹⁷ we chose the MB as experimental model to characterize the contribution of periadventitial fat to vascular resistance in hypertension.

The MB from SHR had a lower weight and volume compared with WKY. This difference was attributable to a reduced perivascular fat mass from smaller adipocytes with a lower total lipid content but without changes in cell number. One possible explanation might be increased sympathetic outflow in SHR,¹⁸,¹⁹ leading to an enhanced lipolysis.²⁰,²¹ In rat adipocytes, catecholamines control lipid mobilization and lipolysis via β₃-adrenoceptors that activate adenylyl cyclase, cAMP-dependent protein kinase, and hormone sensitive lipase. This signaling results in the hydrolysis of triacylglycerols and decreased adipocyte cell size.²² This mechanism is of special importance for the mesenteric adipose tissue because of its dense sympathetic innervation.²³ Moreover, catecholamine-stimulated lipolysis is more effectively inhibited by insulin in adipocytes from the mesentery and omentum than in other depots.²⁴ Thus, the diminished plasma insulin levels in the SHR also favors catecholamine-stimulated lipolysis in mesenteric and lumbar fat depots in this strain.

We next tested the hypothesis that the mesenteric perivascular adipose tissue contributes to the maintenance of vascular tone in SHR. Because this fat controls arterial function by KCa channel activation in normotensive rats,³ we analyzed the effect of 4-AP, a KCa channel inhibitor, on the isolated MB perfusion pressure and on isolated mesenteric artery tone, with (+) fat and without (−) perivascular fat. The fact that 4-AP induced (1) an increase in MB perfusion pressure, (2) a basal contractile effect, and (3) increased serotonin-induced contractions in isolated mesenteric rings provides evidence for an anticontractile effect of perivascular fat through 4-AP-sensitive KCa channels in both WKY and SHR. The anticontractile effect of perivascular fat correlated with the amount of mesenteric fat as shown previously³ but was greater in WKY. Moreover, in mesenteric rings with the same amount of perivascular fat, 4-AP also induced higher contractions in WKY. These findings suggest that both the morphological and functional changes in SHR mesenteric perivascular fat might contribute to the increased basal mesenteric arterial tone and vascular resistance via KCa channels. In fact, the reported differences in the response to 4-AP between strains can be specifically attributed to an anticontractile effect of perivascular fat for the following reasons: (1) they were not dependent on a differential VSMC sensitivity to membrane hyperpolarization; (2) they were not related to unspecific mechanical effects caused by differences in the mesenteric fat mass; and (3) they cannot be attributed to differences in endothelial-dependent dilatation or in contractile capacity of the vessels because of blood pressure. An interesting finding that requires further characterization is that the anticontractile effect of perivascular fat in SHR seems to be dependent on the wall pretension and is more evident at wall pretensions equivalent to 0.9 x the diameter of the vessel, which are closer to the wall tension “in vivo” for each strain.

Because mesenteric leptin was lower in SHR correlating with the lower amount of fat in this strain and because leptin elicits vasodilation through hyperpolarization in mesenteric arteries,⁴ the possibility exists that a lower leptin level might contribute to the increased contractile responses in SHR. The lack of leptin antagonists did not allow us to directly investigate the involvement of leptin. Haynes et al have shown that leptin increases thermogenic sympathetic nerve activity, suggesting a direct leptin effect on sympathetic nerve traffic.²⁵ We did not explore nerve traffic in our experiments. However, because the vasodilatory effects of leptin did not depend on the presence of periadventitial adipose tissue, we suggest that the lower perivascular leptin levels might be responsible for the diminished anticontractile effects of perivascular fat and that they may play a role in vascular dysfunction in SHR. On the other hand, the increased sensitivity of SHR rings to leptin suggests a compensatory mechanism in this strain that precludes judgments as to the net effect.

SHR are not only a hypertensive model but also model the insulin-resistance syndrome with hypertriglyceridemia and glucose intolerance.²⁶ Nevertheless, our SHR strain had lower lipid concentrations than WKY. We performed the plasma extraction in the morning, and the values represent postprandial concentrations because we did not fast the animals. We avoided 24-hour fasting because fasting stimulates lipid mobilization and lipolysis. Both processes reduce mesenteric perivascular adipose tissue. Therefore, our results suggest that the SHR vascular dysfunction was independent of carbohydrate and lipoprotein metabolism perturbations. Furthermore, the functional abnormalities of abdominal fat associ-
ated with lower perivascular fat content might play a role in the development or maintenance of high blood pressure in this strain. Whether or not similar results can be observed in other hypertensive strains or species remains to be determined.

In conclusion, we demonstrated that functional abnormalities in the visceral mesenteric fat of SHR are associated with lower amount of perivascular adipose tissue, smaller adipocytes, and lower lipid content. The findings seem contradictory in terms of obesity-related hypertension. 27 Whether or not obese models or overweight patients are resistant to the vascular anticontractile effect of adipose tissue–derived factors should be explored. A possible shift in the balance between adipose tissue–derived vasodilator and vasoconstrictor factors in obesity warrants investigation, particularly because ADRF appears to be absent in a model of obesity. 28

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References
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**Leptin determination**

Leptin was determined by a specific ELISA kit for rats (Assay Designs, Inc., Michigan, USA). The values were within the detection range of the assays. Mesenteric adipose tissue was homogenized in PSS containing leupeptin (10 µg/mL), aprotinin (2 µg/mL), phenylmethylsulphonyl fluoride (10 mg/mL) and 1% Triton X-100 to maximize leptin extraction from the tissue. Homogenates were centrifuged at 10,000 x g for 20 min and infranatants were used for measuring leptin and protein concentration. Leptin concentrations were corrected for the protein content of each sample, as determined by the bicinchoninic protein assay. The results were expressed as pg per mg of protein.

**Plasma analysis**

Plasma aliquots were used to measure glucose (Glucose oxidase, GOD/PAP method, Roche Diagnostics, Barcelona, Spain), triglycerides (GPO/PAP method Roche Diagnostics, Barcelona, Spain), cholesterol (CHOD/PAP method, Roche Diagnostics, Barcelona, Spain) and glycerol (GPO-Trinder; Sigma-Aldrich, Madrid, Spain) by enzymatic colorimetric tests as indicated. Non-esterified fatty acids (NEFA) were analyzed in EDTA-plasma samples by a colorimetric method (Wako Chemicals GmbH, Neuss, Germany). Insulin (Mercodia, Uppsala, Denmark) was determined by using a specific ELISA kit for rats, being the values within the detection range of the assays.
Figure I: Photographs of freshly isolated adipocytes from the mesenteric fat pad of (a) WKY or (b) SHR. Magnification 10x. (c) Distribution of adipocyte size in mesenteric fat pads from WKY and (d) from SHR. Results are expressed in percentage as mean ± S.E.M. of 100 determinations per animal. Six animals were used from each strain.
**Figure II:** Weights of mesenteric rings. P>0.05. n≥13 for each group.
Figure III: a) Contractile response to 60-mmol/L KCl solution in intact (+) fat mesenteric arteries (open bars) and (-) fat mesenteric arteries without periadventitial fat (closed bars) of WKY and SHR rats. Rings were mounted at resting tension of 1.12 mN/mm. b) Contractile response to 2 mmol/L 4-aminopyridine on resting tension in intact (+) fat mesenteric arteries (open bars) and (-) fat mesenteric arteries without periadventitial fat (closed bars) of WKY (left) and SHR (right) rats (n>11, each). Rings were mounted at resting tension of 1.12 mN/mm. * significant with p<0.05. n.s. not significant with p≥0.05.
**Figure IV:** Cumulative concentration-response curves to U46619 (1nM to 1µM) in intact (+) fat mesenteric arteries (open circles) and (-) fat mesenteric arteries without periadventitial fat (closed circles) of WKY (a) and SHR (b) rats (n>7, each). Rings were mounted at a tension equivalent to that generated at 0.9 times the diameter of the vessel at 100 mmHg. The values were not different (p>0.05).
Figure V: Dose-response curves to leptin in serotonin-precontracted mesenteric rings. Data are expressed as mean ± SEM (n>9 in each group, 100 mmHg). (+) fat and (-) fat mesenteric rings from SHR showed an increased relaxation to leptin (1 pmol/L to 30 nmol/L), compared to (+) fat and (-) fat rings of WKY (ANOVA, p<0.05). The responses between (+) fat and (-) fat rings of either WKY or SHR to leptin (1 pmol/L to 30 nmol/L) were not different. (ANOVA, p>0.05).