Skeletal Muscle Protein Tyrosine Phosphatase Activity and Tyrosine Phosphatase1B Protein Content Are Associated with Insulin Action and Resistance

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Abstract

Particulate and cytosolic protein tyrosine phosphatase (PTPase) activity was measured in skeletal muscle from 15 insulin-sensitive subjects and 5 insulin-resistant nondiabetic subjects, as well as 18 subjects with non–insulin-dependent diabetes mellitus (NIDDM). Approximately 90% of total PTPase activity resided in the particulate fraction. In comparison with lean nondiabetic subjects, particulate PTPase activity was reduced 21% ($P < 0.05$) and 22% ($P < 0.005$) in obese nondiabetic and NIDDM subjects, respectively. PTPase1B protein levels were likewise decreased by 38% in NIDDM subjects ($P < 0.05$). During hyperinsulinemic glucose clamps, glucose disposal rates (GDR) increased approximately sixfold in lean control and twofold in NIDDM subjects, while particulate PTPase activity did not change. However, a strong positive correlation ($r = 0.64$, $P < 0.001$) existed between particulate PTPase activity and insulin-stimulated GDR. In five obese NIDDM subjects, weight loss of ~ 10% body wt resulted in a significant and corresponding increase in both particulate PTPase activity and insulin-stimulated GDR. These findings indicate that skeletal muscle particulate PTPase activity and PTPase1B protein content reflect in vivo insulin sensitivity and are reduced in insulin resistant states. We conclude that skeletal muscle PTPase activity is involved in the chronic, but not acute regulation of insulin action, and that the decreased enzyme activity may have a role in the insulin resistance of obesity and NIDDM. (*J. Clin. Invest.* 1994. 93:1156–1162.) Key words: insulin action • insulin resistance • tyrosine phosphatase • skeletal muscle • non–insulin-dependent diabetes mellitus

Introduction

Insulin resistance is a major pathophysiological abnormality in NIDDM, resulting in reduced rates of insulin-mediated glucose uptake, primarily in skeletal muscle tissue (1). The cellular basis of insulin resistance in non–insulin-dependent diabetes mellitus (NIDDM)[1] has recently received considerable attention (2), and it is now clear that in most cases postinsulin binding defects are responsible for decreased insulin action (3, 4).

Current evidence indicates that insulin receptor autophosphorylation and tyrosine kinase activity are severely impaired in skeletal muscle and other tissues in NIDDM (5–8), suggesting that increased protein tyrosine phosphatase activity may contribute to NIDDM-related insulin resistance. In support of this hypothesis, increased skeletal muscle PTPase activity has been recently reported in insulin resistant, nondiabetic Pima Indians (9).

The purpose of this study was to determine the relationship between PTPase activity and insulin action in insulin-sensitive individuals and subjects with varying degrees of insulin resistance. We measured PTPase activity in needle biopsy specimens of skeletal muscle from 20 lean and obese nondiabetic subjects and from 18 subjects with classical NIDDM under basal (fasting) conditions and during hyperinsulinemic glucose clamps. In addition, in five obese NIDDM subjects, the effects of weight loss on skeletal muscle particulate PTPase activity and insulin stimulated GDR were determined. Since PTPase1B is the most likely of the known PTPases to have a major influence on insulin action (10), we also compared skeletal muscle PTPase1B protein content in control and NIDDM subjects.

Methods

Materials. $\gamma[^{32}P]$ATP (6,000 Ci/mmol) and [^{3}H]3-glucose (17.70 Ci/mmol) were obtained from New England Nuclear (Boston, MA); regular human insulin (Novolin R) was kindly provided by Novo Nordisk Pharmaceuticals, Inc. (Princeton, NJ); wheat germ agglutinin agarose was from Vector Laboratories, Inc. (Burlingame, CA); silicotungstic acid was from J.T. Baker Chemical Co. (Phillipsburg, NJ); Triton X-100 and AG 1-X2 acetate were purchased from Bio-Rad Laboratories, Inc. (Richmond, CA); SEP-PAK C18 cartridges were from Waters Associates (Milford, MA). The synthetic peptide, Thr-Arg-Asp-Ile-Tyr-Glu-Thr-Asp-Tyr-Tyr-Arg-Lys, which contains the amino acid sequence between residues 1142 and 1153 of the insulin receptor β-subunit (11), was obtained from the Peptide Synthesis Core Facility at the University of California (San Diego, CA). Reduced carboxamidomethylated and maleylated lysozyme (RCM-lysozyme) was kindly provided by Dr. Michael F. Cicirelli, Life Technologies, Inc. (GIBCO-BRL, Gaithersburg, MD). Anti-protein tyrosine phosphatase1B monoclonal antibody (FG6-1G) was obtained from Oncogene Science (Uniondale, NY). All other reagents were from Sigma Chemical Co. (St. Louis, MO).

Subjects. 18 subjects with NIDDM and 20 nondiabetic subjects with normal glucose tolerance (12) participated in the studies. The clinical and metabolic characteristics of the subjects are listed in Table I. The NIDDM subjects were healthy, except for diabetes, and did not have significant diabetic complications. All NIDDM subjects were initially treated with either diet ($n = 2$) or oral hypoglycemic agents ($n = 16$). Oral hypoglycemic agents were discontinued ≥ 2 wk before study. No subject was on any other medication known to influence carbohydrate or lipid metabolism.

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1. *Abbreviations used in this paper:* FG6-1G, anti-protein tyrosine phosphatase1B monoclonal antibody; GDR, glucose disposal rate; IRP, a synthetic peptide substrate; NIDDM, non-insulin-dependent diabetes mellitus; RCM-lysozyme, reduced carboxyamidomethylated and maleylated lysozyme; Rd, glucose disappearance rate.

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Table I. Subjects' Characteristics

	Age	Body mass index	Fasting plasma glucose	Fasting plasma insulin	Glucose disposal rate
	yr	*kg·M⁻²*	*mM*	*pM*	*mg·kg⁻¹·min⁻¹*
Lean nondiabetic (n = 15)	53±4.0	23.7±1.4	5.2±0.07	36.6±4.8	10.2±0.4*
NIDDM (n = 18)	56±2.0	30.7±1.2	13.1±0.80	111.0±16.5	5.9±0.4‡
Obese nondiabetic (n = 5)	43±1.2	33.0±1.3	5.2±0.07	129.6±31.8	5.8±0.7

* n = 9; ‡ n = 14.

Determination of glucose disposal rate (GDR). The GDR of each subject was determined by hyperinsulinemic glucose clamp. Procedures of the clamp study have been previously described in detail (13). Briefly, [^{3}H]3-glucose was constantly infused (0.6 μCi/min) during the entire study period, starting ≥ 2.5 hours before insulin infusion. In 15 NIDDM and all nondiabetic subjects, insulin was infused in a primed continuous manner at 720 pmol·M^{-2}·min^{-1} with plasma glucose held constant at 5 mM for 4–5 h. In three NIDDM subjects, insulin was infused at a rate of 300 pmol·M^{-2}·min^{-1}, and glucose was maintained at 10–20 mM. The GDR obtained during these hyperinsulinemic hyperglycemic clamps was comparable to that obtained from hyperinsulinemic (720 pmol·M^{-2}·min^{-1}) euglycemic (5 mM) clamps (13). GDR was calculated during the last 60 min of each clamp study from the isotopically determined glucose disappearance rate (Rd), except when glucose infusion rates were greater than Rd. Rd or glucose infusion rates were corrected for changes in glucose concentration and urinary glucose loss (during hyperglycemic studies) to reflect the actual rate of GDR.

Muscle biopsy. Percutaneous muscle biopsies were obtained from the vastus lateralis muscle using the procedure previously described in detail (14). The specimen was blotted to remove blood, frozen in liquid nitrogen within 10 s, and stored in liquid nitrogen until assayed.

Weight loss of obese NIDDM subjects. A hyperinsulinemic (720 pmol·M^{-2}·min^{-1}) euglycemic (5 mM) clamp and muscle biopsy were performed in five obese NIDDM subjects before and after a period of diet-induced weight loss. The characteristics of this study population are shown in Table II. For ≥ 3 d preceding the pre–weight loss study, subjects consumed a weight maintenance diet (~ 30 kcal/kg per d) containing 55% carbohydrate, 30% fat, and 15% protein. Immediately after the initial studies, weight loss was instituted using a very low calorie diet (The Medibase Weight Management Program, Monterey, CA) as previously described in detail (15). The formula provided 600–800 kcal/d and contained 55% carbohydrate, 42% protein, 3% milk fat, and was fully supplemented with micronutrients according to recommended daily allowances. After losing ~ 10% of initial body weight, subjects were refed a weight maintenance diet (same composition as before weight loss) for ≥ 3 wk before post–weight loss studies.

Preparation of cytosolic and particulate fractions from skeletal muscle of nondiabetic and NIDDM subjects. Tissue homogenization and fractionation into particulate and cytosolic fractions used a slight modification of the method of McGuire et al. (9). Briefly, muscle biopsies were mechanically homogenized in a Potter-Elvejhem homogenizer (Caframo, Wiarton, Ontario, Canada) in 10 vol of buffer A (25 mM imidazole, pH 7.5, 4 mM DTT, 2 mM benzamidine, 0.2 mM PMSF, and 4 μg/ml leupeptin). Homogenates were centrifuged 20 min at 350,000 *g* and supernatants were retained as the "cytosolic fraction". Pellets were resuspended in the same volume of buffer B (buffer A supplemented with 200 mM NaCl and 0.5% Triton X-100), set on ice for 30 min, and recentrifuged 10 min at 14,000 *g*. Supernatants collected after treatment with Triton X-100 were retained as the "particulate fraction." All manipulations were carried out at 4°C.

Determination of protein tyrosine phosphatase (PTPase) activity. PTPase activity was measured using a synthetic peptide substrate (IRP) homologous to the major site of tyrosine autophosphorylation of the human insulin receptor: amino acids 1142–1153 (11). The substrate contains three potential sites of tyrosine phosphorylation. It was phosphorylated in vitro by purified activated human insulin receptor in the presence of γ[^{32}P]ATP and purified as described (16). The dephosphorylation reaction was carried out at 30°C in a final volume of 50 μl, containing 160 μg/ml (particulate) or 1.5 mg/ml (cytosolic) protein, with 1–5 μM ^{32}P-IRP and 20–25 μM nonradioactive P-IRP (an average of 44,000 cpm/reaction), unless otherwise stated, in 25 mM imidazole, pH 7.0, 1 mg/ml BSA, and 0.1% (vol/vol) β-mercaptoethanol. After 6 min, the reaction was terminated by adding a threefold excess volume of ice-cold 10% TCA. ^{32}Pi released from the labeled substrate was measured after organic extraction by the method of Shacter (17). PTPase activity was defined as the percent of original cpm released·min^{-1}·μg protein^{-1}. The reaction was linear with respect to time and amount of PTPase activity present, until ≥ 40% of the ^{32}Pi had been released. All determinations were performed within the linear range. In some cases, PTPase activity was also determined using tyrosine-phosphorylated RCM-lysozyme as the substrate (18). Protein concentrations were determined by the method of Bradford (19).

Immunoblot analysis of PTPase1B protein. To quantitate the amount of PTPase1B in skeletal muscle, particulate proteins were immunoblotted using a monoclonal anti-PTPase1B antibody, FG6-1G (20). The antibody reacts with a conserved epitope in the catalytic domain of PTPase1B (20). Equal amounts of particulate protein (100

Table II. Clinical and Metabolic Characteristics of Obese NIDDM Patients before and after Weight Loss

				Fasting	
(n = 5)	Age	Weight	Body mass index	Serum glucose	Serum insulin
	yr	*kg*	*kg·M⁻²*	*mM*	*pM*
Before	51±3	106.3±3.3	32.6±1.2	13.0±1.5	161±44
After	—	96.5±3.4	29.5±1.3	8.8±1.2	110±24

μg/sample) were resolved by electrophoresis through 10% polyacrylamide gels under reducing conditions as described by Laemmli (21). Resolved proteins were then electrophoretically transferred to nitrocellulose at 15 mA overnight as described by Towbin et al. (22). After transfer, the blot was incubated 1 h in the presence of 0.3% gelatin and then incubated an additional 2 h in the same solution containing FG6-1G (2 μg/ml). Reactive proteins were visualized using alkaline phosphatase conjugated to goat anti-mouse α chain, with BCIP (5-bromo-4-chloro-3-indolyl phosphate)/NBT (nitroblue tetrazolium) for color development. The intensity of the bands was determined by laser densitometric scanning using a Stratascan – 7000 (Stratagene Inc., La Jolla, CA).

Statistical analysis. All data are expressed as the mean±SEM. Data calculations and statistical analysis were performed using the Stat View Program (Brain Power Inc., Calabasas, CA). Statistical significance was determined using Student's *t* test.

Results

Skeletal muscle PTPase activity in nondiabetic and NIDDM subjects. Basal particulate and cytosolic PTPase activities were measured in skeletal muscle from nondiabetic and NIDDM subjects, using phosphorylated IRP as the enzymatic substrate. The results are shown in Fig. 1. Approximately 90% of total PTPase activity was associated with the particulate fraction (0.283±0.014 vs 0.027±0.021% of original cpm released · min^{-1} · μg $protein^{-1}$ in the particulate and cytosolic fractions from nondiabetic subjects, and 0.220±0.011 vs 0.026±0.017% of original cpm released · min^{-1} · μg $protein^{-1}$ in the particulate and cytosolic fractions from NIDDM subjects, respectively). Basal particulate PTPase activity was 22% ($P < 0.005$) lower in NIDDM subjects than in nondiabetic subjects (0.220±0.011 vs 0.283±0.014 percent of original cpm released · min^{-1} · μg $protein^{-1}$, respectively). In contrast, no difference was detected in cytosolic PTPase activity between NIDDM and nondiabetic individuals.

Results similar to those shown in Fig. 1 were also obtained using phosphorylated RCM-lysozyme as the substrate (Table III). Skeletal muscle particulate PTPase activity was 29% lower in NIDDM subjects than in nondiabetic counterparts ($P < 0.001$), while cytosolic PTPase activity was not detectably altered. As a result of these observations, all subsequent studies of human muscle PTPase activity were carried out using the particulate fraction only, and the enzyme activity was measured using phosphorylated IRP.

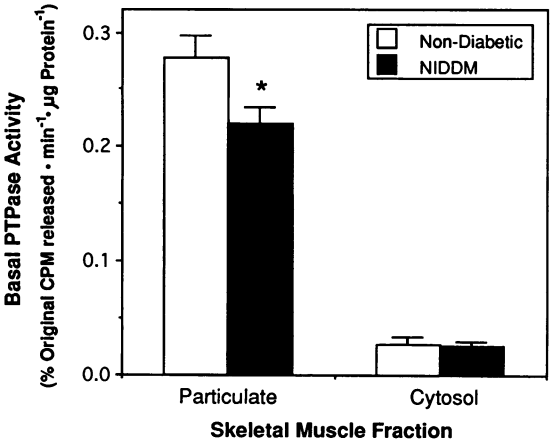

Figure 1. Basal tyrosine phosphatase activity in fractionated skeletal muscle from nondiabetic and NIDDM subjects. Muscle biopsies were obtained and fractionated as described. Particulate (160 μg/ml) and cytosolic (1.5 mg/ml) proteins were incubated with phosphorylated IRP (1–5 μM ^{32}P-IRP and 20–25 μM nonradioactive P-IRP) at 30°C for 6 min. ^{32}Pi released from ^{32}P-IRP was measured after organic extraction of ^{32}Pi (17). The results represent the mean±SEM for 15 lean control subjects and 18 NIDDM subjects. * $P < 0.005$ vs nondiabetic by Student's *t* test.

To determine the effects of insulin resistance alone, basal particulate PTPase activity was measured in skeletal muscle from six lean (insulin-sensitive) and five obese (insulin-resistant) nondiabetic subjects. In comparison with insulin sensitive nondiabetic subjects, PTPase activity was reduced 21% ($P < 0.05$) in insulin resistant nondiabetic subjects.

To confirm the specificity of our assay and to rule out the possible dephosphorylation of either phosphorylated IRP or RCM-lysozyme by nonspecific enzymes contaminating the preparation, we measured PTPase activity in the presence of 100 μM sodium orthovanadate. The presence of vanadate (an inhibitor of PTPases) during exposure of the substrate to particulate or cytosolic PTPases resulted in the inhibition of phosphate release from the tyrosyl residues of IRP and RCM-lysozyme by > 90% (data not shown).

Effect of acute insulin infusion on GDR and PTPase activity. To assess the relationship between PTPase activity and insulin action in vivo, we measured GDR and skeletal muscle particulate PTPase activity in nondiabetic and NIDDM subjects before and during hyperinsulinemic clamps. The insulin-stimulated values were then compared with corresponding values for basal GDR and PTPase activity. As shown in Fig. 2, acute insulin infusion for 4–6 h increased glucose disposal rates 5.5-fold in nondiabetic subjects (from 1.84±0.22 to 10.21±0.98 mg · kg^{-1} · min^{-1}, $P < 0.001$) and 2.2-fold in NIDDM subjects (from 2.37±0.32 to 5.32±0.63 mg · kg^{-1} · min^{-1}, $P < 0.05$). In contrast, particulate PTPase activity was unaltered by the acute insulin treatment. Since determination of PTPase activity at a single time point would have failed to detect changes that might have occurred after shorter or longer infusion periods, particulate PTPase activity was additionally measured in skeletal muscle from six nondiabetic subjects before and after hyperinsulinemic clamps of 1, 3, and 6 h duration. No significant alteration in PTPase activity was observed in response to insulin at any of the times tested (data not shown). Therefore, in subsequent studies, only PTPase activity from basal muscle biopsies was used to compare with insulin stimulated GDR.

Although there was no acute change in PTPase activity in response to insulin, a strong positive correlation (0.64, *P*

Table III. Basal PTPase Activity against Phosphorylated RCM-Lysozyme in Fractions of Skeletal Muscle from Nondiabetic and NIDDM Subjects

	Fraction	
	Particulate	Cytosol
Nondiabetic (n = 9)	0.73±0.03	0.09±0.01
NIDDM (n = 8)	0.52±0.02*	0.08±0.01

Results are expressed as the percent of original cpm released · min^{-1} · μg $protein^{-1}$. Values are mean±SEM. Muscle biopsies were obtained and fractionated as described. * $P < 0.001$ vs nondiabetic by Student's *t* test.

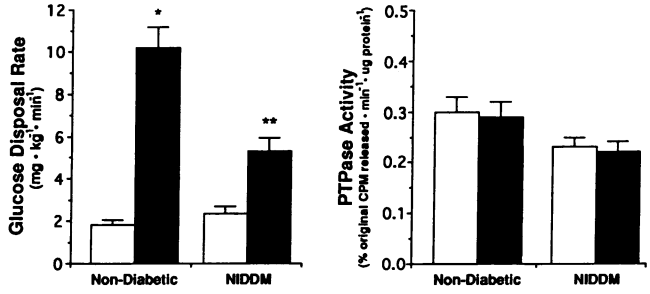

Figure 2. Comparison of GDR and PTPase activity before and during acute hyperinsulinemic clamps of nondiabetic and NIDDM subjects. GDR was determined under basal conditions and during a 4–6 h hyperinsulinemic (720 pmol · M^{-2} · min^{-1}) glucose (5 mM) clamp as described in Methods. Particulate PTPase activity was measured as described in Fig. 1 from muscle biopsies obtained before and during the clamp period. GDR was determined in six nondiabetic and five NIDDM individuals. PTPase activity was measured in eight nondiabetic and five NIDDM individuals. Results represent the mean±SEM. *$P < 0.001$ and **$P < 0.05$ vs basal values by Student's t test. □, Basal; ■, insulin stimulated.

< 0.001) was observed between particulate PTPase activity and insulin-stimulated GDR in control, obese nondiabetic, and NIDDM subjects (Fig. 3).

Effect of weight loss on PTPase activity. The presence of a positive association between insulin stimulated GDR and particulate PTPase activity and the absence of an acute change in PTPase activity in response to insulin infusion together suggested that a more chronic relationship could exist between particulate PTPase activity and in vivo insulin action. To test this hypothesis, skeletal muscle particulate PTPase activity and insulin-stimulated GDR were measured in five obese NIDDM subjects before and after a period of weight reduction (Fig. 4). Weight loss was chosen to assess the potential chronic relationship between these variables because it has been well documented to be an effective means of improving whole body insulin sensitivity, glucose tolerance, and peripheral glucose disposal in obese NIDDM subjects (23, 24). As shown in Fig. 4, skeletal muscle particulate PTPase activity increased signifi-

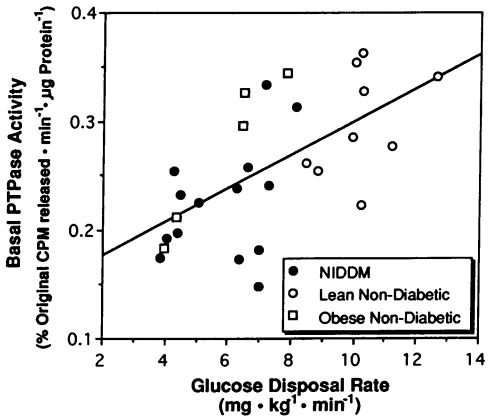

Figure 3. Relationship between in vivo insulin-stimulated GDR and human muscle particulate PTPase activity measured under basal conditions. PTPase activity was determined as described in Fig. 1. GDR was determined as described in Methods. Subjects included nine lean and five obese nondiabetic subjects and 14 NIDDM individuals ($r = 0.64$, $P < 0.001$).

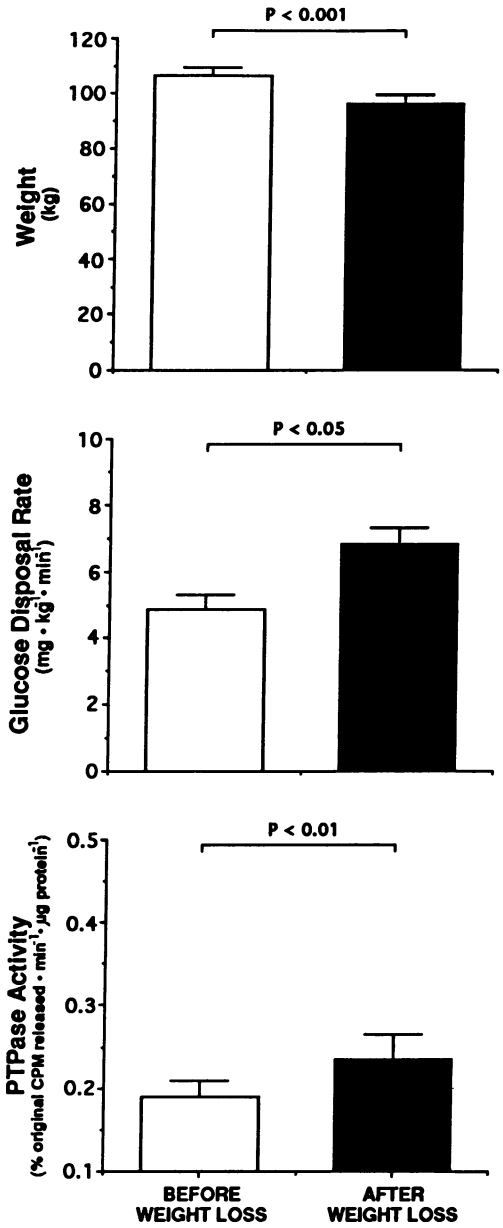

Figure 4. Comparison of weight, insulin-stimulated GDR, and PTPase activity before and after weight loss in five obese NIDDM subjects. Particulate PTPase activity was measured as described in Fig. 1. Insulin stimulated GDR was determined as described in Methods.

cantly after weight loss in NIDDM subjects in a manner that paralleled improved insulin-stimulated GDR: the average amount of weight loss was 9% of original body weight (from 106.3±3.3 to 96.5±3.4 kg, $P < 0.001$), the average increase in insulin-stimulated GDR after weight loss was 41% (from 4.86±0.46 to 6.83±0.47 mg · kg^{-1} · min^{-1}, $P < 0.05$), and the average increase in particulate PTPase activity was 24% (from 0.190±0.02 to 0.235±0.03 percent of original cpm released · min^{-1} · µg $protein^{-1}$, $P < 0.01$).

Expression of PTPase1B protein in skeletal muscle of control and NIDDM subjects. To determine whether decreased particulate PTPase activity in NIDDM could be accounted for by reduced expression of PTPase1B protein, we measured

PTPase1B protein levels in particulate fractions of skeletal muscle from five control and five NIDDM subjects. Identical amounts of total protein were fractionated by SDS-PAGE and transferred to nitrocellulose membrane. PTPase1B was detected using the monoclonal antibody, FG6-1G, reactive against the catalytic domain of human placental PTPase1B. Fig. 5 *A* shows a representative histogram of PTPase1B protein levels in muscle derived from normal and NIDDM subjects. In all subjects, PTPase1B migrated as a doublet of ~ 50 kD. The cellular content of both proteins was decreased in all but one of five diabetic subjects (lane *2*). This subject's clinical characteristics also differed from those of the others. A hyperinsulinemic clamp was not performed on this individual, but the subject was atypically lean, very physically active, and insulinopenic. Based on our experience, we would expect that he would have considerably less peripheral insulin resistance than the other four diabetics, who were obese, hyperinsulinemic, and minimally active. Similarly, one of the control subjects (lane *6*) had a relatively low PTPase1B protein content, similar to that found in diabetics. He likewise differed from the other control subjects by demonstrating moderately severe insulin resistance during hyperinsulinemic glucose clamps. As shown in Fig. 5 *B*, the average amount of PTPase1B was 38% lower in NIDDM subjects than in nondiabetic counterparts ($P < 0.05$).

Discussion

The purpose of this study was to investigate the in vivo relationship between PTPase activity and insulin action. Phosphorylated IRP was used as the enzyme substrate. PTPase activity was determined in particulate and supernatant fractions of skeletal muscle from insulin-sensitive nondiabetic subjects and insulin-resistant NIDDM subjects. We found 90% of total tissue PTPase activity associated with the particulate fraction of skeletal muscle homogenates from both nondiabetic and NIDDM subjects, in agreement with findings reported in a variety of tissues and cell lines (18, 25, 26). Particulate PTPase activity was significantly reduced in skeletal muscle of NIDDM subjects in comparison with nondiabetic counterparts. This result differs from the findings of McGuire et al. (9), who observed a 33% increase in basal particulate PTPase activity in nondiabetic insulin-resistant Pima Indians in comparison with insulin-sensitive controls.

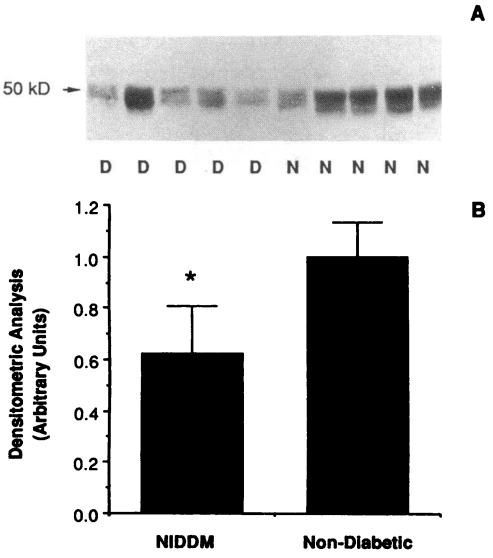

Figure 5. PTPase1B protein levels in skeletal muscle from nondiabetic and NIDDM subjects. Particulate fractions were prepared from skeletal muscle and analyzed on Western blots using antibody specific for PTPase1B as described earlier. (*A*) A representative Western blot from a typical experiment. (*B*) PTPase1B protein levels were quantitated by scanning densitometry and expressed for each individual as a fraction of the mean control value. The data represent the mean±SEM (n = five nondiabetic and five NIDDM). *$P < 0.05$ vs nondiabetic subjects by Student's t test. *N*, nondiabetic; D, NIDDM.

Our study differed from the study of McGuire et al. (9) in both the enzyme substrate used and the subject population studied. To determine whether the differences in our findings resulted from the use of different substrates, we additionally measured PTPase activity in skeletal muscle from control and NIDDM subjects using phosphorylated RCM-lysozyme (the substrate used in the earlier study). Our results were similar using either phosphorylated IRP or RCM-lysozyme as the substrate for tissue PTPase activity, negating this possibility. To rule out obvious differences in the two subject populations (NIDDM vs insulin resistant nondiabetic), we examined particulate PTPase activity in skeletal muscle from insulin resistant, nondiabetic subjects. In comparison with insulin sensitive nondiabetic controls, particulate PTPase activity was also reduced in insulin resistant nondiabetic subjects. These observations indicate that reduced PTPase activity is associated with the insulin resistant state. The differences between our findings and those of McGuire et al. (9) remain unclear. It is possible, however, that the mechanisms underlying insulin resistance in Pima Indians are specific to that ethnic group and differ from those in the general population.

At this point, we can only speculate about the reason for decreased particulate PTPase activity in insulin-resistant states such as NIDDM. Since our data indicate no alteration in cytosolic PTPase activity between control and NIDDM subjects, reduced particulate PTPase activity is not caused by redistribution into the cytosolic fraction. However, a number of other factors could be responsible for reduced particulate PTPase activity. Increased proteolytic degradation or decreased synthesis of particulate PTPases could result in the reduced enzyme activity of the insulin resistant state. Alternatively, the activation state of a PTPase inhibitor could be increased in insulin resistance, resulting in the inactivation of particulate PTPases. Finally, insulin resistance could alter the association of some PTPases with regulatory proteins responsible for controlling their activation state, resulting in decreased particulate PTPase activity.

To evaluate the in vivo relationship between PTPase activity and insulin action, particulate PTPase activity was compared with GDR measured under hyperinsulinemic clamp conditions. A strong positive correlation existed between PTPase activity and insulin-stimulated GDR in all subjects, regardless of diabetic status. This finding indicates a positive association between PTPase activity and insulin action in vivo, and suggests that insulin sensitivity, rather than the presence or absence of NIDDM, determines the level of particulate PTPase activity.

Acute insulin treatment did not influence particulate PTPase activity in muscle obtained from either insulin sensitive or NIDDM subjects. The correlation between PTPase activity and insulin-stimulated GDR thus appears to be caused

by chronic changes in PTPase activity. To test this possibility, basal particulate PTPase activity and insulin stimulated GDR were measured in NIDDM subjects before and after a period of substantial weight loss. In NIDDM subjects weight reduction increased both insulin stimulated GDR and skeletal muscle PTPase activity. The increases in both PTPase activity and GDR with weight loss provide further evidence for a positive association between PTPase activity and insulin action, indicating that PTPase activity is regulated by factors involved in the chronic improvement of GDR.

The reason for the positive association between PTPase activity and insulin action is not clear. However, the association could result from the PTPase-dependent activation of an insulin action cascade. It has been shown that PTPase activity is essential for insulin-stimulated glucose uptake in 3T3-L1 adipocytes (27). It is possible that hormone-induced PTPase activity may mediate insulin action by dephosphorylating phosphotyrosines that negatively regulate the signaling potential of intermediates, such as the Src-type tyrosine kinases (28–30). Thus, the reduced PTPase activity observed in insulin-resistant nondiabetic subjects and NIDDM subjects might be responsible for the impaired insulin action and subsequent insulin resistance in these patients. Alternatively, the positive association between the enzyme activity and insulin action could arise from changes in PTPase activity resulting from differences in insulin action. Experiments that will discriminate between these two possibilities are currently under way in our laboratory.

To test the possibility that the reduced PTPase activity that accompanies NIDDM is caused by decreased expression of PTPase1B, we measured PTPase1B protein levels in skeletal muscle from nondiabetic and NIDDM subjects by Western blot analysis, using a monoclonal antibody against human placental PTPase1B. Two distinct protein bands with molecular masses ~ 50 kD were detected in both subgroups. These two different forms of PTPase1B could result from posttranslational processing of the protein, or they could be encoded by alternatively spliced RNA species. It is also possible that the monoclonal antibody we used recognized a second PTPase, sharing sequence homology with the catalytic domain of PTPase1B. The cellular content of both proteins was decreased in all but one of five diabetic subjects. The reason for higher PTPase1B protein levels in one of the five diabetic subjects may relate to the clinical characteristics of this patient, who differed from the other diabetic subjects as mentioned in Results. In NIDDM patients, the average PTPase1B protein content was 38% lower than in nondiabetic counterparts ($P < 0.05$). These findings can explain, at least in part, the mechanism of reduced PTPase activity in NIDDM. The mechanism through which PTPase1B protein content itself is impaired is not clear, but could be accounted for through either decreased PTPase1B biosynthesis and/or increased degradation of PTPase1B protein.

In summary, basal particulate PTPase activity is significantly reduced in skeletal muscle from obese, insulin-resistant, non-diabetic subjects and NIDDM subjects in comparison with lean, insulin-sensitive, nondiabetic controls, and is associated with a decreased quantity of PTPase1B protein as well. Skeletal muscle PTPase activity varies in nondiabetic and NIDDM subjects in a manner that correlates strongly with GDR, and is increased after weight loss in a manner parallel to the improvement of GDR. In conclusion, skeletal muscle PTPase activity is positively correlated with insulin action and regulated by factors involved in the chronic stimulation of GDR. Reduced PTPase activity might play an important role in the insulin resistance of nondiabetic and NIDDM subjects.

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