

# Skeletal Muscle Insulin Resistance in Normoglycemic Subjects With a Strong Family History of Type 2 Diabetes Is Associated With Decreased Insulin-Stimulated Insulin Receptor Substrate-1 Tyrosine Phosphorylation

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Normoglycemic subjects with a strong family history of type 2 diabetes are insulin resistant, but the mechanism of insulin resistance in skeletal muscle of such individuals is unknown. The present study was undertaken to determine whether abnormalities in insulin-signaling events are present in normoglycemic, nonobese subjects with a strong family history of type 2 diabetes. Hyperinsulinemic-euglycemic clamps with percutaneous muscle biopsies were performed in eight normoglycemic relatives of type 2 diabetic patients (FH<sup>+</sup>) and eight control subjects who had no family history of diabetes (FH<sup>-</sup>), with each group matched for age, sex, body composition, and ethnicity. The FH<sup>+</sup> group had decreased insulin-stimulated glucose disposal ( $6.64 \pm 0.52$  vs.  $8.45 \pm 0.54$  mg · kg<sup>-1</sup> fat-free mass · min<sup>-1</sup>;  $P < 0.05$  vs. FH<sup>-</sup>). In skeletal muscle, the FH<sup>+</sup> and FH<sup>-</sup> groups had equivalent insulin stimulation of insulin receptor tyrosine phosphorylation. In contrast, the FH<sup>+</sup> group had decreased insulin stimulation of insulin receptor substrate (IRS)-1 tyrosine phosphorylation ( $0.522 \pm 0.077$  vs.  $1.328 \pm 0.115$  density units;  $P < 0.01$ ) and association of PI 3-kinase activity with IRS-1 ( $0.299 \pm 0.053$  vs.  $0.466 \pm 0.098$  activity units;  $P < 0.05$ ). PI 3-kinase activity was correlated with the glucose disposal rate ( $r = 0.567$ ,  $P = 0.02$ ). In five subjects with sufficient biopsy material for further study, phosphorylation of Akt was  $0.266 \pm 0.061$  vs.  $0.404 \pm 0.078$  density units ( $P < 0.10$ ) and glycogen synthase activity was  $0.31 \pm 0.06$  vs.  $0.50 \pm 0.12$  ng · min<sup>-1</sup> · mg<sup>-1</sup> ( $P < 0.10$ ) for FH<sup>+</sup> and FH<sup>-</sup> subjects, respectively. Therefore, despite normal insulin receptor phosphorylation, post-

receptor signaling was reduced and was correlated with glucose disposal in muscle of individuals with a strong genetic background for type 2 diabetes. *Diabetes* 50: 2572–2578, 2001

Type 2 diabetes is a common metabolic disorder characterized by insulin resistance and relative or absolute insulin deficiency (1). Strong evidence favors the role of genetic factors in the development of type 2 diabetes. For example, the risk of developing type 2 diabetes is higher in individuals with a strong family history of diabetes (2), and the concordance rate of type 2 diabetes in monozygotic twins ranges from 55 to 90% (3,4). Normoglycemic subjects with a strong family history of type 2 diabetes display insulin resistance (5–9), suggesting that impaired insulin action is a primary factor that contributes to the pathogenesis of type 2 diabetes. In Pima Indians, the insulin resistance displays a trimodal distribution, although the pattern of inheritance in other ethnic groups remains unclear. A number of specific gene mutations have been identified as the cause of diabetes in some families (10–15), but the genetic basis of the majority of type 2 diabetes remains unknown.

Because insulin resistance in skeletal muscle precedes the development of type 2 diabetes (1), it is important to determine the biochemical and molecular abnormalities present in skeletal muscle before the onset of hyperglycemia and other metabolic disturbances that could cause secondary defects in insulin action. Impaired stimulation of nonoxidative glucose metabolism (glycogen synthesis) and glycogen synthase activity in skeletal muscle are consistently found in type 2 diabetic subjects (16–19). The defect in activation of glycogen synthase is also present in normoglycemic subjects with a family history of diabetes (8). Moreover, the defect in insulin-stimulated glycogen synthase activity persists when skeletal muscle cells from type 2 diabetic patients are cultured under “normoglycemic” conditions (20); in that study, Jackson et al. (20) reported that protein expression of insulin receptor substrate (IRS)-1, p85, Akt, and glycogen synthase was normal. However, the defect in insulin activation of glycogen

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Received for publication 26 December 2000 and accepted in revised form 7 August 2001.

ANOVA, analysis of variance; FFA, free fatty acid; FFM, fat-free mass; FH<sup>+</sup>, family history positive for diabetes; FH<sup>-</sup>, family history negative for diabetes; G6P, glucose 6-phosphate; GS, glycogen synthase; IRS, insulin receptor substrate; OGTT, oral glucose tolerance test; PI, phosphatidylinositol.

synthase could have resulted from a proximal defect in the activity of the insulin receptor signal transduction system and not be intrinsic to glycogen synthase.

We and others have shown that insulin stimulation of insulin receptor and IRS-1 tyrosine phosphorylation, as well as the association of phosphatidylinositol (PI) 3-kinase with IRS-1 and its subsequent activation, are dramatically reduced in skeletal muscle in type 2 diabetes (21,22). These defects in insulin signaling have been related to defects in insulin-stimulated glucose disposal (21). It is uncertain whether all or any of these abnormalities are primary to the muscle or whether some or all are acquired secondarily to changes in the diabetic metabolic milieu, especially hyperglycemia. The present study was undertaken to determine which abnormalities in proximal insulin-signaling events are present in normoglycemic, non-obese subjects with a strong family history of type 2 diabetes. To accomplish this, we performed hyperinsulinemic-euglycemic clamps in combination with percutaneous skeletal muscle biopsies in a group of normoglycemic relatives of type 2 diabetic patients and a group of well-matched control subjects with no family history of diabetes.

## RESEARCH DESIGN AND METHODS

Participants in this study included eight subjects with at least two first-degree relatives with type 2 diabetes and eight control subjects with no family history of type 2 diabetes. All subjects had a normal 75-g oral glucose tolerance test (OGTT) and were judged to be in good health on the basis of physical examination, medical history, routine blood work, urinalysis, and electrocardiogram. No subject was taking any medication, and none of the subjects engaged in regular physical exercise. Over the 3 days before the study, subjects were instructed to maintain their usual diet and not to perform any exercise. The purpose, nature, and potential risks of the study were explained to all subjects, and written consent was obtained before their participation. The protocol was approved by the Institutional Review Board of the University of Texas Health Science Center at San Antonio.

**Study design.** All studies were conducted in the General Clinical Research Center of the University of Texas Health Science Center at San Antonio, beginning at 8:00 A.M. after a 10-h overnight fast. Each subject participated in two studies: an OGTT and a hyperinsulinemic-euglycemic clamp. The studies were performed at 3- to 7-day intervals.

At 8:00 A.M. on the day of the OGTT, a catheter was inserted into an antecubital vein for all blood drawing. At  $t = -30$  and 0 min, blood samples were obtained for the measurement of plasma glucose, free fatty acids (FFAs), and insulin concentrations. At  $t = 0$ , subjects ingested 75 g glucose in an orange-flavored solution. Blood samples were subsequently obtained at 30, 60, 90, and 120 min for determination of plasma glucose, FFA, and insulin concentrations.

Then 3-7 days after the OGTT, subjects returned to the General Clinical Research Center at 8:00 A.M. for a hyperinsulinemic-euglycemic clamp with biopsies of the vastus lateralis muscle. An antecubital vein was cannulated for infusion of [ $3\text{-}^3\text{H}$ ]glucose, 20% glucose, and insulin. A hand vein was cannulated retrogradely, and the hand was placed in a heated box ( $55^\circ\text{C}$ ) for sampling of arterialized blood. A primed ( $25\ \mu\text{Ci}$ )-continuous ( $0.25\ \mu\text{Ci}/\text{min}$ ) infusion of [ $3\text{-}^3\text{H}$ ]glucose was begun 2 h before the start of insulin infusion to allow for isotopic equilibration. Blood was drawn every 10 min during the last 30 min of the isotopic equilibration period for measurement of plasma glucose, insulin, and FFA concentrations and tritiated glucose radioactivity. After 60 min of bed rest, a percutaneous muscle biopsy was obtained with a Bergstrom cannula from the vastus lateralis muscle under local anesthesia (23). Muscle biopsy specimens were immediately blotted free of blood, frozen in liquid nitrogen, and stored under liquid nitrogen until processing. After the 2-h isotopic equilibration period, insulin was started at a rate of  $40\ \text{mU} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$ . The plasma glucose concentration was measured every 5 min by a glucose analyzer (Beckman Instruments, Fullerton, CA), and an infusion of 20% glucose was adjusted to maintain euglycemia (24). Blood was obtained every 10-15 min during the insulin clamp for measurement of plasma insulin and FFA concentrations and tritiated glucose radioactivity. A second percutaneous muscle biopsy was obtained from a site about 4 cm distal to the first site 30 min after the start of insulin infusion ( $\sim 90$  min after the initial biopsy). The insulin infusion was continued for 120 min to obtain a measure of the rate

TABLE 1  
Subject characteristics

	FH+	FH-
Age (years)	43 $\pm$ 2	42 $\pm$ 2
Sex (F/M)	5/3	5/3
Ethnicity (Mexican-American/Anglo)	5/3	4/4
BMI ( $\text{kg}/\text{m}^2$ )	27.2 $\pm$ 1.2	26.2 $\pm$ 1.3
Percent lean body mass	69 $\pm$ 3	69 $\pm$ 3
Fasting plasma glucose (mg/dl)	97 $\pm$ 3	95 $\pm$ 2
HbA <sub>1c</sub> (%)	5.1 $\pm$ 0.1	5.2 $\pm$ 0.2
Fasting plasma insulin ( $\mu\text{U}/\text{ml}$ )	8.5 $\pm$ 0.5*	7.0 $\pm$ 0.5
Fasting plasma FFA ( $\mu\text{M}$ )	643 $\pm$ 69	547 $\pm$ 33
Cholesterol (mg/dl)	197 $\pm$ 13	192 $\pm$ 15
LDL cholesterol (mg/dl)	122 $\pm$ 8	120 $\pm$ 9
HDL cholesterol (mg/dl)	54 $\pm$ 5	53 $\pm$ 3
Triglycerides (mg/dl)	109 $\pm$ 23	95 $\pm$ 25

Data are means  $\pm$  SE. \* $P < 0.05$  for FH<sup>+</sup> vs. FH<sup>-</sup>.

of insulin-stimulated glucose disposal during the last 30 min of the study (90-120 min period).

**Insulin receptor signaling and enzyme activity assays.** Insulin receptor and IRS-1 tyrosine phosphorylation were assayed using immunoprecipitation and immunoblot analysis, as previously described (21). Insulin stimulation of the association of PI 3-kinase activity with IRS-1 was assayed by determining the ability of anti-IRS-1 immunoprecipitates to incorporate [ $^{32}\text{P}$ ]ATP into PI (21). Activation of Akt was assessed using immunoblot analysis of 100  $\mu\text{g}$  total muscle protein by determining the phosphorylation of serine 473 using a specific antiphospho-Akt antibody (Cell Signaling Technologies, Beverly, MA). Insulin receptor phosphotyrosine and Akt immunoblots were stripped and probed for protein expression. IRS-1 protein expression was determined using separate immunoblots. Insulin receptor and IRS-1 tyrosine phosphorylation and Akt serine phosphorylation were expressed relative to protein levels. All data were calculated as the percent of the mean insulin-stimulated value in the FH<sup>-</sup> group. Glycogen synthase (GS) activity was determined using 0.1 and 10 mmol/l glucose 6-phosphate (G6P), as previously described (23). The ratio of GS<sub>0.1</sub> to GS<sub>10</sub> activity or the fractional velocity is an indicator of dephosphorylation and activation of the enzyme (23).

**Analytical determinations, calculations, and statistics.** Plasma insulin concentrations were determined by radioimmunoassay (Diagnostics Products, Los Angeles). Plasma FFA concentrations were assayed by an enzymatic method (NEFA-C kit; Wako Pure Chemicals, Osaka, Japan). Body composition was determined by bioimpedance (RJL Bio-106 Spectrum Body Composition Analyzer; RJL Systems, Detroit, MI). Plasma glucose specific activity was determined on plasma samples after barium hydroxide/zinc sulfate precipitation. During the postabsorptive period, the rate of glucose appearance equals the rate of disappearance and is calculated as the tritiated glucose infusion rate (dpm/min) divided by the plasma glucose specific activity (dpm/mg). During the euglycemic insulin clamp, nonsteady-state conditions prevail. In our study, rates of glucose appearance and disappearance were calculated with Steele's nonsteady-state equation using a glucose distribution volume of 0.65 (25). Differences between means were compared by unpaired  $t$  tests or repeated measures of analysis of variance (ANOVA) where appropriate.

## RESULTS

**Subject characteristics and OGTT.** Characteristics of the subjects are given in Table 1. Both groups of subjects were well matched with respect to age, sex, ethnicity, and body composition. The fasting plasma insulin concentration was slightly higher in the family history-positive (FH<sup>+</sup>) group than in the family history-negative (FH<sup>-</sup>) group ( $P < 0.05$ ). Results from the OGTT are shown in Fig. 1. There were no significant differences in the plasma glucose or insulin concentrations between the FH<sup>+</sup> and FH<sup>-</sup> groups during the OGTT, except for the fasting plasma insulin concentration, which was significantly higher in FH<sup>+</sup> subjects (Table 1). However, there were slight delays in the kinetics of both plasma glucose and insulin concentrations in the FH<sup>+</sup> group (Fig. 1). The

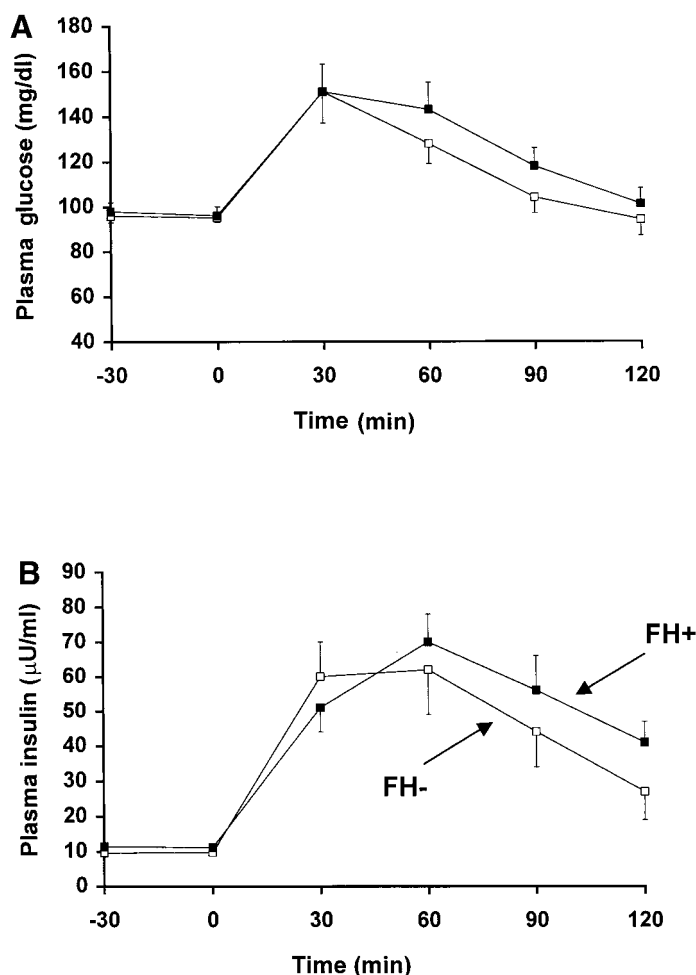


FIG. 1. Plasma glucose (A) and insulin (B) concentrations during the 75-g OGTT in subjects with (■) and without (□) a strong family history of type 2 diabetes. Data are means  $\pm$  SE.

fasting plasma FFA concentrations were slightly but not significantly higher in the FH<sup>+</sup> group. During the OGTT, plasma FFA concentrations decreased similarly in FH<sup>+</sup> and FH<sup>-</sup> individuals (not shown).

**Hyperinsulinemic-euglycemic clamp.** Plasma glucose, insulin, and FFA concentrations during the euglycemic insulin clamp are given in Table 2 for the basal condition, at 30 min into the insulin infusion (time of the second muscle biopsy), and at 90–120 min during the insulin infusion. There were no differences between the FH<sup>+</sup> and FH<sup>-</sup> groups for any of these parameters, except for fasting plasma insulin concentrations, as noted above. Glucose disposal rates were determined using tritiated glucose

specific activities and were expressed relative to fat-free mass (FFM). During the basal state, the rate of glucose disposal (which equals the rate of endogenous glucose production) was similar in the FH<sup>+</sup> and FH<sup>-</sup> groups ( $2.80 \pm 0.08$  vs.  $2.95 \pm 0.24$  mg  $\cdot$  kg<sup>-1</sup> FFM  $\cdot$  min<sup>-1</sup>). During the 90–120 min period of the euglycemic insulin clamp, the rate of whole-body glucose disposal was reduced by  $22 \pm 6\%$  ( $P = 0.01$ ) in the FH<sup>+</sup> versus the FH<sup>-</sup> group ( $6.64 \pm 0.52$  vs.  $8.45 \pm 0.62$  mg  $\cdot$  kg<sup>-1</sup> FFM  $\cdot$  min<sup>-1</sup>;  $P < 0.05$ ).

**Insulin receptor signaling.** Basal and insulin-stimulated values for insulin receptor and IRS-1 tyrosine phosphorylation and IRS-1-associated PI 3-kinase activity are shown in Fig. 2, expressed as the percent of insulin-stimulated values in the FH<sup>-</sup> group. Neither basal nor insulin-stimulated insulin receptor tyrosine phosphorylation differed between the groups. In contrast, both the basal and the insulin-stimulated IRS-1 tyrosine phosphorylation were significantly decreased ( $P < 0.001$  by repeated measures ANOVA) in the FH<sup>+</sup> versus the FH<sup>-</sup> individuals. The association of PI 3-kinase with IRS-1 was also reduced in the FH<sup>+</sup> group ( $P < 0.03$  by repeated measures ANOVA) during both basal and insulin-stimulated conditions ( $P < 0.05$ ). To assess insulin receptor signaling downstream of PI 3-kinase, as well as further downstream effects, we assayed phosphorylation of Akt and glycogen synthase activity in five subjects in each group for whom there was sufficient muscle biopsy material (Fig. 3). Insulin increased Akt phosphorylation and GS activity in both groups ( $P < 0.01$ ). In FH<sup>+</sup> subjects, there was a trend for both Akt phosphorylation and glycogen synthase activity to be decreased, which mirrored the decreases in IRS-1 function and PI 3-kinase activity. Protein expression of the insulin receptor ( $1.41 \pm 0.14$  vs.  $1.75 \pm 0.14$  density units), IRS-1 ( $1.78 \pm 0.17$  vs.  $1.58 \pm 0.22$  density units), and Akt ( $5.49 \pm 0.76$  vs.  $4.70 \pm 0.47$  density units) did not differ between the FH<sup>+</sup> and FH<sup>-</sup> groups, respectively. There were no differences in glycogen synthase activity assayed using 10 nmol/l G6P, which is a measure of total glycogen synthase activity ( $6.76 \pm 2.22$  vs.  $6.41 \pm 2.36$  nmol  $\cdot$  min<sup>-1</sup>  $\cdot$  mg<sup>-1</sup>) in the FH<sup>+</sup> vs. FH<sup>-</sup> groups, respectively).

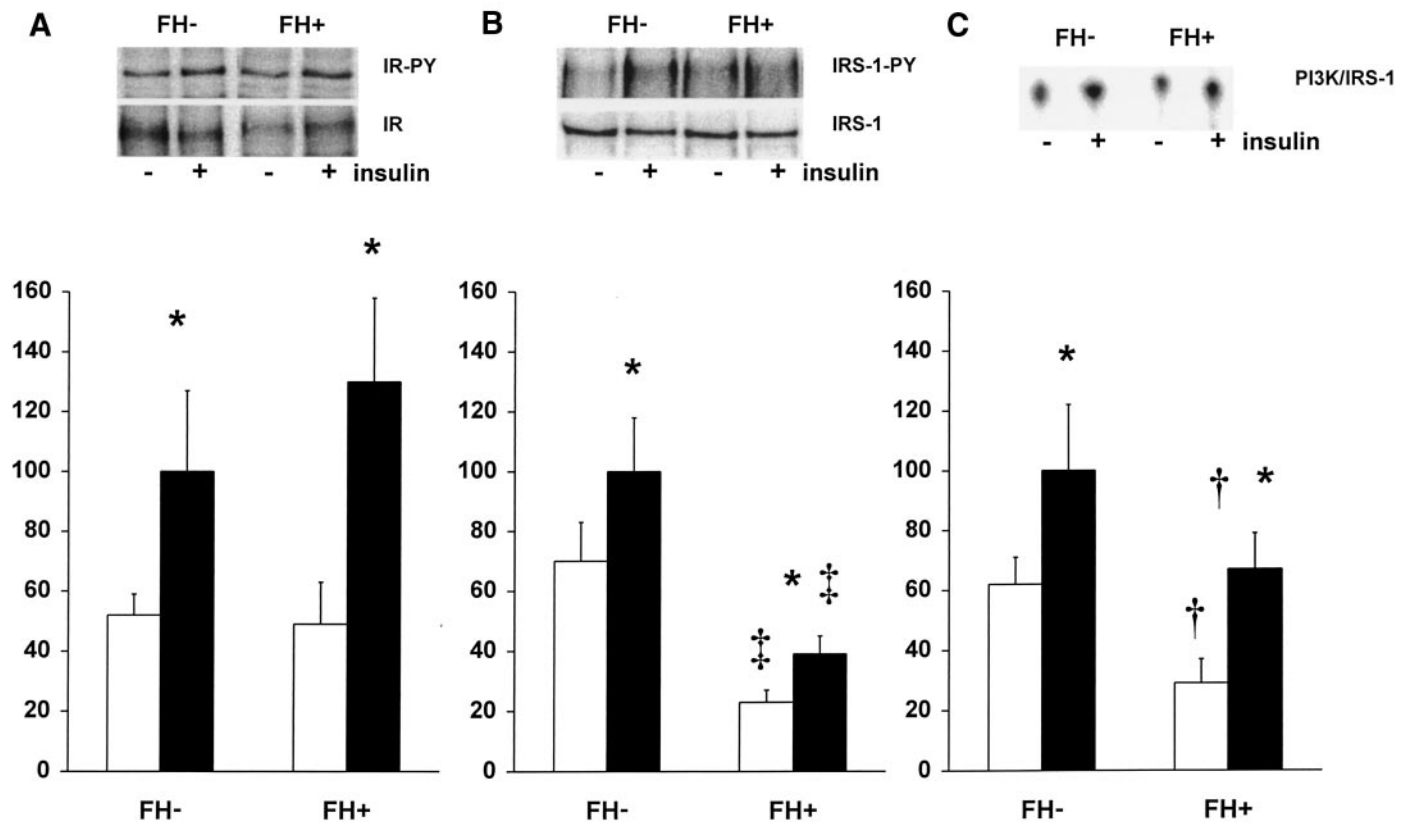
**Correlations.** To determine the association between insulin receptor signaling elements and body composition or insulin-stimulated glucose disposal rates, correlation analysis was performed. The association of PI 3-kinase with IRS-1 was significantly correlated with the rate of glucose disposal ( $r = 0.567$ ,  $P = 0.02$ ). Multiple regression analysis revealed that the association of PI 3-kinase with IRS-1 and IRS-1 tyrosine phosphorylation together accounted for  $>40\%$  of the variability in the rate of insulin-stimulated glucose disposal ( $P < 0.05$ ).

TABLE 2

Plasma glucose, insulin and FFA concentrations during the hyperinsulinemic-euglycemic clamp

	Basal		Biopsy		End of clamp	
	FH <sup>-</sup>	FH <sup>+</sup>	FH <sup>-</sup>	FH <sup>+</sup>	FH <sup>-</sup>	FH <sup>+</sup>
Glucose (mg/dl)	94 $\pm$ 1	94 $\pm$ 2	88 $\pm$ 2	91 $\pm$ 2	91 $\pm$ 2	92 $\pm$ 1
Insulin ( $\mu$ U/ml)	7.0 $\pm$ 0.5	8.5 $\pm$ 0.5*	56 $\pm$ 5	63 $\pm$ 3	56 $\pm$ 3	63 $\pm$ 3
FFA ( $\mu$ mol/l)	733 $\pm$ 33	687 $\pm$ 41	ND	ND	91 $\pm$ 13	82 $\pm$ 14

Data are means  $\pm$  SE. Plasma glucose, insulin, and FFA concentrations during the basal state ( $-30$  to  $0$  min); during the first 30 min of the hyperinsulinemic-euglycemic clamp (10–30 min), at which time a percutaneous muscle biopsy was performed; and at the end of the euglycemic clamp (90–120 min), at which time rates of insulin-stimulated glucose disposal were determined. \* $P < 0.05$  vs. FH<sup>-</sup> group. ND, not done.



**FIG. 2.** Effect of insulin on insulin receptor (IR) tyrosine phosphorylation (IR-PY; **A**); IRS-1 tyrosine phosphorylation (IRS-1-PY; **B**), and association of PI 3-kinase activity with IRS-1 (PI3K/IRS-1; **C**). Determinations were made using percutaneous muscle biopsies taken during the basal state (□) and after 30 min of insulin infusion (■) during a hyperinsulinemic-euglycemic clamp at an insulin infusion rate of  $40 \text{ mU} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$ . In the upper panel of each graph, a representative immunoblot for an FH<sup>+</sup> and FH<sup>-</sup> subject is shown. Data are expressed as percent of the insulin-stimulated value in the subjects with no family history of diabetes. \* $P < 0.01$  vs. basal values; † $P < 0.05$  and ‡ $P < 0.01$  for FH<sup>+</sup> vs. FH<sup>-</sup> groups.

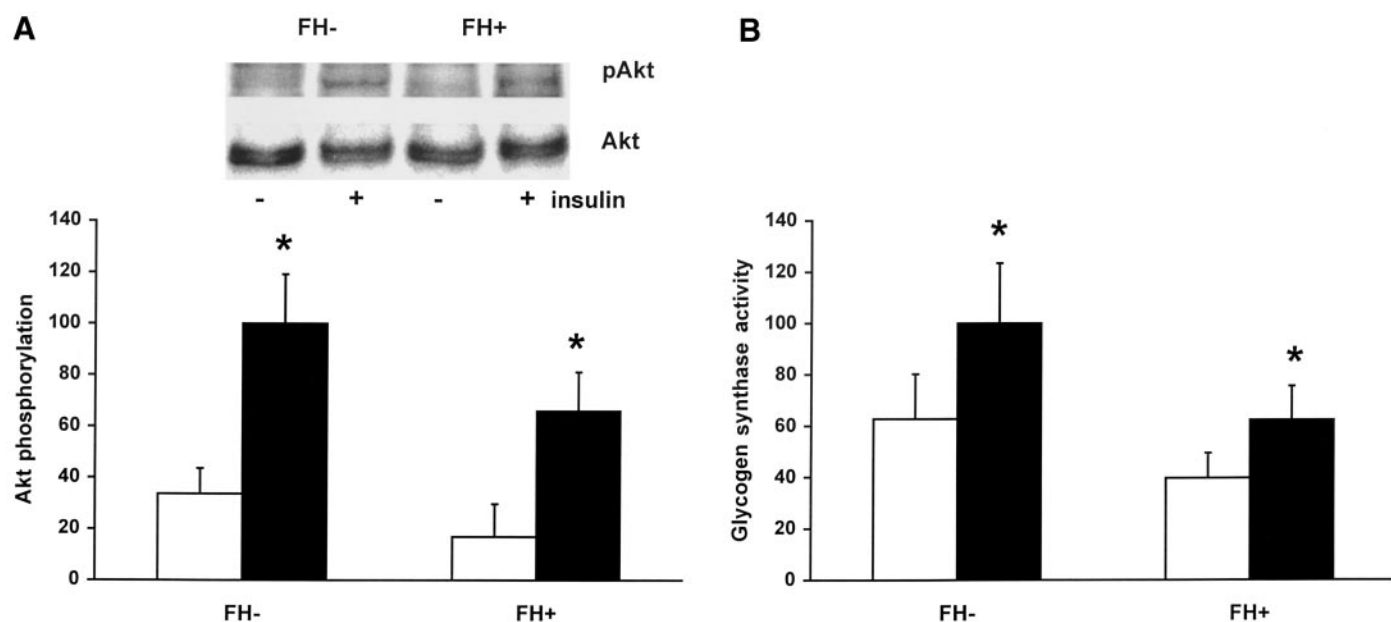
## DISCUSSION

The present study was undertaken to identify abnormalities in insulin receptor–signaling events in normoglycemic, nonobese subjects with a strong family history of type 2 diabetes. This approach should allow for the identification of primary, and possibly hereditary, abnormalities that are related to skeletal muscle insulin resistance before the onset of acquired secondary abnormalities, such as hyperglycemia or elevated FFA levels, that could impair insulin receptor signal transduction. Consistent with a number of previous reports (5–9), our results with the euglycemic clamp confirmed that subjects with a strong family history of type 2 diabetes are insulin resistant. In the present study, subjects with a strong family history of diabetes were well matched to control subjects, thereby excluding age, sex, body composition, and ethnicity as factors responsible for the insulin resistance. Because the subjects were not obese and had similar BMIs, insulin resistance secondary to obesity should not have influenced the present findings.

Previous studies have shown that normoglycemic subjects with a strong family history of type 2 diabetes have decreased insulin-stimulated glucose disposal (5–7), decreased nonoxidative glucose metabolism (6), decreased insulin-mediated activation and expression of glycogen synthase (8), decreased suppression of lipolysis (26), increased intramyocellular lipid levels (27), plasma lipid abnormalities (28), endothelial dysfunction (29), and ab-

normal insulin secretion (30). Taken together, the results of these prior studies indicate that normoglycemic relatives of type 2 diabetic subjects display most of the features of the insulin-resistance syndrome. The FH<sup>+</sup> subjects who took part in the current study were, on average, insulin resistant compared with the FH<sup>-</sup> control subjects, as indicated by decreased insulin-stimulated rates of glucose disposal. Although subjects in the FH<sup>+</sup> group were insulin resistant compared with control subjects, they were still more sensitive to insulin than obese nondiabetic subjects (21). Consistent with this, their fasting plasma insulin levels were significantly, but slightly, increased. Furthermore, although glucose tolerance in the FH<sup>+</sup> group was normal, there were subtle delays in the kinetics of plasma glucose and insulin concentrations during the OGTT that could reflect decreased insulin action.

This study was undertaken to determine whether abnormalities in the insulin-signaling cascade might be associated with skeletal muscle insulin resistance. Our results showed that normoglycemic subjects with a strong family history of type 2 diabetes have normal basal and insulin-stimulated insulin receptor tyrosine phosphorylation, as well as normal insulin receptor protein expression. These results indicate that an abnormality intrinsic to the insulin receptor is unlikely to account for the insulin resistance that characterizes this group of normal glucose tolerant subjects with a strong family history of type 2 diabetes. In contrast, both the basal and the insulin-stimulated IRS-1



**FIG. 3.** Effect of insulin on phosphorylation of serine 473 of Akt (A) and glycogen synthase activity (B). Determinations were made using percutaneous muscle biopsies taken during the basal state (□) and after 30 min of insulin infusion (■) during a hyperinsulinemic-euglycemic clamp at an insulin infusion rate of  $40 \text{ mU} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$ . In the upper panel of the Akt graph, a representative immunoblot for an FH<sup>+</sup> and FH<sup>-</sup> subject is shown. Data are expressed as percent of the insulin-stimulated value in the subjects with no family history of diabetes. \* $P < 0.01$  vs. basal values.

tyrosine phosphorylation were significantly decreased in the subjects with a family history of diabetes, and this was accompanied by decreased association of PI 3-kinase activity with IRS-1. Studies using inhibitors of PI 3-kinase activity have shown that insulin stimulation of PI 3-kinase activity is requisite for stimulation of glucose transport and glycogen synthesis (31–33). Therefore, the present findings are consistent with the hypothesis that insulin resistance in skeletal muscle of normoglycemic subjects with a family history of diabetes can be accounted for by a decrease in insulin-stimulated tyrosine phosphorylation of IRS-1. Because of the close matching of experimental and control subject characteristics, including glucose tolerance, body composition, and plasma lipid concentrations, it can be said that this abnormality is intrinsic to these individuals and not secondary to an altered metabolic status. Thus, this defect can be considered to be primary and possibly hereditary. However, it is a recognized principle that the phenotype is the product of the interaction between the genotype and the environment. The present results do not rule out the possibility that the environment can play some role. However, these subjects were all chosen randomly, so environmental effects should have been randomized between FH<sup>-</sup> and FH<sup>+</sup> subjects.

Both basal and insulin-stimulated values for postreceptor signaling events were decreased in the FH<sup>+</sup> group, and there was significant insulin stimulation of these parameters, despite the lower values in the FH<sup>+</sup> group. To address the significance of this, we examined the correlation between insulin-signaling protein function and insulin stimulation of glucose disposal. We found that the rate of glucose disposal during insulin stimulation was significantly correlated with insulin stimulation of the association of PI 3-kinase activity with IRS-1. In fact, using multiple regression analysis, we found that IRS-1 tyrosine

phosphorylation and the association of PI 3-kinase with IRS-1 together accounted for slightly >40% of the variation in insulin-stimulated glucose disposal. This analysis suggests that the absolute value of these signaling events is relevant and meaningful to glucose disposal.

The results of the present study do not provide evidence regarding the molecular mechanism responsible for decreased tyrosine phosphorylation of IRS-1. Several possibilities exist. It is known that SHP-2, a tyrosine phosphatase that normally associates with IRS-1, serves to dampen insulin-stimulated tyrosine phosphorylation of that protein (34). An increase in SHP-2 activity associated with IRS-1 could thus be involved. On the other hand, even though insulin receptor tyrosine phosphorylation was normal, it is possible that the activity of the insulin receptor tyrosine kinase toward substrates other than the insulin receptor  $\beta$  subunit, such as IRS-1, might be decreased. In support of this notion, Handberg et al. (35) found that insulin receptor tyrosine kinase activity in vitro against a poly Glu:Tyr peptide substrate was decreased in muscle from relatives of type 2 diabetic patients. However, their study was performed using an assay method that differed from the one used in the present study. In the present study, insulin receptors in muscle were stimulated by insulin and tyrosine phosphorylated in vivo. Thus, the substrate being tyrosine phosphorylated was the insulin receptor itself. In the study by Handberg et al. (35), the insulin receptors were partially purified, stimulated with insulin in vitro, and assayed against an artificial peptide substrate. Yet another possibility would be the involvement of a serine kinase. Several serine kinases, including extracellular-regulated kinase, glycogen synthase kinase 3, and protein kinase B, can phosphorylate IRS-1 and interfere with insulin signaling (36–38).

Regardless of the mechanism involved, a more detailed examination of IRS-1 function in individuals with a strong

family history of type 2 diabetes is required. Results of the present study also show that normal glucose tolerant subjects with a strong family history of type 2 diabetes have reduced insulin-stimulated association of PI 3-kinase activity with IRS-1. This defect is likely secondary to decreased insulin-stimulated tyrosine phosphorylation of IRS-1. A separate abnormality in PI 3-kinase cannot be ruled out, but we consider it to be less likely. Yet another possibility is the presence of IRS-1 mutations in the FH<sup>+</sup> subjects. Several investigators have reported that a common mutation in IRS-1 is associated with obesity, type 2 diabetes, and insulin resistance (39–42). The subjects in the present study were not genotyped for IRS-1.

It has also been reported that subjects with a strong family history of type 2 diabetes have reduced  $VO_{2max}$  and an increased proportion of type IIb muscle fibers (43). Although these parameters were not measured in the present group of subjects, such abnormalities could be consistent with the present results. Regardless, it is unclear at present if decreased aerobic capacity is a primary aspect of the physiology of FH<sup>+</sup> subjects or secondary to decreased physical activity.

In summary, the present study confirms that nonobese, normoglycemic subjects with a strong family history of type 2 diabetes have decreased insulin-stimulated glucose disposal. This abnormality is accompanied by and possibly attributable to diminished insulin-stimulated tyrosine phosphorylation of IRS-1, decreased association of PI 3-kinase activity with IRS-1, and diminished Akt phosphorylation and glycogen synthase activity. Because of the close matching of the experimental and control subjects, it is likely that this defect is intrinsic to the skeletal muscle of individuals with a family history of type 2 diabetes.

#### ACKNOWLEDGMENTS

This work was supported by National Institutes of Health Grants DK-41075 (L.J.M.) and DK-24092 (R.A.D.), an American Diabetes Association grant (L.J.M. and R.A.D.), a General Clinical Research Center Grant M01-RR-01346, a Veterans Administration Merit Award (R.A.D.), and the Veterans Affairs Medical Research Fund.

We are indebted to Andrea Barrentine and Jean Finlayson for expert technical assistance, and John Kincaid, Jim King, Norma Diaz, and Patricia Ortiz for invaluable nursing support.

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