Voluntary Running Improves *In Vivo* Insulin Resistance in High-Salt Diet–Fed Rats

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It is well known that exercise training, including voluntary running (VR), improves insulin resistance. However, the effect of VR on insulin resistance induced by high salt intake is unclear. The aim of this study was to determine whether VR would improve the glucose utilization in normal male Sprague-Dawley rats fed a high-salt diet (HSD) on 2-week early prevention and 1-week midway intervention protocols. *In vivo* glucose utilization was measured by euglycemic clamp technique. Further analyses of the possible changes in insulin signaling occurring in skeletal muscle were performed by Western blot and reverse transcription polymerase chain reaction (RT-PCR). The glucose infusion rates (GIRs) after 2 weeks of HSD feeding were decreased (HSD vs. control: 21.5 ± 0.8 vs. 27 ± 0.5 mg/kg body wt/min; $P < 0.05$), and improved by 2 weeks VR to 30.5 ± 1.5 mg/kg body wt/min. Additionally, the GIRs after 3 weeks of HSD feeding were decreased (HSD vs. control: 20.0 ± 0.3 vs. 26.5 ± 0.6 mg/kg body wt/min; $P < 0.05$), and they also improved by the third week of VR (28.5 ± 0.7 mg/kg body wt/min vs. sedentary; $P < 0.01$). There were no differences in skeletal muscle for the total mass of insulin receptor-beta (IR-β), IR substrate-1 (IRS-1), Akt, and glucose transporter 4 (GLUT4) in any of the groups of 2 weeks of HSD loading control and VR. VR did not regulate the enhanced tyrosine phosphorylation of IR-β and IRS-1 by 2 weeks of HSD feeding. However, the enhanced serine phosphorylation of Akt and the tyrosine phosphorylation of GLUT4 were significantly inhibited by the early VR. HSD also impaired GLUT4 content in the plasma membrane and mRNA expression, but the decreases were improved by 2 weeks of VR. These results suggest that early voluntary exercise would prevent the development of insulin resistance induced by an HSD due in part by enhancing the impaired GLUT4 translocation and mRNA expression in skeletal muscle. Exp Biol Med 232:1330–1337, 2007

Key words: high-salt; insulin resistance; voluntary running; Akt; GLUT4

Introduction

Insulin resistance is a key feature of the metabolic syndrome, which is characterized by obesity, atherogenic, dyslipidemia, and hypertension (1). There is increasing evidence that excessive dietary salt induces development not only of hypertension in humans and animal models, but also insulin resistance (2–4). Furthermore, high-salt (HS) loading in Sprague Dawley rats causes insulin resistance, decreases glucose utilization in skeletal muscle, and increases blood pressure (3, 4). Ogihara *et al.* (3) reported that the molecular mechanism of HS diet (HSD)-induced insulin resistance is unique and differs from those mechanisms attributed to other factors, such as high fat (5), aging (6), and obesity (7, 8). Moreover, insulin-induced tyrosine phosphorylation of insulin receptor-beta (IR-β) and insulin receptor substrate-1 (IRS-1) in the skeletal muscle is enhanced by salt loading, which induced insulin resistance with enhanced insulin signaling in rats, and the decreased insulin sensitivity was not reversed by an insulin sensitizer (3).

It is well known that physical exercise improves insulin resistance (9). Epidemiologic studies demonstrate that lifestyle intervention programs can prevent or delay insulin resistance and type 2 diabetes (10). Increasing evidence has shown that exercise training improves insulin resistance induced by streptozotocin (STZ)-induced diabetes (11), fructose feeding (12), obese Zucker rats (13), maturation (14, 15), and high-fat feeding (16, 17). Furthermore, exercise training enhances insulin sensitivity in human skeletal muscle (18, 19). Mechanisms include improvements

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of impaired insulin signaling (14, 18, 20–22). However, little is known regarding the effects of exercise training on insulin resistance induced by HSD feeding.

The first objective of this study is to determine the preventive and midway intervention effects of voluntary running (VR) on the impaired insulin sensitivity in HSD-fed rats. *In vivo* insulin action was measured using the euglycemic clamp technique in the catheterized conscious animals. Our second objective was to investigate in HSD-fed rats the preventive effects of VR on the insulin signaling pathway in the skeletal muscle, the main site of post-absorptive glucose disposal and a major insulin target (23, 24). Our results suggest that VR prevents and improves HS-induced insulin resistance that is associated with the regulation of glucose transporter 4 (GLUT4) translocation and mRNA expression, but not the enhanced IR-β and IRS-1 phosphorylation in skeletal muscle.

Materials and Methods

Materials. Male Sprague-Dawley rats (CLEA Japan, Tokyo, Japan) weighing approximately 150 g were used in experiments. Neutral insulin was purchased from Novo Nordisk (Bagsvaerd, Denmark). Anti–IR-β, anti–IRS-1, antiphosphotyrosine, and anti-GLUT4 antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA), and anti-Akt and anti–phospho-Akt (Ser473) from Upstate (Lake Placid, NY). Hyperfilm was purchased from Amersham (Amersham, UK). All other reagents were of biochemical grade.

Animal Care and Experimental Protocol. Rats were housed individually at 23°C with light from 0800–2000 hrs with free access to laboratory chow diet (CE-2; CLEA Japan) and water for 1 week. Food, water intake, and body weight were measured three times per week. After the baseline period, rats were randomly selected according to the experimental procedure (Fig. 1). Rats in the exercise-trained group were housed in the cage with a running wheel throughout the experiment; thereby, rats were allowed voluntary running exercise. In the experimental protocol A (Fig. 1A), 18 rats were divided into three groups: normal control group (fed standard diet containing 0.3% NaCl [NC]), salt loading group (8% NaCl [HS]), and VR group fed with HSD (HST). Additionally, to investigate the midway intervention effect of VR in HSD-fed rats, the experimental protocol B (Fig. 1B) was performed by other rats. Rats who underwent two weeks of HSD loading were subdivided into two groups: sedentary control (HSC) and voluntary running group (HSR). Daily running distance for exercise-trained rats was calculated from the number of wheel revolutions (1.0 m/revolution). Unless rats ran on average at least 1000 m/day, they were excluded from the training group. Sedentary rats were housed individually in cages without an exercise wheel. All procedures were approved by the Animal Use and Care Committee of Nagoya University.

Euglycemic Clamp Procedures. On the last day of voluntary training, 24 hrs before the clamp, exercising animals were denied access to their running wheels. Two hours later, animals were anesthetized using an intraperitoneal injection of pentobarbital sodium (50 mg/kg body wt). The right jugular vein and the left carotid artery were exposed, and catheters were inserted and exteriorized at the back of the neck and encased in silastic tubing as described (25). After overnight fast (16 hrs), the baseline blood sample was drawn. All rats were then submitted to a euglycemic clamp procedure (26) to valuate tissue sensitivity to exogenous insulin. The euglycemic clamp study was performed essentially as described, with some modifications (15, 27). In brief, the venous catheter was used for the infusion of glucose (20%) and insulin (6 mU/kg/min). During euglycemic clamp, blood glucose concentrations were measured every 10 min, and the euglycemia was maintained at 4.2 ± 0.1 m*M* of baseline. The means of glucose infusion rate (GIR) values from 100 to 120 mins for the euglycemic clamp procedure were regarded as an index of the whole-body insulin action, since a plateau in the GIR was achieved during these periods of time, as reported previously (25, 28). At the end of the euglycemic clamp, the gastrocnemius and soleus were excised under the pentobarbital sodium, made free of blood, and immediately frozen at liquid nitrogen temperature and stored at –80°C until analysis.

Electrophoresis and Immunoblotting Analysis. Procedures for detecting and quantifying the amounts of skeletal IR-β, IRS-1, Akt, GLUT4 protein, and the phosphorylation of IR-β, IRS-1, and Akt were carried out as described previously (25, 27). For immunoprecipitation of GLUT4 and immunoanalysis, the subcellular membrane fractions were treated with lysis buffer (250 m*M* sucrose, 20 m*M* Tris, 0.2 m*M* EDTA, 25 m*M* NaF, 10 m*M* $Na_2P_2O_7$, 100 m*M* NaCl, and 40 m*M* phenylmethylsulfonyl fluoride [PMSF]; 5 μg/ml leupeptin, 5 μg/ml aprotinin, 1 μg/ml pepstatin, and 1% Triton X-100) and incubated with 20 μl/ml protein A sepharose (10% wt/vol) for 1 hr. The protein A sepharose was pelleted at 10,000 *g* for 30 secs, and the supernatant was incubated overnight (gently rocking the reaction mixutre) with 5 μg/ml rabbit polyclonal GLUT4 antibody. After this, 0.3% bovine serum albumin and 50 μl/ml protein A sepharose were added after the overnight incubation and the incubation was continued for an additional 2 hrs. The immune complexes were washed as described (25). Samples were resuspended in treatment buffer containing β-mercaptoethanol, boiled for 5 min, and separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE; 10% acrylamide gel). The membranes were then incubated with the antiphosphotyrosine antibody overnight at 4°C. Bound antibodies were detected by incubation with goat anti-rabbit IgG for 1 hr at room temperature. After washing, blotted proteins were visualized using the Western blotting detection system (ECL Plus; Amersham) and then exposed to Hyperfilm (ECL plus, Amersham UK). Quantification of the band intensity was

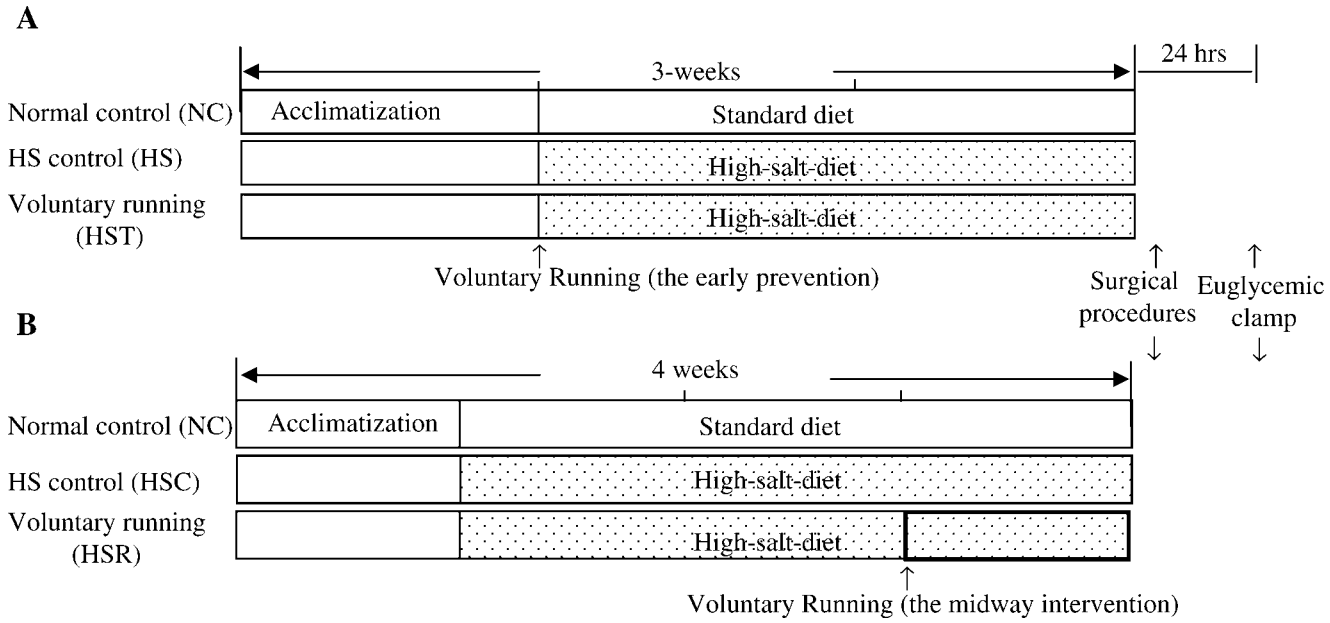

Figure 1. Experimental protocol.

performed using the public domain National Institutes of Health image program.

Membrane Preparation of Skeletal Muscle. Plasma membranes (PMs) were isolated using a previously characterized procedure (29). In brief, the hind limb muscles (about 5 g) were placed in Tris buffer (20 m*M* Tris base, 250 m*M* sucrose, 0.2 m*M* EDTA, and 40 m*M* PMSF; and 5 μg/ml leupeptin, 5 μg/ml aprotinin, and 1 μg/ml pepstatin), pH 7.4, at 4°C, and homogenized with a Polytron homogenizer (Clifton, NJ). Following several differential centrifugation steps, the resulting pellets were resuspended using a homogenizer in buffer, and they were loaded on top of discontinuous sucrose gradients (25%, 32%, and 35%). Following centrifugation at 150,000 *g* for 16 hrs, fractions were collected on the 25% sucrose layer, diluted with buffer, and centrifuged at 190,000 *g* for 1 hr. The resulting pellets were resuspended in appropriate volumes of Tris buffer, were used fresh for protein measurements, and were kept at –80°C until used for Western blot analysis.

Reverse Transcription Polymerase Chain Reaction (RT-PCR) Analysis for GLUT4 mRNA. Total cellular RNA was extracted from powdered soleus muscle (around 30 mg) by a modified guanidinum isothiocyanate method (30) with the ISOGEN reagent (Nippon Gene, Tokyo, Japan) following the instructions of the manufacturer. DNA-free RNA was eluted using diethylpyrocarbonate-treated water. Total RNA concentrations were determined spectrophotometrically. The GLUT4 primers used for PCR were as follows: 5′-AGAGTGCCTGAAACC-3′ and 5′-CCCTAAGTATTCAAGTTCTG-3′. PCR was performed under the following conditions: 95°C ×10 mins, 38 cycles (95°C × 30 secs, 60°C × 1 min). Then, the samples (15 μg) were electrophoretically run in 1.2% (wt/vol) agarose-formaldehyde gels, stained with 0.5 μg/ml ethidium bromide, visualized under an ultraviolet light, and photographed with a UVP gel imaging system (Fuji Film, Tokyo, Japan). The amount of RT-PCR product was quantitatively determined by measuring the density of the specific bands (31).

Blood Assays. Blood glucose (BG) concentrations were determined using a glucose analyzer (model 2300A; Yellow Springs Instruments, Yellow Springs, OH). Serum insulin was assayed with a radioimmunoassay kit (Phadesepa Insulin RIA; Pharmacia AB, Uppsala, Sweden).

Statistical Analysis. All data are presented as means ± SE. Statistical significance was calculated using a two-tailed paired Student's *t* test analysis or one-way ANOVA. *P* values lower than 0.05 were considered significant.

Results

Body Weight, Food and Water Intake, BG, and Serum Insulin Levels. The body weight, diet and water intake, BG, and serum insulin levels before, during, and immediately after the euglycemic clamp are shown in Table 1. The final body weights and diet intake of the HS loading RV rats and the controls were not significantly different (Table 1). However, the water intake was greater in all HS loading groups compared with NC ($P < 0.001$). Additionally, HS loading and RV treatment did not affect the fasting BG and serum insulin levels. Steady-state serum insulin levels during euglycemic clamp procedures were not significantly different among all experimental groups (Table 1).

The Preventive and Midway Intervention Effect of VR on Whole-Body Glucose Uptake in HSD-Fed Rats. The average GIRs for the last 30 mins during euglycemic clamp are shown in Figure 2A and B. Because a

Table 1. Body Weight, Food and Water Intake, and Concentrations of BG and Serum Insulin Before and Immediately After the Euglycemic Clamp Procedure[a]

Group	Body weight (g)	Diet intake (g)	Water intake (g)	Glucose (mg/dl)		Insulin (μU/ml)	
				Basal	Clamp	Basal	Clamp
Preventive effect groups							
Control ($n = 6$)	209 ± 3	19 ± 2	28 ± 3	68 ± 2	70 ± 3	0.8 ± 0.1	78 ± 6
HS diet ($n = 6$)	207 ± 2	21 ± 3	110 ± 9*	71 ± 4	71 ± 2	0.9 ± 0.1	82 ± 8
HS diet + exercise ($n = 6$)	203 ± 3	23 ± 4	113 ± 11*	71 ± 3	68 ± 3	0.9 ± 0.2	76 ± 5
Medical effect groups							
Control ($n = 3$)	214 ± 4	19 ± 3	30 ± 5	67 ± 2	71 ± 3	0.8 ± 0.1	83 ± 8
HS diet ($n = 4$)	212 ± 3	22 ± 2	120 ± 12*	73 ± 4	73 ± 4	0.9 ± 0.1	79 ± 7
HS diet + exercise ($n = 3$)	205 ± 2	21 ± 4	119 ± 8*	71 ± 3	69 ± 2	0.8 ± 0.1	80 ± 4

[a] Data are mean ± SE.
* $P < 0.001$ for control versus HS diet.

plateau GIR was achieved, GIR was used as an indicator of whole-body glucose utilization. In the protocol A study, after 2 weeks of HS loading the GIRs of HS groups were significantly decreased compared with control (HS vs. NC: 21.5 ± 0.8 vs. 27 ± 0.5 mg/kg body wt/min; $P < 0.05$). The GIRs of the HST group showed a significant increase and reached levels similar to those of the control (30.5 ± 1.5 mg/kg body wt/min; $P < 0.01$ vs. HS sedentary; Fig. 2A).

In the protocol B study, we also investigated the midway intervention effect of exercise training on glucose uptake in HSD-fed rats from the third week. After 3 weeks of HS loading, the GIRs of HS groups were significantly decreased compared with NC (HSC vs. NC: 20 ± 0.3 vs. 26.5 ± 0.5 mg/kg body wt/min; $P < 0.05$). The GIRs in 2 weeks of HSD feeding and then midway voluntary exercise group also were greater than those of sedentary (HSR: 28.5 ± 0.7 mg/kg body wt/min; $P < 0.01$ vs. HSC sedentary; Fig. 2B).

These results suggest that VR prevents the decreases of whole-body glucose uptake in synchronal the HSD feeding and VR group and that it improves the impaired insulin sensitivity in the primary HS feeding for 2 weeks and, subsequently, 1 week midway VR group.

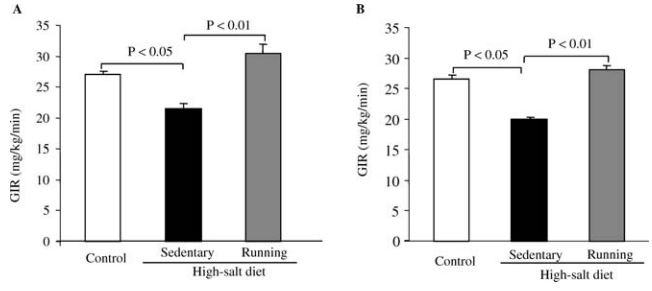

Figure 2. Whole-body insulin action in control and HSD feeding and voluntary running rats during a hyperinsulinemic-euglycemic clamp. Rats were infused with insulin (6.0 mU/kg body wt/min) for 120 mins. The steady-state GIRs were determined between 100 and 120 mins during the clamp. (A) The preventive effect of VR on glucose infusion rate in HSD-fed rats for 2 weeks ($n = 6$ in each group). (B) The midway intervention effect of VR on glucose infusion rate in HSD-fed rats ($n = 3$ or 4 in each group). Data are expressed as mean ± SE.

Effects of Two Weeks of HSD Feeding and VR on the Mass and Tyrosine Phosphorylation of IR-β and IRS-1 in Rat Skeletal Muscle from Euglycemic Clamp Study. The total protein content of IR-β and IRS-1 was measured in gastrocnemius muscles of all rats. No significant differences in the mass of IR-β and IRS-1 were detected by HSD feeding and VR (Fig. 3).

The tyrosine phosphorylation level of IR-β and IRS-1 was determined by immunoblotting the phosphotyrosine antibody immunoprecipitates with the IR-β or IRS-1 antibodies. As shown in Figure 3A, the tyrosine phosphorylation level of IR-β in the gastrocnemius muscle of the HS group was increased compared with normal control (124% of control; $P < 0.05$). The same tendency was found for the IRS-1 tyrosine phosphorylation (130% of control; $P < 0.05$), as shown in Figure 3B. However, we did not find that VR affected these proteins and their tyrosine phosphorylations in HSD feeding and with VR rats.

Effects of Two Weeks of HSD Feeding and VR on the Mass and the Serine Phosphorylation of Akt in Rat Skeletal Muscle from Euglycemic Clamp Study. We investigated the phosphorylation of Ser473 of Akt in the muscle of HSD-fed rats. The amounts of Akt in muscle were assessed by the immunoblotting of anti-Akt immunoprecipitants (Fig. 4). The amounts of Akt in muscle of controls and HS-fed rats were comparable. Insulin-stimulated phosphorylation of Ser473 of Akt (p-Akt) was demonstrated by immunoblotting with the p-Akt–specific antibody. Levels of p-Akt were enhanced about 131% of controls ($P < 0.05$) in the muscle of the HS-fed rats. In contrast to the results, the levels of p-Akt in gastrocnemius muscle were similar in normal controls and HSD feeding exercise-trained rats.

Effects of Two Weeks of HSD Feeding and VR on the Mass, PM, Phosphorylation, and mRNA of GLUT4 in Rat Skeletal Muscle from Euglycemic

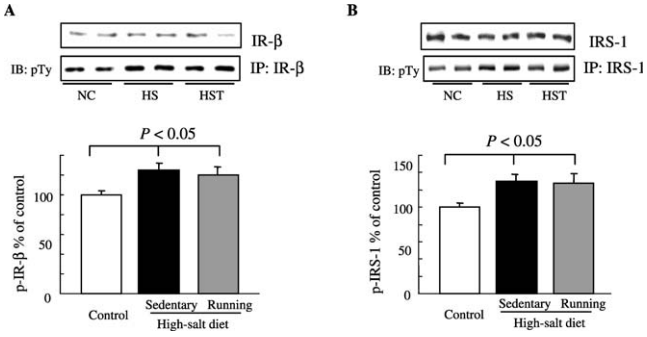

Figure 3. Effects of 2 weeks of HS feeding and voluntary running on the mass and tyrosine phosphorylation of IR-β and IRS-1 in the gastrocnemius of rats. Muscle proteins were resolved by SDS-PAGE, and tyrosine phosphorylation was detected by immunoprecipitation with anti–IR-β or IRS-1 antibody, followed by immunoblot analysis with antiphosphotyrosine antibody. Data are expressed as the mean ± SE ($n = 5$, respectively).

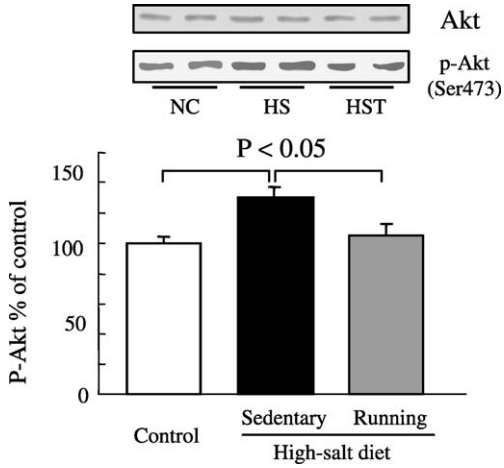

Figure 4. Effects of 2 weeks of HSD feeding and voluntary running on the mass and phosphorylation of Akt in the gastrocnemius of rats. Muscle proteins were resolved by SDS-PAGE, and phosphorylation was detected by immunoblot analysis with anti-Akt phosphorylation antibody. Data are expressed as the mean ± SE ($n = 5$, respectively).

Clamp Study. We assessed the skeletal muscle GLUT4 mass by Western blot analysis, and the GLUT4 mass of gastrocnemius muscle was similar among the three groups (Fig. 5A), indicating the impairment or improvement of insulin-induced glucose uptake in gastrocnemius muscle from 2 weeks of HSD feeding rats or VR rats was not due to reduced expression of GLUT4 total mass.

GLUT4 protein in PM fractions was attenuated in the skeletal muscle of HSD-fed rats (72% of control; $P < 0.05$), and the PM fractions prepared from the skeletal muscle of HSD-fed and RV rats showed a significant increase to the similar levels of the control (Fig. 5B).

Figure 5C shows that the tyrosine phosphorylation of GLUT4 in the HS group was significantly increased compared with the control (130% of control; $P < 0.05$), and the increased phosphorylation of GLUT4 was attenuated by VR treatment in the HT group ($P < 0.05$).

In an RT-PCR study, we found a decrease of 35% of GLUT4 mRNA in the soleus muscle of HSD-fed rats compared with control as shown by the densitometric analysis (Fig. 5D; $P < 0.05$). With the VR treatment, GLUT4 mRNA expression was restored to control values in HT groups.

Discussion

It is well established that exercise training prevents the development of insulin resistance (11–15). However, it is not clear that VR prevents the high-salt loading–induced insulin resistance in rats. Because high-salt loading induces insulin resistance with unique molecular mechanisms, including an apparent paradoxical increase in insulin signaling (3), it differs from other animal models of insulin resistance (6, 7, 11, 14, 15). In the current study, we demonstrated that VR prevents and ameliorates the decreased whole-body insulin action induced by HSD feeding.

Exercise training is generally recommended for the prevention and treatment of insulin resistance. Consequently, it is necessary and important to clarify the mechanisms underlying the improvements of insulin sensitivity by VR. The ameliorated insulin sensitivity after exercise is mainly due to enhanced insulin action on skeletal muscle, the predominant tissue for insulin-stimulated glucose disposal (32). Moreover, the estimation of the whole-body insulin action with euglycemic clamp technique reflects the disposal in skeletal muscle (60%–70%), adipose tissue (10%), and liver (30%; Ref. 24). Therefore, our purpose was to determine whether the improvements in insulin-stimulated whole-body glucose uptake that are associated with VR in HSD feeding are accompanied by an improvement in insulin signaling in skeletal muscle. In agreement with previous reports, the present data confirm that insulin stimulation of the tyrosine phosphorylation of IR-β and IRS-1 is enhanced in the muscle of HSD-fed rats (3, 4). We also found that the tyrosine phosphorylation of IR-β and IRS-1 was markedly enhanced in the 2 weeks of VR group compared with normal controls. In other words, the 2-weeks of VR did not affect the abnormal insulin signaling induced by HSD feeding. This result is in conflict with our previous reports that exercise training prevents a maturation-induced insulin resistance, and it suggests that the improvement of insulin sensitivity by exercise training may exert effects not only on GLUT4 (15), but also on IRS-1 and PI3-kinase (14). Chibalin *et al.* (33), reported that exercise training increased insulin-stimulated insulin receptor and IRS-1 tyrosine phosphorylation as well as IRS-1–associated PI3-kinase activity. However, our data are consistent that exercise training improves muscle insulin resistance but not insulin signaling in obese Zucker rats (34). Our results indicate that VR does not reverse abnormalities in proximal insulin signaling.

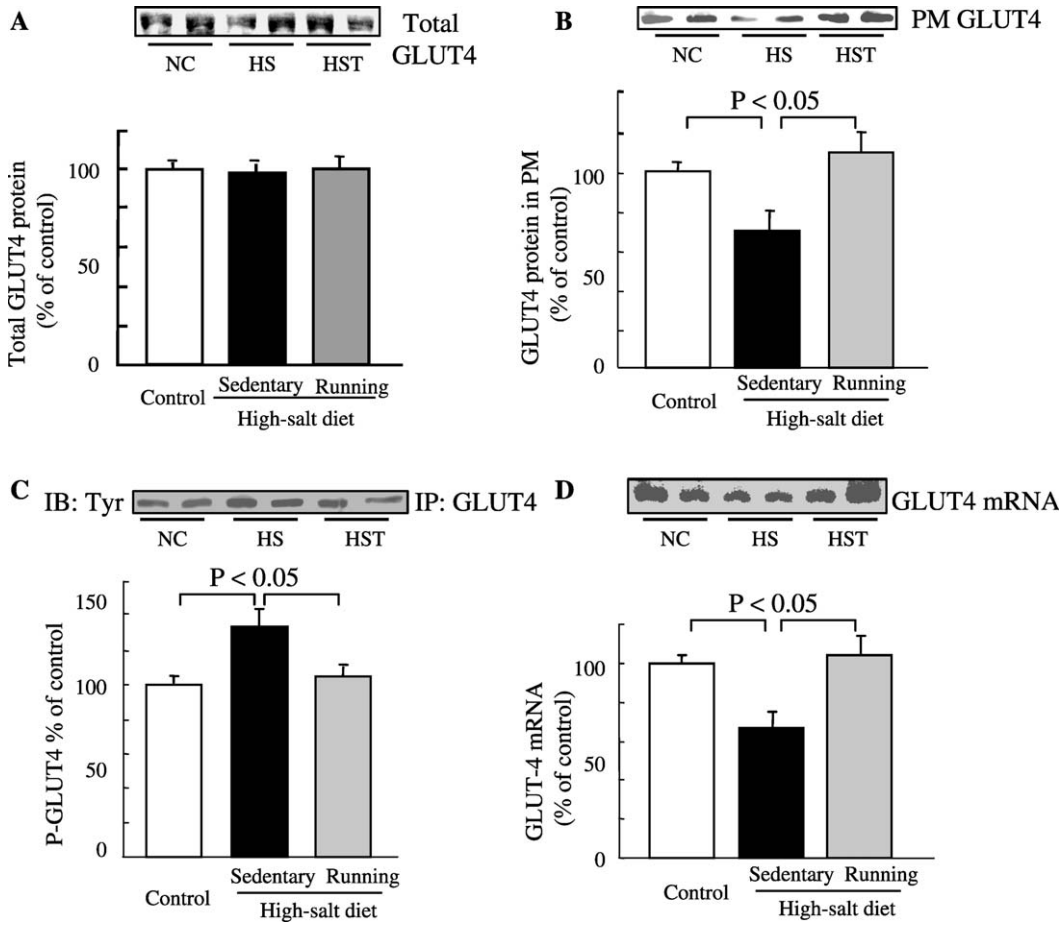

Figure 5. Effects of 2 weeks of HSD feeding and voluntary running on the total mass, plasma membrane, and the phosphorylation in gastrocnemius. (A) GLUT4 total mass. (B) GLUT4 protein in plasma membrane. (C) GLUT4 phosphorylation. (D) GLUT4 mRNA. Analysis of the expression of GLUT4 mRNA in rat soleus by RT-PCR. Values are mean ± SE and are presented as a percentage of control ($n = 5$, respectively).

Ser473 is one of the major phosphorylation sites of Akt (35), which has been linked to glucose transport, based on findings that overexpression of constitutively active Akt leads to enhanced glucose transport in 3T3-l adipocytes and L6 myotubes (36, 37). Our data suggest that HSD feeding induces an abnormal enhanced p-Akt, which is inhibited by VR, and HSD and VR do not affect the total mass. The results are supported by Luciano *et al.* (38) in that Akt phosphorylation represents an important event for glucose uptake in endurance exercise–trained rats, but they are inconsistent with an earlier report (34) showing that exercise training had no effect on Akt expression. Taken together, the experimental conditions (i.e., the type of exercise, including the density, time [acute and endurance], animal model, and *in vivo* or *in vitro*), alter the effects of exercise training on insulin signaling.

The importance of GLUT4 in the regulation of glucose uptake is well known (39, 40). The translocation of GLUT4 from intracellular vesicles to the plasma membrane of skeletal muscle is regulated by insulin; this process is normally mediated by PI3-kinase (41) and also occurs independently of PI3-kinase (42, 43). Numerous studies have ascribed the improved insulin action of exercise to concurrent increases in muscle GLUT4 levels in humans (44) and animals (45). Our data suggest that there was no difference in GLUT4 mass among NC, HS, and HST animals. It may be relative to the running activity, as in our experimental program the rats ran about 1000 m/day compared with a previous report (female rats, 2000∼8000 m/day; Ref. 17). We also found that HSD decreases insulin-stimulated GLUT4 translocation in PM, and the impaired GLUT4 in PM after HSD feeding was improved by 2 weeks of VR, in agreement with previous reports that exercise training increases GLUT4 translocation in humans (22) and animal skeletal muscle (15). Whether GLUT4 phosphorylation regulates glucose transport remains incompletely understood. Reusch *et al.* reported that the elevated $[Ca^{2+}]_i$ diminished insulin-stimulated glucose transport and suggested that increased phosphorylation of GLUT4 in adipocytes with high $[Ca^{2+}]_i$ may alter its intrinsic activity (46). Moreover, STZ-induced diabetes results in increased phosphorylation of GLUT4, despite significant reductions in total GLUT4 protein. Treatment of diabetic animals with insulin restores GLUT4 protein levels and reduces its

phosphorylation to control levels (47). Recently, Cingolani *et al*. reported that impaired insulin-stimulated GLUT4 translocation in adipocytes of hypertensive animals could be related to a deficient GLUT4 phosphorylation of serine (48). Our data with high-salt loading in rats show increased tyrosine phosphorylation of GLUT4 and a concomitant decrease in glucose uptake, both of which are regulated by VR. These findings are consistent with those showing that insulin treatment restored the decreased glucose transport activity and the enhanced GLUT4 phosphorylation in diabetic rats to control levels (47). We also observed that HSD feeding decreases the expression of GLUT4 mRNA, which is improved by a 2-week VR program. GLUT4 mRNA is also decreased in STZ-diabetic (49) and high-fat–fed rats (50). This is consistent with the previous study showing that the transcription of GLUT4 gene in rat skeletal muscle was increased by exercise (51).

In summary, 2 weeks of early prevention and 1 week of midway intervention of a VR program improved the impaired whole-body insulin sensitivity in HSD-fed rats. This improvement was associated with an increase in GLUT4 translocation and mRNA expression, and it was also related to decreases in enhanced Ser473 phosphorylation of Akt and tyrosine phosphorylation of GLUT4, but it was not accompanied by any changes in the response of abnormal IR-β and IRS-1 tyrosine phosphorylation.

In conclusion, the current study suggests that VR would improve *in vivo* insulin-regulated whole-body glucose utilization in HSD-fed rats, in part through improving the activity of GLUT4 in skeletal muscle. Further investigation is necessary to fully clarify the mechanisms of exercise training on HSD-induced insulin resistance.

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