Lipoatrophy and Hypertension

Elevated Blood Pressure in Transgenic Lipoatrophic Mice and Altered Vascular Function

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Abstract—The role of perivascular fat in the control of vascular function was studied using lipoatrophic A-ZIP/F1 transgenic mice. Only a small amount of brown fat was found around the aorta but not around mesenteric arteries. Blood pressure of A-ZIP/F1 mice became higher than wild-type (WT) mice from 10 weeks of age. The presence of perivascular fat reduced the contraction of WT aorta to phenylephrine and serotonin, whereas this effect was either absent or less prominent in A-ZIP/F1 aorta. In WT mice, transfer of solution incubated with aorta with fat to aorta with fat removed caused a relaxation response, but not in A-ZIP/F1 mice, indicating the release of a relaxation factor from perivascular fat in WT aorta. This factor was acting through the activation of calcium-dependent potassium channels. Perfusion of phenylephrine to the isolated mesenteric bed caused a higher increase in perfusion pressure in A-ZIP/F1 than in WT mice. Contractile response of aorta to angiotensin II (Ang II) was mediated by Ang II type 1 receptors and was higher in A-ZIP/F1 than in WT mice. Expression of Ang II type 1 receptors but not Ang II type 2 receptors was higher in aorta of A-ZIP/F1 than WT mice. Treatment with an Ang II type 1 receptor antagonist (TCV 116, 10 mg/kg per day) for 2 weeks normalized the blood pressure of A-ZIP/F1 mice. These results suggest that the absence of perivascular fat tissue, which enhances the contractile response of the blood vessels to agonists, and an upregulation of vascular Ang II type 1 receptors in A-ZIP/F1 mice, are some of the mechanisms underlying the blood pressure elevation in these lipoatrophic mice. (*Hypertension*. 2007;49:365-372.)

Key Words: lipoatrophy ■ hypertension ■ vascular fat function ■ adipose tissue

O besity significantly increases the prevalence of associated risk factors, especially hypertension, and it is considered a major cardiovascular risk determinant,[1] but the mechanisms underlying the observed association between these diseases are poorly understood. It is now generally recognized that adipose tissue is an important endocrine and secretory organ, and some of its products, such as angiotensinogen (AGT), are important regulators of blood pressure.[2–4] In most systemic blood vessels, there is a layer of perivascular adipose tissue (PVAT) surrounding these vessels outside of the adventitial layer that may directly affect vessel wall structure and function. With the exception of the aorta, where there is usually a mixture of white and brown fat tissues, PVAT in most vessels is composed of white fat. It is now known that PVAT in the rat aorta secretes a vasodilatory substance that acts by a tyrosine kinase–dependent activation of K^+ channels on the vascular smooth muscle cells,[5] an effect that we have now demonstrated in PVAT taken from a human thoracic artery.[6] We have recently developed a nongenetic model of adult obesity through fetal and postnatal exposure to

nicotine, where we have found an increase in the amount of fat tissues in various parts of the body, including PVAT, but the PVAT from these obese animals did not cause vasodilation to the same degree as PVAT from the nonobese animals.[7] Taken together, these data suggest that altered function of PVAT may be a link between obesity and hypertension, but the mechanisms by which altered function of the PVAT may influence hypertension are currently not known.

At the other end of the spectrum is lipoatrophy, where humans with a severe decrease in the amount of adipose tissue are known to suffer from insulin resistance, type 2 diabetes, and sometimes hypertension.[8] The A-ZIP/F1 mice were produced as a model for lipoatrophy study.[9] These transgenic mice have no white fat and a drastically reduced amount of brown fat. These mice are diabetic, with reduced leptin and elevated serum glucose, insulin, free fatty acid, and triglycerides and hypertrophy of the heart, kidneys, pancreas, and liver.[9] It was not known whether these mice are also hypertensive. In this study, we examined the blood pressure and vascular function in both wild-type (WT) and lipoatro-
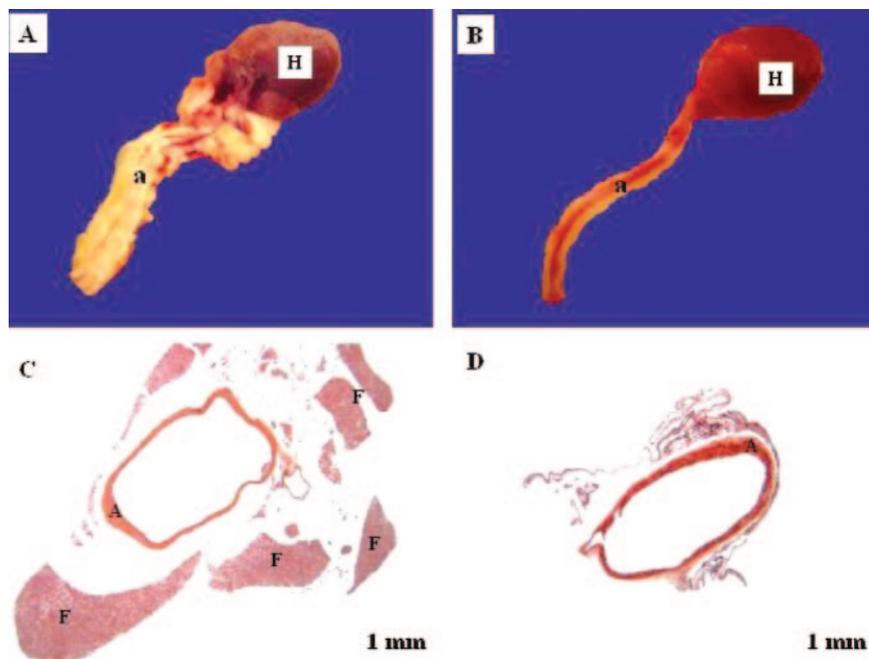
Figure 1. Gross morphology of thoracic aorta from WT surrounded by white adipose tissue (A) as compared with aorta from A-ZIP/F1 mice (B), with only a thin layer of brown adipose tissue. Similar morphology is shown in cross-sections of aorta from WT (C) as compared with A-ZIP/F1 mice (D). H indicates heart; A, aorta; F, fat tissue. Magnification bar=1 mm.

phic mice, and the effects of the vascular renin–angiotensin system in these mice.

Methods

Animals

A breeding colony was established in our central animal facility at McMaster University with breeders provided by Dr C Vinson of the National Institutes of Health. The A-ZIP/F1 mice are hemizygous on the FVB/N background, produced by breeding hemizygous males with WT females.[9] WT controls were littermates. These mice were fed standard chow, given water ad libitum, and maintained in alternating 12-hour cycles of light and dark. Female mice were used in the studies, because male mice were used for breeding purposes. All of the animal experiments were approved by an institutional review committee and conducted in accordance with the guidelines of the Canadian Council on Animal Care.

Blood Pressure and Body Weight Measurement

Tail-cuff systolic blood pressure and body weight were measured from 6 to 20 weeks of age. At 20 weeks of age, the mice were anesthetized with sodium pentobarbital (50 mg/kg IP). Interscapular brown fat, mesenteric artery, and thoracic aorta were dissected out for morphological and functional studies. The weight of interscapular brown fat was also determined.

Treatment With Angiotensin II Type 1 Receptor Antagonist (TCV 116)

WT and A-ZIP/F1 mice were given oral treatment with TCV 116 (10 mg/kg per day) for 2 weeks starting at the age of 16 to 18 weeks. Blood pressure was monitored before and during the treatment period. Contractile response to phenylephrine (PHE), serotonin (5HT), and angiotensin II (Ang II) in the aorta and the mesenteric arterial beds (MABs) were examined at the end of the treatment.

Morphological Study

Samples of thoracic artery and mesenteric arteries were fixed in 10% buffered formalin and embedded in paraffin. Three-micron–thick cross-sections of the arteries were stained with hematoxylin and eosin.

Contractility Study With Aorta

Thoracic aortic samples were stored in 4°C oxygenated physiological salt solution (PSS) with the following composition (in mM): 119 NaCl, 4.7 KCl, 1.2 KH_2PO4, 1.2 $MgSO_4$, 22 $NaHCO_3$, 11.1 glucose, and 1.6 $CaCl_2$. Vascular function was studied using a conventional organ bath system. Two pairs of aortic rings (3- to 4-mm long) were cut consecutively from the middle part of the thoracic aorta. In each pair, PVAT was left intact on 1 ring (Fat+), whereas PVAT was removed in the other ring (Fat−). After 1 hour of equilibration under a resting tension of 1 g at 37°C, these rings were contracted with PHE and 5HT. The cumulative concentration–contraction curve to PHE and 5HT was expressed as a percentage of the contraction to KCl (60 mmol/L), which induces contraction through membrane depolarization. We also investigated the contractile response to Ang II (10^{-7} mol/L and 10^{-6} mol/L) in the presence and absence of an Ang II type 1 (AT_1) receptor antagonist (CV11974, 100 nM, Takeda Pharmaceutical). Involvement of K^+ channels in the anticontractile effects by PVAT was examined in the aorta of WT mice with different K^+ channel blockers. Functional integrity of the endothelial cells was established by the relaxation response to carbamylcholine chloride in the aorta precontracted with PHE (10^{-6} mol/L).

Transfer of Solution Experiments

Fat+ and Fat− aortic rings were prepared as described above. These rings were contracted with 1 μmol/L of PHE to a submaximal tension (70% to 80% of the maximal). When the contraction in response to PHE had reached a plateau, 3 mL of the solution

Body Weight and the Weight of Interscapular Brown Fat in WT and A-ZIP/F1 Mice

Group	Body Weight, g	Interscapular Brown Fat Weight, g	% of Body Weight
WT (N=7)	32.79±1.63	0.13±0.01	0.40±0.02
A-ZIP/F1 (N=10)	30.39±0.95	0.08±0.01	0.26±0.03*
WT (TCV 116; N=6)	25.81±0.53		
A-ZIP/F1 (TCV 116; N=6)	29.13±0.87		

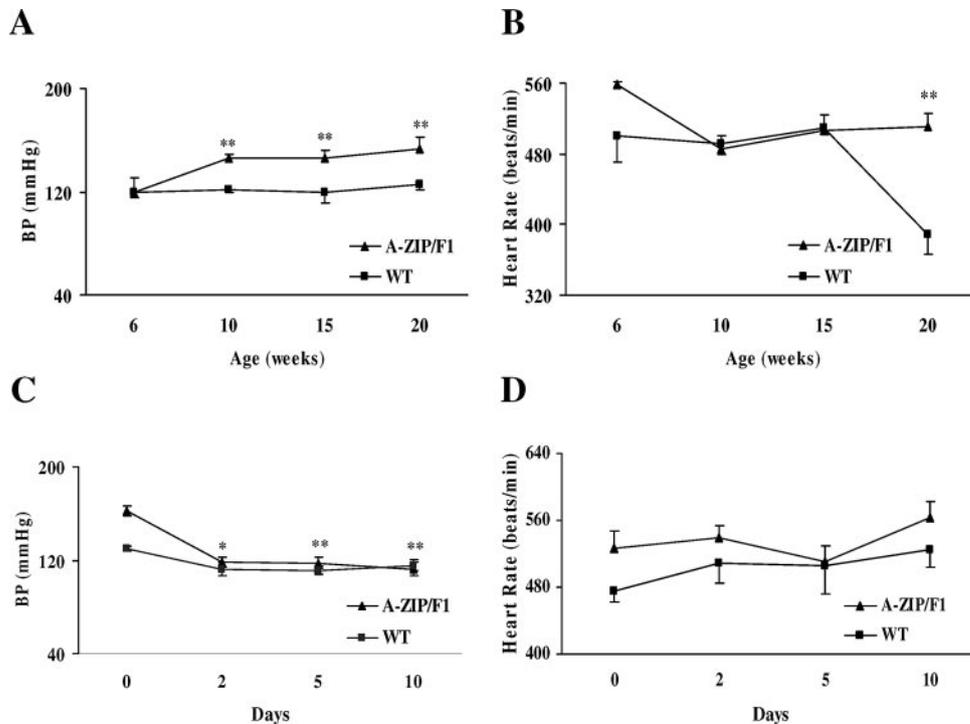The interscapular brown fat content was normalized to body weight. Mean±SEM, *$P<0.01$.

Figure 2. Systolic blood pressure (BP) and heart rate profile of WT and A-ZIP/F1 mice (A and B) and mice treated with TCV 116 for 2 weeks (C and D). *$P<0.05$, **$P<0.01$ vs WT mice. N=7 to 10 in WT and A-ZIP/F1 mice and N=6 each in treated WT and A-ZIP/F1 mice.

incubated with Fat+ ring for 25 minutes (donor) was transferred to the Fat− ring (recipient). The relaxation of the recipient aorta (Fat−) was expressed as a percentage of the precontracted tension. Transferring solution between Fat− rings served as a control. The cumulative concentration–contraction curve to PHE was expressed as a percentage of the contraction to KCl (60 mmol/L). Cross-transfer of solution incubated with Fat+ WT aorta to AZIP/F1 aorta was also carried out.

Perfusion Study With Mesenteric Vascular Bed

WT or A-ZIP/F1 mice at 20 to 28 weeks were used to study vascular response of the MAB using the method of McGregor.[10] The abdomen was opened through a midline incision. MAB was exposed and gently cleared of attached tissues. After clearance of the vascular bed by perfusion with warm oxygenated PSS containing heparin (100U/mL), the MAB was transferred into a warm PSS buffer (37°C [pH 7.4]) in the perfusion chamber. Flow was generated by a syringe pump at a constant flow rate of 1 mL/min. Perfusion pressure was recorded by a pressure transducer. After perfusing MAB with warm PSS for 30 minutes, 120 mmol/L of KCl in PSS was introduced to induce contraction. We have carried out preliminary experiments to establish that 120 mmol/L of KCl was optimal to study the pressure response in MAB of mice. After 30 minutes of the washout period, a dose-dependent curve to PHE was established by bolus injection of PHE.

Expression of Ang II Receptors and Plasma Ang II Levels

Total RNA of thoracic aorta from 20-week-old female WT and A-ZIP/F1 mice was extracted using TRIzol reagent (Invitrogen) according to the manufacturer's instruction. After DNAse treatment, RT-PCR was performed by RETRO script (Ambion) according to the manufacturer's instructions. One microgram of total RNA was reverse transcribed by Moloney murine leukemia virus reverse transcriptase (Ambion). Using 1 μL of cDNA solution, PCR was performed with gene-specific primers for AT_1, Ang II type 2, and GAPDH with the following sequences: AT_1 (forward, 5′-ccattgtccacccgatgaag-3′, reverse, 5′-tgcaggtgactttggccac), Ang II type 2

(forward 5′- cagcagccgtcctttgataa-3′; reverse, 5′-ttatctgtaggtttgtgt-gagcaa-3′), and GAPDH (forward, 5′-cctgcaccaccaactgctta-3′; reverse, 5′-tcatgagcccttccacaatg-3′). PCR products were separated by electrophoresis in 2% agarose gel containing ethidium bromide and identified by fluorescent UV light. Scion Image was used to measure the band intensities and normalized with GAPDH from the same sample of RNA. Plasma Ang II levels were measured using an enzyme immunoassay kit (Peninsula Laboratories Inc).

Statistics

The results were expressed as mean (±SEM). Statistical analyses were performed using ANOVA and paired and unpaired Student's t test. $P\leq0.05$ was considered statistically significant.

Results

Physical Characteristics

At 20 weeks of age, A-ZIP/F1 mice had essentially no white fat tissue throughout their body and a drastically reduced amount of brown fat relative to WT mice. Thoracic aorta from A-ZIP/F1 mice was covered by a small amount of brown fat compared with an abundance of fat tissue in the aorta of WT mice (Figure 1). PVAT was almost absent around mesenteric arteries. Body weight and the weight of interscapular fat were similar between WT and A-ZIP/F1 mice, but when normalized with body weight, A-ZIP/F1 mice have significantly less interscapular fat than WT mice (Table 1). Blood pressure of A-ZIP/F1 mice was similar to WT at 7 weeks but became higher than WT from 10 weeks of age onward (Figure 2A). Heart rate was similar until 20 weeks when heart rate from WT became lower than A-ZIP/F1 mice (Figure 2B). Treatment with TCV 116 normalized the blood pressure in A-ZIP/F1 mice (28±1.2%) to the level of untreated WT
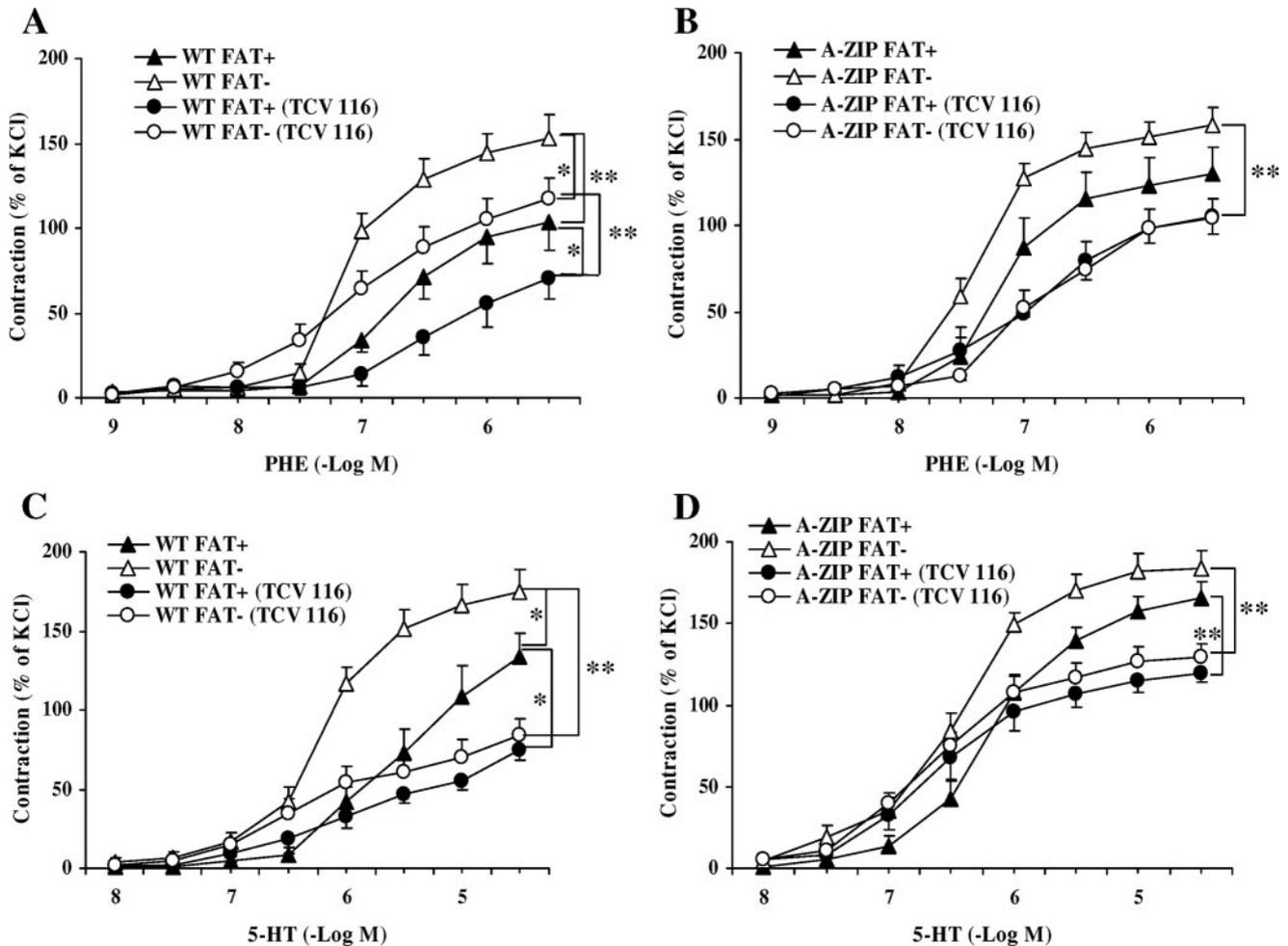
Figure 3. Contractile response of aorta from control and treated WT and A-ZIP/F1 mice with (Fat+) or without (Fat−) perivascular fat tissue to PHE (A and B) and to 5HT (C and D). *P<0.05, **P<0.01. N=7 each in WT and A-ZIP/F1 mice, and N=6 each in treated WT and A-ZIP/F1 mice.

mice, and this treatment also lowered the BP of WT mice (12±0.91%; Figure 2C). Heart rate was not affected by this treatment (Figure 2D).

Functional Studies

Maximal contraction induced by KCl was similar between the aorta from WT and A-ZIP/F1 mice. We have, therefore, normalized the response to agonists to KCl response. The presence of PVAT significantly reduced the contraction to PHE (Figure 3A) and 5HT (Figure 3C) in the aorta of WT, whereas in the A-ZIP/F1 mice, the presence of brown fat only slightly attenuated these contractile responses (Figure 3B and 3D). TCV 116 treatment reduced the contractile response to PHE of WT aorta with or without PVAT, in contrast with aorta from A-ZIP/F1 mice, where only aorta without PVAT was affected. Such a treatment also attenuated the response to 5HT in the aorta from WT and A-ZIP/F1 mice with or without PVAT.

In the presence of PVAT, a higher contractile response was found in the aorta of A-ZIP/F1 than WT at high concentration of Ang II (10^{-6} mol/L) but not at low concentration (10^{-7} mol/L; Figure 4A and 4B). With PVAT removed, aorta from A-ZIP/F1 showed a higher contractile response than WT at

both concentrations. AT_1 receptor antagonist (CV11974) significantly reduced the response of the aorta from A-ZIP/F1 mice both in the presence and absence of PVAT, but the effect of this antagonist on the aorta from WT mice was minimal (Figure 4C and 4D). Chronic treatment with TCV 116 abolished the response of the aorta to Ang II in both A-ZIP/F1 and WT mice (data not shown).

The transfer of incubating solution from Fat+ to Fat− tissue caused a significant relaxation (27%) in WT aorta but not in A-ZIP/F1 mice (Figure 5A). Transfer of incubating solution from Fat+ WT aorta to Fat− A-ZIP/F1 aorta also caused a relaxation response (Figure 5B; 39%), but the difference when compared with Fat+ to Fat− transfer in WT mice was not significant (P=0.10). Contractile response to PHE in the Fat+ WT aorta was enhanced by incubation with calcium-dependent K^+ channel blocker tetraethylammonium chloride (1 mmol/L) but not by voltage-dependent K^+ channel blocker 4-aminopyridine (1 mmol/L), ATP-dependent K^+ channel blocker glipizide (10 μmol/L), and inward rectifier inhibitor barium chloride (100 μmol/L; Figure 6). Baseline tension was transiently increased by 4-aminopyridine but not by other blockers, and these treatments did not affect the contractile response to PHE in the preparations without
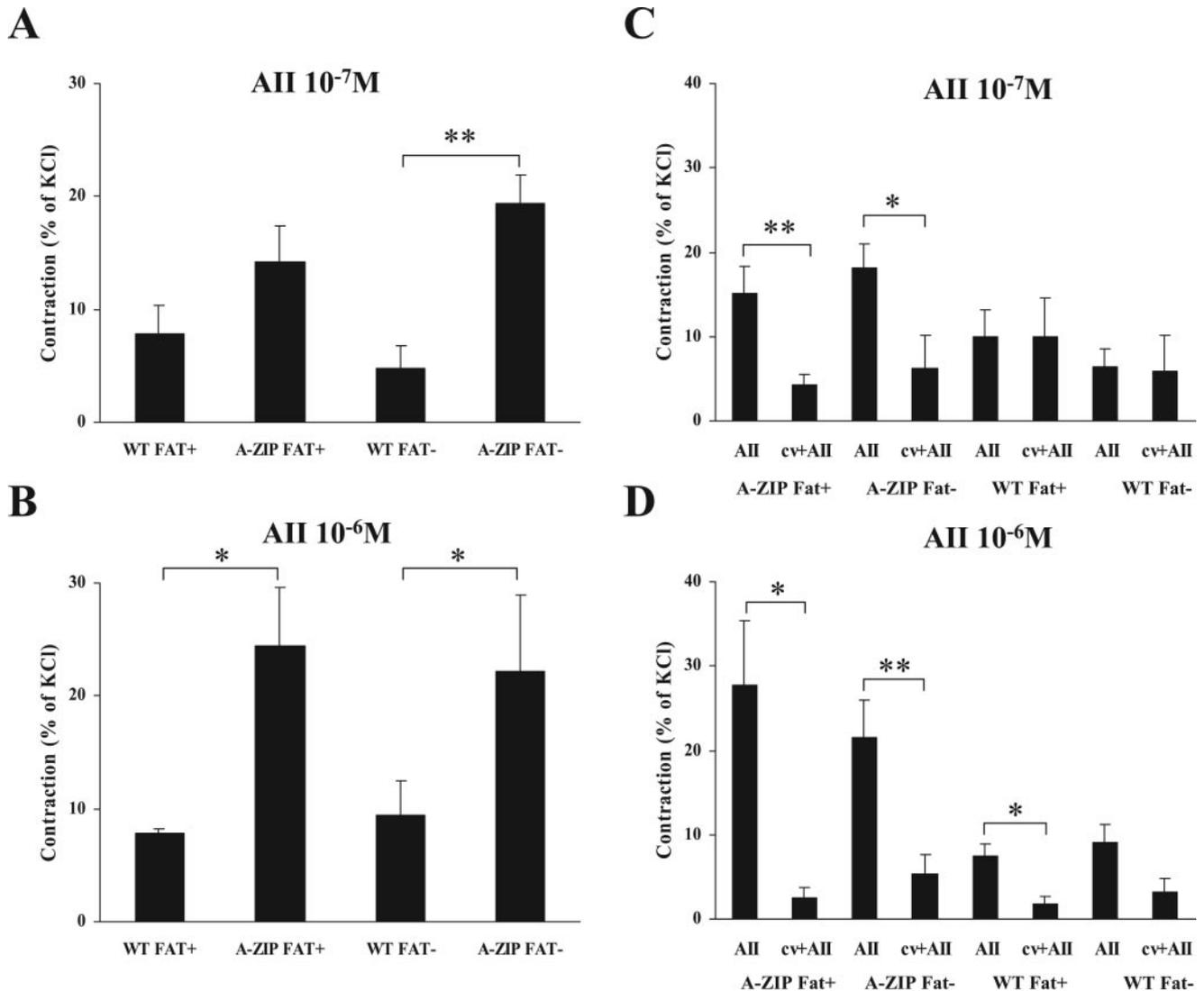
A

B

C

D

Figure 4. Contractile response of aorta from WT and A-ZIP/F1 mice with (Fat+) or without (Fat−) perivascular fat tissue to 2 different concentrations of Ang II (A and B) and effect of AT_1 receptor blocker (CV11974, cv) on the contractile response of aorta to Ang II (C and D). *$P<0.05$, ** $P<0.01$. N=7 each in WT and A-ZIP/F1 mice.

PVAT (data not shown). Relaxation response to carbamyl-choline chloride was concentration dependent, but there was no difference between WT and A-ZIP/F1 aorta with or without PVAT and between Fat+ and Fat− arteries in either WT or A-ZIP/F1 mice (Figure 7).

Perfusion of MAB with KCl caused a higher increase in perfusion pressure in WT than AZIP/A1 mice (Figure 5C). PHE caused a higher increase in perfusion pressure in A-ZIP/F1 than WT mice (Figure 5D). Treatment with TCV 116 reduced the response to PHE in both A-ZIP/F1 and WT mice and in AZIP/F1 mice to the level of untreated WT mice. Contractile response to Ang II was abolished by TCV 116 treatment (data not shown).

Ang II Receptors Expression
The expression of AT_1 receptors but not Ang II type 2 receptors was higher in the aorta of A-ZIP/F1 than WT mice (Figure 8). Plasma level of Ang II in WT mice (88.1±8.5 pg/mL; n=14) was similar to that of A-ZIP/F1 mice (91.5±12.0 pg/mL; N=7).

Discussion
We have shown here for the first time that lipoatrophic A-ZIP/F1 mice developed a higher blood pressure than WT mice from 10 weeks of age. This elevated blood pressure was contributed by ≥2 of the following factors: the absence of fat around the blood vessels, which normally modulate the contractile function of the blood vessels, and an enhanced response of the aorta to Ang II, probably because of an upregulation of AT_1 receptors in the blood vessels.

In blood vessels from rats and humans, contractile response to various agonists is attenuated in the presence of PVAT because of the release of an unknown relaxing agent from the PVAT, which acts through the potassium channels.[5,6,11] In this study, our finding that aorta from WT mice responded less to PHE and 5HT in the presence of PVAT is consistent with the concept that adipose tissue surrounding the aorta releases a vasodilator factor. The existence of this relaxation factor was confirmed by our transfer experiments where donor solution from Fat+ WT aorta caused a relaxation
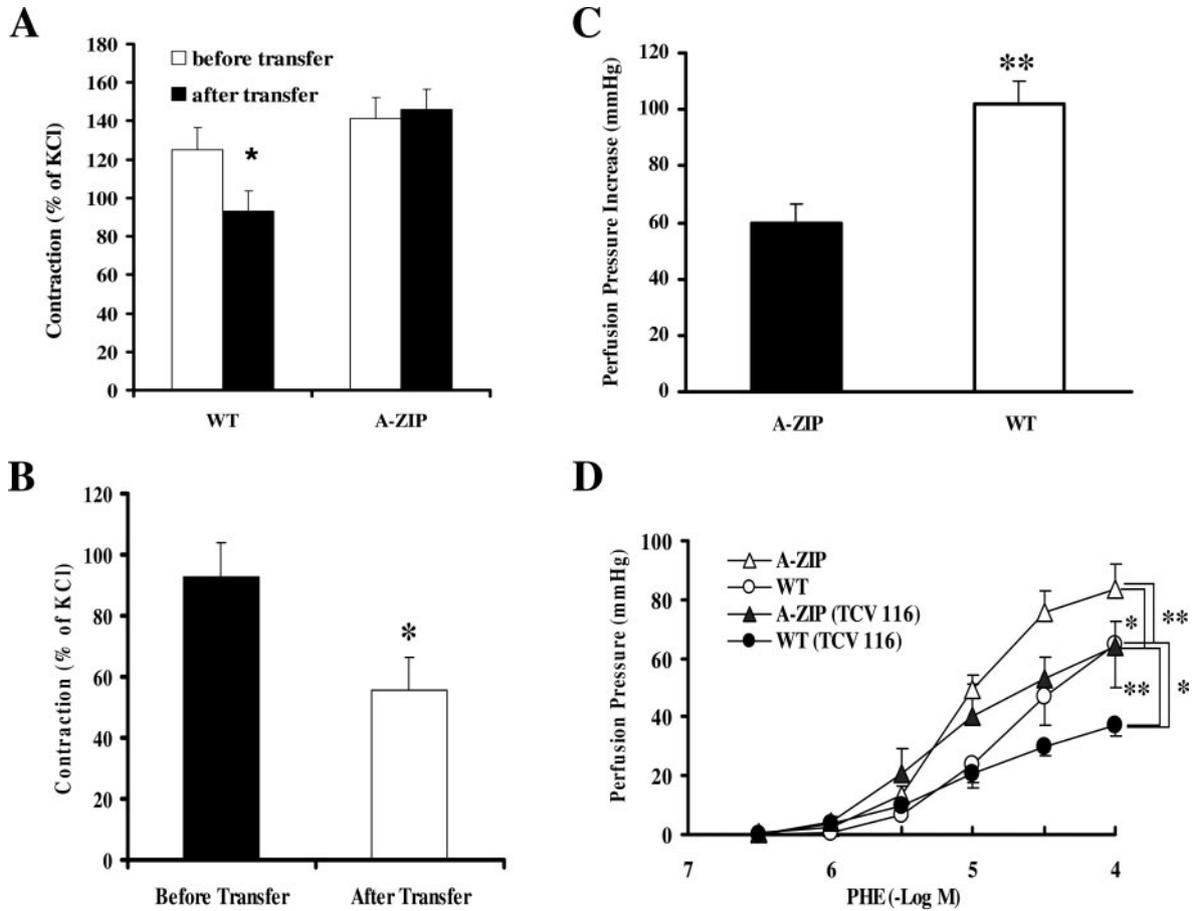
A

B

C

D

Figure 5. A, Transfer of solution incubated with aorta with intact fat tissue to tissue with fat removed in WT and AZIP/F1 mice, N=10 each, *P<0.05. B, Transfer of WT donor to AZIP/F1 recipient aorta, N=4 each, *P<0.05. C, Increase in perfusion pressure caused by infusion of KCl and (D) dose-dependent response to PHE in the mesenteric vascular bed in control and treated mice. N=7 each in the control groups, and N=5 in treated WT and A-ZIP/F1 groups. *P<0.05, **P<0.01.

response in Fat− WT aorta. This is in contrast with A-ZIP/F1 mice where the presence of a small amount of brown fat had no effect on contractile response to PHE or 5HT. To show that this lack of effect in A-ZIP/F1 mice was not because of a change in the response of the aorta to this relaxation factor, we had carried out a cross-transfer experiment. Our results showed that A-ZIP/F1 aorta was still responsive to this

relaxation factor from Fat+ WT aorta. We have also established that this relaxation response to PVAT was mediated through calcium-dependent K+ channels, which is consistent with the results obtained in the human internal thoracic artery[6] but different from rat aorta, where it was mediated through K_{ATP} channels.[5] Our results also showed that the differences in the response of aorta to different agonists between A-ZIP/F1 and WT aorta was not because of an alteration in endothelial functions, because relaxation response to carbam-
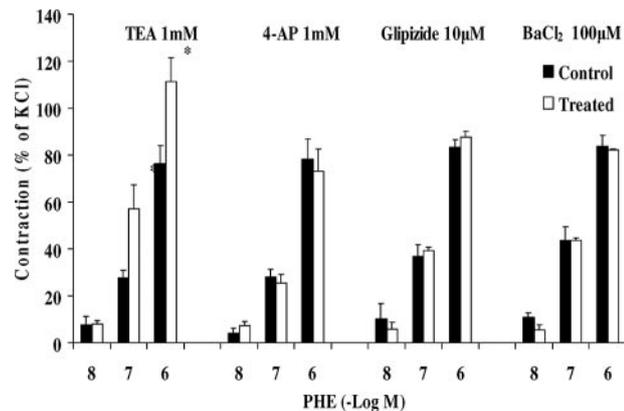
Figure 6. Concentration-dependent contraction of WT aorta with intact fat tissue to PHE with (treated) or without (control) pretreatment with different potassium channel blockers. N=5, *P<0.05 vs control.
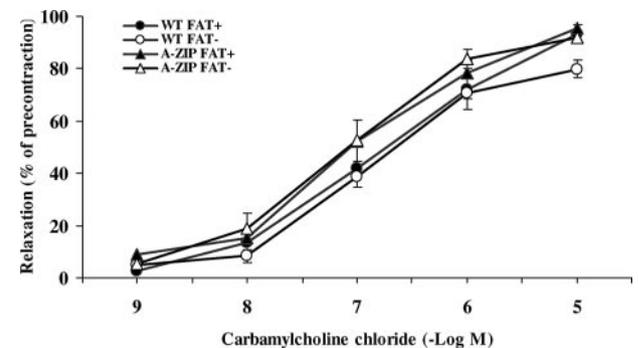
Figure 7. Relaxation response of aorta from WT and A-ZIP/F1 mice in the presence (Fat+) and absence (Fat−) of perivascular fat tissue to carbamylcholine chloride. N=6.
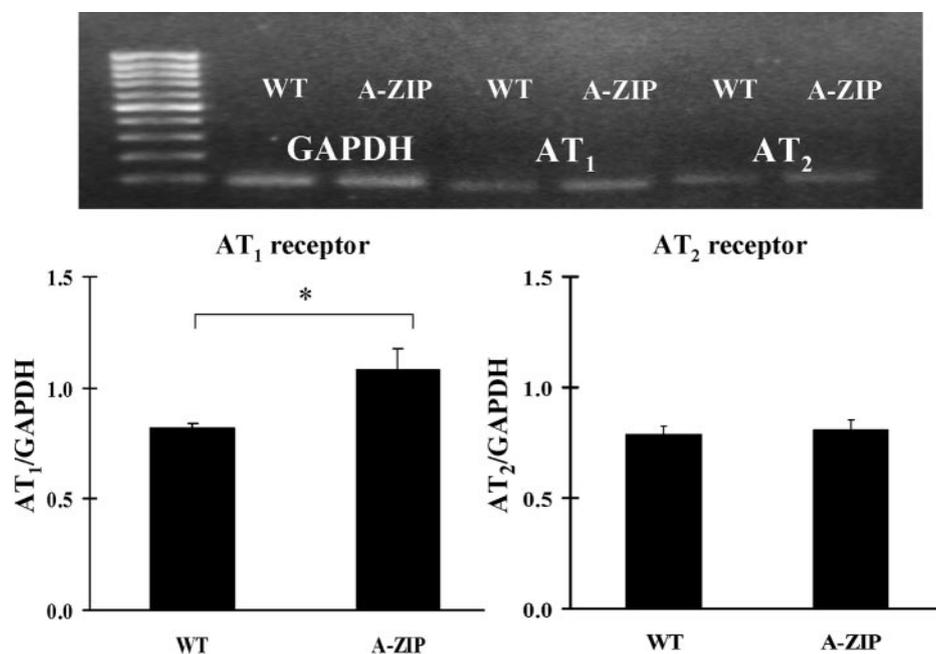
Figure 8. Expression of Ang II type 1 (AT₁) and type 2 (AT₂) receptors in the aorta of WT and A-ZIP/F1 mice. *$P<0.05$. N=7 each in WT and A-ZIP/F1 mice.

ylcholine chloride was not different between them both in the presence and absence of PVAT.

In the MAB, our perfusion study again showed that vascular bed from A-ZIP/F1 was more sensitive to PHE by generating a higher perfusion pressure than WT MAB, probably because of the absence of PVAT. Therefore, in both conduit vessel (aorta) and resistance arteries (MAB), a higher contractile response to various agonists in A-ZIP/F1 is related to the absence of PVAT as compared with WT mice. These results suggest, at least in part, that the elevated blood pressure in the A-ZIP/F1 mice is because of the absence of the modulating effect of PVAT on vascular functions because of the absence of PVAT. A higher response of WT MAB than A-ZIP/F1 MAB to KCl stimulation that we found here is puzzling. It is possible that at this high concentration (120 mmol/L), KCl stimulated the perivascular nerves causing the release of a vasoconstrictor from PVAT. In rat mesenteric artery, we found that stimulation of perivascular nerve with electrical field stimulation causes the release of a constricting factor from PVAT.[12]

White adipose tissue is a rich source of AGT and is probably second only to the liver in the production of this precursor to Ang II.[4] AGT produced by adipose tissue is secreted to the bloodstream, thereby playing a role in the endocrine system.[13] Mice that overexpress adipose AGT have increased levels of circulating AGT compared with WT mice and are hypertensive,[13] suggesting that AGT produced by adipose tissue may play a role in the development of cardiovascular diseases, including hypertension. Paradoxically, in the total absence of white adipose tissue, as in the case of A-ZIP/F1 mice, blood pressure of these mice is elevated. This is probably because the vascular renin–angiotensin system is upregulated in the A-ZIP/F1 mice. In this study, we found that aorta from A-ZIP/F1 mice are more responsive to Ang II than that in the WT mice based

on the results that in the absence of PVAT, aorta from A-ZIP/F1 mice showed a higher contractile response to Ang II than WT aorta.

We also found that the Ang II response was mediated by AT₁ receptors, with aorta from A-ZIP/F1 mice being more sensitive to AT₁ blockade than WT aorta. This functional change is corroborated by an increased expression of AT₁ receptors in aorta of A-ZIP/F1 than WT aorta, with no difference in Ang II type 2 receptor expression. However, the plasma level of Ang II was similar between WT and A-ZIP/F1 mice. This suggests that, in spite of the absence of white adipose tissue, which is a rich source of AGT, to produce Ang II, liver in the A-ZIP/F1 mice is still capable of producing sufficient AGT for Ang II production. Liver in A-ZIP/F1 mice is hypertrophied by 2.1-fold mostly because of lipid deposition.[9] These results suggest that the upregulation of AT₁ receptors was happening at the local vascular level in A-ZIP/F1 mice because of the absence of PVAT. Our treatment study with an AT₁ receptor antagonist, which showed a blood pressure normalization effect, certainly supports the role of AT₁ receptors in the control of blood pressure in A-ZIP/F1 mice. We also found that treatment with TCV 116 abolished the response to Ang II in the aorta and MAB. This may be related to reduced expression of AT₁ receptors in the vessels from treated mice, as shown in a previous study with cerebral microvessels,[14] but the mechanisms for this decreased expression remain unclear. The absence of in vitro response of aorta and mesenteric bed to Ang II from mice treated with TCV 116 showed the potent long-lasting effect of this drug. Treatment with TCV 116 also reduced the contractile response of aorta from WT and AZIP/F1 mice to PHE and 5-HT, although the reduction in the response of AZIP/F1 aorta to PHE did not reach the statistical significance level. This reduction in response was

probably related to the blood pressure–lowering effect of TCV 116 alone, independent of its AT_1 receptor effect.

In summary, we have shown here for the first time that lipoatrophic A-ZIP/F1 mice developed a higher blood pressure than their WT controls. The enhanced response of the blood vessels to agonists because of the absence of PVAT in A-ZIP/F1 mice and, therefore, a reduced release or production of a fat-derived relaxing factor and an upregulation of vascular AT_1 receptors are some of the mechanisms underlying the blood pressure elevation in these lipoatrophic mice.

Perspectives

Because obesity is becoming a worldwide epidemic, many studies have focused on the detrimental effects of fat tissue on cardiovascular functions, including the roles of various adipokines in the control of blood pressure. At the opposite end of the spectrum are patients with a reduced amount or total lack of fat tissue (lipoatrophy). Here we showed that an absence of perivascular fat tissue is also harmful to the health of the individuals, because it can lead to the development of hypertension, in addition to other complications, such as diabetes.

Disclosures

None.

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