Elevated PC-1 Content in Cultured Skin Fibroblasts Correlates With Decreased In Vivo and In Vitro Insulin Action in Nondiabetic Subjects

Evidence That PC-1 May Be an Intrinsic Factor in Impaired Insulin Receptor Signaling

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Membrane glycoprotein PC-1 inhibits insulin receptor (IR) tyrosine kinase activity and subsequent cellular signaling. PC-1 content is elevated in muscle and adipose tissue from insulin-resistant subjects, and its elevation correlates with in vivo insulin resistance. To determine whether elevated PC-1 content is a primary cause of insulin resistance, we have now measured PC-1 content in cultured skin fibroblasts from nonobese nondiabetic insulin-resistant subjects and found that *1*) PC-1 content was significantly higher in these cells when compared with cells from insulin-sensitive subjects (6.7 ± 0.9 vs. 3.1 ± 0.6 ng/0.1 mg protein, mean ± SE, $P < 0.01$); *2*) PC-1 content in fibroblasts was highly correlated with PC-1 content in muscle tissue ($r = 0.95$, $P = 0.01$); *3*) PC-1 content in fibroblasts negatively correlated with both decreased in vivo insulin sensitivity and decreased in vitro IR autophosphorylation; and *4*) in cells from insulin-resistant subjects, insulin stimulation of glycogen synthetase was decreased. These studies indicate, therefore, that the elevation of PC-1 content may be a primary factor in the cause of insulin resistance. *Diabetes* 47:1095–1100, 1998

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ANOVA, analysis of variance; BSA, bovine serum albumin; DMEM, Dulbecco's modified Eagle's medium; DTT, dithiothreitol; EC_{50}, effective concentration, 50%; ELISA, enzyme-linked immunosorbent assay; FBS, fetal bovine serum; FPG, fasting plasma glucose; IR, insulin receptor; ITT, insulin tolerance test; OGTT, oral glucose tolerance test; PBS, phosphate-buffered saline; PI 3-K, phosphatidylinositol 3-kinase; PMSF, phenylmethylsulfonyl fluoride; TBST, Tris-buffered saline with Tween; TLC, thin-layer chromatography.

Resistance to the biological actions of insulin is a major factor in the pathogenesis of NIDDM (1–5). Because the basic biochemical mechanisms causing insulin resistance are unknown, attempts to reduce insulin resistance in these patients have been difficult. A major barrier in understanding these mechanisms is that the secondary metabolic derangements that occur in NIDDM, including hyperglycemia and obesity, most likely are obscuring the primary factors responsible for impaired insulin action.

While NIDDM occurs in ~5% of the populations of the U.S. and Western Europe, insulin resistance is present in a much larger number of individuals. Reaven (1) and colleagues have provided compelling evidence that 25% of nonobese nondiabetic individuals are just as insulin-resistant as NIDDM patients. Studies suggest that insulin resistance in these individuals may have a genetic component (6–9), and that they are prone to develop NIDDM. Because they are not diabetic and have a relative lack of metabolic alterations, these subjects represent an important group for studying the primary mechanisms of insulin resistance.

The cellular response to insulin is mediated through the insulin receptor (IR), a specific glycoprotein in the plasma membrane of target cells. The IR consists of two identical extracellular α-subunits that contain the insulin-binding domain and two transmembrane β-subunits that have ligand-activated tyrosine kinase activity. When insulin binds to the IR, the receptor is first activated by tyrosine autophosphorylation, and then the IR tyrosine phosphorylates various effector molecules, such as IR substrate–1, leading to hormone action (10–15).

Membrane glycoprotein PC-1 is a class II transmembrane protein that is present in most cells, but whose function is unknown. We have reported that PC-1 was overexpressed in fibroblasts from a very insulin-resistant NIDDM patient (13) and in several established cell lines in tissue culture (14). In these cells, IR tyrosine kinase activity was concomitantly decreased (13,14). Moreover, in several cultured cell types, when we overexpressed PC-1 by transfection (13,15), IR tyrosine kinase activity and subsequent IR signaling were

decreased. These studies suggest, therefore, that PC-1 is an important cellular inhibitor of IR signaling.

To understand the role of PC-1 in the early development of human insulin resistance, we studied muscle and fat tissues of insulin-resistant subjects that were neither obese nor diabetic (16,17). PC-1 was elevated in the tissues of these subjects, and the elevation of PC-1 negatively correlated with the ability of insulin to act both in vivo and in vitro. These studies indicated that elevated PC-1 is associated with insulin resistance in the absence of neither NIDDM nor obesity and suggested, therefore, that PC-1 may be a primary factor in the development of insulin resistance.

To test this hypothesis, we have now obtained skin fibroblasts from lean insulin-resistant nondiabetic subjects and compared them with cells of sensitive subjects. Skin fibroblasts in culture are an important model because they can be studied under standard conditions that are independent of in vivo metabolic conditions. In these individuals, we now observe a strong correlation between both fibroblast PC-1 content and muscle content, and between fibroblast PC-1 content, IR tyrosine kinase activity, and whole body insulin sensitivity. These studies provide major support, therefore, for the concept that PC-1 overexpression may play a primary role in the pathogenesis of insulin resistance.

RESEARCH DESIGN AND METHODS

Subjects. Twelve healthy nonobese (BMI <30 kg/m^2) subjects were studied after 8 days on a weight-maintaining diet (50% carbohydrate, 30% lipid, and 20% protein). In accordance with the Declaration of Helsinki, informed consent was obtained. No subject was diabetic, as indicated by fasting plasma glucose (FPG) values <100 mg/dl on two different occasions. Moreover, in 10 of 12 subjects (5 insulin-sensitive and 5 insulin-resistant), an oral glucose tolerance test (OGTT) was performed and indicated normal glucose tolerance.

Whole body insulin sensitivity was measured by either euglycemic clamp (n = 6) or intravenous insulin tolerance test (ITT) (n = 6). Both methods have been shown to give equivalent results (18). Insulin-stimulated glucose disposal measurement by the euglycemic hyperinsulinemic clamp (19) was performed with an infusion of insulin (40 mU · m^{-2} · min^{-1}) and a variable glucose infusion to maintain plasma glucose within 10% of the baseline value. The amount of glucose infused to maintain euglycemia, the M value (mg · kg body wt^{-1} · min^{-1}), ranged between 4.6 and 8.6 (mean ± SE, 6.2 ± 0.67). Intravenous ITT (0.1 U regular insulin/kg body wt) was performed as previously described (18). The constant rate of glucose disappearance (K_{ITT}) was calculated according to the formula $0.693/t_{1/2} \cdot K_{ITT}$ values ranged between 4.2 and 9.4 (6.6 ± 0.8).

According to insulin sensitivity, subjects were subdivided into either insulin-sensitive (n = 6) or insulin-resistant (n = 6) based on having an M or K_{ITT} value higher or lower than the median value obtained in a larger group of nonobese nondiabetic subjects studied by either clamp (n = 64, median M = 5.7) or ITT (n = 67, median K_{ITT} = 6.0).

Cell culture. Cell cultures were established from 4-mm forearm skin-punch biopsies. Fibroblasts were grown in monolayers in Dulbecco's modified Eagle's medium (DMEM) containing 5.5 mmol/l glucose and supplemented with penicillin (100 U/ml), streptomycin (100 mg/ml), amphotericin B (0.25 mg/ml), gentamicin (25 mg/ml), 400 mmol/l L-glutamine, and 10% fetal bovine serum (FBS). Fibroblasts were grown at 37°C in an atmosphere of 5% CO_2, and after 4–6 weeks, they were subcultured using 0.05% Trypsin solution in Ca^{2+}- and Mg^{2+}-free phosphate-buffered saline (PBS). Fibroblasts were used for experiments between the 6th and 12th passages. Cells (800 × 103) were plated in 100-mm petri dishes and grown in 10% FBS DMEM for 5 days. After incubation for 2 h in serum-free DMEM, cells were solubilized in 50 mmol/l HEPES, pH 7.6, containing 1% Triton X-100, 1 mmol/l phenylmethylsulfonyl fluoride (PMSF), 2 mmol/l orthovanadate, and 1% bovine serum albumin (BSA). The material was then centrifuged at 10,000g for 20 min at 4°C, and the supernatants were used for the PC-1 content measurement.

Muscle tissue specimens. Muscle tissue specimens were obtained from the external oblique muscle at elective abdominal surgery (cholecystectomy). After adipose tissue was dissected and blood was removed, specimens were immediately frozen in liquid nitrogen. Soluble extracts were prepared from frozen muscle tissue as previously described (16).

PC-1 content. PC-1 cell content was measured by a specific enzyme-linked immunosorbent assay (ELISA) as previously described (17). Wells in Maxisorb plates (Nunc, Roskilde, Denmark) were precoated (overnight incubation at 4°C) with an affinity-purified polyclonal antibody to PC-1 (provided by Dr. I. Yamashima). After washing with Tris-buffered saline with Tween (TBST) buffer (20 mmol/l Tris, 150 mmol/l NaCl, 0.05% Tween-20) to remove unbound antibody, wells were blocked with 150 µl TBST containing 1% BSA (30 min at 56°C) and washed again with TBST. Then, 100 µl (20–40 µg protein) cell or tissue lysate was added to each well, and PC-1 was allowed to bind overnight at 4°C. After extensive washing with TBST, a biotinylated anti–PC-1 monoclonal antibody was added (50 mmol/l HEPES, pH 7.6, containing 0.05% Tween-20, 1 mmol/l PMSF, 2 mmol/l orthovanadate, 1% BSA, and 1 mg/ml bacitracin). After 2 h at 22°C, the peroxidase-streptavidin method was used (17), and the peroxidase activity was determined colorimetrically (17). Data were normalized for protein content (20).

^{125}I-labeled insulin binding. ^{125}I-insulin (33 pmol/l in 50 mmol/l HEPES buffer, pH 7.8) was added to subconfluent fibroblasts preincubated in 6-well trays in serum-free DMEM (4 h at 37°C, 5% CO_2) in the absence or presence of increasing native insulin concentrations. After 2 h at 22°C, cells were washed three times with cold PBS and solubilized with 0.03% SDS, and the bound radioactivity was counted. ^{125}I-insulin binding in the presence of 0.16 mmol/l native insulin was considered nonspecific and was subtracted to calculate specific binding. Data were normalized for protein content (20).

IR autophosphorylation. IR autophosphorylation was determined by a specific ELISA similar to that used for measuring PC-1 content (21). Cells, exposed for 10 min to increasing insulin concentrations (0–100 nmol/l), were solubilized as described above, and IRs were immunocaptured on plastic wells precoated with a monoclonal antibody specific to the IR. After washing, a biotinylated antiphosphotyrosine antibody (UBI Diagnostics, Lake Placid, NY) was added, followed by peroxidase-conjugated streptavidin. The reaction was developed by the addition of 100 µl *o*-phenylenediamine (0.67 mg/ml in 50 mmol/l citrate-phosphate buffer, with 0.4 ml/ml of 30% H_2O_2) and the absorbance measured at 490 nm. Data were normalized for protein content (20).

In vitro phosphatidylinositol 3-kinase assay. Fibroblasts were incubated with insulin (5 min, 37°C) and then lysed at 4°C in buffer containing 20 mmol/l Tris-HCl, pH 8.0, 137 mmol/l NaCl, 1 mmol/l $MgCl_2$, 1 mmol/l $CaCl_2$, 10% (vol/vol) glycerol, 1% Nonidet P-40, 1 mmol/l dithiothreitol (DTT), 1 mmol/l PMSF, and 0.4 mmol/l Na-orthovanadate (phosphatidylinositol 3-kinase [PI 3-K] lysis buffer). The insoluble material was removed by centrifugation at 13,000g for 15 min. Soluble supernatants were immunoprecipitated with anti-phosphotyrosine monoclonal antibody (a-PY) (UBI diagnostics). The immunoprecipitate was washed twice in PBS containing 1% Nonidet P-40 and 1 mmol/l DTT, twice in 100 mmol/l Tris, pH 7.4, containing 500 mmol/l $LiCl_2$ and 1 mmol/l DTT, and finally twice with 10 mmol/l NaCl and 1 mmol/l DTT. PI 3-K activity was measured directly in these immunoprecipitates in 50 µl of reaction mixture containing 20 mmol/l HEPES, pH 7.1, 0.4 mmol/l EGTA, 0.4 mmol/l sodium phosphate, 10 mmol/l $MgCl_2$, 0.2 mg/ml PI, and [γ-^{32}P]ATP (40 mmol/l, 10 µCi). After 5 min at 25°C, the reaction was stopped by the addition of 15 µl of 4N HCl and 130 µl of $CHCl_3$:methanol (1:1). Samples were centrifuged, and the lower organic phase was applied to a silica gel thin-layer chromatography (TLC) plate that had been coated with 1% potassium oxalate at 100°C for 1 h. TLC was performed in $CHCl_3$:CH_3OH:H_2O:NH_4OH (60:47:11.3:3.2), dried, and visualized by autoradiography.

Glycogen synthesis. The rate of glycogen synthesis was estimated from the incorporation of ^{14}C-glucose into cellular glycogen (22). Fibroblasts (10 × 104) were implanted in 6-well trays and grown for 5 days. After incubation for 16 h in serum-free DMEM, fibroblasts were preincubated with increasing concentrations of insulin (0–100 nmol/l) (30 min at 37°C in 1 ml of serum-free DMEM). ^{14}C-glucose (1.25 µCi, final concentration) was then added for 60 min, and the incorporation was stopped by rapidly washing cells three times with ice-cold PBS. Cells were solubilized in 30% KOH, transferred to separate tubes, and 2 mg glycogen was added. Glycogen was then precipitated by adding 2.2 vol 100% ethanol, and the radioactivity was incorporated into glycogen determined by a β-Beckman L6000TA counter (Fullerton, CA). Data were normalized for protein content (20).

RESULTS

Subjects studied. All subjects were nondiabetic (Table 1). The resistant group had slightly but significantly higher FPG values and much higher fasting plasma insulin values than the sensitive subject group (95.5 ± 12.0 vs. 52.8 ± 12.6 pmol/l, P < 0.001). The insulin-resistant subjects were slightly but not significantly older, and both groups had similar BMI values.

A significant difference was observed in serum cholesterol (4.47 ± 0.23 vs. 5.66 ± 0.35 mmol/l, P < 0.02) in insulin-sensitive and insulin-resistant subjects, respectively, but not in

TABLE 1
Characteristics of the study subjects

	Clinical characteristics							Fibroblasts	
	Sex (M/F)	Age (years)	BMI (kg/m^2)	FPG (mmol/l)	Fasting immunoreactive insulin (pmol/l)	M (mg · kg^{-1} · min^{-1})	K_{ITT}	IR tyrosine kinase EC_{50} (nmol/l)	PC-1 content (ng/0.1 mg protein)
Insulin-sensitive subjects									
1	M	49.0	22.0	4.72	52.8	—	6.4	0.225	3.87
2	M	25.0	19.8	4.62	33.6	—	9.4	0.180	5.60
3	F	28.0	19.1	4.66	49.8	—	8.8	0.025	0.96
4	M	31.0	23.8	4.88	42.0	7.40	—	0.135	2.70
5	M	35.0	25.2	4.94	36.0	6.80	—	0.150	2.34
6	F	31.0	21.9	4.61	43.2	11.6	—	0.120	3.09
Mean ± SE		33.0 ± 3.4	22.0 ± 1.0	4.75 ± 0.06	42.9 ± 3.1	8.6 ± 1.5	8.2 ± 0.9	0.139 ± 0.027	3.09 ± 0.64
Insulin-resistant subjects									
1	M	21.0	20.7	5.22	124.8	—	4.5	0.300	4.60
2	F	21.0	22.5	5.04	93.6	—	4.2	0.400	3.96
3	F	43.0	22.8	5.38	54.6	—	5.4	0.350	7.63
4	M	49.0	26.4	5.22	90.0	4.8	—	0.400	5.53
5	F	55.0	23.9	5.44	78.0	5.0	—	0.300	9.00
6	F	54.0	29.7	5.16	132.0	4.6	—	0.400	9.26
Mean ± SE		40.0 ± 6.4	24.3 ± 1.3	5.24 ± 0.06*	95.5 ± 11.8*	4.8 ± 0.1*	4.7 ± 0.4*	0.358 ± 0.020*	6.66 ± 0.93*

IR tyrosine kinase EC_{50} is the concentration of insulin required to cause a 50% stimulation of IR autophosphorylation in cultured fibroblasts. *P < 0.05 vs. insulin-sensitive subjects.

triglyceride levels (1.40 ± 0.36 vs. 1.93 ± 0.08 mmol/l) and mean blood pressure (95 ± 2 vs. 97 ± 3 mmHg).

PC-1 content in cultured fibroblasts. PC-1 content in solubilized fibroblasts was significantly higher in cells from insulin-resistant subjects (6.7 ± 0.9 vs. 3.1 ± 0.6 vs. ng per 0.1 mg protein, P < 0.01) (Fig. 1). When all subjects were considered, PC-1 content significantly correlated with FPG levels (r = 0.70, P = 0.01) and almost significantly with fasting plasma insulin levels (r = 0.56, P = 0.055).

^{125}I-insulin binding in cultured fibroblasts. Total ^{125}I-insulin binding (expressed as the percentage of bound/total radioactivity) was similar in insulin-resistant and insulin-sensitive subjects (0.66 ± 0.14 and 0.65 ± 0.17 per 0.1 mg of protein, respectively). Competition-inhibition curves indicated similar IC_{50}s (inhibiting concentrations, 50%) (0.26 ± 0.06 vs. 0.35 ± 0.11

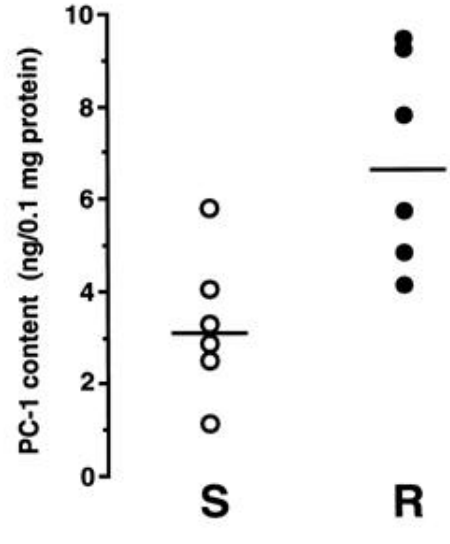

FIG. 1. PC-1 content in cultured skin fibroblasts from insulin-sensitive (○) and insulin-resistant (●) subjects. Horizontal bars indicate mean values. P = 0.01, sensitive vs. resistant subjects.

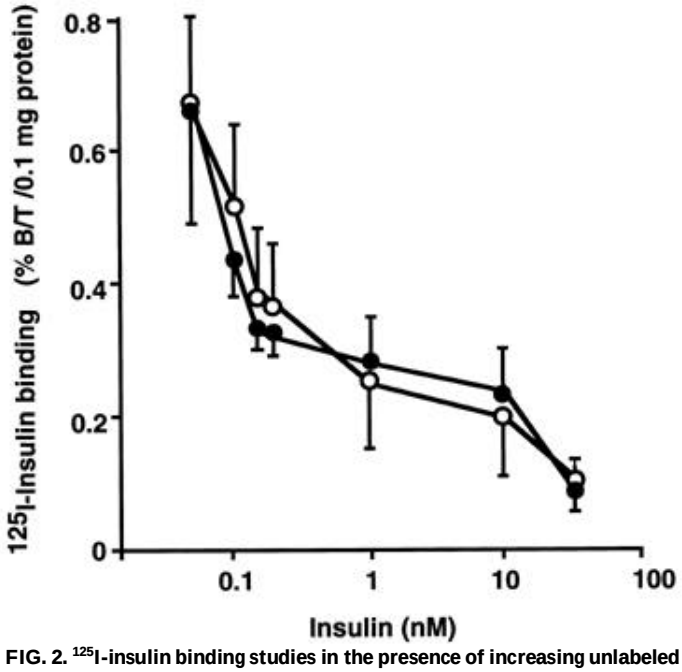

FIG. 2. ^{125}I-insulin binding studies in the presence of increasing unlabeled insulin concentrations in cultured skin fibroblasts of insulin-sensitive (○) and insulin-resistant (●) subjects.

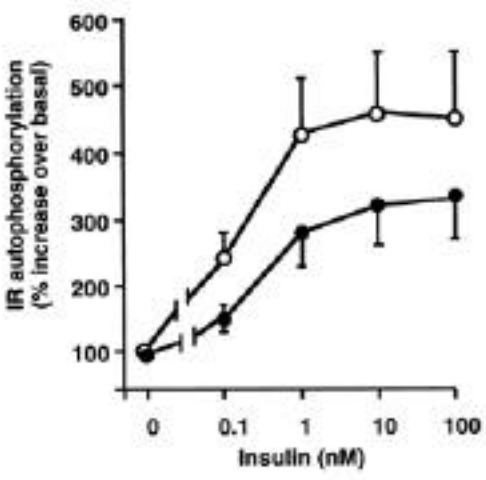

FIG. 3. Insulin stimulation of IR autophosphorylation in cultured skin fibroblasts of insulin-sensitive (○) and insulin-resistant (●) subjects. Data are presented as the percentage increase over basal (P = 0.014 by two-way ANOVA test, sensitive vs. resistant subjects). Basal values (in the absence of insulin) in the two groups were not statistically different (28 ± 8 vs. 35 ± 5, arbitrary densitometric units/0.1 mg protein, respectively).

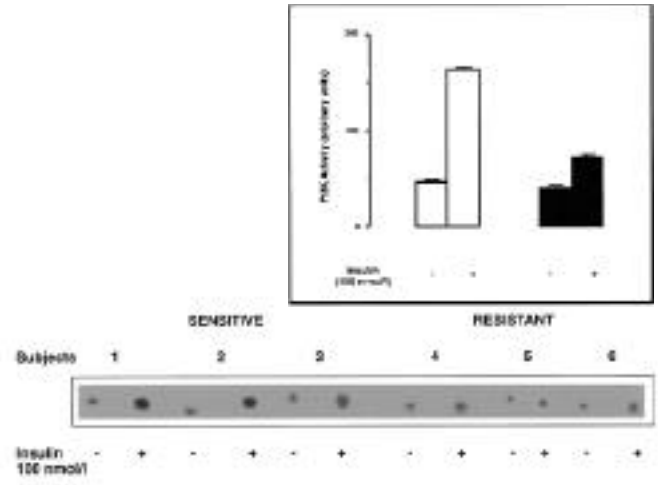

FIG. 5. PI 3-K activity in cultured skin fibroblasts from three (1–3) insulin-sensitive (□) and three (4–6) insulin-resistant (■) subjects in the absence or presence of 100 nmol/l insulin (A). Data are means ± SE. The reaction mixture was spotted onto a silica gel plate and subjected to TLC, and then the plate was exposed for autoradiography (B).

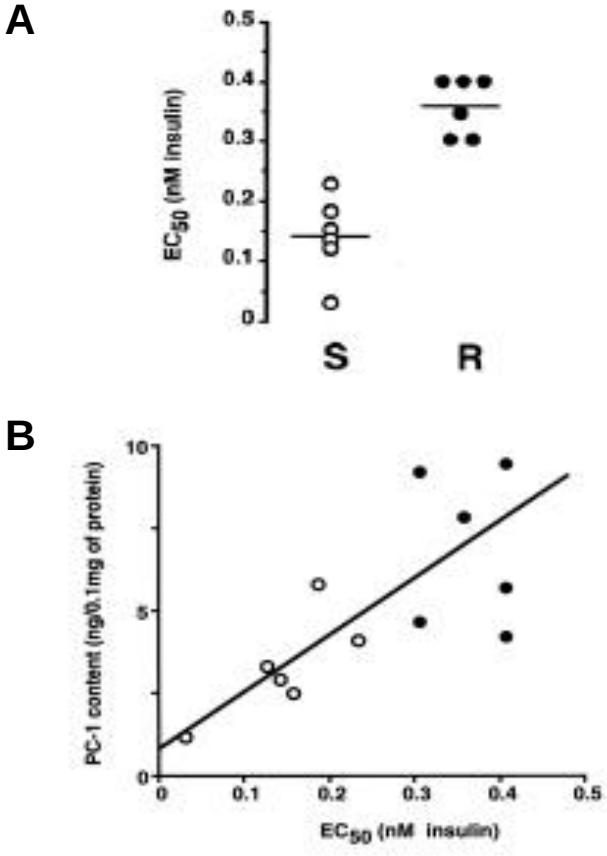

FIG. 4. EC_{50} of insulin-stimulated IR autophosphorylation in cultured skin fibroblasts. A: EC_{50} values in fibroblasts from insulin-sensitive (○) and insulin-resistant (●) subjects. Horizontal bars indicate mean values. P = 0.001, sensitive vs. resistant subjects. B: Correlation between PC-1 content and EC_{50} in cultured skin fibroblasts from insulin-sensitive (○) and insulin-resistant (●) subjects (r = 0.72; P = 0.008).

nmol/l, NS) (Fig. 2). These data indicated, therefore, that cells with high PC-1 content did not have an altered insulin binding.

IR autophosphorylation. In cells from both groups, insulin induced IR autophosphorylation, with a detectable effect at 0.1 nmol/l insulin and a maximal effect at 10–100 nmol/l. At all insulin concentrations, the cells from insulin-resistant subjects were less responsive to insulin than cells from insulin-sensitive subjects (P = 0.014, by two-way analysis of variance [ANOVA] test) (Fig. 3).

In addition to being less responsive, cells from resistant subjects were less sensitive to insulin. In these cells, the EC_{50} (effective concentration, 50%) of IR autophosphorylation was 0.36 ± 0.02 vs. 0.14 ± 0.03 nmol/l, $P < 0.001$ (Fig. 4A).

The EC_{50} of insulin stimulation of IR autophosphorylation in cultured fibroblasts significantly correlated (r = 0.72, P = 0.008) with PC-1 cell content (Fig. 4B). Moreover, a significant negative correlation was observed between the EC_{50} of insulin stimulation of IR autophosphorylation and whole body insulin sensitivity when measured by both insulin clamp studies (as indicated by M, r = –0.87, P = 0.024) and ITT (as indicated by K_{ITT}, r = –0.96, P = 0.0027).

Insulin-stimulated PI 3-K activity in cultured fibroblasts. To evaluate downstream signaling of the IR, we studied insulin stimulation of the enzyme PI 3-K in cultured fibroblasts from three insulin-sensitive and three insulin-resistant subjects. No difference in the unstimulated fibroblast PI 3-K activity was observed. However, insulin-stimulated (100 nmol/l) PI 3-K activity in fibroblasts from insulin-sensitive subjects was significantly higher ($P < 0.05$) than that in fibroblasts from insulin-resistant subjects (Fig. 5).

^{14}C-glucose incorporation into glycogen in cultured fibroblasts. In cells from both groups, insulin stimulated ^{14}C-glucose incorporation into glycogen. An effect was observed at 0.1 nmol/l insulin and progressively increased up to 100 nmol/l insulin (Fig. 6). Basal (unstimulated) glycogen synthesis was not significantly different in the two groups (0.74 ± 0.22 vs. 1.19 ± 0.20 nmol/l · h^{-1} · 0.1 mg^{-1} of protein in insulin-resistant and insulin-sensitive subjects, respectively). The insulin dose-response curves indicated

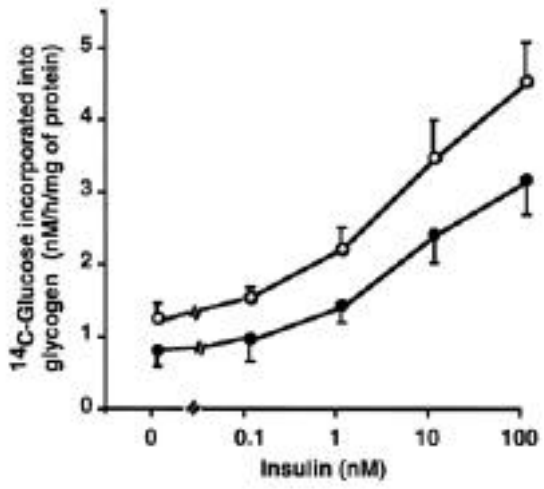

FIG. 6. Insulin stimulation of glycogen synthesis (incorporation of ^{14}C-glucose into glycogen) in cultured skin fibroblasts from insulin-sensitive (○) and insulin-resistant (●) subjects. At all insulin concentrations, including basal, incorporation of ^{14}C-glucose into glycogen was greater in the insulin-sensitive subjects than in the insulin-resistant subjects (P = 0.001 by two-way ANOVA test).

that, at all insulin concentrations, fibroblasts from resistant subjects were significantly less responsive to insulin than fibroblasts from sensitive subjects ($P < 0.001$ by two-way ANOVA test) (Fig. 6). Because higher concentrations of insulin will also activate the related receptor for IGF-I (11), they were not used in these studies, and thus, maximal responsiveness could not be calculated.

Fibroblast versus muscle tissue PC-1 content. In an additional five nonobese (BMI <30 kg/m^2) nondiabetic (by OGTT) subjects, we next compared PC-1 content in both cultured fibroblasts and biopsied internal oblique muscle (Table 2). Over a range of PC-1 values, the content of this protein in cultured fibroblasts closely reflected the PC-1 content in muscle tissues (r = 0.95, P = 0.010). These data indicate, therefore, that cultured skin fibroblasts are an appropriate model for studying PC-1 because they reflect its content in a major insulin-responsive tissue.

DISCUSSION

Insulin resistance is a common clinical condition whose etiology is poorly understood and for which there is no effective treatment. In certain individuals, there is evidence that insulin resistance is inherited and may have a genetic component. Because insulin resistance is a major feature of NIDDM, its reversal could potentially either prevent, delay, or ameliorate hyperglycemia. In addition, insulin resistance per se (independent of hyperglycemia and NIDDM) is associated with and may cause hypertension, dyslipidemia, and coronary heart disease (1). Effective treatment of insulin resistance, therefore, should also benefit these conditions. Thus, it is important to understand the biochemical basis of insulin resistance.

In a variety of cultured cells, the elevation of PC-1 content inhibits IR tyrosine kinase activity and subsequent cellular signaling. In addition, in muscle and adipose tissue of insulin-resistant subjects, we have reported that the content of PC-1 is elevated and that this elevation correlates with both insulin resistance in vivo and decreased IR tyrosine kinase activity and glucose transport in vitro (16,17,23). These data strongly suggest, therefore, that PC-1 overexpression in these insulin-sensitive tissues is a factor in causing insulin resistance.

TABLE 2
Relationship between muscle and fibroblast PC-1 content

Case	Sex	Age (years)	BMI (kg/m^2)	PC-1 content (ng/0.1 mg protein)	
				Muscle	Fibroblast
A	M	53	25.0	2.2	3.7
B	F	21	22.5	2.9	5.0
C	F	16	26.9	5.3	7.5
D	M	31	23.8	5.5	6.5
E	F	60	28.7	9.6	17.7

In those previous studies, however, it was unclear whether the elevation of PC-1 was a primary biochemical defect or was secondary to altered metabolic activities. In the present study, to determine whether the elevation of PC-1 was a primary defect, we examined skin fibroblasts from nonobese subjects who were insulin-resistant, but did not have NIDDM. The content of PC-1 was more than doubled in cells from these subjects when compared with cells from insulin-sensitive subjects. Parallel measurements of PC-1 in muscle and fibroblasts revealed a direct correlation between PC-1 content in these two tissues. This result indicated that the level of PC-1 in fibroblasts mirrors PC-1 content in the major tissue for insulin-mediated glucose disposal. Moreover, PC-1 content in fibroblasts negatively correlated with both in vivo insulin sensitivity and in vitro activation of IR autophosphorylation. Finally, in fibroblasts from insulin-resistant subjects, insulin stimulation of glycogen synthesis was reduced, and, although studied in only three subjects from each group, insulin-stimulated PI 3-K activity was also reduced. These studies suggest, therefore, that the elevation of PC-1 content is a primary factor in the cause of insulin resistance.

How PC-1 regulates IR tyrosine kinase activity is unknown. It is a class II transmembrane glycoprotein that is located both on plasma membrane and in the endoplasmic reticulum. PC-1 exists as a homodimer of 230–260 kDa, depending on the cell type, and is inserted into the membrane such that there is a small cytoplasmic NH_2-terminal and a larger extracellular COOH-terminal (30,31). The extracellular domain of PC-1 cleaves sugar-phosphate, phosphosulfate, pyrophosphate, and phosphodiesterase linkages.

The active enzyme site for phosphodiesterase and pyrophosphatase contains a key threonine residue necessary for these activities (30,31). We have shown that mutation of this residue does not impair the ability of PC-1 to inhibit IR function (15), and thus, these enzyme activities of PC-1 are not involved in its ability to inhibit the IR. Most likely, PC-1 modifies IR signaling by directly binding to the IR. We have observed that PC-1 binds to the IR both in vivo and in vitro, and subsequently, receptor function is inhibited (I.D.G., unpublished observations). It is therefore possible that PC-1 interacts with the IR and alters its ability to activate the β-subunit. It is also possible that protein phosphorylation mediates the interaction of PC-1 with the IR. PC-1 has been reported to be a threonine kinase (32,33), and the

IR tyrosine kinase function is blunted by serine/threonine phosphorylation (10).

There is evidence that insulin resistance has a genetic component. Lean offspring of NIDDM patients are themselves insulin-resistant, and studies of monozygotic twins suggest that insulin resistance is an inherited trait (8,9). In Pima Indians, a group of individuals with a very high prevalence of insulin resistance, insulin resistance may have a codominant pattern of inheritance (6). The PC-1 gene is in chromosomal region 6q22-q23 (30,31), and Duggirala et al. (34) have found that the NIDDM marker D6S290 is located near the PC-1 gene in Mexican-Americans with NIDDM. We now find that PC-1 content is elevated in fibroblasts from nonobese nondiabetic insulin-resistant subjects. Because these fibroblasts were grown under standardized conditions for several passages, their elevated PC-1 content is very likely genetically determined. These data suggest, therefore, that PC-1 may be a candidate gene for the cause of insulin resistance.

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