Improved Lipid and Glucose Metabolism in Transgenic Rats With Increased Circulating Angiotensin-(1-7)

Sérgio Henrique S. Santos, Janaina Felix Braga, Érica Guilhen Mario, Laura Cristina J. Pôrto, Maria da Glória Rodrigues-Machado, Antonela Murari, Leida Maria Botion, Natalia Alenina, Michael Bader, Robson Augusto S. Santos

Objective—Obesity and diabetes remain among the world’s most pervasive health problems. Although the importance of angiotensin II for metabolic regulation is well documented, the role of the angiotensin-(1-7)/Mas axis in this process is poorly understood. The aim of this study was to evaluate the effect of increased angiotensin-(1-7) plasma levels in lipid and glucose metabolism using transgenic rats that express an angiotensin-(1-7)-releasing fusion protein, TGR(A1-7)3292 (TGR).

Methods and Results—The increased angiotensin-(1-7) levels in TGR induced enhanced glucose tolerance, insulin sensitivity, and insulin-stimulated glucose uptake. In addition, TGR presented decreased triglycerides and cholesterol levels, as well as a significant decrease in abdominal fat mass, despite normal food intake. These alterations were accompanied by a marked decrease of angiotensinogen expression and increased Akt in adipose tissue. Furthermore, augmented plasma levels and expression in adipose tissue was observed for adiponectin. Accordingly, angiotensin-(1-7) stimulation increased adiponectin production by primary adipocyte culture, which was blocked by the Mas antagonist A779. Circulating insulin and muscle glycogen content were not altered in TGR.

Conclusion—These results show that increased circulating angiotensin-(1-7) levels lead to prominent changes in glucose and lipid metabolism. (Arterioscler Thromb Vasc Biol. 2010;30:953-961.)

Key Words: angiotensin-(1-7) Mas axis • glucose metabolism • adipose tissue • cholesterol • triglycerides • adiponectin

Excess body fat and insulin resistance are the central signs of metabolic syndrome (MS), a complex state often associated with other diseases. Other features include hyperinsulinemia, dyslipidemia (high triglycerides and total cholesterol plasma levels), hypertension, proinflammatory and prothrombotic status, and microalbuminuria.1,2 The pathogenesis of MS is multifactorial and might be related to a sedentary lifestyle, an unbalanced diet, and genetic predisposition.2–4 However, the molecular mechanisms underlying MS are complex and not fully understood.5

The renin-angiotensin system (RAS) is now recognized to be important for the development of cardiovascular and metabolic disorders.6 The RAS consists primarily of an enzymatic cascade in which angiotensinogen (AGT) is converted to angiotensin (Ang) I and subsequently to Ang II by the actions of renin and angiotensin-converting enzyme (ACE), respectively.7 Angiotensin (Ang)-(1-7) is formed primarily from Ang II by ACE2 and from Ang I by prolylendopeptidase or neutral endopeptidase and, indirectly to a lesser extent, by ACE2.8,9 It is well documented that Ang II, acting via its Ang II type 1 (AT1) receptor, is a potent proinflammatory, pro-oxidant, and prothrombotic agent that interferes with several steps of intracellular insulin signaling.10,11 Increased levels of Ang II have been observed in both obese and diabetic patients.10 The increase in Ang II is closely correlated to insulin resistance.4,5 RAS components, especially AGT, found in adipose tissue, are closely related to the Ang II effects on insulin resistance.10,12

The ACE2/Ang-(1-7)/Mas axis has been suggested as an important counterregulatory arm in the RAS with effects opposite those of ACE/Ang II/AT1. This arm can produce NO-dependent vasodilatation, as well as antiarrhythmic, antiproliferative, and antithrombotic effects.13,14

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From Laboratory of Hypertension, INCT-NanoBiofar (S.H.S.S., J.F.B., M.d.G.R.-M., A.M., R.A.S.S.) and Laboratory of Cellular Metabolism (E.G.M., L.M.B.), Department of Physiology and Biophysics, Biological Sciences Institute, Federal University of Minas Gerais, Belo Horizonte, Minas Gerais, Brazil; Department of Nutrition, Federal University of Lavras, Lavras, Minas Gerais, Brazil (L.C.J.P.); Max-Delbrück-Center for Molecular Medicine, Berlin-Buch, Germany (N.A., M.B.); Programa de Pós-Graduação do Instituto de Cardiologia E Fundação Universitaria de Cardiologia, Porto Alegre, RS/Brazil (R.A.S.S).

Correspondence to: Robson Augusto S. Santos, Laboratório de Hipertensão, Departamento de Fisiologia e Biofísica, Universidade Federal de Minas Gerais, Avenida Antonio Carlos 6627-ICB/Pampulha, 31270-901, Belo Horizonte, Minas Gerais, Brazil. E-mail robsonsant@gmail.com

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The role of Ang-(1-7) in metabolic regulation is poorly understood. Recently, we have demonstrated that Mas deficiency in FVB/N mice induces dyslipidemia, lower glucose tolerance and insulin sensitivity, hyperinsulinemia, hyperleptinemia, decreased glucose uptake in white adipose cells, and an increase in adipose tissue mass. These observations suggest that chronic deficiency in Ang-(1-7)/Mas axis components can lead to an MS-like state.

In this study, we sought to evaluate whether a chronic increase in Ang-(1-7)/Mas activity would influence lipid or glucose metabolism. We used a transgenic rat model, TGR(A1-7)3292 (TGR), which expresses an Ang-(1-7)-producing fusion protein, resulting in a chronic elevation of plasma Ang-(1-7) levels.

**Methods**

**Animals**

Ten- to 12-week-old male TGR and control Sprague-Dawley rats were obtained from the transgenic animal facilities at Laboratory of Hypertension, Federal University of Minas Gerais, Belo Horizonte, Brazil. All experimental protocols were performed in accordance with the international guidelines for animal care and approved by local authorities.

**Blood Measurements**

Serum was obtained after centrifugation (3200 rpm for 10 minutes at 4°C). Total serum cholesterol and triglycerides were assayed using enzymatic kits (Doles, Goiania, Brazil). ELISA kits were used to measure serum adiponectin (AdipoGen, Seoul, Korea), leptin (R&D Systems, Minneapolis, Minn), and insulin (Linco Research Inc) levels. Leptin and adiponectin secretion was calculated as serum levels normalized to fat tissue weight. For measurement of blood angiotensins, arterial blood was collected from an arterial cannula implanted, under tribromoethanol anesthesia (300 mg/kg of body weight), in the abdominal aorta through the femoral artery, 18 to 24 hours before blood draw. Plasma levels of Ang-(1-7) (n = 8 and 5 for Sprague-Dawley and TGR, respectively) and Ang II (n = 5 for both groups) were measured by radioimmunoassay as described previously.

**Histology**

Epididymal fat tissue from TGR and Sprague-Dawley rats was excised, fixed in Bouin solution, and embedded in paraffin. Images using a ×20 objective were captured with an Evolution LC Color light camera (MediaCybernetics), and adipocyte diameters from at least 540 cells for each animal were measured using NIH Image software.

**Muscle Glycogen Content**

Muscle glycogen was extracted according to Sjörgren et al and determined as glucose following acid hydrolysis. Briefly, muscle samples were placed in tubes with 30% KOH saturated with Na₂SO₄. The tubes were placed in a boiling water bath for 1 hour until a homogeneous solution was obtained; they were then removed from the water bath and cooled in ice. Absolute ethanol was added to precipitate the glycogen from the alkaline digest. The samples were left in ice for 10 minutes and then centrifuged at 840g for 20 minutes. The supernatant was carefully aspirated, and the glycogen was washed with distilled water and precipitated with ethanol again. Glycogen precipitates were dissolved in 3.0 mL of distilled water, transferred to a 10-mL volumetric flask, and diluted to the mark with water. The contents of the flasks were thoroughly mixed, and the measured portion was further diluted with water in a second volumetric flask so as to yield a solution with a glycogen concentration of 3 to 30 mg/mL. Amnithone was carefully added to 2-mL aliquots of the final dilution in the ice bath, and the tubes were then placed in a boiling water bath for 10 minutes. After the tubes cooled down, the absorbance of the samples was measured at 620 nm on a spectrophotometer. Glucose at different concentrations was used for a calibration curve.

**Western Blot Analysis**

Proteins were extracted from epididymal adipose tissue samples (~300 mg) of TGR and Sprague-Dawley rats, and 30 µg of protein was resolved on SDS-PAGE gels (10%) and then transferred onto nitrocellulose membranes. The glucose transporter GLUT4 (Cell Signaling Technology), total Akt (Cell Signaling Technology), phosphorylated Akt (Cell Signaling Technology), and β-actin (internal control) (Cell Signaling Technology) were probed with a polyclonal rabbit antibody (1:1000). Goat anti-rabbit IgG conjugated with peroxidase (1:5000) was used as a secondary antibody. The blots were visualized using a chemiluminescence Western blotting detection reagent (ECL; Amersham Pharmacia Biotech) and revealed on a photographic film (Kodak) followed by quantification using TINA 2.08c program (Raytest, Straubenhart, Germany).

**Reverse Transcription and Real-Time Polymerase Chain Reaction**

Total RNA from adipose tissue was prepared using TRizol reagent (Invitrogen Corp, San Diego, Calif), treated with DNase, and reverse transcribed with Moloney murine leukemia virus (Invitrogen) using random hexamer primers. Levels of transforming growth factor-β (TGF-β), tumor necrosis factor-α (TNF-α), lipoprotein-binding protein (aP2), adiponectin, AGT, AT₁, and Mas mRNA were determined by real-time quantitative polymerase chain reaction using SYBR Green reagent (Applied Biosystems) in an ABI Prism 7000 platform (Applied Biosystems) with the following primers: TGF-β-FW, 5'-AAA CGG AAG GGC ACT GGA; TGF-β-RV, 5'-GGG ACT GGC GAG CCT TAG TT; AGT-FW, 5'-ACA CCC CTG CTA CAG TCC AC; AGT-RV, 5'-ACC CCT TCT AGT GTC GAG AAG TT; TNF-α-FW, 5'-TGC CTC AGC CTC TTC TCA TT-3'; TNF-α-RV, 5'-CCC ATT TGG GAA CTT CTC CT-3'; adiponectin-FW, 5'-CTC CAC CCA AGG AAA CCTT GT-3'; and adiponectin-RV, 5'-CTG GTC CAC ATT TTT TTC CT-3'. Gene expression was normalized to the endogenous glyceraldehyde 3-phosphate dehydrogenase (GAPDH) using the comparative CT method of Livak and Schmittgen was applied to compare gene expression levels between groups, using the equation 2⁻ΔΔCT.

**In Vivo Experiments: Glucose Tolerance and Insulin Sensitivity Tests**

For the glucose tolerance test, d-glucose (2 mg/g of body weight) was intraperitoneally injected into overnight fasted rats. Glucose...
levels from tail blood samples were monitored at 0, 15, 30, 60, and 120 minutes after injection using an Accu-Chek glucometer (Roche Diagnostics Corp, Indianapolis, Ind). Insulin sensitivity test was performed in overnight fed rats, after intraperitoneal injection of insulin (0.75 U/kg body weight; Sigma, St Louis, Mo). Tail-blood samples were taken at time points 0, 15, 30, and 60 minutes after injection for measurement of blood glucose levels.

**In Vitro Experiments**

**Glucose Uptake**

Adipocytes from Sprague-Dawley and TGR were isolated from epididymal fat pads according to Rodbell. Tissues were digested with collagenase at 37°C with constant shaking for 45 minutes. Cells were filtered through a 300-μm nylon mesh and washed 3 times with buffer containing 1% BSA and the following (in mmol/L): 137 NaCl, 5 KCl, 4.2 NaHCO3, 1.3 CaCl2, 0.5 MgCl2, 0.5 MgSO4, 0.5 KH2PO4, 20 HEPES (pH 7.4).

**Glucose Transport Assay**

After isolation, adipocytes were incubated in a bath for 45 minutes at 37°C in the presence or absence of insulin (25 ng/mL). The uptake of 2-deoxy-[3H]glucose was used to determine the rate of glucose transport as previously described. Briefly, glucose uptake was initiated by the addition of 2-deoxy-[3H]glucose (0.2 μCi/tube) for 3 minutes. Thereafter, cells were separated by centrifugation through silicone oil, and cell-associated radioactivity was determined by scintillation counting. Nonspecific association of 2-deoxy-[3H]glucose was determined by performing parallel incubations in the presence of 15 mmol/L phloretin, and this value was subtracted from glucose transport activity in each condition. At the end of the analysis, the difference between the baseline uptake and insulin-stimulated uptake in Sprague-Dawley and TGR was calculated for each animal to demonstrate the capacity to respond to insulin stimulation (insulin sensitivity).

**Primary Culture of Adipocytes**

Adipocytes were isolated under sterile conditions from epididymal fat pads of Sprague-Dawley rats by the Rodbell method and maintained in primary culture according to the method of Marshall et al for 3 hours in DMEM containing 5 mmol/L glucose, 25 mmol/L HEPES, 2% fetal bovine serum, 20 U/mL penicillin, 20 mg/mL streptomycin, and 1% BSA. Isolated adipocytes were diluted (1:120 weight/volume) and placed in sterile airtight polypropylene tubes with cells floating on top of the medium in a thin cell layer. The cells were incubated under basal conditions or in the presence of 10^{-6} mol/L d-Ala7-Ang-(1-7) (A779), 10^{-9} mol/L Ang-(1-7), or both (Ang-(1-7)+A779). All tubes were incubated at 37°C. At the end of the incubation period, samples were collected to measure adiponectin. The optimal time of collection was determined in preliminary experiments, and the Ang-(1-7) concentration was chosen according to previous publications. Adiponectin was measured using an ELISA kit (AdipoGen) according to the manufacturer’s instructions.

**Statistical Analysis**

Data are expressed as mean±SEM. The statistical significance of the difference in mean values between TGR and Sprague-Dawley rats was assessed by an unpaired Student t test or 2-way ANOVA (glucose tolerance and insulin sensitivity tests). The data obtained with the primary cell culture of adipocytes were assessed by 1-way ANOVA and the Tukey post test.

**Results**

As previously documented in other studies, we observed that the Ang-(1-7) plasma level was increased in TGR (51.82±6.3 in TGR versus 29.17±8.7 pg/mL in Sprague-Dawley rats, P<0.01). The Ang II plasma level did not differ between Sprague-Dawley and TGR (101.1±18.41 in TGR versus 110.9±14.18 pg/mL in Sprague-Dawley rats).

To examine the functional consequences of increased Ang-(1-7), we first determined the relationship between body weight and fat weight. Twelve-week-old TGR displayed a lower body weight compared with Sprague-Dawley rats (278.3±13.3g in TGR versus 375.7±10.2g in Sprague-Dawley rats) (Figure 1a). Without alteration in food intake (Table). In addition, TGR presented a decrease in epididymal (7.52±0.12 mg/g of body weight in TGR versus 10.82±0.95 mg/g of body weight in Sprague-Dawley rats) (Figure 1b) and retroperitoneal (3.25±0.35 in TGR versus 7.40±1.25 mg/g of body weight in Sprague-Dawley rats) (Figure 1c) adipose tissue, indicating that TGR have a substantial decrease in fat mass in relation to Sprague-Dawley rats. Histological analysis showed a similar diameter of the adipocytes in both rat strains (76.06±1.47 μm in TGR versus 81.80±3.64 μm in Sprague-Dawley rats) (Figure 1d and 1e), indicating that the decrease in adipocyte tissue mass was not due to adipocyte atrophy (Figure 1f).

The analyses of lipid parameters demonstrated that TGR present a marked decrease in plasma levels of triglycerides (14.82±3.77 mg/dL in TGR versus 35.22±3.39 mg/dL in Sprague-Dawley rats, P<0.05) (Figure 2a) and total cholesterol (81.68±4.74 mg/dL in TGR versus 102.7±6.11 mg/dL in Sprague-Dawley rats, P<0.05) (Figure 2b).

Leptin levels were not altered in the serum of transgenic rats (Table); however, the correction by adipose tissue weight showed a high leptin secretion from adipocytes in TGR considering the lower fat mass in these animals (441.2±31.4 pg/mL per g in TGR versus 235.7±15.2 pg/mL per g in Sprague-Dawley rats, P<0.05). On the other hand, adiponectin levels were increased in TGR (Table), and the correction of these values by the adipose tissue weight demonstrated high production per adipocyte (8393±420 ng/mL per g in TGR versus 3864±262 ng/mL per g in Sprague-Dawley rats, P<0.05). To test whether the increase in adiponectin could be due to a direct effect of Ang-(1-7) on adipose tissue, additional experiments were performed in primary cell cultures of adipocytes. As shown in Figure 3, Ang-(1-7) induced a significant increase in adiponectin release by the cells. This effect was blocked by the antagonist A779.

The insulin level was not altered in TGR (Table); however, a higher glucose tolerance and enhanced insulin sensitivity were observed in TGR compared with Sprague-Dawley rats (Figure 2c and 2d). Moreover, in vitro analysis of glucose uptake showed a 2-fold increase in the insulin-stimulated glucose uptake by adipocytes from TGR, indicating a greater response to insulin stimulation (Figure 2e). However, the basal muscular glycogen content (0.3217±0.0615 mg of muscle in TGR versus 0.3729±0.0689 in Sprague-Dawley rats) and the fasted glycemia (Table) were not altered in TGR compared with Sprague-Dawley rats.

To understand the molecular mechanisms underlying changes in glucose and lipid metabolism in TGR, we analyzed the levels of insulin-signaling components in
adipose tissue of these animals. Although the total GLUT4 protein level was not altered (Figure 4a), both total and phosphorylated Akt were increased in adipose tissue from TGR (Figure 4b and 4c), indicating activation of the phosphatidylinositol 3-kinase (PI3K)/Akt pathway by chronic elevation of circulating Ang-(1-7) levels. In addition, real-time polymerase chain reaction analysis revealed a marked decrease in AGT expression in TGR epididymal adipose tissue and an increase in adiponectin. On the other hand, TGF-β, TNF-α, AT1, and Mas receptor were not altered in adipose tissue (Figure 5).

Discussion

Diabetes and dyslipidemia remain among the world’s most pervasive health problems. The role of Ang II in glucose and lipid metabolism is well studied; however, there are few studies dealing with the metabolic actions of Ang-(1-7). This study is the first to evaluate the effect of chronic elevation of Ang-(1-7) plasma levels on the metabolic state of nondiseased animals. For this, we have used TGR, which present an increase in Ang-(1-7) plasma levels of 2.0-fold. In this study, we observed numerous metabolism-related alterations in TGR, such as enhanced glucose tolerance and insulin sensitivity, a decrease in triglyceride and cholesterol plasma levels, an increase in adiponectin levels and secretion, an increase in adipose tissue Akt phosphorylation, lower fat mass and AGT expression in adipose tissue, and an improved responsiveness to insulin stimulation.

These results are in keeping with the data obtained in Mas-knockout mice, which present an MS-like state, with increased adipose tissue mass associated with elevated serum cholesterol and triglyceride levels, high fasting glycemia, hyperinsulinemia, glucose intolerance, decreased insulin sensitivity, and glucose uptake by adipocytes. Considering that comparable data have been obtained in 2 different species, the role of Ang-(1-7) and its receptor in metabolism appears to be very important in mammals in general. It should be pointed out, however, that the effect of Mas deficiency on the glucose and lipid metabolism in mice is dependent on background.

The role of the RAS in glucose metabolism is normally focused on Ang II actions, especially on cross-talk mechanisms between Ang II and insulin. Currently, it is clear that Ang II has prominent effects on insulin actions, increasing the activation of the proliferative pathway (mitogen-activated protein kinase) and decreasing the activation of the metabolic pathway (PI3K/Akt). The effect on the metabolic pathway is mediated by an increase in serine phosphorylation of the insulin receptor, IRS-1, and P85 and a decrease in tyrosine phosphorylation of IRS-1. Ang II also inhibits insulin-stimulated docking between IRS-1 and PI3K, decreasing intrinsic PI3K activ-
ity associated with the P85/P110 complex. Recently, it was described that Ang-(1-7) treatment with osmotic minipumps in rats treated with a high-fructose diet can ameliorate insulin resistance and hypertension; in this model, Ang-(1-7) normalized the decreased activation of insulin receptor/IRS-1/PI3K/Akt signaling, improving the insulin metabolic pathway through a decreased phosphorylation of the inhibitory site IRS-1/Ser307 in adipose tissue. These findings are in accordance with the observation that Mas-knockout mice present impaired glucose metabolism, and in accordance with this study, in which we observed that TGR present a slightly but significantly enhanced glucose tolerance and insulin sensitivity (in vivo experiments) accompanied by a prominent increase in insulin-stimulated glucose uptake by adipocytes (in vitro experiments). Taken together, these data corroborate the

Figure 2. Lipid and glycemic profile of TGR (n=6) and Sprague-Dawley (SD) (n=6) rats. a, Serum levels of triglycerides are decreased in TGR. b, Serum levels of total cholesterol are decreased in TGR. c, Insulin sensitivity test after intraperitoneal injection of insulin (0.75 U/kg body weight) was performed on rats fed a normal diet. Blood samples were collected from the tail at the indicated time points and analyzed for glucose concentration. Results are expressed as mean of plasma glucose levels (mg/dL)±SEM. d, Glucose tolerance test. Overnight-fasted rats were given an intraperitoneal injection of glucose (2 mg/g body weight). Data are presented as mean of plasma glucose levels (mg/dL)±SEM. e, Effects of insulin on glucose uptake. Adipocytes taken from TGR or SD rats were incubated in the presence or absence of 25 ng/mL insulin. Rates of glucose transport were determined by measuring the 2-deoxy-[3H]glucose uptake. Changes in glucose uptake between the basal and insulin-stimulated state in adipocytes from TGR and SD rats are shown. Data are presented as mean±SEM; *P<0.05.

Figure 3. Adiponectin production from primary cell culture of Sprague-Dawley rat adipocytes (n=3). Shown are pure adipocyte cell culture (C), adipocyte incubated with 10⁻⁶ mol/L Mas antagonist A779 (A779), adipocyte incubated with 10⁻⁹ mol/L angiotensin-(1-7) (Ang-(1-7)), and adipocyte incubated with 10⁻⁶ mol/L A779 plus 10⁻⁹ mol/L angiotensin-(1-7) (Ang-(1-7)+A779). Data are presented as mean±SEM; **P<0.05; ***P<0.01.
hypothesis that Ang-(1-7) improves the activation of the insulin metabolic pathway (PI3K/Akt) acting directly on glucose metabolism, increasing IRS-1 phosphorylation. This hypothesis is supported by the increase in total and phosphorylated Akt in TGR adipose tissue. The absence of changes in fasted glycemia and muscular glycogen content in this model indicates that Ang-(1-7) is able to act on glucose metabolism control, particularly in the presence of an alteration in glucose homeostasis, such as that induced by insulin or high glucose stimulation.

Many components of the RAS, including the Ang-(1-7) receptor Mas, are locally produced by adipose tissue. Furthermore, obese Zucker rats show an increased expression and secretion of AGT in adipocytes. Increased expression of AGT is considered an important characteristic of preadipocyte differentiation, especially because Ang II has a fundamental role in this process. It has previously been demonstrated that Mas-deficient mice present a significant increase in AGT expression in adipose tissue, suggesting that the Ang-(1-7)/Mas axis regulates AGT expression in fat tissue. In keeping with these results, a marked decrease in AGT expression was observed in epididymal fat of TGR. This interesting change appears to be limited to the local RAS because it is not correlated with the Ang II plasma levels, which were essentially unchanged in TGR. Accordingly, a tissue-specific change in the RAS has previously been demonstrated in TGR, with decreased AGT expression and local Ang II levels in the heart.

Despite an unchanged food intake, the TGR animals exhibited a considerable decrease in abdominal fat mass (epididymal and retroperitoneal adipose tissue) without adipocyte atrophy. The decreased fat mass can explain the lower body weight observed in this model. The absence of alterations in food intake indicates that the changes in fat mass were not induced by decreased appetite, pointing to changes in metabolic regulation. Accordingly, recently Jayasooriya et al reported that mice lacking ACE present increased energy expenditure with reduced fat mass and also increased Ang-(1-7) plasma levels. These observations suggest a pivotal role of the RAS in fat mass control.

The adipose tissue has been described as an important endocrine organ that releases many different adipokines with relevant actions in lipid and glucose metabolism control and as a modulator of food ingestion. Adiponectin is an adipokine that has been recognized as a key regulator of insulin sensitivity and tissue inflammation; it has direct actions in liver, skeletal muscle, and the vasculature. In contrast to other adipokines, adiponectin secretion and the resulting circulating levels are inversely proportional to body fat content. Levels are further reduced in subjects with diabetes and coronary artery disease. Adiponectin antagonizes many effects of TNF-α, and this in turn suppresses adiponectin production. We have observed a significant increase in adiponectin plasma...
levels in TGR even in the presence of a decreased fat mass and an augmented mRNA expression in adipocytes. This fact can explain, at least in part, the enhanced glucose tolerance and insulin sensitivity in this model, indicating the participation of the Ang-(1-7)/Mas axis in adipokine regulation. In agreement with these data, it has been observed that Mas-knockout mice present a decrease in adiponectin secretion.12

To gain further insight into the role of the Ang-(1-7)/Mas axis in adiponectin production, we performed a primary cell culture of Sprague-Dawley rat adipocytes. The results demonstrated that Ang-(1-7) increased the adiponectin release from adipocytes. This effect was blocked by the Mas antagonist A779, supporting the involvement of the Ang-(1-7)/Mas axis in the modulation of adipokine production.

Despite the fact that TGR exhibited a decrease in fat mass, no changes in the level of leptin, an important adipokine for food intake, body weight, and adipose tissue mass control,36,37 were observed in these animals. However, correction for adipose tissue weight indicated a relative increase in leptin production by adipocytes. This could contribute to keeping leptin plasma levels normal. This fact can explain, at least partially, the absence of alteration in food intake.

The role of the RAS in lipid metabolism has been addressed in many studies, but some aspects are still controversial. It is clear that Ang II/AT1 blockers (ACE inhibitors and Ang II receptor blockers) can prevent atherosclerosis and decrease cholesterol and triglyceride levels.5,10,11,38,39 However, Ang II can stimulate synthesis or lysis of fat tissue, depending on the experimental conditions.5,40–42 On the other hand, as pointed out above, many studies have shown that Ang-(1-7) can counterregulate Ang II actions and participate in the ACE inhibitor effects.12,40,43 Our data showing a decrease in fat mass in TGR suggest that the Ang-(1-7)/Mas axis is an important component in the control of fat accumulation and lipolysis. This is further supported by our previous observation showing an increased fat mass in Mas-knockout mice. The lower cholesterol and triglyceride plasma levels in TGR animals also suggest that Ang-(1-7) can downregulate plasma lipid levels. These changes can be attributed, at least in part, to the marked increase in adiponectin plasma levels, because this adipokine also has an essential role in lipid regulation.35,44 However, we cannot exclude the participation of other factors, including a direct effect of the Ang-(1-7)/Mas axis in adipocytes.

One may argue that the phenotype observed in TGR could be determined only by a counterregulation of AT1-
related actions. However, it has been shown that the blockade of AT1 receptors in Sprague-Dawley rats did not change glucose tolerance or triglycerides plasma levels.45 Furthermore, AT1 blockade in hypertensive diabetic rats did not improve glucose metabolism.46 In addition, in TG(mREN2)27 rats, blockade of AT1 receptor did not increase IRS1/Akt phosphorylation.47 As shown in Figure 5, one of the phenotypic changes observed in TGR is an increase in Akt phosphorylation. This is in keeping with our previous observation that stimulation of Mas with Ang-(1-7) in CHO-Mas transfected cells, which did not express AT1 receptors, produced activation of the PI3K/Akt pathway, which is involved in insulin-induced glucose transport.48 Taken together, these data suggest that the Akt pathway, which is involved in insulin-induced glucose metabolism in the adipocytes of these animals.

Another important protein in adipose tissue metabolism is the adipocyte lipid-binding protein (aP2), which belongs to a family of related cytosolic proteins thought to bind long-chain fatty acids.49–51 This protein is markedly decreased in rats with diabetes induced by streptozotocin51 and is strongly correlated with fatty acid esterification. The high expression of this protein in TGR adipose tissue associated with a decreased adipocyte tissue mass and lower triglyceride plasma levels indicate an enhanced fatty acid metabolism in the adipocytes of these animals.

Taken together, our results demonstrate that chronic Ang-(1-7) elevation in plasma can improve, in the physiological state, the glucose metabolism in vitro and in vivo. Chronically elevated Ang-(1-7) plasma levels also lead to a decrease of adipose tissue mass and lipid plasma levels. The mechanisms involved in these effects appear to involve an augmented action of Akt in adipose tissue, a decreased AGT expression, and increased aP2 expression, as well as a stimulated adipogenesis production. Moreover, our results suggest that chronic increases in plasma Ang-(1-7) levels do not induce tachyphylaxis, decreasing its actions.

These findings have obvious clinical relevance considering that Ang-(1-7) is a natural component of human blood. Our results suggest an important role of Ang-(1-7) in metabolism and open the possibility of using Mas agonists52 for treating cardiovascular and metabolic diseases.

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**Disclosures**

None.

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