Reversal of diet-induced insulin resistance with a single bout of exercise in the rat: the role of PTP1B and IRS-1 serine phosphorylation

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Lifestyle interventions including exercise programmes are cornerstones in the prevention of obesity-related diabetes. In this study, we demonstrate that a single bout of exercise inhibits high-fat diet-induced insulin resistance. Diet-induced obesity (DIO) increased the expression and activity of the protein tyrosine phosphatase 1B (PTP1B) and attenuated insulin signalling in gastrocnemius muscle of rats, a phenomenon which was reversed by a single session of exercise. In addition, DIO was observed to lead to serine phosphorylation of insulin receptor substrate 1 (IRS-1), which was also reversed by exercise in muscle in parallel with a reduction in c-Jun N-terminal kinase (JNK) activity. Thus, acute exercise increased the insulin sensitivity during high-fat feeding in obese rats. Overall, these results provide new insights into the mechanism by which exercise restores insulin sensitivity.

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Insulin resistance of skeletal muscle glucose transport is a key defect in the development of impaired glucose tolerance and type 2 diabetes. It is well established that chronic exercise can have beneficial effects on insulin action in insulin-resistant states (Henriksen, 2002). It is important to note that improvements in glucose tolerance can be observed in people with mild type 2 diabetes mellitus after acute exercise (Azevedo *et al.* 1995; Kennedy *et al.* 1999). The molecular mechanism for enhanced glucose uptake with chronic exercise may be partly related to increased expression and activity of key proteins known to regulate glucose metabolism in skeletal muscle (Hjeltnes *et al.* 1998; Chibalin *et al.* 2000; Zierath, 2002).

The action of insulin is mediated by receptor binding at the surface of insulin-sensitive tissue (Czech & Corvera, 1999). The insulin receptor (IR) is a protein with endogenous tyrosine kinase activity that, following activation by insulin, undergoes rapid auto-phophorylation and subsequently phophorylates intracelular protein substrates, such as insulin receptor substrate 1 and 2 (IRS-1 and IRS-2) (Cheatham & Kahn, 1995). Phosphorylation of IRS-1 and IRS-2 tyrosine residues induces activation of phosphatidylinositol 3-kinase (PI3-K) by binding the p85 subunit and activating the catalytic p110 subunit (White & Kahn, 1994). Activation of a serine/threonine kinase Akt occurs downstream from PI3-K. Once phosphorylated, Akt contributes to various biological processes including regulation of glucose uptake (Virkamaki *et al.* 1999).

Dephosphorylation of IR and IRS-1 or serine phosphorylation of IR substrates are the main mechanisms that suppress the insulin pathway (Ventre *et al.* 1997; Greene *et al.* 2003). Protein tyrosine phosphatases (PTPs) are important regulators of tyrosine phosphorylation-dependent signalling events and may represent novel targets for therapeutic intervention in a variety of human diseases (Tonks, 2003). Several PTPs, including PTPα, PTPε, CD45, SHP2, LAR and PTP1B, have been implicated as negative regulators of insulin signalling (Asante-Appiah & Kennedy, 2003). PTP1B is a major PTP implicated in the regulation of insulin action, including in the insulin-resistant state (Seely *et al.* 1996; Elchebly *et al.* 1999). c-Jun N-terminal kinase (JNK) is a member of the mitogen-activated protein (MAP) kinase family (Weston *et al.* 2002) and can be activated by tumour necrosis factor α (TNFα) (Hirosumi *et al.* 2002) and interleukin 1β (IL 1β) (Major & Wolf, 2001). In addition, JNK might serve as a feedback inhibitor during insulin stimulation (Lee *et al.* 2003). Three JNK isoforms have been described, JNK1, 2 and 3 (Ip & Davis, 1998), of which JNK1 is most involved in the pathophysiology of obesity and insulin resistance (Hirosumi *et al.* 2002).

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Table 1. Components of rat diet and rat chow

Ingredients	Standard chow (g)	High-fat diet (g)
Casein	202	200
Sucrose	100	100
Cornstarch	397	115.5
Dextrinated starch	130.5	132
Lard	—	312
Soybean oil	70	40
Cellulose	50	50
Mineral mix American Institute of Nutrition (AIN)-93	35	35
Vitamin mix AIN-93	10	10
L-cystine	3	3
Choline	2.5	2.5

JNK activation induces inhibitory serine 307 (Ser307) phosphorylation of IRS-1, (Aguirre *et al.* 2000; Lee *et al.* 2003). Ser307 is located next to the PTB domain in IRS-1 and its phosphorylation inhibits the interaction of the PTB domain with the phosphorylated NPEY motif in the activated insulin receptor, causing insulin resistance (Aguirre *et al.* 2002). Previous studies suggest that, in addition to JNK, IκB kinase beta (IKKβ) phoshorylation also increases serine phosphorylation of IRS-1. Thus, the IKK complex appears to be another candidate that plays a key role in the phosphorylation of IRS-1 and in the regulation of insulin sensitivity.

As much of the molecular basis underlying the beneficial effects of exercise in the insulin-resistant state remains unclear, the current study was designed to investigate the effects of a single bout of exercise on PTP1B activity and IRS-1 serine phosphorylation associated with insulin resistance induced by DIO.

Methods

Experimental animals and diet

Male Wistar rats from the University of Campinas Central Animal Breeding Center were used in the experiments. All experiments were approved by the Ethics Committee of the State University of Campinas (UNICAMP).

The 4-week-old Wistar rats were divided into three groups, control rats fed standard rodent chow (Table 1), obese rats fed on an obesity-inducing diet for 3 months (DIO) (Table 1) and DIO rats submitted to a single bout of exercise (DIO + EXE).

Exercise protocol

Rats were acclimated to 10 min swimming for 2 days. The animals swam for two 3 h bouts separated by a 45 min rest period and the water temperature was maintained at ∼34°C. This exercise protocol was adaptated from a published procedure (Chibalin *et al.* 2000). After the last bout of exercise, animals were fed *ad libitum* and food was withdrawn 6 h before the tissue extraction. The rats were anaesthetized with intraperitoneal injection of sodium thiopental (40 mg (kg body weight)$^{-1}$) 8 and 16 h after the exercise protocol. Following the experimental procedures, the rats were killed under anaesthesia (200 mg kg^{-1} thiopental) following the recommendations of the NIH.

Insulin tolerance test, serum insulin quantification and glycogen formation

The rats were given an insulin tolerance test (ITT; 1.5 IU insulin (kg body weight)$^{-1}$) 16 h after the exercise protocol. Briefly, 1.5 IU kg^{-1} human recombinant insulin (Humulin R) from Eli Lilly (Indianapolis, IN, USA) was infused intraperitoneally to anaesthetized rats, the blood samples were collected at 0, 5, 10, 15, 20, 25 and 30 min from the tail for serum glucose determination. The rate constant for plasma glucose disappearance (K_{itt}) was calculated using the formula 0.693/biological half life (t_{γ_2}). The plasma glucose (t_{γ_2}) was calculated from the slope of last square analysis of the plasma glucose concentration during the linear phase of decline (Bonora *et al.* 1989). Plasma glucose level was determined using a glucose meter (Advantage, Boehringer Mannheim, USA). Plasma was separated by centrifugation (1100 *g*) for 15 min at 4°C and stored at −80°C until assayed. Radioimmunoassay was employed to measure serum insulin level, according to a previous description (Scott *et al.* 1981). Glycogen content in gastrocnemius muscle fragments was measured, according to a previously described method (Pimenta *et al.* 1989).

Hyperinsulinaemic–euglycaemic clamp procedures

HPLC-purified 2-deoxy-D-[1-^{14}C]glucose (2-[^{14}C]DG) was obtained from Amersham Biosciences Group (UK). The Harvard apparatus (model 11) and Harvard compact infusion pumps (model 975) were obtained from South Natick, MA, USA.

After 6 h of fasting, animals were anaesthetized intraperitoneally and catheters were then inserted into the left jugular vein (for tracer infusions) and carotid artery (for blood sampling), as previously described (Prada *et al.* 2000). Experiments were started when glycaemia had returned to stable levels, 30 min after the end of the surgical procedure. A 120 min hyperinsulinaemic–euglycaemic clamp procedure was conducted in anaesthetized catheterized rats, as shown previously (Prada *et al.* 2000, 2005), with continuous infusion of human insulin at a rate of 3.6 mU (kg body wt) $^{-1}$ min^{-1} to raise the plasma insulin concentration to approximately 800–900 pmol l^{-1}. Blood samples (20 μl) were collected at 5 min intervals for the immediate measurement of plasma glucose

concentrations, and 10% unlabelled glucose was infused at variable rates to maintain plasma glucose at fasting levels. To estimate insulin-stimulated glucose transport and metabolism in skeletal muscle, (2-[^{14}C]DG) was administered as a bolus (10 μCi) 45 min before the end of the clamp procedure. All infusions were performed using Harvard infusion pumps. At the end of the clamp procedure, animals were killed by an intravenous injection of ketamin and diazepam. Within 2 min, both portions of gastrocnemius from hindlimbs were removed. Each tissue, once exposed, was dissected out within 2 s, weighed, frozen with liquid N_2 and stored at -80°C for later analysis.

Analytical procedures for hyperinsulinemic-euglycemic clamping

Plasma glucose was measured using a glucometer (Advantage, Boehringer Mannheim, USA). The whole blood glucose uptake was obtained from averaged rates of the last 30 min of 10% unlabelled glucose infusion during clamp procedures. Glucose transport activity in skeletal muscle was calculated from the tissue 2-deoxy-D-glucose (2DG) profile, as described before (Ferre *et al.* 1985; McGuinness & Mari, 1997; Prada *et al.* 2005).

Protein analysis by immunoblotting

As soon as anaesthesia was assured by the loss of pedal and corneal reflexes, the abdominal cavity was opened, the cava vein exposed, and 0.2 ml normal saline or insulin (10^{-9} M) injected. At 90 s after insulin injection, both portions of gastrocnemius were ablated, pooled, minced coarsely and homogenized immediately in extraction buffer containing (mM): Tris 100 (pH 7.4), sodium pyrophosphate 100, sodium fluoride 100, EDTA 10, sodium vanadate 10 and phenylmethylsulfonyl fluoride (PMSF) 2, and 0.1 mg aprotinin ml^{-1} and 1% Triton-X 100 at 4°C with a Polytron PTA 20S generator (Brinkmann Instruments model PT 10/35) operated at maximum speed for 30 s. The extracts were centrifuged at 15 000 r.p.m. (9000 *g*) and 4°C in a Beckman 70.1 Ti rotor (Palo Alto, CA, USA) for 45 min to remove insoluble material, and the supernatants of these tissues were used for protein quantification using the Bradford method (Bradford, 1976).

Proteins were denaturated by boiling in Laemmli (Laemmli, 1970) sample buffer containing 100 mM DTT, run on SDS-PAGE, transferred to nitrocellulose membranes, which were blocked, probed and developed as previously described (Saad *et al.* 1997). The β subunit of the IR (IRβ), IRS-1 and IRS-2 were immunoprecipitated from rat muscle with or without previous insulin infusion. Antibodies used for immunoblotting were anti-phosphotyrosine, anti-IR, anti-IRS-1, anti-IRS-2, anti-PTP1B, anti-PI3-K, antiphosphoserine-IRS-1307 (Upstate Biotechnology, NY, USA), antiphospho-Akt (Cell Signalling Technology, MA, USA), anti-Akt, anti-JNK, antiphospho-JNK, antiphospho-c-jun, anti-IκBα and anti-SOCS3 (Santa Cruz Biotechnology Inc., CA, USA). Blots were exposed to preflashed Kodak XAR film with Cronex Lightning Plus intensifying screens at -80°C for 12–48 h. Band intensities were quantified by optical desitometry (Scion Image software, ScionCorp, Frederick, MD, USA) of the developed autoradiographs.

Protein tyrosine phosphatase activity assay

The gastrocnemius muscles were removed and homogenized in the solubilization buffer containing (mM): Tris 20 (pH 7.6), EDTA 5, PMSF 2, EGTA 1 and NaCl 130, and 0.1 mg aprotinin ml^{-1} and 1% Triton X-100. The lysates were centrifuged (15 000 *g* for 25 min at 4°C) and the supernatants were collected for immunoprecipitation, as previously described. Immunoprecipitates were washed in PTP assay buffer containing (mM): Hepes 100 (pH 7.6), EDTA 2, DTT 1 and NaCl 150, and 0.5 mg ml^{-1} bovine serum albumin. The pp60$^{c\text{-}src}$ C-terminal phosphoregulatory peptide (TSTEPQpYQPGENL; Biomol) was added to a final concentration of 200 μM in a total reaction volume of 60 μl in a PTP assay buffer for the immunoprecipitation. The reaction was then allowed to proceed for 1 h at 30°C. At the end of the reaction, 40 μl aliquots were placed into a 96-well plate, 100 μl Biomol Green reagent (Biomol) was added, and absorbance was measured at 630 nm (Taghibiglou *et al.* 2002).

Statistical analysis

Where appropriate, the results were expressed as the means ± S.E.M. Differences between the control group and DIO and between DIO and DIO + EXE were evaluated using one-way analysis of variance (ANOVA). When the ANOVA indicated significance, a Bonferroni *post hoc* test was performed.

Results

Physiological and metabolic parameters

Table 2 shows comparative data regarding control, DIO and DIO + EXE rats. Rats fed on the high-fat diet for 12 weeks had a greater body weight, epididymal fat and fasting serum insulin than age-matched controls. No significant variations were found in body weight, epididymal fat and fasting serum insulin in DIO + EXE compared to DIO rats. The fasting glucose concentration was similar between the groups; however, the decrease in the glucose disappearance rate (K_{itt}), induced by the

Table 2. Characteristics of Wistar rats after 3 months on a high-fat diet (DIO), DIO rats submitted to acute exercise (DIO + EXE) and their age-matched controls

Groups	Number of rats (*n*)	Body weight (g)	Epididimal fat (g)	Fasting insulin (ng ml^{-1})	Plasma glucose (mg dl^{-1})	K_{itt} (% min^{-1})
Control	6	403.4 ± 21.0	5.95 ± 0.97	3.28 ± 0.15	73.7 ± 6.9	3.79 ± 0.2
DIO	8	544.7 ± 32.1*	11.85 ± 1.47**	7.97 ± 0.88*	84.4 ± 7.3	1.90 ± 0.4**
DIO + EXE	8	546.2 ± 25.2*	12.1 ± 1.34**	6.39 ± 1.9*	82.1 ± 10.0	4.69 ± 0.6#

*$P < 0.01$, **$P < 0.001$ *versus* control group and #$P < 0.001$ *versus* DIO.

high-fat diet, returned to the basal levels 16 h after acute exercise.

A hyperinsulinaemic–euglycaemic clamp procedure with tracer infusions was performed to examine the effects of acute exercise on the metabolism of glucose in skeletal muscle. The glucose infusion rate needed to clamp glycaemia at fasting levels in the presence of a constant infusion of insulin (3.6 mU (kg body weight)$^{-1}$ min^{-1}) was 4-fold lower in DIO rats than in controls and returned to control levels in DIO + EXE rats (Fig. 1*A*).

Using 2DG uptake analysis, the insulin-stimulated glucose uptake in skeletal muscle was quantified. As shown in Fig. 1*B*, DIO rats presented a significant reduction in glucose uptake in the skeletal muscle when compared to control group. In contrast, 16 h after the exercise protocol, insulin induced an increased glucose uptake of 33.8% in the muscle of DIO + EXE rats when compared to DIO rats. In addition, we evaluated the relative quantities of muscular glycogen in controls, DIO and DIO + EXE rats. The high-fat diet decreased glycogen levels in the gastrocnemius of DIO rats when compared to the control group, and returned to control levels 16 h after a single bout of exercise (Fig. 1*C*).

A single bout of exercise improves insulin signalling in the muscle of DIO rats

The effect of *in vivo* intravenous insulin infusion on IR tyrosine phosphorylation was examined in the gastrocnemius muscle of control, DIO and DIO + EXE rats. The muscles were immunoprecipitated with anti-IR antibody and then blotted with anti-phosphotyrosine antibody. Insulin induced an increase in IR tyrosine phosphorylation levels in muscle from control, DIO and DIO + EXE rats. In the control animals, insulin increased IR tyrosine phosphorylation by 9.6-fold, compared with 3.1-fold increases in the muscle of DIO rats, representing reductions in IR tyrosine phosphorylation of 4.0-fold. Insulin increased IR tyrosine phosphorylation by 6.6-fold in the muscle from DIO + EXE rats, representing an increase in IR tyrosine phosphorylation of 2.6-fold compared with DIO rats (Fig. 2*A* upper panel). There was no difference in basal levels of IR tyrosine phosphorylation between the three groups (data not shown). The protein expression of IR in the gastrocnemius muscle of control, DIO and DIO + EXE rats was quantified by immunoprecipitation and immunoblotting with anti-IR antibody.

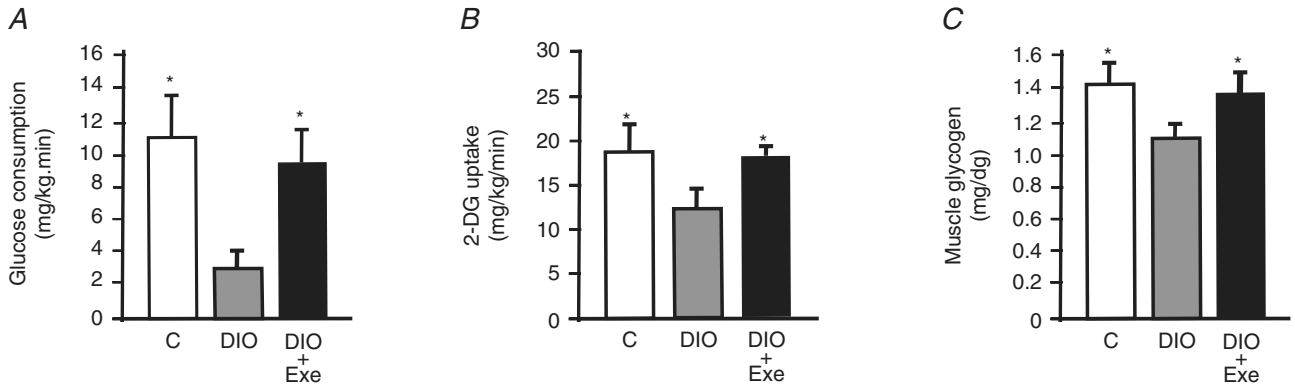

Figure 1. Effects of acute exercise on glucose uptake and glycogen content in control, DIO and DIO + EXE rats

A, steady-state glucose infusion rates obtained from averaged rates of 90–120 min of 10% unlabelled glucose infusion during hyperinsulinaemic–euglycaemic clamp procedures in the control, DIO and DIO rats submitted to acute exercise. *B*, glucose transport in gastrocnemius muscle was evaluated by 2-deoxy-D-glucose uptake during the last 45 min of the hyperinsulinaemic–euglycaemic clamp studies. *C*, muscular glycogen content is expressed as mg (100 g tissue)$^{-1}$. Bars represent means ± S.E.M. of $n = 5$ rats. *$P < 0.05$, *versus* DIO rats.

The IR protein levels were not different between the groups (Fig. 2*A* lower panel).

IRS-1 tyrosine phosphorylation and IRS-1–PI-3 kinase association increased in control animals by 10.6- and 10.1-fold following insulin administration, respectively, compared with 2.5- and 2.6-fold increases in the muscle of DIO rats (representing reductions in IRS-1 tyrosine phosphorylation and IRS-1–PI3K association of 6.4- and 5.6-fold, respectively), and increases of 4.7- and 5.0-fold in the muscle of DIO + EXE rats (representing increases in IRS-1 tyrosine phosphorylation and IRS-1–PI3K association of 2.4- and 2.5-fold, respectively, compared with DIO rats) (Fig. 2*B* upper and middle panel). IRS-2 tyrosine phosphorylation and IRS-2–PI-3 kinase association increased in control animals by 9.2- and 8.5-fold following insulin administration, respectively, compared with 2.6- and 3.0-fold increases in the muscle of DIO rats (representing reductions in IRS-2 tyrosine phosphorylation and in IRS-2–PI3K association of 5.1- and 3.7-fold, respectively), and increased 5.1- and 5.9-fold in the muscle of DIO + EXE rats (representing increases in IRS-2 tyrosine phosphorylation and in IRS-2–PI3K association of 2.5 and 2.4-fold, respectively, compared with DIO rats) (Fig. 2*C* upper and middle panel). There were no differences in basal levels of IRS-1 and IRS-2 tyrosine phosphorylation between the three groups (data not shown). The protein expression of IRS-1 and IRS-2 in the gastrocnemius muscle from control, DIO and DIO + EXE rats were quantified by immunoprecipitation and immunoblotting with anti-IRS-1 or anti-IRS-2 antibodies. The IRS-1 and IRS-2 protein levels were not different between the groups (Fig. 2*B* and *C* lower panels). Finally, in gastrocnemius muscle from control rats, insulin increased Akt serine phosphorylation by 9.3-fold, compared with 2.8-fold increase in the muscle from DIO rats (representing reductions in Akt serine phosphorylation of 4.6-fold) and increases of 5.8-fold in the muscle of DIO + EXE rats (representing an increase in Akt serine phosphorylation of 2.6-fold compared with DIO rats) (Fig. 2*D* upper panel). There were no differences between the basal levels of Akt serine phosphorylation in the three groups (data not shown). The protein expression of Akt in the gastrocnemius muscle of control, DIO and DIO + EXE rats was quantified by immunoblotting with anti-Akt antibodies. The Akt protein levels were not different between the groups (Fig. 2*D* lower panel).

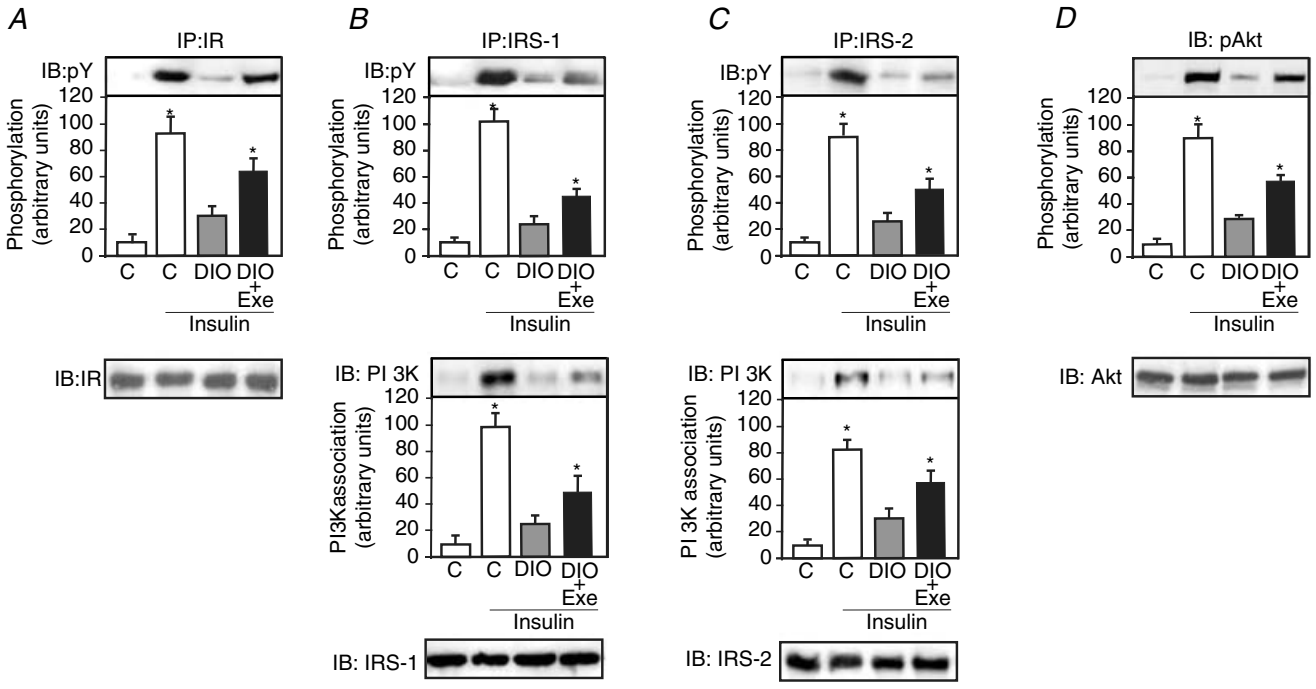

Figure 2. Insulin signalling in muscle of controls, DIO and DIO + EXE rats
Muscle extracts from rats injected with saline or insulin were prepared as described in the Methods. *A*, tissue extracts were immunoprecipitated (IP) with anti-IRβ antibody and immunoblotting (IB) with anti-PY antibody (upper panel) or anti-IRβ antibody (lower panel). *B* and *C*, tissue extracts were also IP with anti-IRS-1 and anti-IRS-2 antibodies and IB with anti-PY antibody (upper panels), anti-PI3K antibodies (middle panels) or anti-IRS-1, anti-IRS-2 antibody (lower panel). *D*, muscle extracts were IB with anti-phospho Akt and anti-Akt antibody (upper and lower panel, respectively). The results of scanning densitometry were expressed as arbitrary units. Bars represent means ± S.E.M. of $n = 6$–8 rats. *$P < 0.05$, *versus* DIO rats.

Acute exercise-mediated suppression of PTP1B activity in DIO rats

Obesity induced by diet increased the expression of PTP1B in DIO rats by 2.0-fold compared to control rats, a phenomenon that was reversed by acute exercise (Fig. 3*A*). Figure 3*B* shows that PTP1B activity increased in the muscle of DIO rats by 95% when compared to controls and acute exercise decreased PTP1B by 61% compared to DIO rats. To further explore the possibility that acute exercise mediated suppression of PTP1B activity in DIO rats, we observed that insulin induced IR tyrosine phosphorylation and IR/PTP1B interaction in muscle from DIO + EXE rats. The high-fat diet increased the IR/PTP1B association by 10.6-fold in the gastrocnemius muscle of DIO rats when compared with control rats and, in the muscle of DIO + EXE rats, IR/PTP1B association was decreased by 2.1-fold when compared with DIO rats (Fig. 3*C* upper panel). The IR protein levels were not different between the groups (Fig. 3*C* lower panel). As shown in Fig. 3*D*, insulin, in a time-dependent manner, induced increases in IR tyrosine phosphorylation in muscle from DIO rats after the exercise protocol, with a concomitant reduction of IR–PTP1B association. We also evaluated the IRS-1–PTP1B association in muscle from controls, DIO and DIO + EXE rats. The high-fat diet induced an increase in IRS-1–PTP1B association by 8.8-fold in gastrocnemius muscle of DIO rats when compared with control rats, and

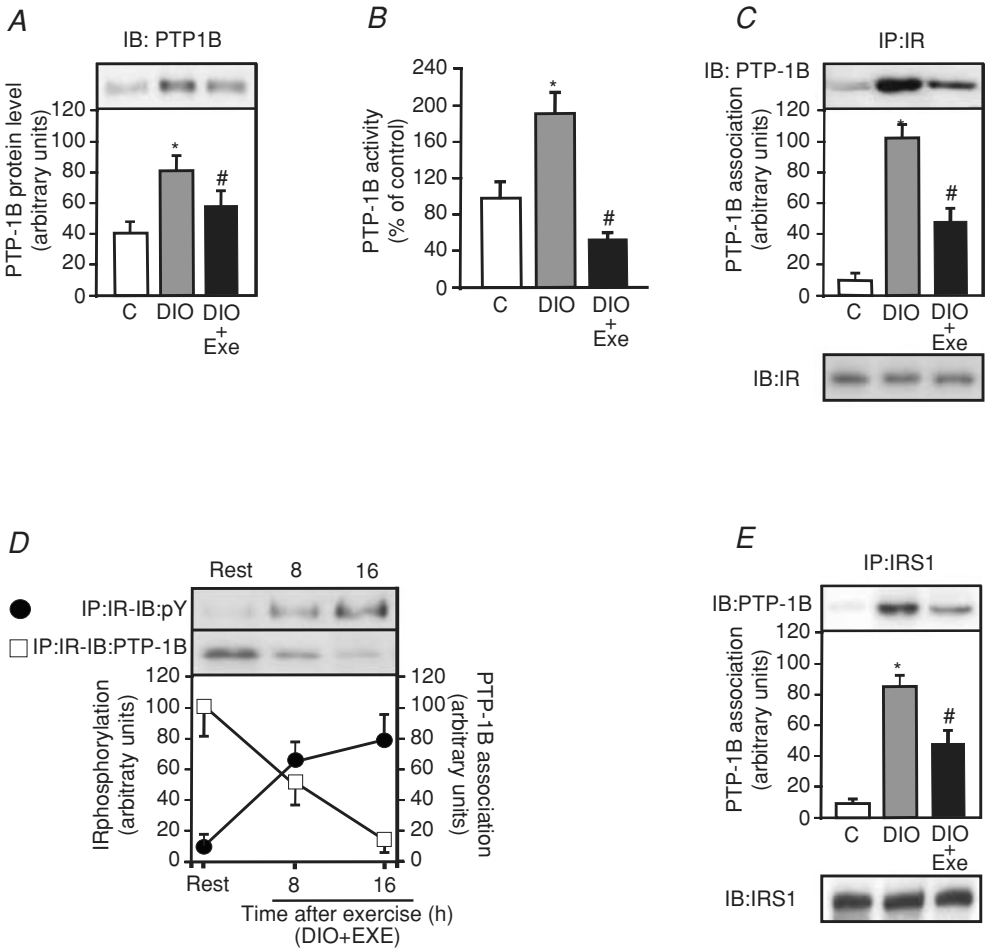

Figure 3. Effect of acute exercise on PTP1B protein levels, activity and PTP1B association with IRβ and IRS-1

A, PTP1B protein level in DIO and DIO + EXE rats were compared with control group. *B*, PTP1B assay was performed as described in the Methods. *C*, tissue extracts were immunoprecipitated (IP) with anti-IRβ followed by immunoblotting (IB) with anti-PTP1B antibody or anti-IRβ antibody (upper and lower panels). *D*, insulin-stimulated IRβ phosphorylation (●) and the IRβ–PTP1B association (□) were determined using IP with anti-IRβ and IB with anti-PY antibody and IP with anti-IRβ followed by IB with anti-PTP1B antibody. *E*, IP with anti-IRS-1 followed by IB with anti-PTP1B antibody to evaluated the IRS-1–PTP1B association (upper panel). Muscle extracts were also IP with anti-IRS-1 and IB with anti-IRS-1 antibody (lower panels). The results of scanning densitometry were expressed as arbitrary units. Bars represent means ± S.E.M. of $n = 6$–8 rats. $^*P < 0.05$, *versus* control and $\#P < 0.05$, DIO + EXE *versus* DIO.

in the muscle of DIO + EXE rats IRS-1–PTP1B association was decreased by 1.7-fold when compared with DIO rats (Fig. 3*E* upper panel). The IRS-1 protein levels were not different between the groups (Fig. 3*E* lower panel).

A single bout of exercise inhibits Ser307 phosphorylation of IRS-1, JNK activity and IκBα degradation in DIO rats

Among the serine residues that become phosphorylated in response to risk factors of insulin resistance, Ser307 has been studied extensively and Ser307 phosphorylation has become a molecular indicator of insulin resistance (Eldar-Finkelman & Krebs, 1997; Aguirre *et al.* 2002; Hirosumi *et al.* 2002; Lee *et al.* 2003); however, the effect of acute exercise on high-fat diet-induced IRS-1 serine phosphorylation has not been identified. To address this issue, we tested Ser307 phosphorylation in the gastrocnemius muscle of control, DIO and DIO + EXE rats. The muscles were blotted with anti-IRS-1 phosphoserine antibody. The high-fat diet increased IRS-1 serine phosphorylation levels in the muscle of DIO rats by 4.5-fold when compared with control rats. In the muscle of DIO + EXE rats, IRS-1 serine phosphorylation decreased by 1.7-fold when compared with DIO rats (Fig. 4*A*).

JNK activation was determined by monitoring phosphorylation of JNK (Thr183 and Tyr185) and c-Jun (Ser63), which is a substrate of JNK. The high-fat diet induced an increase in JNK phosphorylation in the muscle of DIO rats by 7.2-fold when compared with control rats. In the muscle of DIO + EXE rats, JNK serine phosphorylation decreased by 2.0-fold when compared with DIO rats (Fig. 4*B* upper panel). The JNK protein levels were not different between the groups (Fig. 4*B* lower panel). Consistent with JNK activation, c-Jun phosphorylation was 3.1-fold higher in the muscle of DIO rats when compared with control rats. In the muscle of DIO rats submitted to acute exercise, c-Jun phosphorylation decreased by 1.7-fold when compared with DIO rats

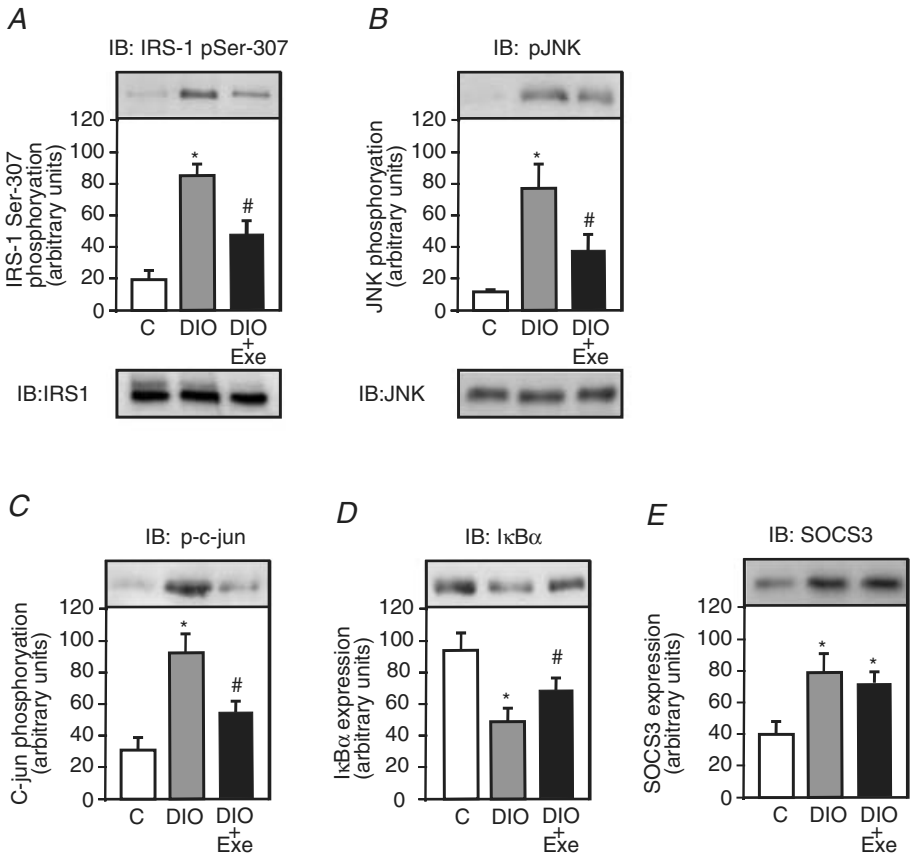

Figure 4. Effect of acute exercise on IRS-1 serine phosphorylation, JNK activity, IκBα degradation and IRS-1 and JNK protein levels in muscle of controls, DIO and DIO + EXE rats
Tissue extracts were immunoblotted (IB) with anti-IRS-1307 phosphoserine antibody (*A* upper panel), anti IRS-1 antibody (*A* lower panel), anti-phospho JNK antibody (*B* upper panel), anti-JNK antibody (*B* lower panel), antiphospho-c-Jun antibody (*C*), anti-IκBα antibody (*D*) and anti-SOCS3 (*E*) in control, DIO and DIO + EXE rats. The results of scanning densitometry were expressed as arbitrary units. Bars represent means ± S.E.M. of $n = 6$–8 rats. *$P < 0.05$, *versus* control and #$P < 0.05$, DIO + EXE *versus* DIO.

(Fig. 4*C*). Finally, we examined the IKK–NF-κB pathway, an important regulator of inflammation, in obesity- and inflammation-induced insulin resistance. The main function of the IKK complex is the activation of NF-κB through phosphorylation and degradation of IκBα (Hevener *et al.* 2003; Greten *et al.* 2004; Viatour *et al.* 2005). Thus, to assess NF-κB activation, we observed IκBα degradation in the muscle of control, DIO and DIO + EXE rats. The high-fat diet led to a decrease in IκBα expression levels in the muscle of DIO rats by 1.9-fold, compared with control rats. However, in the muscle of DIO + EXE rats, IκBα degradation was decreased by 1.4-fold when compared to DIO rats (Fig. 4*D*). The high-fat diet increased SOCS 3 expression in the muscle of DIO rats by 2.0-fold when compared to the control; however, acute exercise did not change the high-fat diet-induced modulation of SOCS 3 expression in this tissue (Fig. 4*E*).

Discussion

Impaired insulin action on whole-body glucose uptake is a hallmark feature of type 2 diabetes mellitus. Physical exercise has been linked to improved glucose homeostasis and enhanced insulin sensitivity immediately after an acute bout of exercise in humans (Devlin *et al.* 1987; Zierath, 1995) and rodents (Richter *et al.* 1982; Wallberg-Henriksson, 1987; Wallberg-Henriksson *et al.* 1988). In this study, we demonstrate that a single bout of exercise partially restored the insulin signalling in muscle of obese rats by different mechanisms. High-fat diet was observed to lead to an increase in the PTP1B protein level and in the activity and serine phosphorylation of IRS-1; it is interesting that acute exercise reversed these parameters in parallel with a reduction in JNK activity and IκBα degradation. However, the acute exercise had no effect on high-fat diet-induced SOCS3 expression.

Several mechanisms may be involved in the pathogenesis of insulin resistance in muscle. The ability of PTP1B to negatively regulate insulin receptor kinase has been established at the molecular level (Myers *et al.* 2001) and ablation of the *PTP1B* gene yields mice displaying characteristics which suggest that inhibition of PTP1B function may be an effective strategy for the treatment of diabetes and obesity (Elchebly *et al.* 1999). In accordance with this, our results show decreased activity and expression of PTP1B in DIO rats after a single bout of exercise. Furthermore, the reduction of PTP1B activity in rats submitted to acute exercise was accompanied by increased insulin sensitivity in skeletal muscle and correlates with increases in tyrosyl phosphorylation of IR, IRS-1 and IRS-2 and with reduction of IR–PTP1B and IRS-1–PTP1B association in skeletal muscle. In contrast to our results, it has been recently was reported that the amount of PTP1B associated with IR-β is not different in the muscle of normal rats at 5, 29 and 53 h after cessation of chronic voluntary exercise (Kump & Booth, 2005). These apparent contradictory results may be related to the protocol of exercise and changes in physiological and metabolic parameters in DIO rats.

Serine phosphorylation of IRS proteins is believed to be a major mechanism of suppression of IRS-1 and IRS-2 activity that contributes to insulin resistance (Saltiel & Olefsky, 1996; Saltiel & Kahn, 2001). Regulation of serine phosphorylation of IR, IRS-1 and IRS-2 proteins has been a focus of investigation in the search for the molecular mechanism of insulin resistance. Our results show a marked reduction in IRS-1 serine phosphorylation, 16 h after acute exercise in DIO rats in parallel with an increase in IR autophosphorylation. A previous study demonstrated that treatment of cultured murine adipocytes with TNF-α induces serine phosphorylation of IRS-1 and converts it into an inhibitor of the IR tyrosine kinase activity *in vitro* (Hotamisligil *et al.* 1996). The IRS-1-mediated inhibition of IR tyrosine kinase activity could occur by direct or indirect interactions between the IR and IRS-1 (Backer *et al.* 1993; O'Neill *et al.* 1994). Serine-phosphorylated IRS-1 might associate with the IR to block the autophosphorylation reaction; alternatively, serine-phosphorylated IRS-1 might act indirectly on the IR through an association with an inhibitor that acts on the IR in a stoichometric or catalytic fashion (Hotamisligil *et al.* 1996). Taken together, these data suggest that a high-fat diet mediates insulin resistance, at least in part, by inducing IRS-1 serine phosphorylation and decreasing IRS-1 and IRS-2 tyrosine phosphorylation and that this effect is inhibited by acute exercise. Studies suggest that over-expression of SOCS3 decreases insulin-induced IRS-1 and IRS-2 tyrosine phosphorylation levels, inducing insulin resistance (Ueki *et al.* 2004). However, this modulation of SOCS3 by DIO was not reversed by acute exercise. As the IR–IRS-1/2 pathway is involved in glucose uptake and glycogen synthesis in muscle, we suggest that acute exercise, by acting on this pathway, reverses insulin resistance of DIO animals.

Activation of inflammatory signalling, including of the IκB–NFκB pathway may also contribute to mediated the serine phosphorylation of IRS-1(Gao *et al.* 2002). However, few studies have examined the effect of acute exercise on the IκB–NFκB pathway. In rats, exercise activates IκB–NFκB signalling in muscle (Ji *et al.* 2004), and acute fatiguing exercise in humans reduces NFκB activity. Similar to a recent study showing that 8 weeks of aerobic exercise training reduced IκB–NFκB signalling in vastus lateralis muscle from subjects with type 2 diabetes (Sriwijitkamol *et al.* 2006), our results show that the high levels of IRS-1, phosphorylated at Ser307, in DIO rats correlated with the disappearance of IκBα. This finding is an indication of IKK activation and suggests that acute

exercise is able to reduce IKK activation and restore the IκBα expression.

Recently, JNK has been linked to the regulation of insulin signalling by several studies (Aguirre *et al.* 2000, 2002; Rui *et al.* 2001; Hirosumi *et al.* 2002; Lee *et al.* 2003). It has been suggested that JNK contributes to insulin resistance by phosphorylating IRS-1 at Ser307, and this phosphorylation leads to inhibition of the IRS-1 function (Aguirre *et al.* 2000, 2002; Rui *et al.* 2001; Lee *et al.* 2003; Prattali *et al.* 2005). However, the effect of exercise on JNK activity remains unclear. Several studies suggest that the activity of JNK intracellular signalling cascade is increased following prolonged running exercise (Boppart *et al.* 2000; Thompson *et al.* 2003). In contrast, JNK phosphorylation was reduced after resistance exercise in old men (Williamson *et al.* 2003). In this study, we observed that a single bout of exercise inhibited DIO-induced JNK activity, and that this inhibition was accompanied by a reduction in IRS-1 serine phosphorylation at Ser307.

In accordance with the results of Oakes *et al.* (1997) we observed that a single bout of exercise completely normalized the insulin action in the diet-induced obese state; however, our data show only a partial amelioration of insulin signalling. Taken together, these data suggest that the complete normalization, by acute exercise, of the insulin action in obesity induced by diet may be caused by other factors. One possibility may be associated with the increase in other insulin-independent signalling pathways. It has been postulated that AMP kinase is a important mediator of acute exercise-induced glucose uptake in muscle (Sakamoto & Goodyear, 2002; Wojtaszewski *et al.* 2002; Krook *et al.* 2004). In addition, in human subjects with type 2 diabetes, where there is impaired insulin signalling in skeletal muscle, acute exercise results in normal activation of AMP kinase (Musi *et al.* 2001; Koistinen *et al.* 2003).

In summary, a single bout of exercise improves insulin sensitivity in DIO rats by reversing high-fat diet-induced decreases in insulin-stimulated IR, IRS-1 and IRS-2 tyrosine phosphorylation. The effect of acute exercise on insulin action is further supported by our findings that DIO + EXE rats show a reduction in PTP1B activity and IRS-1 serine phosphorylation, mechanisms by which a single session of exercise may protect against high-fat diet-induced insulin resistance. Overall, these results provide new insights into the mechanism by which physical activity restores insulin sensitivity.

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