

ORIGINAL ARTICLE

Losartan modulates muscular capillary density and reverses thiazide diuretic-exacerbated insulin resistance in fructose-fed rats

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The renin–angiotensin system (RAS) is involved in the pathogenesis of insulin sensitivity (IS). The role of RAS in insulin resistance and muscular circulation has yet to be elucidated. Therefore, this study sought to determine the mechanisms of angiotensin II receptor blockers (ARBs) and/or diuretics on IS and capillary density (CD) in fructose-fed rats (FFRs). Sprague-Dawley rats were fed either normal chow (control group) or fructose-rich chow for 8 weeks. For the last 4 weeks, FFRs were allocated to four groups: an FFR group and groups treated with the thiazide diuretic hydrochlorothiazide (HCTZ), with the ARB losartan, or both. IS was evaluated by the euglycemic hyperinsulinemic glucose clamp technique at week 8. In addition, CD in the extensor digitorum longus muscle was evaluated. Blood pressure was significantly higher in the FFRs than in the controls. HCTZ, losartan and their combination significantly lowered blood pressure. IS was significantly lower in the FFR group than in the controls and was even lower in the HCTZ group. Losartan alone or combined with HCTZ significantly increased IS. In all cases, IS was associated with muscular CD, but not with plasma adiponectin or lipids. These results indicate that losartan reverses HCTZ-exacerbated insulin resistance, which can be mediated through the modulation of muscular circulation in rats with impaired glucose metabolism.

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INTRODUCTION

Recently, insulin resistance and hyperinsulinemia have been shown to be common in patients with essential hypertension or metabolic syndrome.^{1,2} These impairments of glucose metabolism are associated with a high risk of cardiovascular diseases, and improving insulin sensitivity (IS) has become recognized as an important component of cardiovascular risk reduction in patients with essential hypertension.^{2,3} Antihypertensive drugs are expected to have a beneficial effect on IS. It has been consistently shown that angiotensin II receptor blockers (ARBs) and angiotensin-converting enzyme inhibitors have beneficial effects on glucose metabolism, whereas the thiazide diuretics have an adverse effect, resulting in a worsening of IS.⁴ At the same time, the results of previous clinical studies examining the effects of ARBs on IS have been equivocal, with IS variously reported as being improved⁵ or unchanged.⁶ In addition, some recent clinical and animal studies have reported that the antihypertensive and cardiovascular protective effects of ARBs are strongly reinforced by concomitant administration of thiazides.^{7,8} It would thus be of great interest to determine whether and how ARBs modulate thiazide diuretic-exacerbated IS in hypertension and metabolic syndrome.

Skeletal muscle is considered a primary site of insulin resistance in essential hypertension.⁹ Euglycemic hyperinsulinemic glucose clamp studies have demonstrated that skeletal muscle accounts for over 80% of glucose disposal under hyperinsulinemic conditions in humans. Insulin-mediated glucose uptake has been shown to be correlated with capillary density (CD).¹⁰ CD in skeletal muscle has been shown to be decreased in patients with hypertension,¹¹ non-insulin-dependent diabetes mellitus¹² and obesity,¹³ as well as in experimental animal models.^{14–16} It has also been suggested that the diffusion distance from capillaries to muscle cells and/or some biochemical changes related to the diffusion distance contribute to a decrease in IS.¹⁷

Thus, we hypothesized that an ARB would reverse the thiazide diuretic-induced decrease in IS by modulating the muscle CD in hypertension. This study was designed to test this hypothesis by examining whether a thiazide diuretic (hydrochlorothiazide: HCTZ) or an ARB (losartan) alters IS in fructose-fed rats (FFRs), and if so, whether CD in skeletal muscle is involved in such changes. We selected the FFR model because a high-fructose diet in rats causes metabolic derangements, including hypertension and insulin resistance.^{18,19} Similarly, studies have demonstrated that the consumption of high

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amounts of fructose also causes hypertension and insulin resistance in humans.²⁰

METHODS

General protocol

Six-week-old male Sprague-Dawley rats (SLC, Shizuoka, Japan) were housed in a controlled temperature (24 °C) room with a 12-h light–dark cycle. All procedures were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and the protocols were approved by the Animal Committee of Tohoku University. The rats were divided into two groups at the start of study: one fed standard chow containing 60% starch, 5% fat and 21% protein (Oriental Yeast, Tokyo, Japan; control; $n=10$) and the other fed fructose-rich chow containing 60% fructose, 5% fat and 21% protein (F2HFrD; Oriental Yeast; FFR; $n=33$) for 8 weeks. For the last 4 weeks, the FFRs were allocated to four groups: the FFR group (FFR; $n=9$), HCTZ (Merck, Whitehouse Station, NJ, USA) group (FFR+HCTZ; $n=9$), losartan (Merck) group (FFR+LOS; $n=7$) and HCTZ plus losartan group (FFR+HCTZ+LOS; $n=8$). HCTZ dissolved in 0.5% carboxymethylcellulose was administered by gavage daily at a dose of 80 mg kg⁻¹ day⁻¹ in the FFR+HCTZ and FFR+HCTZ+LOS groups. Because one aim of the study was to examine the effect of losartan on IS exacerbated by HCTZ, we selected the highest dosage of HCTZ that has been commonly used in other rat studies^{8,21} to determine the maximum adverse effect on glucose metabolism. Losartan dissolved in water was given by gavage daily at a dose of 10 mg kg⁻¹ day⁻¹ in the FFR+LOS and FFR+HCTZ+LOS groups. Rats were pair-fed to ensure equivalent caloric intake, thereby avoiding the influence of different food volumes on metabolic abnormalities. Body weight was measured weekly. Systolic blood pressure (SBP) was measured every week from 6–14 weeks of age in conscious rats by the indirect tail-cuff method (Model MK-2000A; Muromachi, Tokyo, Japan).

Surgical procedures and drawing blood

At the age of 14 weeks, after an overnight fast and under ether anesthesia, the rats were implanted with an arterial catheter (PE 100, Becton Dickinson, Sparks, MD, USA) and venous catheter (PE 20), each filled with heparinized saline (100 IU ml⁻¹), into the left carotid artery and right jugular vein, respectively. The free ends of these catheters were brought subcutaneously to the back of the neck. During surgery, a 0.5 ml blood sample was collected from the left carotid artery for biochemical analysis in all rats. Serum triglycerides were measured by a standard autoanalysis technique (Synchro-CX-3; Beckman Coulter, Fullerton, CA, USA). Serum adiponectin levels were measured using the enzyme-linked immunosorbent assay kit (Otsuka Pharmaceutical, Tokyo, Japan). The rats were returned to their individual cages and allowed to recover for 3 days after surgery.

Euglycemic hyperinsulinemic glucose clamp technique

After overnight fasting, conscious rats were examined for IS by a euglycemic hyperinsulinemic glucose clamp technique, as described previously.^{22,23} Briefly, before the start of the glucose clamp, fasting blood glucose was measured by a blood glucose test meter (A790820; GUNZE, Kyoto, Japan). An initial bolus of insulin (25 mU kg⁻¹ of Humalin R, U-100; Eli Lilly Japan, Kobe, Japan) was infused through a venous catheter, and this step was followed by a constant infusion of insulin at a rate of 4 mU kg⁻¹ min⁻¹ for 147 min. During the glucose clamp, 12.5% glucose solution was infused as required to maintain the blood glucose at the fasting level. A volume of 10 µl samples of arterial blood was collected through an arterial catheter at 7-min intervals for the determination of blood glucose. The average rate of glucose infusion (mg kg⁻¹ min⁻¹) for the last 35 min was taken as an index of IS (M value).^{22,23}

Tissue preparation and histological examination

After the IS test, the rats were killed by intraperitoneal injection of pentobarbital (100 mg kg⁻¹). The extensor digitorum longus (EDL) muscle was rapidly dissected and weighed. Each EDL muscle was sliced transversally in the midbelly region into 6-mm sections. These samples were rapidly frozen in isopentane cooled by dry ice and stored at -80 °C until use. Serial transverse cross-sections (10 µm thick) near the mid-belly portion of the EDL were cut in a microtome cryostat at -22 °C, mounted on glass slides and air-dried. CD was

determined by the methods described previously.²² The capillary-to-fiber ratio (C/F) and CD were estimated using an image processing software package (WinROOF ver. 5.0; Mitani Corporation, Tokyo, Japan). For each muscle, at least 200 fibers and their associated capillaries were measured using the maximal number of non-overlapping fields.

Data analysis

All results are expressed as means ± s.e.m. All data analyses were performed by Bonferroni/Dunn testing for multiple comparisons after one-way analysis of variance. Pearson's correlation coefficient and regression analysis were used to compare the relationship between M values and CD or C/F. To identify determinants of change in M values, we performed multiple stepwise regression analysis to compare the relationship between M values as the dependent variables and five variables (CD or C/F and SBP, BW, serum adiponectin and triglycerides) as independent variables. Values of $P < 0.05$ were considered to indicate statistical significance.

RESULTS

Blood pressure

Rats were pair-fed to ensure equivalent caloric intake throughout the experimental period. However, in the first 4 weeks, the food volume in the control rats was actually greater than that in the FFR groups; thus, we had to restrict the food volume in the control rats to achieve caloric equivalence among the groups. However, for the last 4 weeks, the food volume in the FFR+HCTZ+LOS rats was the smallest among the groups, such that we had to restrict the food volume in the control, FFR, FFR+LOS and FFR+HCTZ groups. Therefore, the SBP in FFRs progressively increased throughout the first 4 weeks, but the SBP was not significantly changed during the last 4 weeks in FFRs, as shown in Figure 1. HCTZ alone, losartan alone, and the two drugs in combination completely attenuated the development of hypertension after the 4-week treatment. In addition, the SBP in the FFR+HCTZ+LOS rats at weeks 6, 7 and 8 of the experimental period was lowest among the five groups.

Insulin sensitivity

Figure 2 shows the time course of the blood glucose level (upper panel), insulin infusion rate (middle panel) and glucose infusion rate (lower panel) of the glucose clamp in each group. Steady-state blood

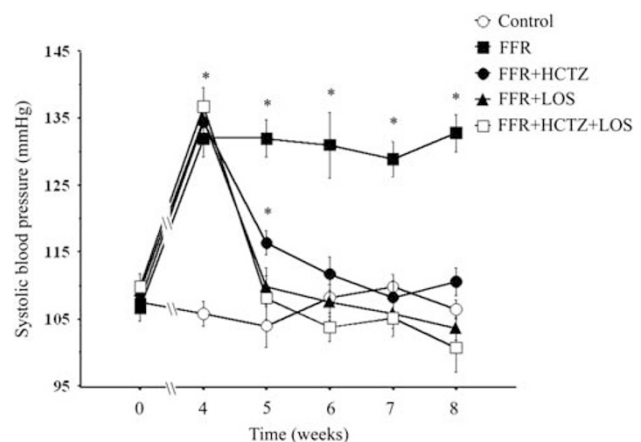


Figure 1 Sequential systolic blood pressure values during the 8-week experimental period in the five groups: control ($n=10$), normal diet group; fructose-fed rat (FFR) ($n=9$), fructose-fed rats group; FFR+HCTZ ($n=9$), fructose-fed rats and hydrochlorothiazide group; FFR+LOS ($n=7$), fructose-fed rats and losartan group and FFR+HCTZ+LOS ($n=8$), fructose-fed rats and hydrochlorothiazide plus losartan group. Data are the means ± s.e.m. * $P < 0.01$ vs. control.

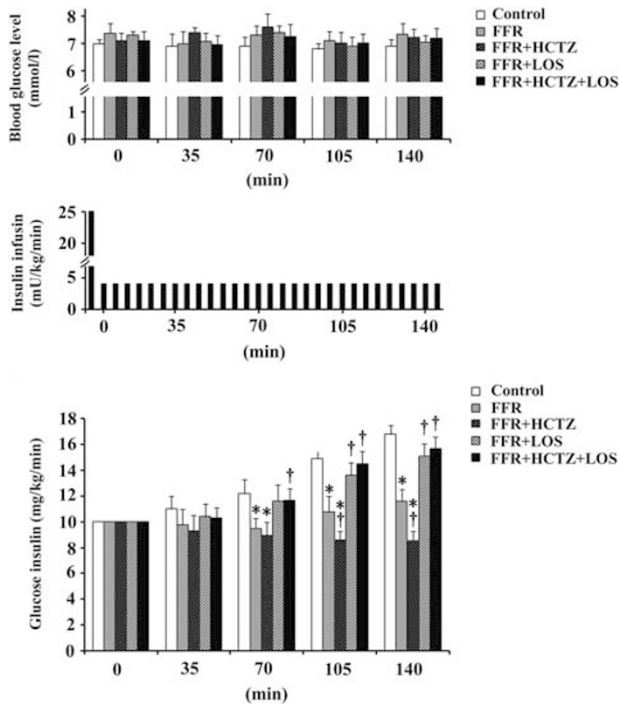


Figure 2 Time course of blood glucose level (upper panel), insulin infusion rate (middle panel) and glucose infusion rate (lower panel) of the glucose clamp in five groups: control ($n=10$), normal diet group; FFR ($n=9$), fructose-fed rats group; FFR+HCTZ ($n=9$), fructose-fed rats and hydrochlorothiazide group; FFR+LOS ($n=7$), fructose-fed rats and losartan group and FFR+HCTZ+LOS ($n=8$), fructose-fed rats and hydrochlorothiazide plus losartan group. Data are the means \pm s.e.m. * $P < 0.05$ vs. control; † $P < 0.05$ vs. FFR.

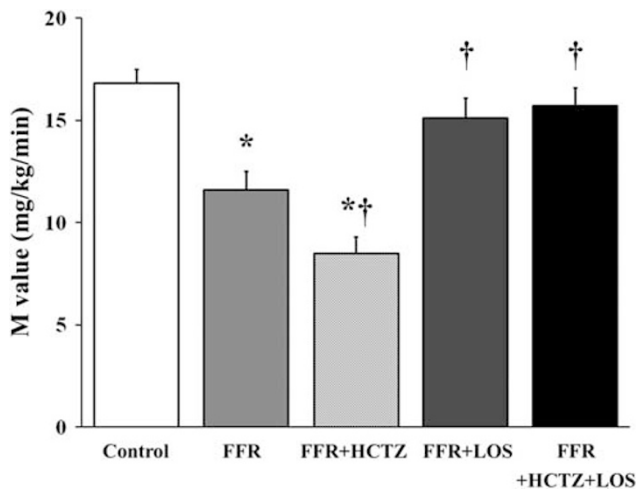


Figure 3 Comparisons of the index of insulin sensitivity (M value) in the five groups: control ($n=10$), normal diet group; FFR ($n=9$), fructose-fed rats group; FFR+HCTZ ($n=9$), fructose-fed rats and hydrochlorothiazide group; FFR+LOS ($n=7$), fructose-fed rats and losartan group and FFR+HCTZ+LOS ($n=8$), fructose-fed rats and hydrochlorothiazide plus losartan group. Data are the means \pm s.e.m. * $P < 0.01$ vs. control; † $P < 0.01$ vs. FFR.

glucose levels during the glucose clamp were similar in the five experimental groups. The mean coefficient of variation (%) = $SD / \text{mean} \times 100$) of the plasma glucose level during the clamps in the five

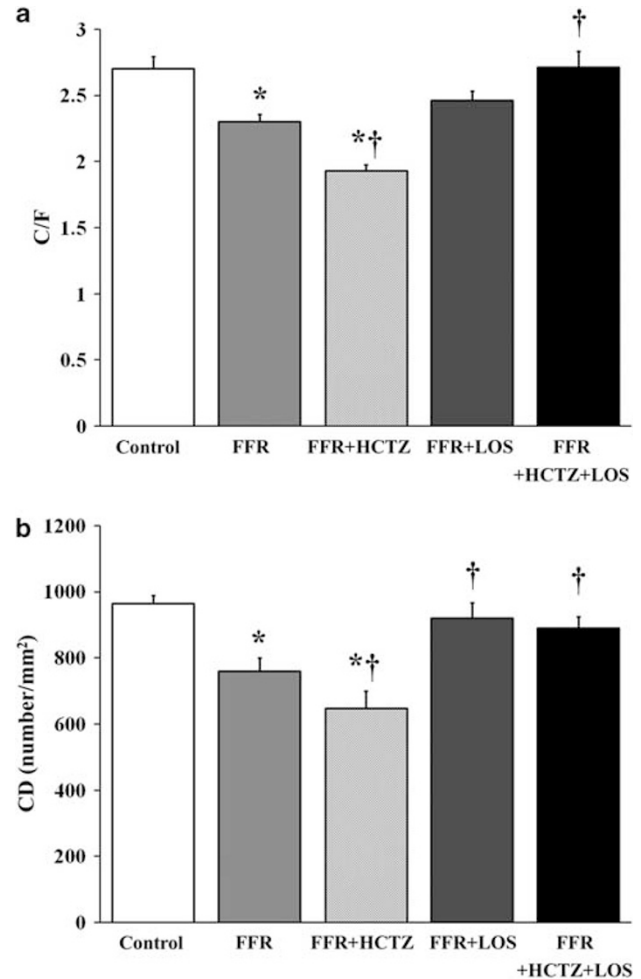


Figure 4 Comparisons of the capillary-to-fiber ratio (C/F) (a) and capillary density (CD) (b) in the five groups: control ($n=10$), normal diet group; FFR ($n=9$), fructose-fed rats group; FFR+HCTZ ($n=9$), fructose-fed rats and hydrochlorothiazide group; FFR+LOS ($n=7$), fructose-fed rats and losartan group and FFR+HCTZ+LOS ($n=8$), fructose-fed rats and hydrochlorothiazide plus losartan group. Data are the means \pm s.e.m. * $P < 0.01$ vs. control; † $P < 0.05$ vs. FFR.

experimental groups was 3%. The M values in the five groups are shown in Figure 3. M values were significantly lower in the FFR groups ($11.6 \pm 0.9 \text{ mg kg}^{-1} \text{ min}^{-1}$) than in the control group ($16.8 \pm 0.7 \text{ mg kg}^{-1} \text{ min}^{-1}$). HCTZ further reduced the M values in the FFR+HCTZ group ($8.5 \pm 0.8 \text{ mg kg}^{-1} \text{ min}^{-1}$) to less than those of the FFR group. However, losartan significantly increased the M values in the FFR+LOS ($15.1 \pm 0.9 \text{ mg kg}^{-1} \text{ min}^{-1}$) and FFR+HCTZ+LOS ($15.7 \pm 0.9 \text{ mg kg}^{-1} \text{ min}^{-1}$) groups compared with those of the FFR group.

CD and regression analyses

The effects of treatment on C/F and CD are shown in Figure 4. The C/F and CD in the FFR groups were significantly lower than in the control group. The CD in the FFR+LOS group and the C/F and CD in the FFR+HCTZ+LOS group were significantly higher than those in the FFR group. Although it did not reach the level of significance ($P=0.12$), the C/F tended to be higher in the FFR+LOS group than in the FFR group. However, the C/F and CD in the FFR+HCTZ group were significantly lower than those in the FFR group (Figure 5).

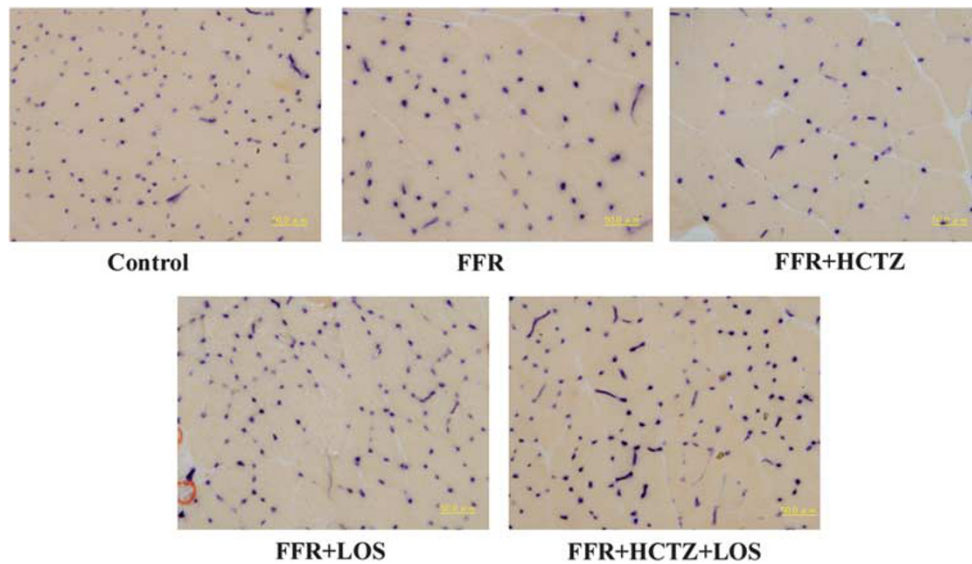


Figure 5 Representative cross-sections of extensor digitorum longus muscle in the five groups. The capillaries stained blue. Scale bar 50 μm . Control, normal diet group; FFR, fructose-fed rats group; FFR+HCTZ, fructose-fed rats and hydrochlorothiazide group; FFR+LOS, fructose-fed rats and losartan group and FFR+HCTZ+LOS, fructose-fed rats and hydrochlorothiazide plus losartan group.

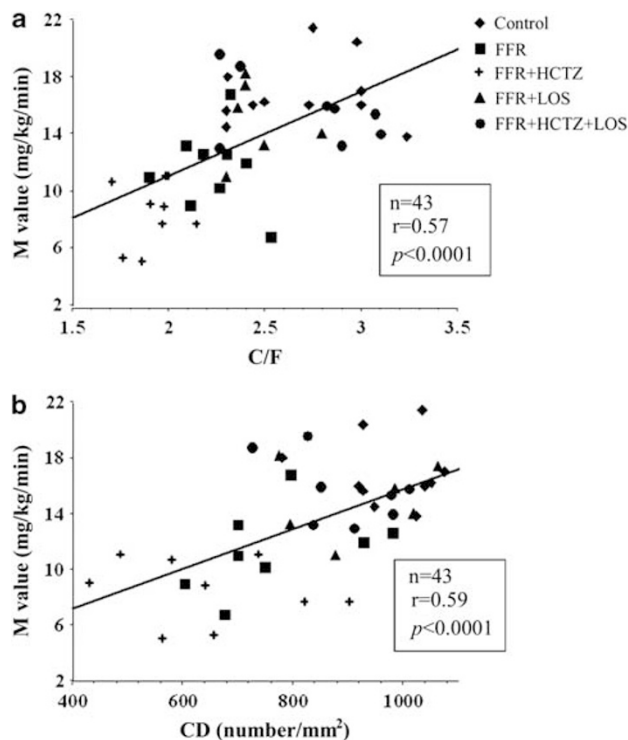


Figure 6 Correlation between M value and capillary-to-fiber ratio (C/F) (a) and capillary density (CD) (b).

There were significantly positive correlations between the M values and C/F (Figure 6a) and CD (Figure 6b). In addition, we performed multiple stepwise regression analysis to compare the relationship between M values as dependent variables, five variables with CD (CD, SBP, BW, serum adiponectin and triglycerides) as independent variables and M values and five variables with C/F (C/F, SBP, BW,

Table 1 Body weight (BW) and serum triglyceride and adiponectin levels at week 8 of the experimental period

| Groups (n) | BW (g) | Serum triglyceride (mg dl^{-1}) | Serum adiponectin ($\mu\text{g ml}^{-1}$) |
|------------------|-------------|--|---|
| Control (10) | 390 \pm 7 | 64.6 \pm 14.6 | 3.44 \pm 0.52 |
| FFR (9) | 388 \pm 9 | 86.0 \pm 11.3 | 2.42 \pm 0.18* |
| FFR+HCTZ (9) | 374 \pm 9 | 96.0 \pm 23.6 | 1.13 \pm 0.12*† |
| FFR+LOS (7) | 384 \pm 5 | 95.5 \pm 12.2 | 2.12 \pm 0.23* |
| FFR+HCTZ+LOS (8) | 378 \pm 8 | 97.3 \pm 15.8 | 1.01 \pm 0.08*† |

Abbreviations: Control, normal diet group; FFR, fructose-fed rats group; FFR+HCTZ, fructose-fed rats and hydrochlorothiazide group; FFR+LOS, fructose-fed rats and losartan group; FFR+HCTZ+LOS, fructose-fed rats and hydrochlorothiazide plus losartan group. Data are the means \pm s.e.m.

* $P < 0.01$ vs. control; † $P < 0.01$ vs. FFR.

serum adiponectin and triglycerides), respectively. The data indicated that the M values were predicted by the CD ($R^2=0.35$, $F=21.7$, $P < 0.0001$) and C/F ($R^2=0.33$, $F=20.5$, $P < 0.0001$), respectively, but not by SBP, BW, serum adiponectin or triglycerides, at week 8 in all rats.

Body weight and serum compositions

There were no significant intergroup differences in BW or serum triglycerides at week 8 (Table 1). The serum adiponectin level in the FFR groups was significantly lower than that in the control group. The serum adiponectin levels in the FFR+HCTZ and FFR+HCTZ+LOS groups were significantly lower than those in the FFR group. However, there were no statistically significant differences in the serum adiponectin levels between the FFR+HCTZ and FFR+HCTZ+LOS groups or between the FFR and FFR+LOS groups.

DISCUSSION

This study confirmed previous reports that a fructose-rich diet reduces IS in normal rats and leads to hypertension.^{18,19,24} The CD of skeletal muscle was lower in FFRs than in the controls, and HCTZ further

decreased IS and CD in FFRs, although it has significant hypotensive effects. However, treatment with losartan recovered IS and CD in both the FFR and the FFR+HCTZ groups to the level seen in the control rats. Multiple regression analysis indicated that the M value, a marker of IS, was predicted by CD in the EDL independent of SBP, BW and serum adiponectin and triglycerides in this study. These results indicate that CD has a role in the modulation of IS by a fructose diet and HCTZ and/or losartan treatment. To the best of our knowledge, this study is the first to investigate CD in FFRs after treatment with HCTZ and/or losartan.

Effects of thiazide diuretics and ARBs on IS

It has been shown that high doses of thiazide diuretics have an adverse effect on glucose metabolism. Consistent with the previous study,¹⁸ the results in this study demonstrated that HCTZ significantly worsened the fructose-induced decrease in IS in FFRs. Conversely, the present findings showed that losartan significantly improved IS in FFRs. Several previous studies failed to demonstrate any losartan-induced improvement in IS in hypertensive or normotensive subjects^{6,25} or in type 2 diabetic rats (IS was assessed by oral glucose tolerance test).²⁶ Moan *et al.*²⁷ found that losartan improved IS in patients with severe essential hypertension but had little effect on IS in those patients with mild essential hypertension. Although our recent results showed that the ARB olmesartan did not significantly improve (less effect) IS in spontaneously hypertensive rats,²² which was consistent with our present results, Higashiura *et al.*²⁸ reported that olmesartan significantly improved IS in FFRs (IS was assessed by the euglycemic hyperinsulinemic glucose clamp technique). These different findings concerning the effects of ARBs on IS may be related to differences in the subjects or rat strains, as well as differences in the measurement methods for IS analysis. In our present and previous studies, we used the euglycemic hyperinsulinemic clamp technique, a gold standard method for investigating and quantifying IS, because it measures the amount of glucose necessary to compensate for an increased insulin level without causing hypoglycemia.²⁹ This technique is a better evaluation method than other methods, such as the oral glucose tolerance test. Although the mechanisms by which ARBs improve IS are not fully understood, a possible mechanism by which ARBs improve IS is through increased cellular cyclic adenosine 3',5'-monophosphate levels and the production of insulin-like growth factor-I.³⁰ In addition, losartan might improve IS by activating insulin-sensitizing peroxisome proliferators-activated receptor (PPAR)- γ .³¹ However, the ability of other ARBs (such as olmesartan) to activate PPAR- γ receptors tested in this setting was less.³²

Capillary density in skeletal muscle is decreased in insulin-resistant rats

Several reports have shown the relationship between skeletal muscle CD and IS/resistance in humans.^{11–13,33–35} Likewise, rodent studies have demonstrated a direct effect of capillarization on insulin action in skeletal muscles.^{14–16,36,37} A decrease in CD is associated with a longer diffusion distance between the nutritional blood vessels and the skeletal muscle cells. This change would impede the delivery of glucose to the muscle cells and thereby decrease IS.¹⁷ In this study, there was a significant positive correlation between IS and CD values in the EDL muscle, and IS was predicted by CD in the EDL independent of SBP, BW, serum adiponectin and triglycerides in all the rats. Thus, fructose, HCTZ and losartan affected IS, at least in part by changing the CD.

In this study, CD in the EDL was significantly lower in FFRs than in the controls. However, Takada *et al.*¹⁹ reported that the CD in the soleus muscle in FFRs was not altered by fructose-rich chow. The

reason for the disparate results between the previous study and this study is not clear, but differences in the kind of skeletal muscle may be involved. Skeletal muscle consists of type I, type IIa and type IIb fibers. Type I and type IIa fibers have high capillary densities, whereas type IIb fibers have a low CD.^{38,39} It is thought that an increase in type IIb fibers contributes to the reduction in CD. As reported by Higashiura *et al.*,²⁸ the percentage of type IIb fibers in the soleus muscle is <1%, and thus there is no significant difference in the amount of type IIb fibers in the soleus muscle between FFRs and control rats. However, there are a significant decrease in the percentage of type I fibers and a significant increase in the percentage of type IIa fibers in FFRs relative to controls. The EDL muscle has more type IIb fibers than the soleus muscle, so it may be easier to detect differences in CD based on changes in type IIb fibers in the EDL than in the soleus muscle. Thus, we evaluated CD in the EDL in this study, and our results showed that a fructose-rich diet in normal rats reduced CD in the EDL. Similarly, our results showed that the CD in skeletal muscle decreased in an experimental animal model of insulin resistance.^{14–16} Frisbee *et al.*¹⁵ suggested that microvessel rarefaction in the skeletal muscles of obese Zucker rats manifesting insulin resistance did not depend on an elevated mean arterial pressure and that other factors associated with insulin resistance may underlie the progressive reduction in skeletal muscle microvessel density in these animals. These results suggest that there is an association between IS and CD in skeletal muscle, although the mechanisms are not clearly understood. Ellis *et al.*¹⁶ found that insulin attenuates the O₂-dependent release of adenosine triphosphate from the red blood cells and suggested that this defect in red blood cell physiology could contribute to a failure in the regulation of the O₂ supply to meet the demand in the EDL in a model of insulin resistance using diabetic fatty Zucker rats fed a high-fat diet. In addition, a high fructose diet also causes mitochondrial dysfunction in skeletal muscle,^{40,41} and mitochondrial dysfunction has been associated with insulin resistance in skeletal muscle.⁴² Brownlee suggested that mitochondrial dysfunction occurs as a 'unifying mechanism' for microvascular and macrovascular complications through reactive oxygen species production.⁴³ Hyperglycemia induced by endothelial dysfunction is inhibited by blocking ROS production from mitochondria and by overexpression of uncoupling proteins or manganese superoxide dismutase.⁴³ Furthermore, endothelial NO synthase in vascular endothelial cells seems to have an important role in insulin-stimulated NO production and vasodilation.⁴⁴

Mechanisms of thiazide diuretics and ARBs on capillary density in skeletal muscle

Capillary rarefaction associated with hypertension has been considered a form of structural autoregulation, reflecting the long-term adaptation of the microcirculation to elevated blood pressure. It has been shown that vasodilatation and increased blood flow promote angiogenesis in skeletal muscles.⁴⁵ Thus, losartan may increase the CD through its vasodilator action or hypotensive effect.⁴⁶ In addition to the vasodilator or hypotensive effect, the proangiogenic effect of losartan may also be mediated by the following mechanism: ARB blocks AT1 receptors, whereas unbound angiotensin II stimulates AT2 receptors, which in turn increase angiogenic factors, such as vascular endothelial growth factor receptors, Tie-2 expression and the angiotensin-1/angiotensin-2 ratio.⁴⁷ In addition, You *et al.*⁴⁸ reported that losartan administration resulted in an improvement in hindlimb ischemia-induced capillary rarefaction, and this improvement was associated with a restoration of progenitor cell-related function in spontaneously hypertensive rat. In contrast, although HCTZ and

losartan had similar hypotensive effects in this study, treatment with HCTZ decreased the CD, indicating that blood pressure was not involved in the changes in CD and IS. A possible mechanism by which HCTZ decreased the CD is that HCTZ reduced blood pressure through decreased cardiac output,⁴⁹ and decreased cardiac output associated with a decreased peripheral blood flow would contribute to the induction of capillary rarefaction in skeletal muscle.⁴⁵ Capillary rarefaction would enable the tissue blood flow to be regulated without consumption of the energy necessary for active vasoconstriction.¹⁴

Serum adiponectin cannot mediate the beneficial effects of ARBs on IS in FFR

Conversely, consistent with previous reports,^{50,51} the results in this study indicated that the decreases in IS induced by a fructose-rich diet or administration of HCTZ are associated with decreased serum adiponectin, a protein with levels that are known to increase with existing PPAR- γ agonists and to correlate with IS.⁵² However, in this study, losartan-increased IS in the FFR+LOS and FFR+HCTZ+LOS groups was not coupled with increased serum adiponectin levels. Recently, several studies, but not all, have revealed that ARBs improved hypertension with increasing adiponectin.^{24,51} Consistent with our present results, Kamari *et al.*²⁴ reported that telmisartan, an ARB with greater PPAR- γ activity, effectively improved fructose-induced hypertension, hyperinsulinemia and hypertriglyceridemia, but did not affect serum adiponectin in FFRs. In addition, Sharabi *et al.*⁵³ showed in FFRs that the PPAR- γ agonist rosiglitazone improved the metabolic profile to a degree similar to telmisartan, but with increases in serum adiponectin levels and gene expression. Therefore, it is unlikely that adiponectin could always mediate the beneficial effects of ARBs on IS in FFRs.

Pair-feeding can eliminate effects of food volume on BW and serum triglyceride

IS is negatively correlated with BW. However, the FFR model is associated with weight loss, although it is a commonly used model that mimics human metabolic syndrome in many respects, including hypertension and insulin resistance.¹⁹ In addition, food volume can also influence glucose, insulin and triglyceride levels. In this study, therefore, all rats were pair-fed to eliminate these effects related to food volume. There were no significant intergroup differences in BW or serum triglycerides at week 8 of this study. We therefore concluded that the intergroup differences in IS were not related to differences in food volume.

In summary, we have demonstrated that HCTZ decreased IS, but losartan ameliorated the HCTZ-induced decrease in IS in FFRs, and these changes in IS were at least partially attributable to a modulation of CD in skeletal muscles. If these findings are confirmed in humans, then the combination of losartan and HCTZ may be recommended for the treatment of hypertensive subjects with insulin resistance, without considering the adverse effect of thiazide diuretics on glucose metabolism.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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