RESEARCH

Chronic AT1 blockade improves glucose homeostasis in obese OLETF rats

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Abstract

Obesity is associated with the inappropriate activation of the renin-angiotensin system (RAS), which increases arterial pressure, impairs insulin secretion and decreases peripheral tissue insulin sensitivity. RAS blockade reverses these detriments; however, it is not clear whether the disease state of the organism and treatment duration determine the beneficial effects of RAS inhibition on insulin secretion and insulin sensitivity. Therefore, the objective of this study was to compare the benefits of acute vs chronic angiotensin receptor type 1 (AT1) blockade started after the onset of obesity, hyperglycemia and hypertension on pancreatic function and peripheral insulin resistance. We assessed adipocyte morphology, glucose intolerance, pancreatic redox balance and insulin secretion after 2 and 11 weeks of AT1 blockade in the following groups of rats: (1) untreated Long-Evans Tokushima Otsuka (lean control; n = 10), (2) untreated Otsuka Long-Evans Tokushima Fatty (OLETF; n = 12) and (3) OLETF + ARB (ARB; 10 mg olmesartan/kg/day by oral gavage; n = 12). Regardless of treatment duration, AT1 blockade decreased systolic blood pressure and fasting plasma triglycerides, whereas chronic AT1 blockade decreased fasting plasma glucose, glucose intolerance and the relative abundance of large adipocytes by 22, 36 and 70%, respectively. AT1 blockade, however, did not improve pancreatic oxidative stress or reverse impaired insulin secretion. Collectively, these data show that AT1 blockade after the onset of obesity, hyperglycemia and hypertension improves peripheral tissue insulin sensitivity, but cannot completely reverse the metabolic derangement characterized by impaired insulin secretion once it has been compromised.

Key Words

- insulin resistance
- hypertension
- renin-angiotensin system
- adiposity
- reactive oxygen species

Introduction

Obesity affects 35% of males and 40% of females in the United States (Flegal et al. 2016) and predisposes individuals to the development of cardiovascular disease (CVD) (Gaal et al. 2006) and type 2 diabetes mellitus (T2DM) (Kahn et al. 2006). Another detriment of obesity is the inappropriate activation of the renin-angiotensin system (RAS) (Engeli et al. 2005). Inappropriately activated RAS disrupts the actions of insulin in peripheral tissues. In L6 and primary myotubes, elevated angiotensin II (Ang II) levels decrease insulin-stimulated glucose uptake and glucose transporter 4
In primary human preadipocytes, elevated Ang II levels increase hepatic glucose output (Rao 1996). Ultimately these events may contribute to the development of T2DM. On the contrary, angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin receptor blockers (ARBs) reduced the onset of T2DM in individuals with cardiovascular risk factors or CVD (McMurray et al. 2010, Tocci et al. 2011). Moreover, in individuals without CVD, but with impaired fasting glucose or glucose intolerance, treatment with an ACEi increased the regression to normoglycemia (Bosch et al. 2006), suggesting that the state of the disease at the onset of RAS inhibition may determine the effectiveness of the intervention on glucose regulation.

Healthy β-cells compensate for glucose intolerance and insulin resistance by insulin hypersecretion. However, β-cell dysfunction, the inability of β-cells to sustain this compensatory response, ultimately leads to the development of T2DM. Many factors such as elevated Ang II levels may result in β-cell dysfunction (Chu et al. 2006, Lastra et al. 2007, Habibi et al. 2008, Chhabra et al. 2013, Sauter et al. 2015). Elevated Ang II levels also increase the expression and activity of the oxidant-generating enzyme NADPH oxidase 2 (Nox2) in L6 myotubes (Wei et al. 2006, Lastra et al. 2007). To manage tolerable levels of oxidants, antioxidant enzymes such as superoxide dismutase (SOD) neutralize elevated levels of superoxide. Pancreatic islets of male Wistar rats contain moderate but physiologically sufficient levels of SOD in the cytoplasm and mitochondria (Tiedge et al. 1997). However, levels of the hydrogen peroxide removing enzymes, glutathione peroxidase (GPx) and catalase, are extremely low in pancreatic islets, composing less than 1% of the expression levels in the liver (Tiedge et al. 1997). Consequently, excessive generation of reactive oxygen species impairs β-cell function, leading to decreased glucose-stimulated insulin secretion (GSIS) (Li et al. 2012). Nevertheless, this condition was reversed by AT1 blockade in young db/db mice (Chu et al. 2007), suggesting that activation of AT1 contributes to the manifestation of insulin resistance via oxidative injury to the pancreas and associated impaired GSIS. However, the degree to which AT1 blockade can correct the pancreatic dysfunction present during the later progression of insulin resistance and early onset T2DM is not well established.

Although there has been substantial progress in delineating the mechanisms by which RAS activation impairs pancreatic β-cell function and peripheral insulin signaling, whether the disease state of the organism and treatment duration determine the beneficial effects of RAS inhibition is unknown. The objective of this study was to compare the benefits of acute and chronic AT1 blockade started after the onset of obesity, hyperglycemia and hypertension on pancreatic function and peripheral insulin resistance. Using Otsuka Long-Evans Tokushima Fatty (OLETF) rats, a model characterized by hyperphagia, obesity, hyperglycemia, hypertension, dyslipidemia and elevated RAS (Kawano et al. 1992, Nishiyama et al. 2008, Montez et al. 2012), we tested the hypothesis that chronic AT1 blockade after the onset of obesity, hyperglycemia and hypertension decreases fasting plasma glucose and glucose intolerance by improving adipocyte morphology, and that these effects are independent of improvements in pancreatic function.

**Materials and methods**

Detailed methods are available in the Supplementary Methods (see section on supplementary data given at the end of this article).

All experimental procedures were reviewed and approved by the institutional animal care and use committees of Kagawa Medical University (Kagawa, Japan) and the University of California, Merced (Merced, CA, USA).

**Animals**

Eight-week-old male, Long-Evans Tokushima Otsuka (LETO) and OLETF rats were studied (Japan SLC Inc., Hamamatsu, Japan). Rats were randomly assigned to their study groups based on body mass (BM), so that mean BM in each group was within 5% of each other at the onset. The study groups were: (1) untreated LETO (lean control; n=5/time point) + vehicle (0.5% methylcellulose by oral gavage once daily; 1μL/g), (2) untreated OLETF (n=6/time point) + vehicle and (3) OLETF + ARB (ARB; 10mg olmesartan/kg/day by oral gavage at a volume of 1μL/g; for 2 or 11 weeks; n=6/time point). For ARB administration, olmesartan was suspended in distilled water using 0.5% methylcellulose to achieve a concentration of 10mg/mL and was kept at 4°C for less than 5 days. The two ARB dosing regimens represented acute (2 weeks) and chronic (11 weeks) treatments. All animals were housed in a specific pathogen-free facility under controlled temperature (23°C) and humidity (55%) with a 12-h light,
12-h darkness cycle. All animals had free access to water and standard laboratory rat chow consisting of 5% fat, 24% protein and 54% carbohydrates (MF; Oriental Yeast Corp., Tokyo, Japan).

Oral glucose tolerance test (oGTT)

At –4, 2 and 11 weeks, following a 12-h fast (21:00–09:00 h), oGTTs were performed 09:00–12:00 h to assess glucose tolerance as previously described (Rodriguez et al. 2012). The positive incremental areas under the curve for glucose (AUC\textsubscript{glucose}) and insulin (AUC\textsubscript{insulin}) were calculated by the trapezoidal method (Allison et al. 1995) and used to calculate the insulin resistance index (IRI).

Pancreatic insulin and insulin secretion

For the measurement of total pancreatic insulin, 80 mg of frozen pancreatic tissue was homogenized in 250 µL of cold RIPA buffer, containing PIC (Thermo Fisher Scientific, Waltham, MA). The homogenized tissue was centrifuged (20,000 g × 10 min at 4°C), and the aqueous layer was transferred to a separate tube and stored at −80°C for later analysis. Insulin secretion was calculated using the total area under the curve (AUC) for insulin divided by the total AUC for glucose from the oGTT as previously described (Retnakaran et al. 2008, Maki et al. 2009).

Western blot

Cytosolic and membrane proteins were extracted as previously described (Viscarra et al. 2011) and assayed as described in the Supplementary Methods. Membranes were scanned in an Odyssey infrared imager (LI-COR Biosciences, Lincoln, NE, USA).

Statistics

Means (±s.e.) were calculated using all samples unless otherwise noted. Baseline measurements were compared using an independent sample t-test. We used a one-way ANOVA at each time point for adiposity measurements with treatment group as a between-subject factor. For non-esterified fatty acids (NEFA) measurements following an oGTT, we used a three-factor ANOVA with group and time (weeks) as between-subject factors and time after administration as a within-subject factor. For all other data, we used a two-factor ANOVA with group and time as between-subject factors unless otherwise specified in the figure legend. When significant differences were observed, pairwise comparisons were carried out using a Bonferroni correction. Glucose tolerance was assessed by comparing mean AUC values obtained from the glucose profiles during the oGTTs. Statistical significance was set at P<0.05. Statistical analyses were performed with SPSS version 24 (IBM).

Results

Baseline characteristics of OLETF rats

Food intake, fasting plasma glucose (FPG), triglycerides (TG), NEFA, glucose tolerance, insulin secretion and the IRI were measured to assess the disease state at the onset of the study before intervention. At baseline, OLETF rats were characterized by higher food intake and FPG as compared to LETO (Supplementary Table 1).

Effects of AT\textsubscript{1} blockade on SBP and heart rate

SBP and heart rate were measured to assess the effects of AT\textsubscript{1} blockade on cardiovascular function. SBP measured by tail cuff: SBP was greater at baseline in OLETF compared to LETO and remained elevated for the duration of the study. AT\textsubscript{1} blockade decreased SBP at 2 weeks and remained lower throughout the study (Fig. 1A). Heart rate measure by tail cuff: There was a significant time, but not group effect on heart rate. Mean heart rate decreased with time (Fig. 1B). SBP measured by telemetry: SBP was greater at –3 weeks in OLETF compared to LETO, and AT\textsubscript{1} blockade normalized it (Supplementary Fig. 1). However, because of the loss of battery life in most telemeters, comparisons could only be made until 5 weeks (Supplementary Fig. 1).

Chronic AT\textsubscript{1} blockade decreases fasting plasma glucose

FPG, TG, NEFA, adiponectin and IRI were measured to assess whether the timing of AT\textsubscript{1} blockade influenced the biochemical parameters of metabolic syndrome and systemic insulin resistance. At 2 weeks, mean FPG was 51% greater in OLETF compared to LETO, and ARB had no significant effect. At 11 weeks, mean FPG was 63% higher in OLETF compared to LETO, and ARB reduced it 22% compared to OLETF (Table 1). There was a significant group, but not time effect on TG. Mean TG was greater in OLETF compared to LETO, while ARB reduced them (Table 1). At 2 weeks, mean plasma NEFA were 56% higher in OLETF compared to LETO, while ARB had no significant effect (Table 1). At 11 weeks, mean plasma
NEFA were not different among the groups (Table 1). There was a significant time, but not group effect on plasma adiponectin. Mean plasma adiponectin decreased with time (Table 1). At 2 weeks, mean IRI was not different among the groups (Table 1). At 11 weeks, mean IRI was 3.7-fold greater in OLETF compared to LETO, and ARB normalized it (Table 1). Collectively, these data demonstrate that the improvements of parameters related to metabolic syndrome are independent of the timing or duration of AT3 blockade, except FPG and IRI, which improved only after chronic blockade.

**The metabolic syndrome-like phenotype is associated with oxidative stress and impaired antioxidant capacity in the pancreas**

Markers of oxidative damage and antioxidant enzyme activities were measured to assess the effects of the metabolic syndrome-like phenotype and AT3 signaling on pancreatic redox balance. There was a significant group, but not time effect on pancreatic lipid peroxidation (4-HNE levels). Mean 4-HNE levels were higher in OLETF compared to LETO, and ARB had no significant effect (Fig. 2A). Mean pancreatic nitrotyrosine levels did not change among the groups or different time points (data not shown). There was a significant group, but not time effect on pancreatic SOD, catalase and GPx activities. Mean SOD, catalase and GPx activities decreased in OLETF compared to LETO, and ARB had no significant effect (Fig. 2B, C and D). Collectively, these data demonstrate that the metabolic syndrome-like phenotype is associated with suppression of pancreatic antioxidant capacity and increased lipid peroxidation and is unaffected by AT3 blockade.

**The metabolic syndrome-like phenotype is associated with blunted insulin secretion**

Pancreatic insulin content, glucose transporter 2 (Glut 2) expression and insulin secretion were measured to assess the effects of a pro-oxidant environment and AT3 signaling on pancreatic function. There were no group or time effects on pancreatic Glut 2 protein expression (data not shown). At 2 weeks, mean pancreatic insulin content was 2.9-fold greater in OLETF compared to LETO,

### Table 1 Mean (± s.e.) morphometrical, biochemical and hormone measurements in LETO, OLETF and OLETF + ARB male rats.

<table>
<thead>
<tr>
<th></th>
<th>LETO (n=5)</th>
<th>OLETF (n=6)</th>
<th>OLETF ARB (n=6)</th>
<th>LETO (n=5)</th>
<th>OLETF (n=6)</th>
<th>OLETF ARB (n=6)</th>
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<tbody>
<tr>
<td>FI (g)</td>
<td>17.7 ± 0.5</td>
<td>25.2 ± 0.7a</td>
<td>24.3 ± 0.9a</td>
<td>19.4 ± 0.5</td>
<td>29.3 ± 1.1a</td>
<td>26.8 ± 0.3a</td>
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<tr>
<td>Retro fat mass (g)*</td>
<td>4.5 ± 0.4</td>
<td>11.4 ± 1.1c</td>
<td>10.8 ± 1.2c</td>
<td>7.7 ± 0.5</td>
<td>23.9 ± 2.0c</td>
<td>16.8 ± 1.8c</td>
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<tr>
<td>Epi fat mass (g)*</td>
<td>4.6 ± 0.6</td>
<td>9.7 ± 0.9c</td>
<td>7.1 ± 1.0</td>
<td>7.8 ± 0.7</td>
<td>13.9 ± 1.oa</td>
<td>11.6 ± 1.1</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>4.1 ± 0.3</td>
<td>6.2 ± 0.1c</td>
<td>6.4 ± 0.4c</td>
<td>4.1 ± 0.3</td>
<td>6.7 ± 0.6c</td>
<td>5.2 ± 0.2c</td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>0.6 ± 0.1</td>
<td>1.1 ± 0.1b</td>
<td>0.8 ± 0.1a</td>
<td>0.4 ± 0.1</td>
<td>1.3 ± 0.2a</td>
<td>0.9 ± 0.1a</td>
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<tr>
<td>NEFA (mEq/L)</td>
<td>0.57 ± 0.05</td>
<td>0.89 ± 0.07c</td>
<td>0.79 ± 0.04c</td>
<td>0.63 ± 0.05</td>
<td>0.71 ± 0.04c</td>
<td>0.80 ± 0.10</td>
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<tr>
<td>Adiponectin (µg/mL)</td>
<td>2.43 ± 0.11</td>
<td>2.92 ± 0.32c</td>
<td>3.08 ± 0.32</td>
<td>1.90 ± 0.15</td>
<td>2.50 ± 0.18c</td>
<td>2.07 ± 0.13b</td>
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<tr>
<td>IRI (r.u. x 10⁹)</td>
<td>4.2 ± 1.9</td>
<td>4.9 ± 1.4</td>
<td>9.1 ± 3.3</td>
<td>3.5 ± 8.6</td>
<td>16.5 ± 4.8c</td>
<td>4.2 ± 1.6c</td>
</tr>
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Comparisons were assessed using a two-factor ANOVA with group and time as between-subjects factors; *, comparisons were assessed using a one-way ANOVA at each time point.

Main effects: *P<0.05 vs LETO; **P<0.05 vs OLETF; ***P<0.05 vs 2 weeks. Pairwise comparisons: #P<0.05 vs LETO 2 weeks; $P<0.05 vs LETO 11 weeks; &P<0.05 vs OLETF 11 weeks; §P<0.05, OLETF 2 weeks vs OLETF 11 weeks; ¶P<0.05, OLETF ARB 2 weeks vs OLETF ARB 11 weeks.

BM, body mass; FI, food intake; IRI, insulin resistance index; NEFA, non-esterified fatty acids; TG, triglycerides.
and ARB normalized it (Fig. 3A). There was a significant group, but not time effect on insulin secretion. Mean insulin secretion was reduced in OLETF compared to LETO, and ARB had no significant effect (Fig. 3B). Collectively, these results suggest that AT$_1$ blockade started after the onset of obesity, hyperglycemia and hypertension is not able to recover impaired pancreatic insulin secretory capacity.

**Effects of AT$_1$ blockade on adiposity and adipocyte morphology**

BM, Food intake, retroperitoneal and epididymal fat masses, and adipocyte morphology were measured to assess whether the timing of AT$_1$ blockade influenced the beneficial effects on BM and adipocyte morphology. Mean weekly BM was greater at −4 weeks in OLETF compared to LETO, remaining so for the duration of the study (Fig. 4A). AT$_1$ blockade regardless of treatment duration had no significant effect compared to OLETF (Fig. 4B). At 2 weeks, retroperitoneal and epididymal fat masses were greater in OLETF compared to LETO, and ARB had no significant effect compared to OLETF (Table 1). At 11 weeks, retroperitoneal and epididymal fat masses were greater in OLETF compared to LETO, while ARB reduced retroperitoneal fat mass 30% (Table 1). There was a significant group, but not time effect on food intake. Mean food intake was greater in OLETF compared to LETO (Table 1). At 2 weeks, the relative abundance of adipocytes between 25–50 and 51–100 µm was 53% and 22%, respectively, lower in OLETF compared to LETO. The relative abundance of adipocytes between 51 and 100 µm was normalized in ARB (Fig. 5A and C). The relative abundance of adipocytes between 101 and 200 µm was 9-fold greater in OLETF compared to LETO, and ARB reduced it 70% compared to OLETF (Fig. 5A and C). Collectively, these data demonstrate that ARB treatment reduced gain in BM regardless of treatment duration, but only chronic ARB decreased retroperitoneal fat mass and improved adipocyte morphology.
TNF-α does not contribute to insulin resistance in OLETF rats

Plasma tumor necrosis factor-alpha (TNF-α) and epididymal fat transmembrane TNF-α were measured to assess the effects of decreased adiposity associated with chronic AT₁ blockade on systemic and local inflammation. There were no group or time effects on plasma TNF-α (Fig. 6A). There were significant group and time effects on transmembrane TNF-α. Mean transmembrane TNF-α was not different between LETO and OLETF; nonetheless, ARB increased it compared to LETO (Fig. 6B). These results suggest that neither systemic nor local TNF-α contribute to the development of insulin resistance in obese OLETF rats.

Chronic AT₁ blockade ameliorates the progression of glucose intolerance in OLETF rats

oGTTs were performed to determine whether the improvements in adiposity and adipocyte morphology associated with chronic ARB translated to an improvement in glucose intolerance. At 2 weeks, mean AUC_glucose was not different between LETO and OLETF (Fig. 7A and E). At 11 weeks, mean AUC_glucose was 2.2-fold greater in OLETF compared to LETO, and chronic ARB decreased
it 36% (Fig. 7C and E). At 2 weeks, mean AUC$_{\text{insulin}}$ was not different among the groups (Fig. 7B and F). At 11 weeks, mean AUC$_{\text{insulin}}$ was not different between LETO and OLETF; nevertheless, ARB reduced it 72% compared to OLETF (Fig. 7B, D and F). These data suggest that chronic AT$_1$ blockade protects against the progression of glucose intolerance in OLETF rats but is not sufficient to completely reverse the impairment.

**Chronic AT$_1$ blockade decreases the NEFA response to an oGTT**

Plasma NEFA were measured during oGTT to assess the effects of improvements in adiposity and adipocyte morphology associated with chronic AT$_1$ blockade on lipid metabolism. At 2 weeks, there was no difference in plasma NEFA in response to the glucose challenge between any of the groups (Fig. 8A). At 11 weeks, plasma NEFA was greater at 15, 30 and 60 min following the glucose challenge in OLETF compared to LETO, and this effect was reversed in ARB (Fig. 8B). These results demonstrate that the development of insulin resistance in OLETF rats is associated with the inability to suppress NEFA levels in response to glucose administration. Furthermore, chronic, but not acute AT$_1$ blockade normalized NEFA levels in response to a glucose challenge, suggesting that activation of AT$_1$ contributes to impaired lipid metabolism.
Hepatic PEPCK and G6Pase protein expression are not modulated by AT₁ signaling in OLETF rats

Fasting plasma insulin (FPI) along with hepatic proteins involved in insulin signaling and gluconeogenesis was measured to assess the impact of AT1 blockade on hepatic insulin signaling. There was a significant group, but not time effect on FPI and hepatic phosphorylated (p)-insulin receptor (IR):IR ratio. Mean FPI and p-IR:IR ratio were greater in OLETF compared to LETO; however, ARB had no significant effect (Supplementary Fig. 2A and B). There was no group or time effects on the mean expressions of hepatic phosphoenolpyruvate carboxykinase (PEPCK-C) and glucose 6-phosphatase (G6Pase) (Supplementary Fig. 2C and D). Collectively, these results suggest that altered expression of hepatic PEPCK-C and G6Pase may not contribute to the increase in FPG in OLETF rats.

Discussion

Inappropriately elevated RAS contributes to the dysregulation of glucose homeostasis in part by impairing β-cell function and peripheral insulin signaling (Favre et al. 2015). Conversely, inhibition of RAS improves β-cell function and peripheral insulin signaling (Henriksen et al. 2001, Shiuchi et al. 2004, Chu et al. 2006, Wei et al. 2006, Nagai et al. 2009), which can delay the onset of T2DM (Tocci et al. 2011). Nonetheless, it is not clear whether the disease state of the organism and treatment duration determine the beneficial effects of RAS inhibition on pancreatic function and insulin sensitivity. Therefore, the aim of this study was to determine whether acute and chronic AT₁ blockade started after the onset of obesity, hyperglycemia and hypertension would have beneficial effects on pancreatic function and peripheral insulin resistance. To this end, the present study demonstrates that regardless of treatment duration, AT₁ blockade decreases SBP, BM and fasting plasma TG. Moreover, chronic AT₁ blockade was associated with the additional benefits of decreased FPG, AUC$_{\text{glucose}}$, AUC$_{\text{insulin}}$, IRI and retroperitoneal fat mass and a beneficial shift in adipocyte size. Despite these benefits, chronic AT₁ blockade did not have an effect on pancreatic oxidative stress or insulin secretion in our rat model. These results suggest that...
ARB improves hyperglycemia

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Collectively, these data demonstrate that early in the development of metabolic syndrome, OLETF rats are afflicted by an increased pro-oxidant state in the pancreas and decreased plasma GLP-1, which would decrease plasma β-cell function; however, these beneficial effects were not replicated in 16-week-old db/db mice (Bindom et al. 2010). Similarly, pioglitazone and/or liraglutide treatment in 7- to 9-week-old db/db mice increased β-cell function and mass, and increased the expression of various genes involved in the regulation of β-cell function; however, again, these effects were attenuated in 16- to 18-week-old db/db mice (Kimura et al. 2015). Collectively, these data suggest that early events that harm the pancreas may be sufficiently detrimental to hinder the ability of targeted treatments to reverse impaired pancreatic function.

Inappropriately elevated Ang II levels result in β-cell dysfunction in C57BL/6N mice (Lastra et al. 2007, Sauter et al. 2015), and increase the expression and activity of Nox2 in L6 myotubes (Wei et al. 2006). In the pancreas, an increase in oxidant production shifts the oxidant/antioxidant balance to a pro-oxidant state, leading to an increase in lipid peroxidation since islets have low levels of antioxidant enzymes (Tiedge et al. 1997). 4-HNE, a by-product of lipid peroxidation, decreases islet insulin and DNA content (Suarez-Pinzon et al. 1996). In the present study, pancreatic 4-HNE levels were increased in OLETF rats, and pancreatic SOD, catalase and GPx enzyme activities were decreased. Collectively, these results demonstrate a chronic pro-oxidant state of the pancreas in obese pre-diabetic OLETF rats. Furthermore, these results suggest that the decrease in pancreatic insulin content in OLETF may be a result of the pro-oxidant state of the pancreas. Moreover, OLETF rats also exhibited decreased insulin secretion despite increased insulin levels after 2 weeks of treatment, suggesting that defective glucose sensing may be responsible for this impairment. To this effect, β-cell glucose toxicity decreases the expression of GLP-1r and GLUT2, leading to impaired insulin secretion (Thorens et al. 1992, Xu et al. 2007, Kawashima et al. 2011). Additionally, 10-week-old, OLETF rats have increased levels of plasma dipeptidyl peptidase-4 activity compared to food-restricted OLETF rats when fed ad libitum (Kirino et al. 2011), which would decrease plasma GLP-1 (Rodriguez et al. 2012). Collectively, these data demonstrate that early in the development of metabolic syndrome, OLETF rats are afflicted by an increased pro-oxidant state in the pancreas and decreased plasma GLP-1, regardless of the disease state, AT1 blockade can improve peripheral insulin resistance but cannot reverse impaired pancreatic function.

We previously demonstrated that 6 weeks of ARB treatment in 9-week old OLETF rats, when the initial detriments of the metabolic syndrome are just appearing in the phenotype, increased insulin secretion associated with a rise in pancreatic GLP-1r protein expression (Rodriguez et al. 2012). An improvement in insulin secretion is one of many avenues by which disruption of RAS can improve glucose intolerance (Chu et al. 2006, Cole et al. 2010). Notwithstanding, in the present study (treatment started at 13 weeks of age), the improvement in glucose tolerance after chronic AT1 blockade was independent of increased insulin secretion. This disparity between the two studies is an important distinction because it may highlight the significance of the timing of treatment on pancreatic function. In support of this view, ACE2 overexpression in 8-week-old db/db mice improved glucose tolerance, pancreatic function and prevented β-cell apoptosis; however, these beneficial effects were not replicated in 16-week-old db/db mice (Bindom et al. 2010). Similarly, pioglitazone and/or liraglutide treatment in 7- to 9-week-old db/db mice increased β-cell function and mass, and increased the expression of various genes involved in the regulation of β-cell function; however, again, these effects were attenuated in 16- to 18-week-old db/db mice (Kimura et al. 2015). Collectively, these data suggest that early events that harm the pancreas may be sufficiently detrimental to hinder the ability of targeted treatments to reverse impaired pancreatic function.

Figure 8
Effects of glucose administration on plasma NEFA. The response of NEFA to an oral glucose tolerance test following an overnight fast (A) after 2 weeks of treatment in Long-Evans Tokushima Otsuka (LETO; n=5), Otsuka Long-Evans Tokushima Fatty (OLETF; n=6) and OLETF + ARB (n=6), and (B) after 11 weeks of treatment in LETO (n=5), OLETF (n=6), and OLETF + ARB (n=6). Comparisons were assessed using a three-factor ANOVA with group and time (weeks) as between-subject factors and time after administration as a within-subjects factor. *P<0.05 vs LETO 11 weeks; †P<0.05 vs OLETF 11 weeks; ‡P<0.05, OLETF 2 weeks vs OLETF 11 weeks.

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leading to an impairment in GSIS. It is important to note that while acute or chronic AT1 blockade cannot reverse these detriments, RAS inhibition before the onset of dysregulated insulin secretion is protective (Nakayama et al. 2005, Rodriguez et al. 2012, Zhang et al. 2013), suggesting that these detriments are irreversible without sufficiently early intervention. If so, identifying appropriate targets for improving therapies to reverse dysregulated pancreatic function associated with the manifestation of metabolic syndrome will be especially necessary.

Obesity inappropriately activates RAS in animal models and humans (Boustany et al. 2004, Engeli et al. 2005). RAS inhibition decreases BM and visceral fat mass in animal models of metabolic syndrome (Benson et al. 2004, Miesel et al. 2012, Müller-Fielitz et al. 2012, 2014, 2015). These beneficial effects on BM and visceral fat mass are partially mediated by Mas receptor activation (Schuchard et al. 2015) and are attributed to multiple factors including the prevention of leptin resistance (Muller-Fielitz et al. 2014, 2015) and increased circulating levels of adiponectin (Zorad et al. 2006, Weisinger et al. 2009). In the present study, we did not observe a reduction in BM regardless of treatment duration; nevertheless, ARB treatment blunted BM gain during the treatment period. The difference in our findings may be attributed to the diets used in the aforementioned studies, which were higher in carbohydrates and fat as compared to the standard chow used in our study. Likewise, 4 weeks of telmisartan treatment did not reduce BM in spontaneously hypertensive (SHR) rats fed a standard chow (Li et al. 2006). Moreover, the BM reducing effects of ARB are dependent on the ARB dose and intact leptin signaling, with higher doses decreasing food intake and BM when leptin signaling is normal (Muller-Fielitz et al. 2011). Although OLETF rats have intact leptin signaling at 5 weeks of age, by 8 weeks of age, they develop peripheral but not central leptin resistance (Niimi et al. 1999), which may be a result of an acquired impairment in the transport of leptin across the blood–brain barrier (Banks et al. 2003). While AT1 blockade regardless of treatment duration did not decrease BM, we found that chronic AT1 blockade decreased retro fat mass and the relative abundance of large adipocytes and normalized the relative abundance of medium adipocytes indicative of a beneficial shift in adipocyte morphology. Nevertheless, acute AT1 blockade had no detectable effect on adipocyte morphology, glucose intolerance or FPG, suggesting that: (1) at this stage in the development of metabolic syndrome, other factors beyond AT1 activation contribute to a greater extent or (2) at this stage of the condition, acute disruption of RAS is insufficient to overcome the progression of the metabolic syndrome. Although we only observed an improvement in adipocyte morphology after chronic AT1 blockade, previous studies have shown similar results regardless of treatment duration (Furuhashi et al. 2004, Mori et al. 2007, Tomono et al. 2008, Munoz et al. 2009, Nagai et al. 2009, Iwai et al. 2010, Muller-Fielitz et al. 2012). This discrepancy may be due to the use of different animal models and/or RAS inhibitor, or the time frame of treatment necessary to detect an effect.

Adipose expansion as seen in obesity is mediated by adipocyte hypertrophy, hyperplasia or both. Similarly, the detrimental effects of obesity on metabolic and cardiovascular health are associated with a higher abundance of hypertrophic adipocytes (Choe et al. 2016). These large adipocytes have lower GLUT4 translocation in response to insulin (Franck et al. 2007), higher lipolysis rates (Laurencikiene et al. 2011) and higher pro-inflammatory adipokine expression and secretion (Skurk et al. 2007). Therefore, large adipocytes are associated with the development of insulin resistance and type 2 diabetes (Acosta et al. 2015, Kim et al. 2015). In the present study, chronic AT1 blockade decreased the relative abundance of large adipocytes. This positive shift in adipocyte morphology may partially explain the improvement in glucose homeostasis. Recent evidence shows that these effects are independent of the blood pressure lowering effects of AT1 blockade (Muller-Fielitz et al. 2014). However, the improvement in glucose homeostasis may be independent of increased glucose uptake into adipocytes as adipocyte itself makes a minor contribution to glucose disposal in response to feeding (Baron et al. 1988). In support of this view, a previous study found that glucose uptake in adipocytes was not altered by telmisartan treatment in Sprague Dawley rats or transgenic rats with low brain angiotensinogen fed a cafeteria diet (Winkler et al. 2016). Collectively, these results suggest that the improvement in glucose homeostasis may be the result of other factors associated with a positive shift in adipocyte morphology such as increased expression and secretion of anti-inflammatory adipokines and decreased expression and secretion of pro-inflammatory adipokines (Skurk et al. 2007). In the present study, OLETF rats did not show increased systemic or local inflammation. Additionally, adiponectin levels decreased with time, which may be a result of increased BM as obesity is associated with decreased circulating levels of adiponectin (Arita et al. 1999). This is significant because adiponectin is important for increasing peripheral insulin sensitivity (Yamauchi et al. 2006).
et al. 2001), suggesting that the observed improvement in glucose homeostasis after chronic AT₁ blockade is not mediated by adiponectin. In the same way, a previous study found that AT₁ blockade improves glucose homeostasis in SHR rats and that these effects are not mediated by peroxisome proliferator-activated receptor (PPAR)-γ (Müller-Fielitz et al. 2012), a transcriptional regulator of adiponectin (Iwaki et al. 2003). Furthermore, we found that at 11 weeks, a glucose challenge did not suppress circulating NEFA levels and that chronic AT₁ blockade reversed this impairment, suggesting that the improvement in glucose homeostasis may result from increased adipogenesis, decreased lipolysis or both, in smaller insulin-sensitive adipocytes (Laforest et al. 2015). Previous studies have demonstrated that Ang II inhibits lipolysis (Goossens et al. 2006); however, a different study demonstrated that in individuals with impaired glycemia, valsartan treatment for 26 weeks suppressed postprandial free fatty acids (Moors et al. 2013), suggesting a suppression of lipolysis. Furthermore, a study in primary cultured human preadipocytes demonstrated that AT₁ blockade increased the lipid accumulation and differentiation of these cells (Janke et al. 2002). Collectively, these results suggest that AT₁ blockade may protect against ectopic lipid deposition in skeletal muscle by suppressing lipolysis and increasing preadipocyte differentiation, leading to an improvement in insulin sensitivity and ultimately on glycemia (Sharma et al. 2002). Nonetheless, we cannot rule out that other mechanisms such as blunting of the hypothalamic pituitary axis activity in response to stress may contribute to the AT₁-associated improvements in glucose homeostasis (Armando et al. 2001, Miesel et al. 2012).

Conclusion

In summary, the present study demonstrates that after the onset of obesity, hyperglycemia and hypertension in OLETF rats, chronic AT₁ blockade results in a beneficial shift in adipocyte morphology, and improved FPG and glucose intolerance regardless of the glycemic state at the onset of the treatment. Despite this, AT₁ blockade cannot reverse impaired insulin secretion, suggesting that once the pancreas has been compromised, recovery will be particularly challenging, and the severity of the condition may be masked by the apparent improvement in systemic glucose tolerance. Should these findings in a rat model recapitulate in humans, early RAS inhibition may be crucial to preserving pancreatic function, and identifying the other factors that contribute to the defined impairments in pancreatic function will be critical for proper management of metabolic syndrome especially as it relates to the timing of treatment.

Supplementary data
This is linked to the online version of the paper at https://doi.org/10.1530/JOE-17-0678.

Declaration of Interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement
R R, J P V-M, D N, A N and R M O designed the research. R R and J N M performed the animal experiments and analyzed the samples and data. R R, J P V-M, D G P, S H A and R M O interpreted the results. R R wrote the original draft of the manuscript. R R, J P V-M, D N, D G P, A N and R M O edited and revised the manuscript. All authors approved the final version of the manuscript for submission.

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ARB improves hyperglycemia


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