Rapid Publication

Abnormal Regulation of Protein Tyrosine Phosphatase Activities in Skeletal Muscle of Insulin-Resistant Humans

MARY C. McGUIRE, ROSE M. FIELDS, BULANGU L. NYOMBA, ITAMAR RAZ, CLIFTON BOGARDUS, NICHOLAS K. TONKS, AND JAMES SOMMERCORN

Insulin resistance in skeletal muscle may be an expression of the genetic basis of a common form of non-insulin-dependent diabetes mellitus (NIDDM) in humans. Impaired insulin action results from an apparent postreceptor defect in insulin signal transduction that limits the influence of the hormone on various protein serine/threonine kinases and phosphatases that are thought to contribute to the mechanism by which insulin affects intracellular events. The fact that numerous responses to insulin are affected suggests that the cause of insulin resistance involves an early step in insulin action. Therefore, we examined the influence of insulin on protein tyrosine phosphatase (PTPase) activities, which may counteract the protein tyrosine kinase activity of the insulin receptor in skeletal muscle of insulin-sensitive and insulin-resistant humans. Insulin infusion in vivo produced a rapid 25% suppression of soluble-PTPase activity in muscle of insulin-sensitive subjects, but this response was severely impaired in subjects who were insulin resistant. Insulin did not affect PTPase activity in the particulate fraction of muscle from either group, but basal particulate activity was 33% higher in resistant subjects than in sensitive subjects. Either or both of these abnormal characteristics of PTPase activities could be central to the causes of insulin resistance and NIDDM.
***Diabetes* 40:939–42, 1991**

Longitudinal studies of the development of non-insulin-dependent diabetes mellitus (NIDDM) have demonstrated that subjects with normal glucose tolerance who subsequently become diabetic first develop impaired glucose tolerance, a condition characterized by a reduced capacity of skeletal muscle to respond to insulin (1). Therefore, insulin resistance may be an early manifestation of a genetic lesion that causes NIDDM in Pima Indians (2) and other racial groups (3). Insulin resistance apparently results from a postreceptor defect in signal transduction (4–6) that limits the ability of the hormone to influence activities of various enzymes, including S6 kinase, S6 peptide kinase, kemptide kinase, myelin basic protein kinase, and type 1 protein phosphatase (7,8; R. Maeda, J.S., unpublished observations), which are thought to mediate its action intracellularly. Because numerous responses are affected, the cause of insulin resistance likely involves an early step in the mechanism of insulin action.

Among the early events in signal transduction after insulin binds to its receptor is the activation of the receptor protein tyrosine kinase (PTK) and the subsequent phosphorylation of certain proteins on tyrosyl residues, which are essential for insulin action (9,10). The extent of tyrosine phosphorylation of these proteins reflects a balance between the opposing activities of PTKs and protein tyrosine phosphatases (PTPases). Recent studies showed that microinjection of a soluble PTPase (PTPase 1B) into *Xenopus* oocytes diminished insulin-stimulated phosphorylation of tyrosyl residues in endogenous proteins, including one of approximately the same molecular weight as the β-subunit of the insulin receptor, and blocked the short-term activation of a serine/threonine-specific protein kinase by insulin (11). In addition, insulin-induced oocyte maturation was delayed in cells that were injected with PTPase (12). Because the effects of excessive intracellular PTPase activity on insulin signal transduction in *Xenopus* oocytes are similar to the consequences of insulin resistance on insulin action in human muscle, we undertook studies to determine whether abnormal properties or regulation of PTPases could contribute to the mechanism of insulin resistance in humans.

RESEARCH DESIGN AND METHODS

After giving written informed consent, volunteers were admitted to the clinical research ward, and after a minimum of

From the Clinical Diabetes and Nutrition Section, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Phoenix, Arizona; and Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
Address correspondence and reprint requests to James Sommercorn, PhD, Clinical Diabetes and Nutrition Section, National Institutes of Health, 4212 North 16th Street, Phoenix, AZ 85016.
Received for publication 4 April 1991 and accepted 2 May 1991.

2 days on a standard diet, each underwent a 75-g oral glucose tolerance test and underwater weighing to determine body composition. Hyperinsulinemic euglycemic clamps at a maximally stimulating plasma insulin concentration and multiple biopsies of the vastus lateralis muscle were performed as described (13). After the first biopsy ($t=0$), a primed continuous insulin infusion (Velosin, Nordisk, Rockville, MD) was given for 2 h. Based on the rate of glucose disposal during the last 40 min of the clamp and on criteria established by the World Health Organization (14), the subjects were classified as nondiabetic and either insulin sensitive (glucose disposal rate ≥9 mg · min^{-1} · kg^{-1} fat free mass) or insulin resistant (glucose disposal rate <9 mg · min^{-1} · kg^{-1} fat free mass).

The muscle obtained by biopsy was frozen in liquid N_2 within 15 s and stored at −70°C before being processed as described (13). Dried muscle powder was homogenized in 25 mM imidazole, pH 7.5, containing 4 mM dithiothreitol, 2 mM benzamidine, 0.2 mM phenylmethylsulfonyl fluoride, and 4 μg leupeptin/ml at a ratio of 50 μl buffer/mg muscle with an Omni 1000 homogenizer (Waterbury, CT). The homogenates were centrifuged at 350,000 × *g* for 20 min, and the supernatant (soluble) fractions were retained and kept at 4°C. The pellet was rehomogenized in the original volume of buffer that was supplemented with 200 mM NaCl and 0.5% Triton X-100. After 30 min at 4°C, the samples were again centrifuged, and the supernatant (particulate) fractions were retained and kept at 4°C. The fractions were assayed for PTPase activity in the presence of 5 mM EDTA with [^{32}P-Tyr]RCM-lysozyme as substrate (15,16).

RESULTS

Table 1 shows characteristics of the subjects who participated in these studies. Compared with insulin-sensitive subjects, insulin-resistant subjects had a higher percentage of body fat and higher plasma glucose at 2 h during an oral glucose tolerance test. Because these values were between 7.8 and 11.2 mM, the insulin-resistant subjects are considered to have impaired glucose tolerance but are not diabetic (14). Insulin-resistant subjects also had higher fasting insulin concentrations and much lower rates of glucose disposal in response to insulin (Table 2). This latter criterion defines these subjects as insulin resistant.

TABLE 1
Characteristics of insulin-sensitive and insulin-resistant subjects

	Sensitive	Resistant
n	10	7
Age (yr)	29 ± 2	31 ± 2
Height (cm)	176 ± 3	168 ± 5
Weight (kg)	78.6 ± 6.7	95.6 ± 12.3
Body mass index (kg/m²)	25.1 ± 1.6	35.7 ± 3.3*
Body fat (%)	17 ± 1	30 ± 4*
OGTT (mM)		
FPG	5.2 ± 0.1	5.3 ± 0.2
2-h PG	5.7 ± 0.6	9.2 ± 0.7*

Values are means ± SE. Insulin sensitivity was based on glucose disposal rate during the last 40 min of a hyperinsulinemic-euglycemic clamp (Table 2). Sensitive subjects were either white (n = 8) or black (n = 2) men. Six of the resistant subjects were Pima Indians (4 men, 2 women), and 1 was a white man. OGTT, oral glucose tolerance test; FPG, fasting plasma glucose; 2-h PG, plasma glucose 2 h after ingestion of 75 g of glucose.
*$P < 0.01$ vs. sensitive by Student's *t* test.

TABLE 2
Results of hyperinsulinemic-euglycemic clamps in insulin-sensitive and insulin-resistant subjects

	Sensitive	Resistant
n	10	7
Glucose (mM)		
Before	5.3 ± 0.2	5.8 ± 0.2*
Steady state	5.5 ± 0.1	5.8 ± 0.1
Plasma insulin (pM)		
Before	36 ± 6	150 ± 48†
Steady state	11,274 ± 534	13,548 ± 1134
Glucose disposal rate (mg · min^{-1} · kg^{-1} fat-free mass)	12 ± 1	6 ± 1†

Values are means ± SE. The coefficient of variation of the values averaged to estimate the steady-state glucose concentration for individuals ranged from 1 to 4.7. Fat-free mass was determined by underwater weighing.
*$P < 0.05$, †$P < 0.01$, vs. sensitive by Student's *t* test.

Basal PTPase activities measured in soluble fractions of muscle from insulin-sensitive and insulin-resistant subjects were not significantly different (Table 3). In insulin-sensitive subjects, infusion of insulin resulted in a rapid suppression of soluble-PTPase activity that was maximal by 15 min and persisted throughout the period examined (Fig. 1). Although the magnitude of the effect averaged 25% of the basal activity, it is possible that more than one PTPase contributes to the total measured activity and that insulin may selectively inhibit only one of these enzymes. In contrast, there was very little influence of insulin on soluble PTPase activity in subjects who were insulin resistant (Fig. 1). Furthermore, the time course of the response was different, especially at 15 min when PTPase activity was maximally inhibited in insulin-sensitive subjects, whereas activity in the insulin-resistant subjects was unaffected by insulin. These results suggest that the mechanism by which the intracellular signal for insulin is generated involves both the activation of a PTK and the inhibition of a PTPase. This dual response would maximize both the rate and extent of signal production.

DISCUSSION

Although mutations in the insulin-receptor gene are associated with insulin resistance and NIDDM in rare disease

TABLE 3
Basal protein tyrosine phosphatase (PTPase) activities (pmol · min^{-1} · mg^{-1} dry muscle) in fractions of skeletal muscle from insulin-sensitive and insulin-resistant subjects

		Fraction	
	n	Soluble	Particulate
Sensitive	10	9.9 ± 1.1	31.9 ± 3.0
Resistant	7	11.3 ± 0.7	42.5 ± 3.7*

Values are means ± SE. Muscle fractions were prepared and PTPase activity was assayed as described in METHODS.
*$P < 0.05$ vs. sensitive by Student's *t* test.

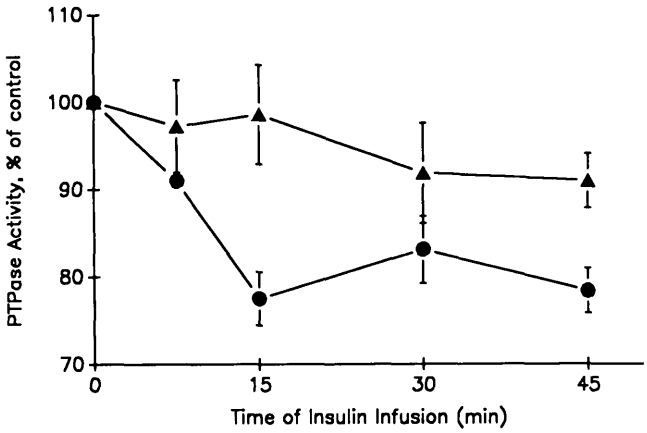

FIG. 1. Influence of insulin on soluble–protein tyrosine phosphatase (PTPase) activity in skeletal muscle of insulin-sensitive and insulin-resistant subjects. Muscle was obtained by needle biopsy from 10 insulin-sensitive (●) and 7 insulin-resistant (▲) subjects at indicated times during hyperinsulinemic-euglycemic clamp. Muscle was processed and PTPase activity was measured as described in METHODS. Values are means ± SE and are expressed as percentage of basal activity (t = 0), which was not different between 2 groups (Table 3). Data were subjected to 2-way repeated-measures analysis of variance, which revealed significant effect of time on PTPase activity ($P < 0.0001$) and that activity in insulin-sensitive and insulin-resistant groups changed differently with time ($P < 0.05$).

syndromes (17), the predicted primary structure of the receptor is normal in subjects with the more common form of NIDDM (4,5). Furthermore, studies of insulin binding and regulation of the PTK activity of the receptor from muscle of Pima Indians have not revealed abnormalities that could account for insulin resistance (6). Some aspects of signal generation by the insulin-receptor function normally in insulin resistance as evidenced by the fact that activation of casein kinase II by insulin is not impaired in skeletal muscle of insulin-resistant subjects (13). Therefore, it is possible that the inability to adequately suppress PTPase activity is the major limitation to insulin signal transduction in muscle of insulin-resistant subjects. The basis for abnormal regulation of soluble-PTPase activity may lie in the structure of the PTPase itself or in a component of the mechanism by which insulin regulates its activity.

Insulin did not appear to influence particulate-PTPase activity in muscle obtained from either insulin-sensitive or insulin-resistant subjects (data not shown), but the basal activity in muscle from insulin-resistant subjects was 33% higher than that of insulin-sensitive subjects (Table 3). Elevated particulate-PTPase activity also could limit insulin signal transduction and therefore contribute to the mechanism of insulin resistance. Particulate-PTPase activity in human muscle may be attributable to a member of a family of receptorlike PTPases (18–21). It is likely that these PTPases contribute to transmembrane signaling in that one member of the family, CD45 (22), plays a crucial role in signal transduction through the T-lymphocyte receptor (23). It is possible that a similar PTPase is chronically activated by an unknown mechanism or that it is overexpressed in muscle of insulin-resistant subjects. Alternatively, low-molecular-weight PTPases, which can also be recovered from particulate fractions (24), may be responsible for the enhanced particulate activity in insulin-resistant subjects.

Our results show that activity of at least one soluble PTPase is under hormonal control and that suppression of its activity likely contributes to normal insulin signal transduction. Furthermore, either abnormal regulation of this enzyme by insulin or excessive activity of a PTPase associated with the particulate fraction of muscle, or possibly both characteristics, may be central to the cause of insulin resistance and NIDDM in humans.

ACKNOWLEDGMENTS

This work was supported by intramural funds from the National Institute of Diabetes and Digestive and Kidney Diseases and Grant 82-1702 from the Arizona Disease Control Research Commission.

We are grateful to Carol Lamkin and the Nursing Staff for care of the subjects and assistance in performing clinical procedures and Curtis D. Diltz for assistance in preparing RCM-lysozyme. These studies were begun while N.K.T. was at the University of Washington, Seattle, WA.

REFERENCES

1. Bogardus C: Perspective: does insulin resistance primarily affect skeletal muscle? *Diabetes Metab Rev* 5:567–68, 1989
2. Yamashita T, Mackay W, Rushforth NB, Bennett PH, Houser H: Pedigree analysis of noninsulin-dependent diabetes mellitus in the Pima Indians suggests a dominant mode of inheritance (Abstract). *Am J Hum Genet* 36 (Suppl.):183s, 1984
3. Eriksson J, Franssila-Kallunki A, Ekstrand A, Saloranta C, Widen E, Schalin C, Groop L: Early metabolic defects in persons at increased risk for noninsulin-dependent diabetes mellitus. *N Engl J Med* 321:337–43, 1989
4. Moller DE, Yokota A, Flier JS: Normal insulin-receptor cDNA sequence in Pima Indians with NIDDM. *Diabetes* 38:1496–500, 1989
5. Cama A, Patterson AP, Kadowaki T, Kadowaki H, Siegel G, Dambrosio D, Lillioja S, Roth J, Taylor SI: The amino acid sequence of the insulin receptor is normal in an insulin-resistant Pima Indian. *J Clin Endocrinol Metab* 70:1155–61, 1990
6. Nyomba BL, Ossowski VM, Bogardus C, Mott DM: Insulin-sensitive tyrosine kinase—relationship with in vivo insulin action on humans. *Am J Physiol* 258:E964–74, 1990
7. Freymond D, Bogardus C, Okubo M, Stone K, Mott D: Impaired insulin-stimulated muscle glycogen synthase activation in vivo in man is related to low fasting glycogen synthase phosphatase activity. *J Clin Invest* 82:1503–509, 1988
8. Kida YA, Esposito-Del Puente A, Bogardus C, Mott DM: Insulin resistance is associated with reduced fasting and insulin-stimulated glycogen synthase phosphatase activity in human skeletal muscle. *J Clin Invest* 85:476–81, 1990
9. Chou CK, Dull TJ, Russell DS, Gherzi R, Lebwohl D, Ullrich A, Rosen OM: Human insulin receptors mutated at the ATP-binding site lack protein tyrosine kinase activity and fail to mediate postreceptor effects of insulin. *J Biol Chem* 262:1842–47, 1987
10. Morgan DO, Roth RA: Acute insulin action requires insulin receptor kinase activity: introduction of an inhibitor monoclonal antibody into mammalian cells blocks the rapid effects of insulin. *Proc Natl Acad Sci USA* 84:41–45, 1987
11. Cicirelli MF, Tonks NK, Diltz CD, Weiel JE, Fischer EH, Krebs EG: Microinjection of a protein-tryosine-phosphatase inhibits insulin action in *Xenopus* oocytes. *Proc Natl Acad Sci USA* 87:5514–18, 1990
12. Tonks NK, Cicirelli MF, Diltz CD, Krebs EG, Fisher EH: Effect of microinjection of a low-*M*, human placenta protein tryosine phosphatase on induction of meiotic cell division in *Xenopus* oocytes. *Mol Cell Biol* 10: 458–63, 1990
13. Maeda R, Raz I, Zurlo F, Sommercorn J: Activation of skeletal muscle casein kinase II by insulin is not diminished in subjects with insulin resistance. *J Clin Invest* 87:1017–22, 1991
14. World Health Organization: *Diabetes Mellitus: Report of a WHO Study Group*. Geneva, World Health Org., 1985 (Tech. Rep. Ser., no. 727)
15. Tonks NK, Diltz CD, Fischer EH: Purification of major protein-tyrosine-phosphatases of human placenta. *J Biol Chem* 263:6722–30, 1988
16. Tonks NK, Diltz CD, Fischer EH: Purification of protein tyrosine phosphatases from human placenta. *Methods Enzymol* 201:427–42, 1991
17. Taylor SI, Kadowaki T, Kadowaki H, Accili D, Cama A, McKeon C: Mutations in insulin-receptor gene in insulin-resistant patients. *Diabetes Care* 13:257–79, 1990
18. Tonks NK: Protein phosphatases: key players in regulation of cell function.

Curr Opin Cell Biol 2:1114–24, 1990
19. Streuli M, Krueger NX, Hall LR, Schlossman SF, Saito H: A new member of the immunoglobulin superfamily that has a cytoplasmic region homologous to the leukocyte common antigen. *J Exp Med* 168:1553–62, 1988
20. Kaplan R, Morse B, Huebner K, Croce C, Howk R, Ricca G, Jaye M, Schlessinger J: Cloning of three human tyrosine phosphatases reveals a multigene family of receptor-like protein-tyrosine-phosphatases expressed in brain. *Proc Natl Acad Sci USA* 87:7000–7004, 1990
21. Krueger NX, Streuli M, Saito H: Structural diversity and evolution of human receptor-like protein tyrosine phosphatases. *EMBO J* 9:3241–52, 1990
22. Tonks NK, Charbonneau H, Diltz CD, Fischer EH, Walsh KA: Demonstration that the leukocyte common antigen CD45 is a protein tyrosine phosphatase. *Biochemistry* 27:8695–701, 1988
23. Koretzky GA, Picus J, Thomas ML, Weiss A: Tyrosine phosphatase CD45 is essential for coupling T-cell antigen receptor to the phosphatidyl inositol pathway. *Nature* (Lond) 346:66–68, 1990
24. Cool DE, Tonks NK, Charbonneau H, Fischer EH, Krebs EG: Expression of a human T-cell protein-tyrosine-phosphatase in baby hamster kidney cells. *Proc Natl Acad Sci USA* 87:7280–84, 1990