Available online at www.sciencedirect.com

SCIENCE DIRECT

Metabolism Clinical and Experimental 54 (2005) 1659–1668

Metabolism
Clinical and Experimental

www.elsevier.com/locate/metabol

Selective angiotensin II receptor antagonism enhances whole-body insulin sensitivity and muscle glucose transport in hypertensive TG(mREN2)27 rats

Julie A. Sloniger, Vitoon Saengsirisuwan, Cody J. Diehl, John S. Kim, Erik J. Henriksen*

Muscle Metabolism Laboratory, Department of Physiology, University of Arizona College of Medicine, Tucson, AZ 85721-0093, USA

Received 4 March 2005; accepted 13 June 2005

Abstract

Essential hypertension is frequently associated with insulin resistance of skeletal muscle glucose transport, and angiotensin II (ANGII) can contribute to the pathogenesis of both conditions. The male heterozygous TG(mREN2)27 rat (TGR) harbors the mouse transgene for renin, exhibits local tissue elevations in ANGII and is an excellent model of both hypertension and insulin resistance associated with defective insulin signaling. The present study was designed to assess the specific role of ANGII in the insulin resistance of the male heterozygous TGR. TGRs were treated with either vehicle or the ANGII (AT_1-specific) receptor antagonist, irbesartan (50 mg/kg body weight), for 21 consecutive days. Compared with vehicle-treated TGRs, whole-body insulin sensitivity was increased 35% ($P < .05$) in the irbesartan-treated group, and insulin-mediated glucose transport was increased ($P < .05$) in both type IIb epitrochlearis (80%) and type I soleus (59%) muscles after irbesartan treatment. Moreover, glycogen synthase activation due to insulin was increased 58% ($P < .05$) in the soleus of the irbesartan-treated TGRs. However, no significant improvements were observed for functionality of insulin-signaling elements (tyrosine phosphorylation of insulin receptor and insulin receptor substrate 1 [IRS1], IRS1 associated with the p85 regulatory subunit of phosphatidylinositol 3′-kinase, and Ser473 of Akt) in muscle of irbesartan-treated animals, except for a 25% increase ($P < .05$) in IRS1 tyrosine phosphorylation in soleus. Collectively, these data indicate that the improvements in whole-body and skeletal muscle insulin action after long-term antagonism of ANGII action in TGRs occur independently of modulation of the functionality of these insulin-signaling elements.

1. Introduction

The insulin resistance syndrome is a condition characterized by insulin resistance of skeletal muscle glucose metabolism and is often accompanied by additional metabolic and cardiovascular abnormalities, including glucose intolerance, hyperinsulinemia, essential hypertension, dyslipidemia, and central obesity [1]. Increased cardiovascular mortality associated with this condition has been directly attributed to the insulin resistance and compensatory hyperinsulinemia [2,3].

Angiotensin II (ANGII), a component of the renin-angiotensin system (RAS), is known to modify blood pressure homeostasis as well as vascular structure and function. It is well established that ANGII contributes to the pathogenesis of hypertension and is an appropriate target for pharmacologic blockade [4]. In addition, elevations in ANGII are associated with the development of insulin resistance of skeletal muscle glucose transport [5,6]. Short- or long-term infusion of ANGII has been shown to cause insulin resistance of skeletal muscle glucose disposal that could not be explained by hemodynamic influences, and it was concluded that ANGII directly affects the muscle glucose transport system [5,6]. Moreover, long-term administration of ANGII receptor antagonists to insulin-resistant obese Zucker rats [7], fructose-fed rats [8,9], or spontaneously hypertensive rats [10] improves whole-body insulin sensitivity, possibly because of the amelioration of skeletal muscle insulin resistance [7].

The TG(mREN2)27 rat (TGR) harbors the mouse *Ren-2* renin gene [11] and provides an excellent monogenetic model of both hypertension and insulin resistance. The TGR displays severe hypertension, left ventricular hypertrophy, and cardiac failure of clearly defined monogenetic origin [12-15]. Increases in the local RAS, as demonstrated by

* Corresponding author.

0026-0495/$ – see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2005.06.016

elevated tissue ANGII levels [13,16-18], likely contribute to the hypertension exhibited by this transgenic animal. In addition, the male heterozygous TGR is also insulin-resistant at the whole-body [19-22] and skeletal muscle levels [20-22], associated with defects in several critical elements of the insulin-signaling cascade, including tyrosine phosphorylation of insulin receptor (IR) and insulin receptor substrate 1 (IRS1), IRS1 associated with the p85 subunit of phosphatidylinositol 3′-kinase (PI3K), and serine phosphorylation of Akt and glycogen synthase kinase 3β [21]. It has recently been reported that long-term administration of the ANGII receptor antagonist valsartan to the TGR improved whole-body and skeletal muscle insulin action [22]. However, the specific role of ANGII in the pathogenesis of the defects in skeletal muscle insulin signaling in the TGR is presently unclear.

In this context, the overall purpose of the present investigation was to assess the specific role of ANGII in the pathogenesis of insulin resistance in the hypertensive male heterozygous TGR. By treating these hypertensive and insulin-resistant animals (21 days) with an AT_1-selective ANGII receptor antagonist on a long-term basis (irbesartan, 50 mg/kg body weight), we tested the following hypotheses: (1) ANGII receptor antagonism will enhance whole-body glucose tolerance and skeletal muscle insulin action on glucose transport and glycogen synthase activity in the insulin-resistant TGR; (2) the ANGII receptor antagonist–induced increases in skeletal muscle glucose transport and glycogen synthase will be associated with improved functionality of critical elements of the insulin-signaling cascade, including IR, IRS1, PI3K, and Akt.

2. Materials and methods

2.1. Animals and treatments

Male heterozygous TGRs were obtained from the Hypertension and Vascular Disease Center of Bowman Gray School of Medicine at Wake Forest University (Winston-Salem, NC) at approximately 5 to 6 weeks of age. TGRs were randomly assigned to groups receiving either a vehicle (water) or irbesartan (50 mg/kg body weight) (Bristol-Meyers Squibb, Princeton, NJ) by gavage for 21 consecutive days, as done previously in obese Zucker rats [7]. All animals were housed in a temperature-controlled room (20 °C-22 °C) with a 12:12-h light-dark cycle (lights on from 7:00 AM to 7:00 PM) at the Central Animal Facility of the University of Arizona. The animals had free access to rodent chow (Ralston Purina 5001, St. Louis, MO; sodium content 0.4%) and water. All of the procedures were approved by the University of Arizona Animal Care and Use Committee.

2.2. Measurement of blood pressure

Resting systolic blood pressure (SBP) and heart rate were measured after 16 days of treatment using a tail-cuff plethysmography system (NIBP-8 Non-invasive Blood Pressure Monitor, Columbus Instruments, Columbus, OH). Animals were acclimated to the tail-cuff plethysmography apparatus for at least 1 week by daily placement of the animal in a holding tube and placement of the pressure cuff at the base of the animal's tail.

2.3. Oral glucose tolerance tests

After 18 days, animals were food-restricted (4 g of chow given at 5:00 PM) the evening before the oral glucose tolerance test (OGTT). At 8 AM, approximately 12 to 15 hours after the most recent treatment, animals underwent the OGTT using a 1-g/kg body weight glucose feeding by gavage. Blood (0.25 mL) was collected from the tip of the tail immediately before and 15, 30, 60, and 120 minutes after administration of the glucose. Whole blood was mixed thoroughly with EDTA (18 mmol/L final concentration) and centrifuged at 13 000 *g* to isolate the plasma. Plasma was stored at −80 °C and subsequently assayed for glucose (Sigma, St. Louis, MO), insulin (Linco Research, St. Charles, MO), and free fatty acids (FFAs) (Wako, Richmond, VA). Immediately after completion of the OGTT, animals received a 2.5-mL subcutaneous injection of 0.9% sterile saline to compensate for plasma loss. Treatments resumed that afternoon for 3 further days.

Table 1
Body weights, heart weights, fasting plasma variables, and resting SBPs and heart rates in TGV and TGI TGRs

Group	Body weight (g)	Heart weight (g)	Heart weight (g/100 g body weight)	SBP (mm Hg)	Heart rate (beats/min)
TGV	313 ± 9	1.28 ± 0.05	0.407 ± 0.012	203 ± 4	446 ± 14
TGI	301 ± 8	0.93 ± 0.03*	0.309 ± 0.002*	151 ± 3*	437 ± 11

Group	Glucose (mg/dL)	Insulin (μU/mL)	FFA (mmol/L)
TGV	113 ± 5	25.9 ± 5.0	0.87 ± 0.08
TGI	105 ± 3	24.6 ± 1.7	0.95 ± 0.06

Values are means ± SE for 4 to 10 animals per group, except for SBP and heart rate, which are derived from 13 to 20 animals. TGV indicates vehicle-treated; TGI, irbesartan-treated.

* $P < .05$, TGI vs TGV.

2.4. In vitro skeletal muscle glucose transport activity

Seventy-two hours after the OGTT, the animals were again food-restricted as described above. At 8 AM, approximately 12 to 15 hours after the final vehicle or irbesartan treatment, the animals were weighed and deeply anesthetized with an intraperitoneal injection of pentobarbital sodium (50 mg/kg body weight). Both soleus and both epitrochlearis muscles were dissected and prepared for in vitro incubation. Whereas the epitrochlearis muscles were incubated intact, the 2 soleus muscles were divided into 3 strips each. Four of the soleus strips (~30 mg) were incubated, whereas the other 2 were quickly frozen in liquid nitrogen for later use in biochemical assays. Glucose transport activity, assessed as 2-deoxyglucose (2-DG) uptake, was determined in the absence or presence of insulin (2 mU/mL) exactly as described previously [23]. This method for assessing glucose transport activity in isolated muscle has been validated [24,25].

2.5. Glycogen synthase activity and biochemical assays

Glycogen synthase activity was assessed as the activity ratio (activity in the absence of glucose-6-phosphate divided by the activity in the presence of 5 mmol/L glucose-6-phosphate) using the filter paper assay of Thomas et al [26], as modified by Henriksen et al [27]. Remaining pieces (~15 mg) of epitrochlearis and soleus muscle were homogenized in 30 volumes of ice-cold 20 mmol/L HEPES (pH 7.4) containing 1 mmol/L EDTA and 250 mmol/L sucrose. These homogenates were used for the determination of total protein content using the bicinchoninic acid method (Sigma), GLUT-4 protein level [23], total hexokinase activity [28], and citrate synthase activity [29].

2.6. Assessment of insulin signaling

Assessment of the functionality and protein expression of insulin-signaling elements was done as described by Sloniger et al [21]. The remaining pieces of muscles were

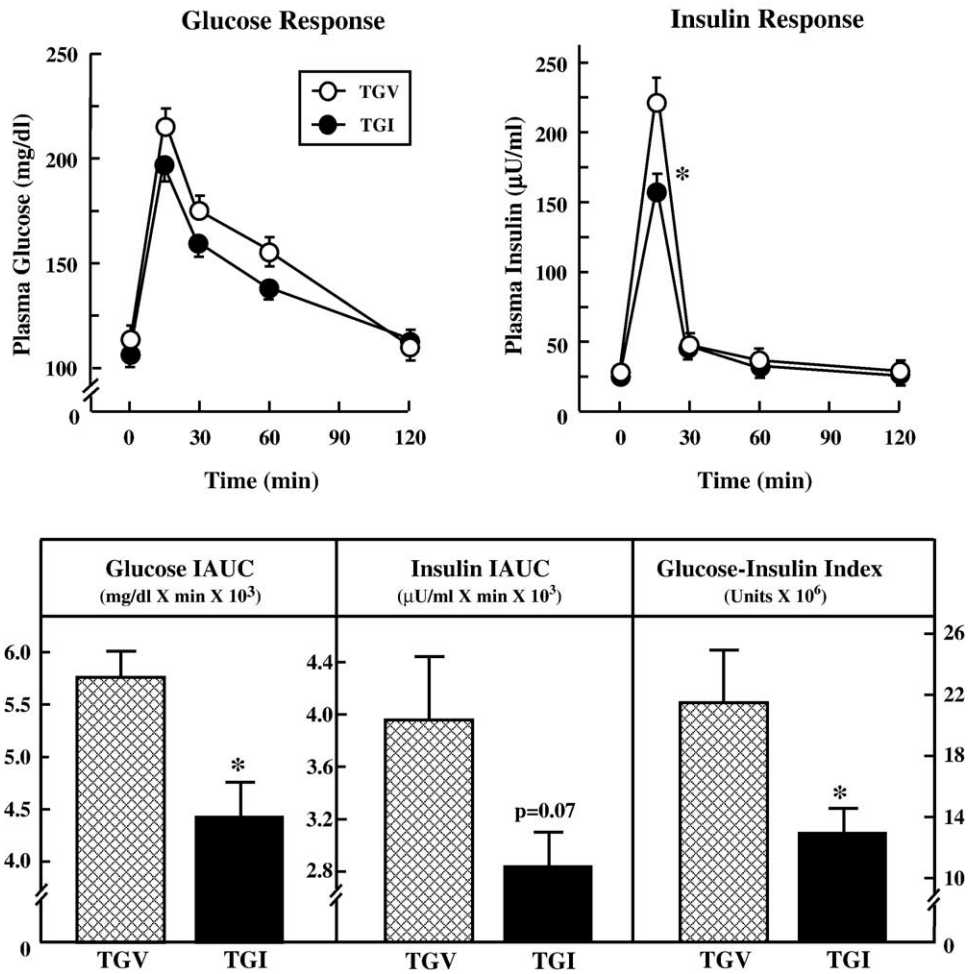

Fig. 1. Top, Glucose and insulin responses to an OGTT in TGRs treated with either vehicle (TGV) or 50 mg/kg irbesartan (TGI) for 21 days. Bottom, The IAUCs for glucose and insulin and the glucose-insulin index. The glucose-insulin index was calculated as the product of the glucose IAUC and the insulin IAUC for each animal. Values are means ± SE for 8 to 10 animals per group. *$P < .05$, TGI vs TGV.

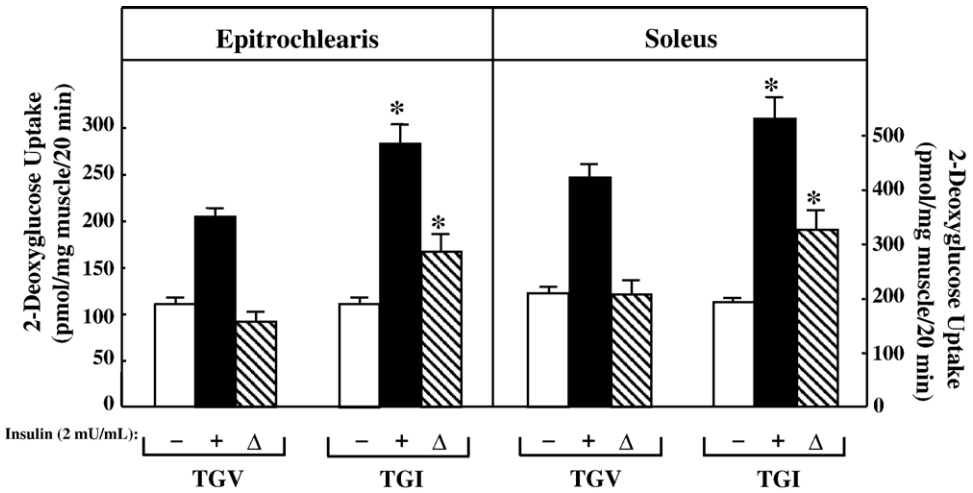

Fig. 2. Glucose transport activity in epitrochlearis and soleus muscles of TGRs treated with either vehicle (TGV) or 50 mg/kg irbesartan (TGI) for 21 days. 2-DG uptake was assessed in the absence (−) or presence (+) of insulin (2 mU/mL). The increase above basal rate due to insulin for 2-DG uptake is also presented (Δ). Values are means ± SE for 4 to 7 animals per group. *$P < .05$, TGI vs TGV.

homogenized in 8 volumes of ice-cold lysis buffer (50 mmol/L HEPES, 150 mmol/L NaCl, 20 mmol/L Na pyrophosphate, 20 mmol/L β-glycerophosphate, 10 mmol/L NaF, 2 mmol/L Na_3VO_4, 2 mmol/L EDTA, 1% Triton X-100, 10% glycerol, 1 mmol/L $MgCl_2$, 1 mmol/L $CaCl_2$, 10 μg/mL aprotinin, 10 μg/mL leupeptin, 0.5 μg/mL pepstatin, and 2 mmol/L phenylmethanesulfonyl fluoride [PMSF]). Homogenates were incubated on ice for 20 minutes and then centrifuged at 13 000g for 20 minutes at 4 °C. Protein concentration was determined using the bicinchoninic acid method (Sigma). Insulin-signaling proteins were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis on 7.5% or 12% polyacrylamide gels (Bio-Rad Laboratories, Hercules, CA) and transferred to nitrocellulose membranes. To determine protein expression of insulin-signaling factors, the blots were incubated with the appropriate dilution of commercially available antibodies against IR β-subunit (IR-β), IRS1, the p85 regulatory subunit of PI3K, and Akt1/2 (Cell Signaling Technology, Beverly, MA).

Muscle incubated in the absence or presence of insulin was used for evaluation of Akt serine phosphorylation.

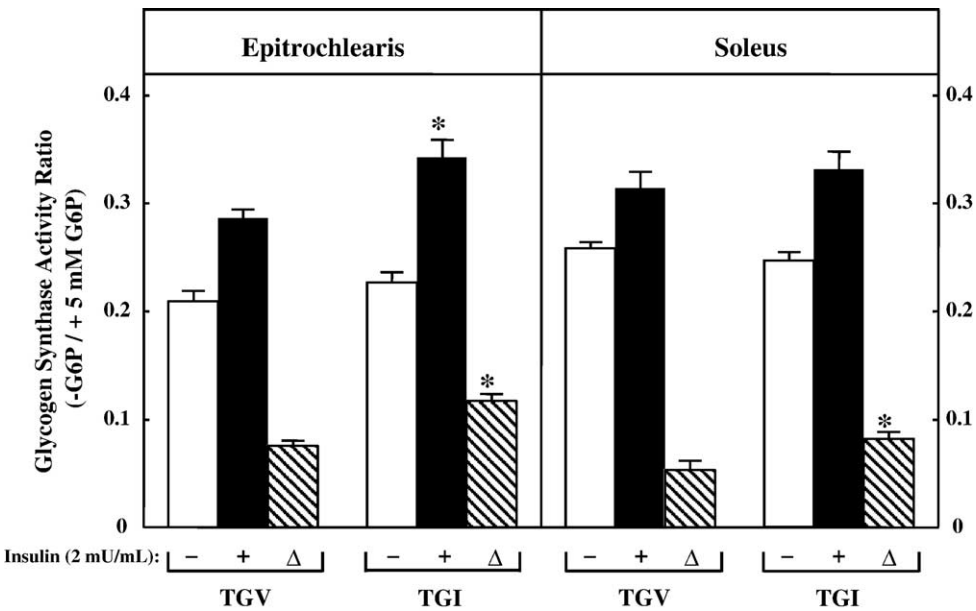

Fig. 3. Glycogen synthase activity in epitrochlearis and soleus muscles of TGRs treated with either vehicle (TGV) or 50 mg/kg irbesartan (TGI) for 21 days. The glycogen synthase activity ratio (activity in the absence of glucose-6-phosphate divided by the activity in the presence of 5 mmol/L glucose-6-phosphate) was assessed in the absence (−) or in the presence (+) of insulin (2 mU/mL). The increase above basal due to insulin for 2-DG uptake is also presented (Δ). Values are means ± SE for 5 animals per group. *$P < .05$, TGI vs TGV.

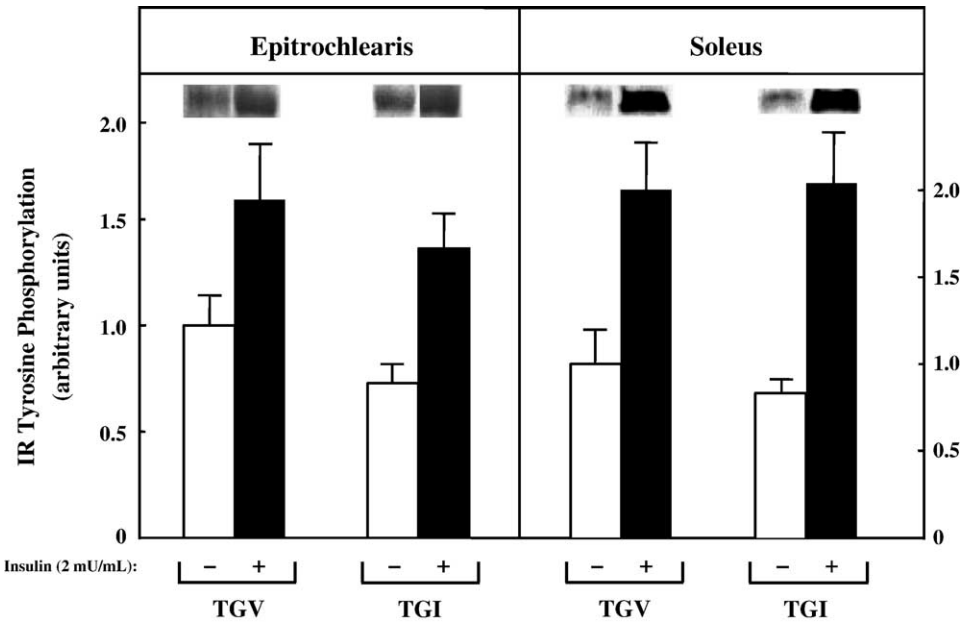

Fig. 4. Tyrosine phosphorylation of IR in epitrochlearis and soleus muscles of TGRs treated with either vehicle (TGV) or 50 mg/kg irbesartan (TGI) for 21 days. IR tyrosine phosphorylation was assessed in the absence (−) and in the presence (+) of insulin (2 mU/mL). Representative bands are shown. Values are ± SE for 4 to 5 animals per group.

Blots were incubated with antibodies against Akt-Ser473 (Cell Signaling Technology). Membranes were then incubated with secondary goat antirabbit antibody conjugated with HRP (Chemicon, Temecula, CA). The proteins were visualized on Kodak X-Omat AR film (Kodak, Rochester, NY) using an enhanced chemiluminescence detection system (Amersham Pharmacia, Piscataway, NJ). Band intensities on the autoradiographs were quantified using an imaging densitometer (Bio-Rad Model GS-800) and Quantity One software.

For measurement of tyrosine-phosphorylated IR-β (IR/pY) and IRS1 (IRS1/pY) and for IRS1-associated p85

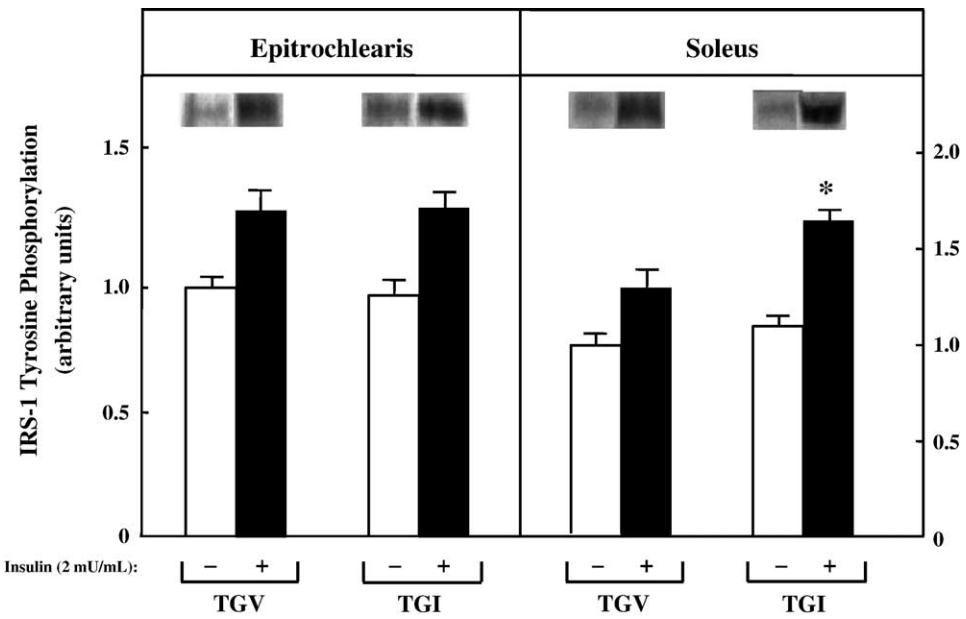

Fig. 5. Tyrosine phosphorylation of IRS1 in epitrochlearis and soleus muscles of TGRs treated with either vehicle (TGV) or 50 mg/kg irbesartan (TGI) for 21 days. IRS1 tyrosine phosphorylation was assessed in the absence (−) and in the presence (+) of insulin (2 mU/mL). Representative bands are shown. Values are ± SE for 4 to 5 animals per group. *$P < .05$, TGI vs TGV.

(IRS1/p85), immunoprecipitations and subsequent immunoblotting were performed. Muscle pieces were homogenized in 1 mL of ice-cold lysis buffer, and protein concentration was determined. Samples were diluted to 2 mg/mL (IR/pY and IRS1/pY) or 3 mg/mL (IRS1/p85). For the assessment of IR/pY, 0.5 mL of diluted homogenate was immunoprecipitated with 15 μL of recombinant agarose-conjugated antiphosphotyrosine antibody (4G10, Upstate Biotechnology, Lake Placid, NY). For analysis of IRS1/pY and IRS1/p85, 0.5 mL of diluted homogenate was immunoprecipitated with 25 μL of agarose-conjugated anti–IRS1 antibody (Upstate Biotechnology). After an overnight incubation at 4 °C, samples were centrifuged and the supernatant was removed. The beads were washed 3 times with ice-cold phosphate-buffered saline, mixed with sodium dodecyl sulfate sample buffer, and boiled for 5 minutes. Proteins were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis on 7.5% polyacrylamide gels (Bio-Rad Laboratories) and transferred to nitrocellulose membranes. Immunoblotting for the detection of IR/pY and IRS1/p85 was completed as described above for the detection of protein expression of IR-β and p85. For the analysis of IRS1/pY, the nitrocellulose membrane was incubated in antiphosphotyrosine antibody (PY99, Santa Cruz Biotechnology, Santa Cruz, CA). Thereafter, the membranes were incubated with secondary goat antimouse antibody conjugated with HRP (Santa Cruz Biotechnology). Protein bands of interest were exposed, visualized, and quantified as described above.

2.7. Statistical analysis

All values are expressed as means ± SE. Differences between the 2 groups were determined using an unpaired Student t test. A level of $P < .05$ was set for statistical significance.

3. Results

The final average body weights of the vehicle- and irbesartan-treated TGRs were not different (Table 1). The efficacy of the AT_1-selective antagonism was evidenced both by the significantly lower ($P < .05$) heart weight of irbesartan-treated transgenic rats, expressed in either absolute (27%) or relative terms (g/100 g body weight) (24%), and by the markedly diminished ($P < .05$) resting SBP (26%) in these animals. Resting heart rate and fasting plasma glucose, insulin, and FFA were not different between groups (Table 1).

The results of the OGTT are shown in Fig. 1. Although the glucose values at any given time point did not differ significantly between the vehicle- and irbesartan-treated TGR groups (Fig. 1, top left panel), the incremental area under the curve (IAUC) for glucose was significantly lower (19%, $P < .05$) in the irbesartan-treated group (Fig. 1, lower left panel). The insulin response during the OGTT was reduced in the irbesartan-treated TGRs, as evidenced by the 23% lower insulin value at the 15-minute time point (Fig. 1, top right panel) and the 22% decrease in the insulin IAUC due to the irbesartan treatment approached statistical significance ($P = .07$) (Fig. 1, lower center panel). The glucose-insulin index is defined as the product of glucose IAUC and insulin IAUC and is inversely related to an increase in whole-body peripheral insulin sensitivity [30]. The glucose-insulin index was 40% smaller ($P < .05$) in the irbesartan-treated TGRs compared with the vehicle-treated transgenic animals (Fig. 1, lower right panel).

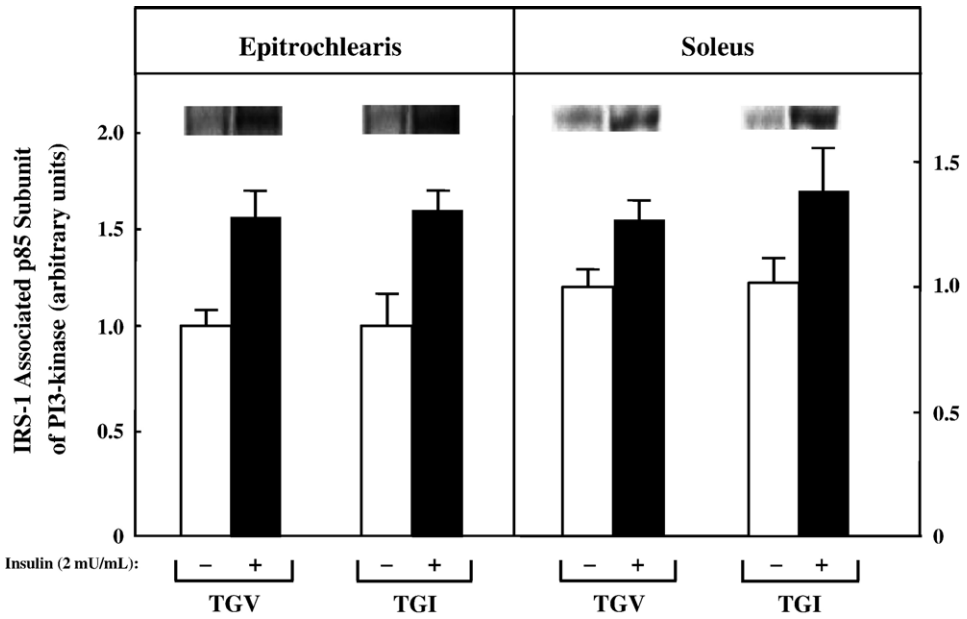

Fig. 6. IRS1 associated with the p85 regulatory subunit of PI3K in epitrochlearis and soleus muscles of TGRs treated with either vehicle (TGV) or 50 mg/kg irbesartan (TGI) for 21 days. IRS1 associated with PI3K was assessed in the absence (−) and in the presence (+) of insulin (2 mU/mL). Representative bands are shown. Values are ± SE for 4 to 5 animals per group.

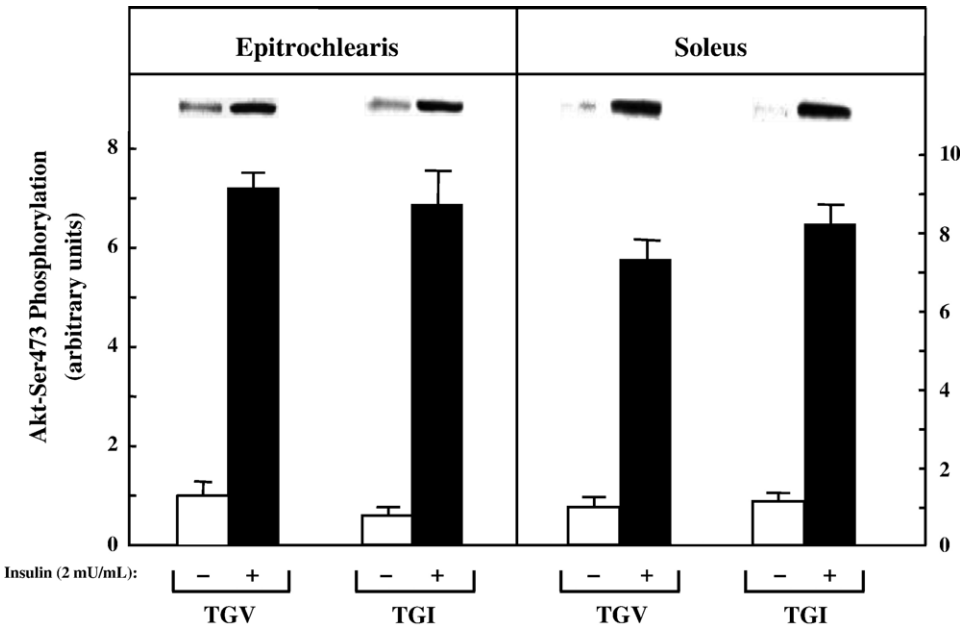

Fig. 7. Akt-Ser473 phosphorylation in epitrochlearis and soleus muscles of TGRs treated with either vehicle (TGV) or 50 mg/kg irbesartan (TGI) for 21 days. Akt phosphorylation was assessed in the absence (−) and in the presence (+) of insulin (2 mU/mL). Representative bands are shown. Values are ± SE for 4 to 5 animals per group.

A cellular locus for this improved insulin sensitivity after long-term treatment with the selective AT_1 antagonist was investigated by assessing insulin-stimulated glucose transport activity in isolated skeletal muscle (Fig. 2). The basal rate of 2-DG uptake was not affected by the irbesartan treatment in the TGRs for either epitrochlearis or soleus muscle. In contrast, the irbesartan treatment was associated with significant increases in the insulin-stimulated rate of 2-DG uptake in the epitrochlearis (30%) and soleus (22%). Moreover, the increases in 2-DG uptake above basal rate due to insulin in muscles of the irbesartan-treated TGR were 80% and 59%, respectively. Finally, significant enhancements of glycogen synthase activity due to insulin were observed in both epitrochlearis (62%) and soleus (58%) muscles of the TGRs treated with the AT_1-selective antagonist (Fig. 3, hatched bars). Total glycogen synthase activity, the whole homogenate level of GLUT-4 protein, and the activities of total hexokinase and citrate synthase were not altered by the irbesartan treatment in either muscle type (data not shown).

Irbesartan treatment of TGRs did not alter protein expression of insulin-signaling elements in muscle, except for a small (24%) decrease in IR-β in epitrochlearis (data not shown). Likewise, long-term ANGII receptor antagonism in TGRs had little effect on the functionality of insulin-signaling factors (Figs. 4-7). In the epitrochlearis, no alterations due to irbesartan treatment for insulin action on tyrosine phosphorylation of IR and IRS1 (Figs. 4 and 5), IRS1 associated with PI3K (Fig. 6), or Ser473 phosphorylation of Akt (Fig. 7) were observed. In the soleus of these animals, only insulin-stimulated IRS1 tyrosine phosphorylation was significantly elevated (25%, $P < .05$) compared with the vehicle-treated group.

4. Discussion

In the present investigation, we have made the finding that long-term treatment of male heterozygous TGRs with the selective ANGII (AT_1 subtype) receptor antagonist irbesartan leads to a significant enhancement of whole-body insulin sensitivity, as assessed using an OGTT (Fig. 1). The TGR is a monogenetic model of hypertension [11] caused by local elevations in ANGII levels [13,16-18]. Treatment with irbesartan was clearly effective in eliciting favorable changes in resting SBP and heart weight (Table 1), in agreement with previous studies using selective ANGII receptor antagonists in this animal model [18,31]. These data supporting a role of ANGII receptor antagonism as a means of improving insulin sensitivity in the TGR are in agreement with those of Blendea et al [22], who demonstrated that long-term treatment of TGRs with the ANGII receptor antagonist valsartan also improved whole-body and skeletal muscle insulin sensitivity. Although a portion of this improvement in whole-body insulin action is likely due to the well-documented hemodynamic modifications elicited by ANGII receptor antagonists (reviewed in References [32-35]), we have also demonstrated that the irbesartan treatment leads to an increase in insulin-stimulated glucose transport activity in isolated skeletal muscle (Fig. 2), in which any potential hemodynamic influences have been eliminated. These results reinforce our previous finding that long-term selective AT_1 antagonism in the obese Zucker rat, an obesity-associated model of marked insulin resistance, is

associated with significant improvements of whole-body and skeletal muscle insulin action [7].

We showed that long-term irbesartan treatment causes small, but significant, improvements in insulin action on glycogen synthase activity in isolated skeletal muscle of the male heterozygous TGR (Fig. 3). We have previously demonstrated that insulin action on glycogen synthase activity is defective in muscle of these hypertensive animals, possibly related to overactivity of the serine/threonine kinase glycogen synthase kinase 3β [21]. Interestingly, defects in insulin action on glycogen synthase activity are also seen in skeletal muscle of both animal models of obesity-associated insulin resistance and type 2 diabetes [36] and in human type 2 diabetic subjects [37,38]. Our results indicate that long-term ANGII receptor antagonism can overcome at least part of the defect in glycogen synthase activity that exists in the skeletal muscle of these insulin-resistant hypertensive transgenic rats.

A critical novel finding in the present investigation is that the irbesartan-mediated enhancement of insulin action on skeletal muscle glucose transport activity and glycogen synthase activity was elicited without clear-cut improvements in insulin stimulation of critical elements of the insulin-signaling cascade (Figs. 4-7). The only significant improvement in insulin signaling was the 25% increase in insulin-stimulated IRS1 tyrosine phosphorylation in soleus muscle of the irbesartan-treated transgenic rats (Fig. 5). This was not observed in the epitrochlearis muscle of these same animals. Moreover, insulin action on the IR tyrosine phosphorylation, IRS1 associated with the p85 subunit of PI3K, and serine phosphorylation of Akt was likewise not up-regulated in either soleus or epitrochlearis muscle after long-term irbesartan treatment. These data support the contention that the improvements in insulin-stimulated glucose transport activity mediated by long-term selective AT_1 antagonism are likely due to some as yet unidentified adaptive response distal to these insulin-signaling elements.

Our finding that improvements in skeletal muscle insulin action on glucose transport mediated by AT_1-selective antagonism are not associated with up-regulation of insulin signaling complement previous investigations on the role of ANGII in modulation of insulin signaling and action in rodent models. Short-term stimulation of heart tissue [39] and cultured aortic smooth muscle cells [40] with ANGII has been shown to induce tyrosine phosphorylation of IRS1 by JAK2 kinase (associated with the AT_1 receptor), leading to a reduction in insulin-stimulated activation of PI3K associated with IRS1. These findings suggest that the reduction in PI3K activity occurs via cross-talk between ANGII and insulin-signaling pathways [39]. However, Ogihara et al [6] have shown more recently that long-term ANGII infusion into normal rats induces hypertension and insulin resistance, but, in contrast to the findings of Folli et al [40], insulin-stimulated tyrosine phosphorylation of IRS, PI3K activation, and Akt serine phosphorylation were actually significantly enhanced in muscle of the ANGII-infused rats. Moreover, the insulin resistance induced by the ANGII infusion in normal rats could be exacerbated by high-salt loading [6], indicating a potential role of total sodium load in the etiology of insulin resistance. The differences in results among the present study, the study of Ogihara et al [6], and the study of Velloso et al [39] may be because of the different experimental models, different tissues, or the differential effects of long- vs short-term modulation of ANGII levels and action. Clearly, further investigations are needed to more clearly assess the role and mechanism of ANGII action in the induction of skeletal muscle insulin resistance. The additional possibility that irbesartan may have inhibited ANGII-mediated renal tubular sodium reabsorption and aldosterone secretion, inducing a reduction in total body sodium and potentially contributing to improved whole-body and muscle insulin sensitivity, warrants further study.

Oxidative stress caused by ANGII is a key feature of ANGII-induced hypertension [41]. Recent evidence suggests that insulin resistance, which often accompanies ANGII-induced hypertension, is related to this ANGII-associated oxidative stress [6,22]. Long-term ANGII infusion into normal rats induced the accumulation of plasma cholesterylester hydroperoxide levels, indicating increased oxidative stress, and this alteration was normalized by treatment with tempol, a membrane-permeable superoxide dismutase mimetic [6]. The tempol treatment reduced insulin resistance of glucose transport and diminished insulin-stimulated PI3K activity in skeletal muscle of ANGII-infused rats. Moreover, it has recently been reported that treatment of TGRs with tempol leads to amelioration of the insulin-resistant state of these animals [22]. Collectively, these findings suggest that the mechanism underlying ANGII-induced insulin resistance involves oxidative stress, which may impair insulin signaling at a point distal to PI3K activation. In contrast, we have observed no improvement of whole-body insulin sensitivity and minimal alterations in skeletal muscle glucose transport in TGRs treated on a long-term basis with the antioxidant *R*-α-lipoic acid (JA Sloniger and EJ Henriksen, unpublished data). Nevertheless, one potential mechanism for the improvement in insulin-stimulated muscle glucose transport after long-term treatment of TGRs with a selective ANGII receptor antagonist is a reduction in muscle oxidative stress, as shown by Blendea et al [22]. In addition, recent evidence suggests that ANGII receptor antagonists can activate peroxisome proliferator–activated receptor γ, the target for thiazolidinedione insulin sensitizers [42].

The metabolic improvements seen in the irbesartan-treated TGRs cannot be attributed to an up-regulation of GLUT-4, as no change in skeletal muscle GLUT-4 protein expression was seen after treatment. This negative finding contrasts with the small, but significant, increase in skeletal and cardiac muscle GLUT-4 previously reported in obese Zucker rats treated with irbesartan [7]. It is possible that skeletal muscle GLUT-4 biosynthesis may be, for unknown

reasons, compromised in the TGR. Increases in skeletal muscle GLUT-4 are absent or minimal in this transgenic animal, even after a period of high-volume exercise training [20], which normally induces large increases in this variable in normotensive animal models [43].

In summary, the present investigation has demonstrated that long-term treatment of hypertensive and insulin-resistant male heterozygous TGRs with the selective ANGII (AT_1 subtype) receptor antagonist irbesartan causes improvements in whole-body insulin sensitivity and insulin-stimulated glucose transport and glycogen synthase activity in skeletal muscle. However, these irbesartan-induced improvements in insulin action on muscle glucose transport in the TGR were elicited without general increases in insulin action on critical elements of the IRS1-dependent insulin-signaling cascade. It is likely that the irbesartan-induced enhancement of insulin action on muscle glucose transport in this transgenic model involves an as yet unidentified adaptive response distal to Akt serine phosphorylation.

Acknowledgment

This study was supported in part by grant-in-aid 0256016Z from the American Heart Association to EJH.

References

[1] Defronzo RA, Ferrannini E. Insulin resistance: a multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidemia, and atherosclerotic cardiovascular disease. Diabetes Care 1991;14:173-94.

[2] Ferrannini E, Haffner SM, Mitchell BD, Stern MP. Hyperinsulinaemia: the key feature of a cardiovascular and metabolic syndrome. Diabetologia 1991;34:416-22.

[3] Reaven GM. Are insulin resistance and/or compensatory hyperinsulinemia involved in the etiology and clinical course of patients with hypertension? Int J Obes 1995;19:S2-S5.

[4] Weir M, Dzau V. The renin-angiotensin-aldosterone system: a specific target for hypertension management. Am J Hypertens 1999;12:205S-13S.

[5] Richey JM, Ader M, Moore D, Bergman RN. Angiotensin II induces insulin resistance independent of changes in interstitial insulin. Am J Physiol Endocrinol Metab 1999;277:E920-6.

[6] Ogihara T, Asano T, Katsuyuki A, et al. Angiotensin II–induced insulin resistance is associated with enhanced insulin signaling. Hypertension 2002;40:872-9.

[7] Henriksen EJ, Jacob S, Kinnick TR, Teachey MK, Krekler M. Selective angiotensin II receptor antagonism reduces insulin resistance in obese Zucker rats. Hypertension 2001;38:884-90.

[8] Iimura O, Shimamoto K, Matsuda K, et al. Effects of angiotensin receptor antagonist and angiotensin converting enzyme inhibitor on insulin sensitivity in fructose-fed hypertensive rats and essential hypertensives. Am J Hypertens 1995;8:353-7.

[9] Higashiura K, Ura N, Takada T, et al. The effects of an angiotensin-converting enzyme inhibitor and an angiotensin II receptor antagonist on insulin resistance in fructose-fed rats. Am J Hypertens 2000; 13:290-7.

[10] Sasaki K, Kushiro T, Nakagawa S, Kanmatsuse K. Effects of angiotensin II receptor antagonism on insulin sensitivity and sympathetic activity in spontaneously hypertensive rats. Jpn J Nephrol 1999;41:692-6.

[11] Mullins JJ, Peters J, Ganten D. Fulminant hypertension in transgenic rats harboring the *Ren-2* gene. Nature 1990;344:541-4.

[12] Sander M, Bader M, Djavidani B, Maser-Gluth C, Vecsei P, Mullins J. The role of the adrenal gland in hypertensive transgenic rat TGR(mREN2)27. Endocrinology 1992;131:807-14.

[13] Lee MA, Böhm M, Kim S, Bachmann S, Bachmann J, Bader M, et al. Differential gene expression of renin and angiotensinogen in the TGR(mREN2)27 transgenic rat. Hypertension 1995;25:570-80.

[14] Lee MA, Böhm M, Paul M, Bader M, Ganten U, Ganten D. Physiological characterization of the hypertensive transgenic rat TGR(mREN2)27. Am J Physiol Endocrinol Metab 1996;270: E919-29.

[15] Langheinrich M, Lee MA, Böhm M, Pinto YM, Ganten D, Paul M. The hypertensive Ren-2 transgenic rat TGR(mREN2)27 in hypertension research: characteristics and functional aspects. Am J Hypertens 1996;9:506-12.

[16] Senanayake PD, Moriguchi A, Kumagai H, Ganten D, Ferrario M, Brosnihan KB. Increased expression of angiotensin peptides in the brain of transgenic hypertensive rats. Peptides 1994;15:919-26.

[17] Campbell DJ, Rong P, Kladis A, Rees B, Skinner SL. Angiotensin and bradykinin peptides in the TGR(mREN2)27 rat. Hypertension 1995;25:1014-20.

[18] Flesch M, Schiffer F, Zolk O, et al. Angiotensin receptor antagonism and angiotensin converting enzyme inhibition improve diastolic dysfunction and Ca^{2+}-ATPase expression in the sarcoplasmic reticulum in hypertensive cardiomyopathy. J Hypertens 1997;15:1001-9.

[19] Holness MJ, Sugden MC. The impact of genetic hypertension on insulin secretion and glucoregulatory control in vivo: studies with the TGR(mREN2)27 transgenic rat. J Hypertens 1998;16:369-76.

[20] Kinnick TR, Youngblood EB, O'Keefe MO, Saengsirisuwan V, Teachey MK, Henriksen EJ. Modulation of insulin resistance and hypertension by voluntary exercise training in the TG(mREN2)27 rat. J Appl Physiol 2002;93:805-12.

[21] Sloniger JA, Saengsirisuwan V, Diehl CJ, et al. Defective insulin signaling in skeletal muscle of the hypertensive TG(mREN2)27 rat. Am J Physiol Endocrinol Metab 2005;288:E1074-81.

[22] Blendea MC, Jacobs D, Stump CS, et al. Abrogation of oxidative stress improves insulin sensitivity in the Ren-2 rat model of tissue angiotensin II overexpression. Am J Physiol Endocrinol Metab 2005;288:E353-9.

[23] Henriksen EJ, Halseth AE. Early alterations in soleus GLUT-4, glucose transport, and glycogen in voluntary running rats. J Appl Physiol 1994;76:1862-7.

[24] Gulve EA, Henriksen EJ, Rodnick KJ, Youn JH, Holloszy JO. Glucose transporters and glucose transport in skeletal muscles of 1 to 25 month old rats. Am J Physiol Endocrinol Metab 1993;264:E319-27.

[25] Hansen PA, Gulve EA, Holloszy JO. Suitability of 2-deoxyglucose for in vitro measurement of glucose transport activity in skeletal muscle. J Appl Physiol 1994;76:979-85.

[26] Thomas JA, Schlender KK, Larner J. A rapid filter paper assay for UDP glucose-glycogen glucosyltransferase, including an improved biosynthesis of UDP-^{14}C-glucose. Anal Biochem 1968;25:486-99.

[27] Henriksen EJ, Tischler ME, Johnson DG. Increased response to insulin of glucose metabolism in the 6-day unloaded rat soleus muscle. J Biol Chem 1986;261:10707-12.

[28] Uyeda K, Racker E. Regulatory mechanisms in carbohydrate metabolism. VII. Hexokinase and phosphofructokinase. J Biol Chem 1965;240:4682-8.

[29] Srere PA. Citrate synthase. Methods Enzymol 1969;13:3-10.

[30] Cortez MY, Torgan CE, Brozinick JT, Ivy JL. Insulin resistance of obese Zucker rats exercise trained at two different intensities. Am J Physiol Endocrinol Metab 1991;261:E613-9.

[31] Böhm M, Lee M, Kreutz R, et al. Angiotensin II receptor blockade in TGR(mREN2)27: effects of renin-angiotensin-system gene expression and cardiovascular functions. J Hypertens 1995;13:891-9.

[32] Weber MA. Interrupting the renin-angiotensin system: the role of angiotensin-converting enzyme inhibitors and angiotensin II receptor antagonists in the treatment of hypertension. Am J Hypertens 1999; 12:189S-94S.

[33] Burnier M, Brunner HR. Angiotensin II receptor antagonists. Lancet 2000;355:637-45.

[34] Israili ZH. Clinical pharmakokinetics of angiotensin II (AT_1) receptor blockers in hypertension. J Hum Hypertens 2000;14(Suppl 1):S73-S86.

[35] Henriksen EJ, Jacob S. Modulation of metabolic control by angiotensin converting enzyme (ACE) inhibition. J Cell Physiol 2003;196:171-9.

[36] Henriksen EJ, Kinnick TR, Teachey MK, O'Keefe MP, Ring D, Johnson KW, et al. Modulation of muscle insulin resistance by selective glycogen synthase kinase–3 inhibition in Zucker diabetic fatty rats. Am J Physiol Endocrinol Metab 2003;284: E892-E900.

[37] Thornburn AW, Gunbiner B, Bulacan F, Brechtel G, Henry RR. Multiple defects in muscle glycogen synthase activity contribute to reduced glycogen synthesis in non–insulin dependent diabetes mellitus. J Clin Invest 1991;85:522-9.

[38] Henry RR, Ciaraldi TP, Abrams-Carter L, Mudaliar S, Soo Park K, Nikoulina S. Glycogen synthase activity is reduced in cultured skeletal muscle cells of non–insulin-dependent diabetes mellitus subjects. J Clin Invest 1998;98:1231-6.

[39] Velloso LA, Folli F, Sun XJ, White MF, Saad MJ, Kahn CR. Cross-talk between the insulin and angiotensin signaling systems. Proc Natl Acad Sci U S A 1996;93:12490-5.

[40] Folli F, Kahn CR, Hansen H, Bouchie JL, Feener EP. Angiotensin II inhibits insulin signaling in aortic smooth muscle cells at multiple levels. A potential role for serine phosphorylation in insulin/angiotensin II crosstalk. J Clin Invest 1997;100:2158-69.

[41] Romero JC, Reckelhoff JF. State-of-the-art lecture. Role of angiotensin and oxidative stress in essential hypertension. Hypertension 1999;34:943-9.

[42] Kurtz TW, Pravenec M. Antidiabetic mechanisms of angiotensin-converting enzyme inhibitors and angiotensin II receptor antagonists: beyond the renin-angiotensin system. J Hypertens 2004;22:2253-61.

[43] Rodnick KJ, Henriksen EJ, James DE, Holloszy JO. Exercise training, glucose transporters and glucose transport in rat skeletal muscles. Am J Physiol Cell Physiol 1992;262:C9-C14.