Mechanism of improving effect of losartan on insulin sensitivity of non-insulin-dependent diabetes mellitus rats

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Abstract: The specific inhibition of angiotensin II action at AT1 receptors by losartan has been shown to decrease peripheral insulin resistance in type 2 diabetic patients and animal models. We examined the effect of losartan on the expression of insulin receptor substrate 1 (IRS-1), protein kinase B (PKB) and glucose transporter 4 (GLUT4), as well as the phosphorylation status of IRS-1 and the association between IRS-1 and phosphatidylinositol (PI) 3-kinase in skeletal muscle from fat-fed and-streptozotocin (STZ)-treated rats, an animal model of type 2 diabetes mellitus. In addition, the effects of losartan on GLUT4 translocation in muscle cells and on insulin sensitivity were also evaluated. Muscle tissues were isolated from male losartan-treated and untreated normal or non-insulin-dependent diabetes mellitus (NIDDM) rats with a dose of 4 mg/kg per day for 6 weeks. Oral administration of losartan improved insulin sensitivity, which was determined by an oral glucose tolerance test (OGTT). In skeletal muscles, the protein levels of IRS-1, PKB and GLUT4 in NIDDM rats were not significantly different from those of the control rats, and they were not affected by losartan. The levels of IRS-1 tyrosine phosphorylation, PI 3-kinase activity associated with IRS-1 and PKB activation after stimulation with insulin in muscle tissue of NIDDM rats were significantly decreased (P<0.01) compared with those in the control rats, while they were not increased by losartan. Losartan had a major effect on GLUT4 translocation in myocytes, as it significantly increased (P<0.05) the insulin-induced amounts of GLUT4 in plasma membrane (PM) and T-tubules (TT) in myocytes from NIDDM rats. Consistent with these results, the plasma glucose level in losartan-treated NIDDM rats was decreased (P<0.05) compared with that in untreated NIDDM rats. Our results suggest that losartan may exert beneficial effects on insulin resistance by increasing the translocation of GLUT4 in muscle tissue, which is probably associated with a non-PI 3-kinase-dependent mechanism.

Key word: losartan; insulin sensitivity; non-insulin-dependent diabetes mellitus (NIDDM); GLUT4 translocation; streptozotocin
Insulin action is initiated through hormone binding to cell surface insulin receptors, which activates the protein kinase associated with the β subunit\textsuperscript{[15]}\textsuperscript{[15]}. The activated insulin receptor binds to and catalyzes the tyrosine phosphorylation of the insulin receptor substrates (IRSs)\textsuperscript{[3-6]}. Tyrosine phosphorylation of these substrates allows them to interact with SH\textsubscript{2}-domain-containing proteins including the regulatory subunit of phosphatidylinositol 3-kinase (PI) 3-kinase\textsuperscript{[7]}\textsuperscript{[7]}. Activation of PI 3-kinase leads to activation of PDK 1 and 2 (phosphatidylinositol 3,4,5-triphosphate-dependent protein kinase)\textsuperscript{[8]}\textsuperscript{[8]}, that in turn activates the serine/threonine protein kinase B (PKB) and PKC\textsubscript{ξ}, which have been implicated in the insulin signaling pathway, leading to GLUT4 gene expression\textsuperscript{[9]}\textsuperscript{[9]} and translocation to the plasma membrane and glucose uptake\textsuperscript{[10,11]}\textsuperscript{[10,11]}. Another important biological action of insulin is mitogenesis, which proceeds through Grb2/son of sevenless (Sos) and Ras, leading to activation of the mitogen-activated protein (MAP) kinase isoforms extracellular signal-regulated kinase (ERK1/2)\textsuperscript{[12]}\textsuperscript{[12]}. Insulin resistance, i.e. impairment of insulin-stimulated glucose uptake, is a known feature of subjects with hypertension\textsuperscript{[13]}\textsuperscript{[13]} and non-insulin-dependent type 2 diabetes mellitus\textsuperscript{[14]}\textsuperscript{[14]}. However, the mechanism of insulin resistance remains unclear\textsuperscript{[15]}\textsuperscript{[15]}. Alterations in insulin receptor binding and function, insulin signal transduction such as decrease in IRS-1 protein phosphorylation and IRS-1 associated PI3-kinase activity\textsuperscript{[16,17]}\textsuperscript{[16,17]} as well as alterations in the translocation of GLUT4 and the total number or intrinsic function of GLUT4 may be involved.

Skeletal muscle accounts for nearly 40% of body mass and is the main tissue involved in the insulin-induced stimulation of glucose uptake. Several studies have shown by using the euglycemic-hyperinsulinemic clamp that at circulating levels of insulin in the upper physiological range most of the infused glucose is taken up by skeletal muscle and converted mainly into glycogen\textsuperscript{[18]}\textsuperscript{[18]}. Based on its quantitative role in glucose uptake, alterations in muscle insulin sensitivity have a profound impact on whole body glucose disposal. In this regard, non-insulin-dependent diabetic patients show deficient insulin-induced glucose transport in skeletal muscle\textsuperscript{[19,20]}\textsuperscript{[19,20]} and insulin resistance of skeletal muscle glucose transport represents a major defect in the normal maintenance of euglycaemia\textsuperscript{[21]}\textsuperscript{[21]}. Thus, This subject is concerned chiefly with the study of the defects of insulin-signalling factors in skeletal muscle from insulin-resistant animal models.

Losartan, a prototype selective non-peptide AT\textsubscript{1} receptor antagonist, is a potent and orally active agent\textsuperscript{[22]}\textsuperscript{[22]}. By inhibiting the binding of angiotensin II at AT\textsubscript{1} receptors, losartan and its active metabolite (E3174) block the vasoconstritor and aldosterone secreting effects of angiotensin II, regardless of the source of secretion of angiotensin II. It is controversial whether losartan improves insulin sensitivity in type 2 diabetes mellitus\textsuperscript{[23,24]}\textsuperscript{[23,24]}. Some studies carried out in human volunteers indicate that losartan improves insulin sensitivity\textsuperscript{[25,26]}\textsuperscript{[25,26]}. Whereas other studies suggest that losartan has neutral effect on insulin sensitivity in genetically hypertensive and fructose-fed rats\textsuperscript{[27,28]}\textsuperscript{[27,28]}. However, the effects of losartan on insulin sensitivity and glucose tolerance have not been investigated in the fat-fed, STZ-induced diabetic model. With this in mind, the present investigation was undertaken to study the effects of chronic treatment with losartan on insulin sensitivity and glucose tolerance in the non-insulin-dependent diabetes mellitus (NIDDM) model. Moreover, we investigated whether losartan treatment affects intracellular signaling in skeletal muscle of the NIDDM rats.

1 MATERIALS AND METHODS

1.1 Materials. The reagents for SDS-PAGE and immuno-blotting were obtained from Biovision (Palo Alto, CA, USA) and apparatus from Liu Yi Co. (Beijing, China). Tris, NP-40, porcine insulin, and nitrocellulose (NC) membranes were obtained from Sigma (St. Louis, MO, USA). Protein A-Sepharose 6 MB was from Pharmacia (Uppsala, Sweden). The monoclonal anti-phosphotyrosine antibody (αPY, PY99) and Akt were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The anti-rat carboxy-terminal IRS-1 antibody (Clone 8-63) was from NeoMarkers (Fremont, CA, USA). The anti-rat carboxy-terminal IRS-1 antibody (Clone 8-63) was from NeoMarkers (Fremont, CA, USA). The monoclonal anti-phosphotyrosine antibody (αPY, PY99) and Akt were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The anti-rat carboxy-terminal IRS-1 antibody (Clone 8-63) was from NeoMarkers (Fremont, CA, USA). The monoclonal Anti-rat Glut4 was from R & D Systems (Minneapolis, AL, USA). Enhanced
Chemiluminescence (ECL) detection reagents were from KPL (Gaithersburg, MA, USA). One Touch II Glucose Analyzer was from Janson & Janson, and insulin RIA Kit from Beifang Biotech Research Center (Beijing, China). All other chemicals were of the highest analytical grade.

1.2 NIDDM rat model and treatment protocol. Male Sprague-Dawley rats (the experimental animal center of Wuhan University) aged 8 weeks, and weighing approximately 200 g, were used for all studies. They were housed 5 per cage in a room with a 12/12-hour light/dark cycle and an ambient temperature of 22 to 25°C. Animals were fed either a normal chow diet consisting (as a percentage of total kcal) of 12% fat, 60% carbohydrate, and 28% protein or a high-fat diet consisting of 41% fat, 41% carbohydrate, and 18% protein. After 2 weeks on either diet, animals (with the exception of noninjected controls) were anesthetized with ketamine (65 mg/kg) and xylazine (7 mg/kg) after an overnight fast and injected with STZ (30 mg/kg) into the tail vein via a temporary indwelling 24-gauge catheter. Animals had free access to food and water after the STZ injection, and both STZ-injected and noninjected animals were continued on their original diets (chow or fat) for the duration of the study. The animals showing fasting glucose levels >6.7 mmol/L at 72 h after STZ injection were considered as diabetic. Rats that received saline were considered as control animals.

The control and diabetic groups were then further subdivided into treated and untreated groups: control (C, n=10), control treated (CT, n=10), NIDDM (D, n=12), NIDDM treated (DT, n=12). Animals were treated with losartan for 6 weeks (final dose of 4 mg/kg/day administered in drinking water). The control group received an equal volume of the vehicle. At the end of the treatment rats were fasted overnight (16 h) and then anesthetized by pentobarbital (100 mg/kg i.p.). Skeletal muscle was quickly removed from hind legs (fast-and slow-twitch muscle) before and 15 min after insulin injection (5 U/kg, i.v.), freeze-clamped, powdered under liquid nitrogen, and stored at -70°C before further processing.

1.3 Blood sample collection and OGTT. At the end of 6 weeks study animals were fasted for 18 h. Blood samples were collected from the tail vein to record glucose and insulin levels. Another blood sample was collected on the same day 2 h after giving the standard feed. During the oral glucose tolerance test (OGTT), glucose (1.5 mg/kg) was administered to 18 h fasted rats. Blood samples (100–200 µl) were collected from the tail vein at 0, 10, 20, 30, 60 and 120 min under light ether anesthesia. Serum was separated immediately and analyzed for glucose by using One Touch II Glucose Analyzer and insulin by RIA kits. The results were expressed as integrated area under the curve (AUC) for glucose and insulin, that was calculated by the trapezoid rule [AUC=C1+C2/2×(t1-t2)] and changes in glucose and insulin concentrations during OGTT were expressed as AUCglucose (mg·dl·min⁻¹) and AUCinsulin (µU/ml·min⁻¹) respectively.

1.4 Calculation of insulin sensitivity. The index of insulin sensitivity was calculated using the formula (100/square root of [fasting plasma glucose×fasting plasma insulin]×[mean glucose×mean insulin during OGTT]) recently developed by Matsuda and De Fronzo[29].

1.5 Subcellular fractionation. Plasma membranes, T-tubules, and GLUT4-enriched intracellular membranes were isolated from muscles (8–10 g, mixed gastrocnemius and quadriceps) using a procedure as described[30]. This subcellular fractionation protocol has been extensively characterized with immunologic and enzymatic markers[30]. In brief, this technique allows the simultaneous and separated isolation of plasma membrane, T-tubules, and intracellular membrane vesicles from the same muscle homogenate. GLUT4 content was determined in fractions obtained from saline- or insulin-infused rats by Western blotting, as described below.

1.6 Western blotting. Aliquots (10 µg) of membrane fractions or aliquots (50 µg) of muscle homogenate were subjected to SDS-PAGE (7.5% gel) and electrophoretically transferred to nitrocellulose (NC) filter membranes for 5 h. NC membranes were then blocked for 2 h at room temperature with Block Solution provided by the ECL kits. This step was followed by overnight incubation at 4°C with primary antibodies, as described in the figure legends. The NC membranes were then washed for 30 min using Wash Solution (ECL kits), followed by a 1-h incubation with either anti-mouse or anti-rabbit IgG conjugated to horseradish-peroxidase in Block Solution. The NC membranes were washed for 30 min in Wash Solution, and the immunoreactive bands were detected by the enhanced chemiluminescence method. A muscle standard (an unrelated crude membrane fraction) was run on every gel for comparison of samples from different immunoblots.

1.7 Tyrosine phosphorylation of IRS-1 and association of IRS-1 with p85 regulatory subunit of PI 3-kinase. Muscles were immediately homogenized in a 10× volume of ice-cold buffer [in mmol/L: 50 Hepes (pH 7.5), 137 NaCl, 1 MgCl2, 1 CaCl2, 10 Na2HPO4, 50 NaF, 2 EDTA, 40β-glycerophosphate, 2 Na2VO4, 0.2 PMSF, and 1% NP-40,
10% glycerol, and 2 μg/ml aprotinin] and centrifuged at 15,000 g at 4°C for 30 min. Protein was determined using a kit from Bio-Rad (Richmond, CA). Muscle lysates (1 mg of protein) were immunoprecipitated (IP) with 2 μg of anti-phosphotyrosine (PY99) or anti-IRS-1 coupled to protein A-Sepharose overnight at 4°C. The immune complex was washed three times in phosphate-buffered saline (PBS) (pH 7.4) containing 1% NP-40 and 2 mmol/L Na3VO4, re-suspended in Laemmli buffer, and boiled for 5 min. Proteins were resolved on SDS-PAGE (6% gel) and processed for Western blot analysis as described above.

1.8 PKB/Akt activity. The cell lysates (described as above) from skeletal muscle were subjected to 10% SDS-PAGE and immunoblotted with an anti-Akt antibody or phosphospecific Akt (Ser 473) antibody. Band intensities were quantitated by densitometric analysis.

1.9 Statistical analysis. All results are expressed as means±SEM. The data were analyzed statistically using analysis of variance (ANOVA) followed by Tukey’s test. Student’s t test was used when the values of two groups were analyzed. The level of significance was set at P<0.05.

2 RESULTS

2.1 Serum glucose and insulin levels

Both fasting and fed glucose levels were significantly higher in the NIDDM control as compared to the Sprague-Dawley control. Losartan treatment significantly prevented the raise in blood glucose levels in both fasting and fed NIDDM animals. However, losartan did not alter both fasting and fed glucose levels in non-diabetic control animals (Table 1). The insulin levels in the NIDDM control group was significantly high as compared to the SD control group. Treatment with losartan significantly decreased the insulin level in the NIDDM-treated (DT) group.

The AUCinsulin was significantly greater for the NIDDM control group. Despite high insulin levels, the AUCglucose was found to be high in these rats (Table 1). Treatment with losartan was not found to alter the AUCinsulin (Table I) in the non-diabetic rats. However, the AUCinsulin in the NIDDM rats treated with losartan was significantly reduced. This was associated with a significant reduction in the AUCglucose for the NIDDM rats treated with losartan.

2.2 Insulin sensitivity

The insulin sensitivity in the NIDDM rats was significantly lower as compared to the SD control group. The impaired insulin sensitivity in the NIDDM rats was improved following losartan treatment (Fig. 1).
rosine phosphorylation reached after stimulation with insulin was reduced by 47% ($P<0.05$) as compared with their control rats (Fig. 2 A and B). Treatment with losartan did not improve the state of IRS-1 tyrosine phosphorylation stimulated by insulin. As evaluated by scanning densitometry, there was no difference in the levels of IRS-1 proteins in each group.

**2.4 PI 3-kinase activity associated with IRS-1**

In control rats, insulin stimulation resulted in an important increase in the association of p85 with IRS-1 (Fig. 3 A and B). Compared with control rats, NIDDM rats exhibited a reduced response to insulin at this level. After insulin stimulation the amount of p85 associated with IRS-1 was reduced by 54% ($P<0.05$) compared with control animals. Treatment with losartan did not affect the level of p85 associated with IRS-1 in control and NIDDM rats.

**2.5 Akt activation**

In control rats, insulin administration resulted in a noticeable increase in the phosphorylation of Akt at Ser 473 (Fig. 4A and B). NIDDM rats exhibited a reduced response to insulin, and the level of phosphorylation of Akt after insulin stimulation was decreased by 40% ($P<0.02$) compared with values measured in control rats (Fig. 4A and B). Losartan administration did not improve the level of phosphorylation of Akt induced by insulin both in control and NIDDM rats. Among the four groups, there was no statistically significant change in expression of Akt (Fig. 4C and D).

**2.6 Protein expression and insulin-induced translocation of GLUT4**

Total GLUT4 protein levels were not significantly different among the C, CT, D and DT groups (Fig. 5A). We next investigated the effect of fat-feeding, STZ-treatment and losartan administration on GLUT4 translocation to both cell surface compartments of muscle cells (i.e., the plasma membrane and the T-tubules) to precisely determine the locus of insulin resistance in this animal model and the mechanism underlying the action of losartan (Fig. 5B–D). insulin stimulation induced translocation of GLUT4 from the intracellular membranes (~35%, from 328 ± 54 to 212

![Image](image_url)
± 25 relative densitometric units [RDU], $P<0.05$) to the plasma membrane (+100%, from 95±15 to 193 ± 25 RDU, $P<0.05$) and the T-tubules (+35%, from 51 ± 6 to 69 ± 4 RDU, $P<0.05$) in control rats fed a standard chow diet. In marked contrast, insulin failed to induce GLUT4 translocation to either the plasma membrane or the T-tubules in skeletal muscle of fat-fed, STZ-treated rats (NIDDM) (Fig. 5 B–D). The impaired translocation of GLUT4 to cell surface compartments in NIDDM rats was reversed by losartan treatment. These results clearly show that the reduced insulin-stimulated glucose uptake in muscle of fat-fed and STZ-treated rats is linked to a defective translocation of GLUT4 glucose transporters to both cell surface compartments of skeletal muscle cells and improved by losartan administration.

3 DISCUSSION

In the present study we developed a novel animal model for type 2 diabetes, fat-fed/STZ rats, which provide a relatively inexpensive and easily accessible rodent model that is not extremely obese and simulates the natural history and metabolic characteristics of patients with type 2 diabetes[31]. Clinical and epidemiological studies suggest that β-cell dysfunction and insulin resistance are two central, interrelated defects in the pathophysiology of type 2 dia-
When insulin resistance, a state of reduced insulin sensitivity, occurs, in order to maintain glucose homeostasis, β-cells increase insulin secretion and release, and enhanced insulin levels can compensate for insulin resistance. With the development of insulin resistance, pancreatic β-cell function is progressively and irreversibly impaired by over-secretion, chronic hyperglycemia and other detrimental factors. At this period, β-cells decompensate and reduce insulin secretion, and then, hyperglycemia and overt diabetes emerge. Recent reports [33] have indicated that insulin resistance may be caused by excess nutrient supply. Both excess glucose and fat can cause insulin resistance in muscle and fat tissue, while excess fat can cause impaired suppression of endogenous glucose production. The present animal model was based on the rationale described above. At first, we used high-fat diets to induce insulin resistance in SD rats. The serum glucose concentrations were similar in chow-fed and fat-fed rats after 2 weeks of high-fat diet, whereas insulin concentrations in fat-fed rats were significantly higher than their chow-fed controls (data not shown). The association of normoglycemia and hyperinsulinemia suggested that fat-fed rats were insulin resistant. Conversion of prediabetes to frank hyperglycemia in patients with type 2 diabetes is associated with a decline in the secretory capacity of the pancreatic β-cell[34,35], but this failure of β-cell compensation is relative, not absolute. We attempted to simulate this evolution from a state of insulin resistance and absolute hyperinsulinemia to “rela-
tive” hypoinsulinemia by injecting insulin-resistant, fat-fed rats with a moderate amount of STZ, that lowers the serum insulin concentration to relatively lower level, although which was still higher than normal, chow-fed rats. Comparison of the data in Table 1 indicates that we were successful in this attempt.

In this study, the NIDDM control rats showed significantly higher levels of fasting and fed glucose levels as compared to non-diabetic control rats. This is in consistent with earlier reports[36,37]. We also found an increase in insulin levels and AUC insulin after glucose load in fat-fed/STZ-diabetic rats. Despite high insulin levels the AUC glucose of the diabetic animals was greater than non-diabetic rats. However, the AUC insulin of NIDDM rats treated with losartan was found to be significantly lower as compared to the NIDDM control rats. This suggests that in normal animals losartan does not alter the release of insulin, but in conditions like hyperinsulinemia, it increases the insulin sensitivity for effective glucose disposal. In a clinical study it has been reported that losartan blocks Ang- II induced vasoconstriction and thereby increases glucose delivery to skeletal muscle by improving insulin sensitivity[38].

Numerous clinical investigations have shown that angiotensin converting enzyme (ACE) inhibitor can improve insulin action on whole-body and skeletal muscle glucose disposal in insulin-resistant and hypertensive subjects[39,40]. However, it is controversial whether insulin sensitivity in type 2 diabetes mellitus is improved by losartan, which lowered blood pressure through a different mechanism from ACE inhibitors[41]. Initial studies investigating the effect of acute antagonism of angiotensin II action in insulin-resistant obese Zucker rats[42] or aged rats[43] reported no modification of insulin-stimulated glucose transport activity or insulin signaling in skeletal muscle. However, recent evidence has been more enlightening. The acute administration of the AT1-specific angiotensin II receptor blocker to insulin-resistant obese Zucker rats leads to a dose-dependent increase in glucose tolerance and whole-body insulin sensitivity[44]. The chronic administration of AT1 receptor antagonists to insulin-resistant fructose-fed rats[45] or spontaneously hypertensive rats[46] also leads to significant improvements in whole-body insulin sensitivity. In the present study, the index of insulin sensitivity was found to be significantly lower in fat-fed/STZ-diabetic rats as compared to controls. This indicates that NIDDM rats are insulin resistant. The specific mechanisms underlying insulin resistant states are heterogeneous and may include a receptor defect or post receptor defect or combination of both[47,48]. Losartan treatment significantly increased the index of insulin sensitivity in the NIDDM rats. It is difficult to explain the discrepancy of the effect of losartan on insulin sensitivities in these investigations. However, one possibility is the differences in animal models. Different animal models of insulin-resistant rats attribute to different pathogenesis. Another possible explanation is that some studies emphasized particularly on the systemic effect of losartan on insulin resistance and others on the local effect of losartan in each organ. There are distinctions between experiments in vivo and in vitro because of the absence of influence of blood contents in experiments with isolated skeletal muscle and fat cells. The effect of losartan on insulin resistance differs between systemic and local in insulin-resistant rats. Therefore, the discrepancy between the local and systemic effects of losartan may explain previous contradictory findings. The third possible explanation is the dose range. Since the dose of losartan used in the present study may be relative high compared with other pertinent investigations, the possibility cannot be denied that the lack of an improving effect of losartan on the OGTT and the glucose infusion rate in some studies may be attributable to their low dose treatments.

The intracellular signaling mechanisms after insulin receptor stimulation have been investigated extensively in recent years. Our results suggest that the protein levels of IRS-1, PKB and GLUT4 were not significantly different in skeletal muscle between NIDDM and control rats, but the levels of IRS-1 tyrosine phosphorylation, PI 3-kinase activity associated with IRS-1 and PKB activation reached significantly higher levels of IRS-1 tyrosine phosphorylation, PI 3-kinase activity associated with IRS-1 and PKB activation reached after stimulation with insulin in muscle tissue from NIDDM rats were significantly decreased. The PI 3-kinase/Akt pathway has been recognized as a major pathway in insulin signaling. In the present study, we found that losartan treatment had no significant effect on insulin –stimulated IRS-1 phosphorylation, PI 3-kinase activity associated with IRS-1 and PKB/Akt activity in skeletal muscle from NIDDM rats, although glucose uptake was increased in this setting. These results are surprising because activation of Akt is believed to cause an increase in glucose uptake[49,50]. The influence of losartan on PI 3-kinase remains to be elucidated.

It was reported that GLUT4 activation was important for glucose transport into cells[51]. We, therefore, examined the expression and translocation of GLUT4 protein in the skeletal muscle and found that there were no significant differences in the expression of GLUT4 among the control, NIDDM and losartan-treated NIDDM groups.
However, losartan treatment may facilitate GLUT4 translocation to both T-tubules, the principal component of the muscle cell surface, and plasma membrane in skeletal muscle from NIDDM rats. Recent work in our laboratory suggests that there are two divergent responses of GLUT4 initiated by insulin stimulation. The first response involves the recruitment of GLUT4 transporters from intracellular reserves and their subsequent insertion into the plasma membrane. The second pathway results in an increase in the intrinsic activity of the transporters. Recent findings suggest that p38 MAPK may be involved in regulation of the intrinsic activity of the transporter and the activities of MAP kinase including ERK1/2 and p38 MAP kinase were increased by losartan. Based on the above findings, we considered that the enhancing effect of angiotensin AT1 receptor antagonist on skeletal muscle glucose uptake may be attributable to MAP-kinase activation, which improves the intrinsic activity of GLUT4. However, whether p38 MAPK activation contributes to increase in GLUT4 translocation induced by losartan administration has yet to be clearly identified.

In conclusion our study represents the first report that losartan improves insulin sensitivity through increasing the translocation of GLUT4 in muscle tissue, which is probably associated with a non-PI 3-kinase-dependent mechanism.

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