

## Insulin Receptor Substrate-2 Is Not Necessary for Insulin- and Exercise-stimulated Glucose Transport in Skeletal Muscle\*

(Received for publication, March 17, 1999, and in revised form, May 4, 1999)

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Insulin receptor substrate-2-deficient (IRS2<sup>-/-</sup>) mice develop type 2 diabetes. The purpose of this study was to determine whether there is a defect in basal, insulin-, and exercise-stimulated glucose transport in the skeletal muscle of these animals. IRS2<sup>-/-</sup> and wild-type (WT) mice (male, 8–10 weeks) exercised on a treadmill for 1 h or remained sedentary. 2-Deoxyglucose (2DG) uptake was measured in isolated soleus muscles incubated *in vitro* in the presence or absence of insulin. Resting blood glucose concentration in IRS2<sup>-/-</sup> mice (10.3 mM) was higher than WT animals (4.1 mM), but there was a wide range among the IRS2<sup>-/-</sup> mice (3–25 mM). Therefore, IRS2<sup>-/-</sup> mice were divided into two subgroups based on blood glucose concentrations (IRS2<sup>-/-</sup>-L < 7.2 mM, IRS2<sup>-/-</sup>-H > 7.2 mM). Only IRS2<sup>-/-</sup>-H had lower basal, exercise-, and submaximally insulin-stimulated 2DG uptake, while maximal insulin-stimulated 2DG uptake was similar among the three groups. The ED<sub>50</sub> for insulin to stimulate 2DG uptake above basal in IRS2<sup>-/-</sup>-H was higher than WT and IRS2<sup>-/-</sup>-L mice, suggesting insulin resistance in the skeletal muscle from the IRS2<sup>-/-</sup> mice with high blood glucose concentrations. Furthermore, resting blood glucose concentrations from all groups were negatively correlated to submaximally insulin-stimulated 2DG uptake ( $r^2 = 0.33$ ,  $p < 0.01$ ). Muscle GLUT4 content was significantly lower in IRS2<sup>-/-</sup>-H mice compared with WT and IRS2<sup>-/-</sup>-L mice. These results demonstrate that the IRS2 protein in muscle is not necessary for insulin- or exercise-stimulated glucose transport, suggesting that the onset of diabetes in the IRS2<sup>-/-</sup> mice is not due to a defect in skeletal muscle glucose transport; hyperglycemia may cause insulin resistance in the muscle of IRS2<sup>-/-</sup> mice.

Conditions of impaired glucose tolerance and type 2 diabetes are characterized by defects in glucose handling by skeletal muscle, liver, and adipose tissue and an increase in pancreatic insulin secretion to compensate for the impaired insulin action

\* This work was supported by National Institute of Arthritis and Musculoskeletal and Skin Diseases Grants AR-45670 and AR-42238 (to L. J. G.) and by National Institutes of Health Grant DK-43808 (to M. F. W.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

§ Supported by the Ministry of Education, Science, Sports and Culture of Japan (Saga Medical School).

¶ Supported by a postdoctoral fellowship from the Alfred Benzon's Foundation of Denmark.

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in the periphery (1). Overt type 2 diabetes typically occurs when insulin secretion becomes insufficient to fully compensate for decreased insulin action in the peripheral tissues (1). Since impaired insulin action in peripheral tissues has been associated with defects in insulin signaling molecules leading to glucose transport (2–5), there has been an intensive investigation of these signaling proteins (6, 7). It is now well established that activation of phosphoinositide 3-kinase (PI<sup>3</sup>-kinase) is necessary to elicit insulin effects on glucose transport (8, 9), and the major mechanism activating PI 3-kinase appears to be via the insulin receptor substrate (IRS) proteins (10, 11). IRS1-deficient mice have normal blood glucose concentrations despite peripheral insulin resistance, suggesting adequate  $\beta$ -cell compensation (12, 13). On the other hand, IRS2-deficient mice exhibit both peripheral insulin resistance and impaired pancreatic  $\beta$ -cell function and develop severe diabetes (14). It is not known if insulin resistance in skeletal muscle is the primary factor leading to the development of diabetes in the IRS2-deficient animals.

Glucose transport is the rate-limiting step for glucose disposal in skeletal muscle under most condition (15). Insulin stimulation and physical exercise are the most physiologically relevant stimulators of transport in muscle, and both stimuli increase transport through the translocation of the GLUT4 glucose transporters to the cell surface (16, 17). The activation of PI 3-kinase is necessary for insulin-stimulated glucose transport in skeletal muscle, whereas exercise stimulates GLUT4 translocation through a PI 3-kinase-independent mechanism (18–21). It is not known if IRS2 is necessary for insulin- or exercise-stimulated glucose transport in skeletal muscle, although one report has suggested that IRS2 functions as a signaling mechanism leading to contraction-stimulated glucose transport in cardiac myocytes (22).

In the current study we determined if skeletal muscles from IRS2-deficient mice have altered rates of glucose transport under basal conditions and in response to submaximal and maximal insulin stimulation. We also investigated whether IRS2 is required for exercise to increase glucose transport in skeletal muscle. Our results show that IRS2 is not necessary for increases in glucose transport in response to insulin or exercise and suggest that it is the onset of hyperglycemia that results in skeletal muscle insulin resistance in IRS2-deficient mice.

### EXPERIMENTAL PROCEDURES

*Experimental Animals*—Male insulin receptor substrate-2-deficient (IRS2<sup>-/-</sup>,  $n = 55$ ) and wild-type (WT,  $n = 46$ ) mice aged 8–10 weeks were studied. The generation of these animals and many of the physiological characteristics of the mice have been described in detail (14).

<sup>1</sup> The abbreviations used are: PI, phosphoinositide; IRS, insulin receptor substrate; WT, wild-type; KRB, Krebs-Ringer bicarbonate buffer.

Animals were housed in an animal room maintained at 23 °C with a 12-h light/12-h dark cycle and fed standard laboratory chow and water *ad libitum*.

**Insulin Experiments**—Following a 15-h fast, mice were killed by decapitation, and blood was collected for the measurement of blood glucose and plasma insulin concentrations. The soleus muscles were rapidly dissected, and both ends of each muscle were tied with suture (silk 4-0) and mounted on an incubation apparatus as described previously (23). Muscles were preincubated in 6 ml of Krebs-Ringer bicarbonate buffer (KRB) (117 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl<sub>2</sub>, 1.2 mM KH<sub>2</sub>PO<sub>4</sub>, 1.2 mM MgSO<sub>4</sub>, 24.6 mM NaHCO<sub>3</sub>) containing 8 mM D-glucose at 37 °C for 20 min. The muscles were then incubated for 20 min in KRB containing 8 mM D-glucose in the absence or presence of 0.9, 1.8, or 120 nM insulin. After this incubation period, muscles were rinsed in 7.5 ml of KRB containing 8 mM D-mannitol at 30 °C for 10 min, and 2DG uptake was measured in 2 ml of KRB containing 1 mM 2-deoxy-D-[1,2-<sup>3</sup>H]glucose (1.5 μCi/ml) and 7 mM D-[<sup>14</sup>C]mannitol (0.45 mCi/ml)(NEN Life Science Products) at 30 °C for 20 min. Insulin was added to the buffer if present during the previous incubation period. The buffers were continuously gassed with 95% O<sub>2</sub>, 5% CO<sub>2</sub>. Muscles were processed, radioactivity determined by liquid scintillation counting for dual labels, and 2DG uptake was calculated as described previously (24).

**Exercise Experiments**—IRS2<sup>-/-</sup> and WT mice were fasted for 15 h. Blood glucose concentrations were measured in blood taken from the tail prior to the exercise. Mice ran on a rodent treadmill (Quinton Instruments Co., Seattle, WA) for 1 h at 0.7 mph up a 10% incline. Animals were killed immediately after exercise, and blood was collected for the measurement of blood glucose and plasma insulin concentrations. The soleus muscles were rapidly dissected and mounted on the incubation apparatus. The muscles were incubated for 20 min in KRB containing 8 mM D-mannitol at 30 °C in the absence or presence of insulin (1.8 or 120 nM), and 2DG uptake was measured as described above.

**GLUT4 Immunoblotting**—Gastrocnemius muscles were homogenized in lysis buffer containing 20 mM Hepes, 2 mM EGTA, 50 mM β-glycerophosphate, 1 mM dithiothreitol, 1 mM Na<sub>3</sub>VO<sub>4</sub>, 1% Triton X-100, 10% glycerol, 10 μM leupeptin, 3 mM benzamide, 5 μM pepstatin A, 10 μg/ml aprotinin, 1 mM phenylmethylsulfonyl fluoride, and 200 μg/ml soybean trypsin inhibitor, pH 7.4. Homogenates were mixed for 1 h at 4 °C and centrifuged at 15,000 × g for 1 h at 4 °C. The supernatants were collected and stored at -80 °C until analyzed. Aliquots of protein (100 μg) were separated by SDS-PAGE and immunoblotted for GLUT4 as described previously (25).

**Blood Glucose, Plasma Insulin, and Muscle Glycogen**—Blood glucose concentrations were measured using a ONE TOUCH PROFILE blood glucose meter (Lifescan, Milpitas, CA). Plasma insulin concentrations were measured by radio immunoassay using rat insulin as a standard (Linco, Charles, MO). For measurement of muscle glycogen, gastrocnemius muscles were dissolved in 30% KOH and 5% Na<sub>2</sub>SO<sub>4</sub> at 70 °C for 15 min. Glycogen was then precipitated by mixing with 3 × volume of absolute alcohol and stored at -20 °C overnight. The precipitates were collected by centrifugation at 13,000 × g for 5 min. The glycogen was hydrolyzed in 6 N H<sub>2</sub>SO<sub>4</sub> at 100 °C for 45 min and cooled. Samples were neutralized with 1 N NaOH, and glucose was measured using the glucose (HK) reagent (Sigma).

**Statistical Analysis**—Data are expressed as means ± S.E. Comparisons between WT and IRS2<sup>-/-</sup> mice were performed by an unpaired Student's *t* test. Comparisons of parameters before and after exercise were performed by Student's *t* test (paired and unpaired as appropriate) or Mann-Whitney *U* test. Comparisons among WT, IRS2<sup>-/-</sup>L, and IRS2<sup>-/-</sup>H mice were performed by a one-way analysis of variance with Fisher's protected least significant difference test.

## RESULTS

**Glucose and Insulin Concentrations and Body Weights**—Blood glucose concentrations in the IRS2<sup>-/-</sup> mice were significantly higher compared with WT mice (Table I). Since IRS2<sup>-/-</sup> mice had such a wide range of blood glucose concentrations (3.1–24.8 mM), we divided these mice into two subgroups: mice from one group had blood glucose concentrations within a 2 S.D. distribution of the WT mice (<7.2 mM, IRS2<sup>-/-</sup>L), and mice from the other group had blood glucose concentrations greater than 2 S.D. of WT mice (>7.2 mM, IRS2<sup>-/-</sup>H). Plasma insulin concentrations were elevated in IRS2<sup>-/-</sup> mice compared with WT, but were not different between the IRS2<sup>-/-</sup>L and IRS2<sup>-/-</sup>H mice. Body weight was approximately 10%

TABLE I  
Body weight, blood glucose, and plasma insulin concentrations

	Wild-type, n = 46	IRS2 <sup>-/-</sup>		
		Total, n = 55	Subgroups	
			IRS2 <sup>-/-</sup> L, n = 30	IRS2 <sup>-/-</sup> H, n = 25
Body weight (g)	25.4 ± 0.6	22.6 ± 0.5 <sup>a</sup>	22.8 ± 0.7	22.2 ± 0.7
Blood glucose (mM)	4.1 ± 0.2	10.3 ± 1.0 <sup>a</sup> (range 3.1–24.8)	5.5 ± 0.2 (<7.2)	16.1 ± 1.2 (>7.2)
Plasma insulin (pM)	108 ± 10	195 ± 27 <sup>a</sup>	215 ± 48	171 ± 21

<sup>a</sup> *p* < 0.05 versus wild-type. Data are means ± S.E.

lower in IRS2<sup>-/-</sup> mice compared with WT and was not statistically different between the IRS2<sup>-/-</sup>L and IRS2<sup>-/-</sup>H mice.

**Insulin-stimulated Glucose Transport**—To determine whether there is a defect in basal and insulin-stimulated glucose transport in skeletal muscle from IRS2<sup>-/-</sup> mice, isolated soleus muscles were incubated in KRB buffer in the absence or the presence of insulin. IRS2<sup>-/-</sup> mice in the absence of hyperglycemia had normal 2DG uptake under basal conditions (Fig. 1). However, in the IRS2<sup>-/-</sup>H mice, where mean blood glucose concentration was 16.1 mM, there was a significant reduction in basal 2DG uptake. 2DG uptake in response to two different submaximal insulin concentrations was also significantly lower in the IRS2<sup>-/-</sup>H mice, but not the IRS2<sup>-/-</sup>L mice (Fig. 1). In contrast, incubation with a maximal dose of insulin induced similar rates of 2DG uptake in all three groups (Fig. 1). Using the data shown in Fig. 1, we calculated the insulin dose that results in 50% of the maximal insulin-stimulated 2DG uptake (ED<sub>50</sub>). Since basal 2DG uptake in IRS2<sup>-/-</sup>H mice was lower than those of WT and IRS2<sup>-/-</sup>L mice, we calculated the ED<sub>50</sub> with and without subtraction of basal 2DG uptake for each individual group. The ED<sub>50</sub> without subtraction of basal 2DG uptake in IRS2<sup>-/-</sup>H mice (1.5 nM) was greater than WT (0.8 nM) and IRS2<sup>-/-</sup>L mice (0.7 nM). Similarly, the ED<sub>50</sub> with subtraction of basal 2DG uptake in IRS2<sup>-/-</sup>H mice (1.9 nM) was greater than WT (1.4 nM) and IRS2<sup>-/-</sup>L mice (1.3 nM), suggesting that only the IRS2<sup>-/-</sup>H mice are insulin-resistant in skeletal muscle. Fasting blood glucose concentrations from all groups were negatively correlated with basal 2DG uptake (*r*<sup>2</sup> = 0.21, *p* < 0.05) and submaximally insulin-stimulated (1.8 nM) 2DG uptake (*r*<sup>2</sup> = 0.33, *p* < 0.01).

**Glucose, Insulin, and Muscle Glycogen Levels before and after Exercise**—Blood glucose concentrations in the WT and IRS2<sup>-/-</sup>H mice did not change in response to exercise, while there was a slight, but statistically significant, decrease in blood glucose in the IRS2<sup>-/-</sup>L mice (Fig. 2A). Interestingly, plasma insulin concentrations dramatically decreased with exercise in both groups of IRS2<sup>-/-</sup> mice, resulting in similar insulin levels compared with the WT (Fig. 2B). Since blood glucose concentrations among the three groups were significantly different, the alcohol precipitation method for glycogen was used in order to avoid measuring glucose associated with blood and tissue. Muscle glycogen content at rest was similar among the WT, IRS2<sup>-/-</sup>L, and IRS2<sup>-/-</sup>H groups and was significantly decreased after exercise in all three groups (Fig. 2C). However, the percent decrease in glycogen after exercise was lower in IRS2<sup>-/-</sup>H mice compared with IRS2<sup>-/-</sup>L mice (47.7 versus 68.2%, respectively, *p* < 0.05), suggesting less reliance on muscle glycogen as a fuel source during exercise in the IRS2<sup>-/-</sup>H mice.

**Exercise-stimulated Glucose Transport**—We next determined if IRS2 is necessary for the effects of exercise *per se* to increase glucose transport and to determine whether IRS2 is required for the additive effects of exercise plus insulin on glucose transport in skeletal muscle. 2DG uptake following

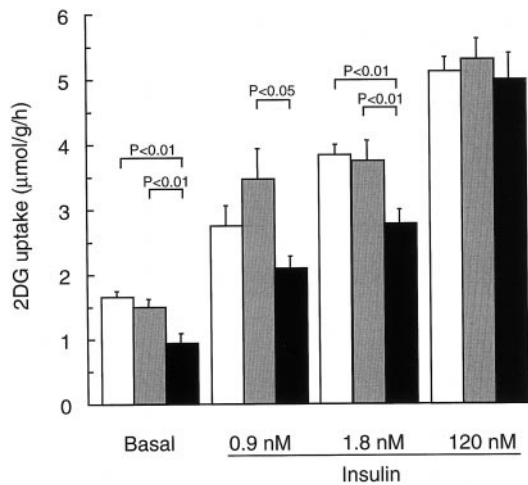


FIG. 1. Insulin stimulated glucose transport in isolated soleus muscle from WT (open bars), IRS2<sup>-/-</sup>L (shaded bars), and IRS2<sup>-/-</sup>H mice (filled bars). 2DG uptake was measured in the absence or presence of 0.9, 1.8, or 120 nM insulin as described under "Experimental Procedures." Data are means  $\pm$  S.E.;  $n = 6$ –20 per group.

exercise in the absence of insulin was not statistically decreased in the IRS2<sup>-/-</sup>L mice, but was lower in the IRS2<sup>-/-</sup>H mice (Fig. 3). The -fold increase above basal in IRS2<sup>-/-</sup>H mice (2.4-fold) was similar to the WT mice (2.1-fold), suggesting that the lower exercise-induced increase in 2 DG uptake may be due to impaired basal rates of glucose transport in the IRS2<sup>-/-</sup>H mice. 2DG uptake with the combination of exercise and insulin was not altered in the IRS2<sup>-/-</sup>L mice, while 2DG uptake in the IRS2<sup>-/-</sup>H mice was lower compared with the WT and IRS2<sup>-/-</sup>L mice (Fig. 3). The partially or fully additive effects of exercise plus insulin (submaximal and maximal insulin dose) on 2DG uptake in the WT and IRS2<sup>-/-</sup>L animals was not present in the hyperglycemic IRS2<sup>-/-</sup>H mice.

**GLUT4 Protein in Muscle**—To determine whether IRS2 deficiency alters GLUT4 expression in skeletal muscle, we measured GLUT4 protein in WT and IRS2<sup>-/-</sup> mice. Fig. 4 shows that GLUT4 content in the gastrocnemius muscles was not different between the IRS2<sup>-/-</sup>L and the WT mice. However, the hyperglycemic IRS2<sup>-/-</sup>H mice had 18 and 28% less GLUT4 compared with the WT and IRS2<sup>-/-</sup>L mice, respectively.

#### DISCUSSION

Of the four members of the IRS family that have now been identified, IRS1 (26) and IRS2 (27) have the widest tissue distribution, including expression in skeletal muscle, liver, and adipose tissue. In contrast, neither IRS3 (28) nor IRS4 (29) are expressed in adult skeletal muscle. Both IRS1 and IRS2 are tyrosine-phosphorylated in response to insulin, and binding of tyrosine-phosphorylated IRS1 to the p85 regulatory subunit of PI 3-kinase and subsequent activation of the enzyme are thought to be important for insulin-stimulated glucose transport (8, 9). Recent studies using "knockout" mouse models have advanced our understanding of the role of the insulin receptor and IRS proteins in insulin-stimulated glucose transport. Muscle-specific insulin receptor knockout mice have a dramatic impairment in insulin-stimulated glucose uptake in skeletal muscle, demonstrating that the receptor is necessary for insulin-stimulated glucose transport (24). IRS1-deficient mice have normal blood glucose concentrations despite peripheral insulin resistance (13). In the current study, we observed that IRS2<sup>-/-</sup> mice with near normal glycemia have normal rates of basal and insulin-stimulated glucose uptake in skeletal muscle. These findings demonstrate that IRS2 is not necessary for glucose

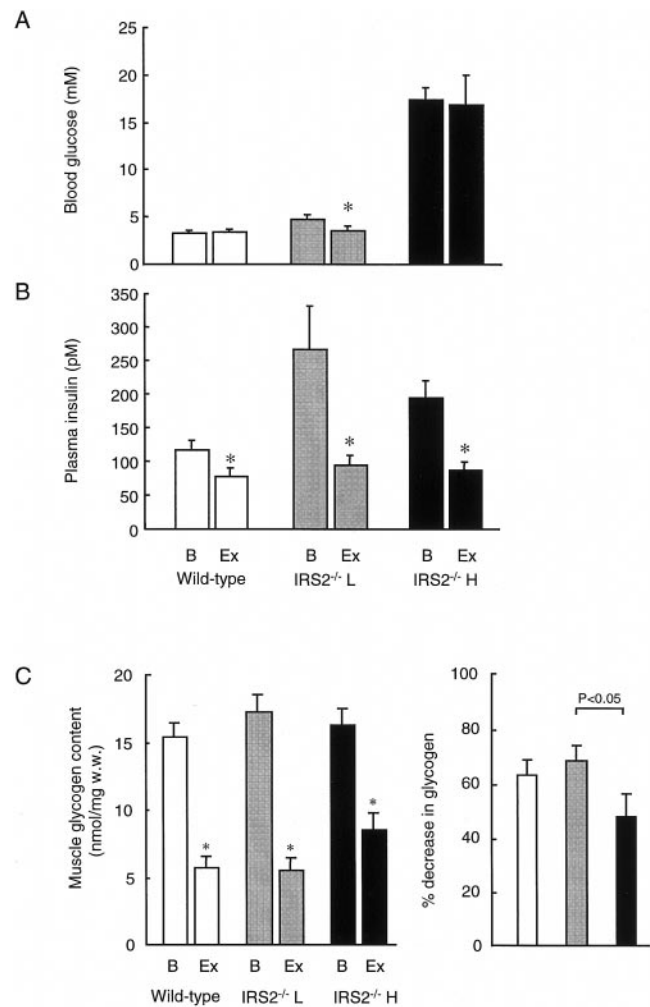


FIG. 2. Effects of 1-h treadmill exercise on blood glucose and plasma insulin concentrations and muscle glycogen content in WT (open bars), IRS2<sup>-/-</sup>L (shaded bars), and IRS2<sup>-/-</sup>H mice (filled bars). A, blood glucose concentrations; B, plasma insulin concentrations; C, muscle glycogen content (left panel) and the percent decrease in glycogen content (right panel). B, basal condition. Ex, exercised condition. Data are means  $\pm$  S.E. \*,  $p < 0.05$  versus basal condition;  $n = 5$ –35 per group.

transport in skeletal muscle and suggest that signaling through other molecules such as IRS1 are sufficient to mediate insulin's effects on glucose transport in this tissue.

There is good evidence that the first detectable defect in human patients predisposed to type 2 diabetes is insulin resistance in skeletal muscle (30). IRS2<sup>-/-</sup> mice develop overt type 2 diabetes, usually by the age of 10 weeks, and these animals are characterized by impaired  $\beta$ -cell function and peripheral insulin resistance as measured by the euglycemic hyperinsulinemic clamp (14). In the current study, only the IRS2<sup>-/-</sup> mice that had developed severe hyperglycemia had impaired glucose uptake in isolated skeletal muscles. Furthermore, fasting blood glucose concentrations were negatively correlated to submaximally insulin-stimulated glucose uptake in the muscle. Thus, hyperglycemia resulting from hepatic insulin resistance and  $\beta$ -cell failure, and not the lack of IRS2 in muscle, is likely to cause insulin resistance in the skeletal muscle of IRS2<sup>-/-</sup> mice.

Both hyperglycemia and hyperinsulinemia typically accompany the development of type 2 diabetes, and it is difficult to determine their priority. In the IRS2<sup>-/-</sup> mice the precursor leading to insulin resistance and type 2 diabetes may be hyperinsulinemia, since IRS2<sup>-/-</sup> mice with near normal glycemia

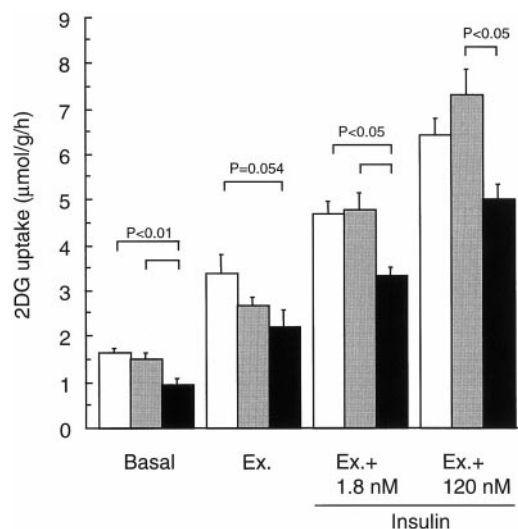


FIG. 3. Effects of 1-h treadmill exercise on glucose transport in isolated soleus muscle from the WT (open bars), *IRS2*<sup>-/-</sup>L (shaded bars), and *IRS2*<sup>-/-</sup>H mice (filled bars). 2DG uptake was measured in the absence or presence of 1.8 or 120 nM insulin as described under "Experimental Procedures." Data are means  $\pm$  S.E.;  $n = 3$ –20 per group.

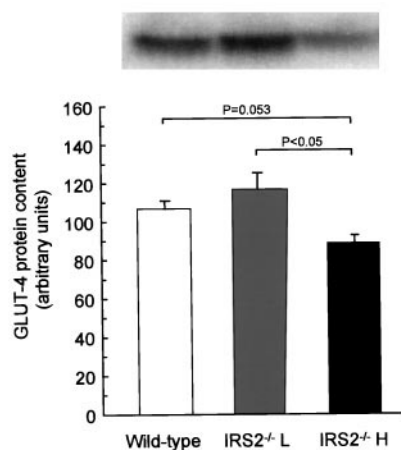


FIG. 4. Muscle GLUT4 content in the WT (open bars), *IRS2*<sup>-/-</sup>L (shaded bars), and *IRS2*<sup>-/-</sup>H mice (filled bars). The results are expressed as arbitrary units of a reference standard for comparisons among different blots. Data are means  $\pm$  S.E.;  $n = 8$  per group.

and normal insulin-stimulated glucose uptake in skeletal muscle were already hyperinsulinemic. Furthermore, there is the wide range of fasting insulin concentrations in *IRS2*<sup>-/-</sup>L mice with near normal glycemia (Table I). These findings also suggest that insulin resistance in skeletal muscle is not causing the hyperinsulinemia in these animals.

Although 6–8-week-old *IRS2*<sup>-/-</sup> mice are hyperinsulinemic, the rate of hepatic glucose production in the fasted state is similar to WT mice (14). However, low dose insulin infusion during an insulin-glucose clamp does not suppress hepatic glucose production in *IRS2*<sup>-/-</sup> mice (14), and these animals also display decreased levels of liver glycogen.<sup>2</sup> These findings suggest that the liver is insulin-resistant for the suppression of glucose production and/or that there is impairment in glycogenesis. In contrast, in skeletal muscle, glycogen levels in the basal state were not altered in either group of *IRS2*<sup>-/-</sup> mice. The difference between these tissues provides further evidence that *IRS2* plays a more limited role in regulating carbohydrate

metabolism in skeletal muscle compared with other tissues and that insulin resistance in skeletal muscle occurs secondary to defects in glucose homeostasis in other tissues such as the liver and pancreas.

It has been suggested recently that contraction-induced signaling leading to glucose transport in isolated rat cardiac myocytes involves *IRS2* (22). Therefore, we investigated whether *IRS2* is necessary for the effects of exercise to increase glucose transport in skeletal muscle and if the protein is necessary for the post-exercise increase in insulin-stimulated glucose transport. Similar to the effects of insulin, only the hyperglycemic *IRS2*<sup>-/-</sup> mice had impaired glucose uptake in response to exercise. For the effects of exercise "per se," the lower rate of glucose uptake in the hyperglycemic *IRS2*<sup>-/-</sup> mice was probably due to lower basal rates of transport, since the -fold increment above basal was normal in these animals. The fact that the defect in insulin-stimulated glucose uptake in the post-exercise state only occurred in the *IRS2*<sup>-/-</sup>H mice is consistent with our hypothesis that hyperglycemia is the cause of defects in glucose transport in the muscle from *IRS2*<sup>-/-</sup> mice animals. Impaired insulin-stimulated glucose uptake after exercise in hyperglycemic *IRS2*<sup>-/-</sup> mice could be due to decreased glycogenolysis during exercise, since post-exercise glycogen concentrations are negatively correlated with glucose transport in rat epitrochlearis muscle (31). The decrease in GLUT4 content in skeletal muscle from hyperglycemic *IRS2*<sup>-/-</sup> mice could also be a factor leading to an impaired insulin-stimulated glucose transport after exercise. However, the decrease in GLUT4 content in skeletal muscle did not result in impaired maximal insulin-stimulated glucose transport.

Given that hyperinsulinemia may be the precursor leading to insulin resistance in the *IRS2*<sup>-/-</sup> mice, it is of interest that there was a dramatic decrease in plasma insulin concentrations following exercise in both the *IRS2*<sup>-/-</sup>L and *IRS2*<sup>-/-</sup>H mice, regardless of prevailing blood glucose concentrations. In addition, *IRS2*<sup>-/-</sup>L mice with near normal glycemia had a significant decrease in blood glucose concentrations after 1 h of exercise. Although 4-week-old *IRS2*<sup>-/-</sup> mice were already hyperinsulinemic, the insulin response to a glucose load is nearly normal (14). Interestingly, glucose-stimulated insulin secretion deteriorates with aging, and then hyperglycemia results in impaired  $\beta$ -cell function. Given that *IRS2*<sup>-/-</sup> mice with near normal glycemia showed normal glucose transport and insulin response, it is possible that performance of regular exercise from an early age may have a beneficial effect on glucose homeostasis in these animals.

In summary, the current study demonstrates that expression of the *IRS2* protein is not necessary for the stimulation of glucose transport in skeletal muscle in response to insulin or exercise. The initial onset of diabetes in *IRS2*<sup>-/-</sup> mice is not due to a defect in skeletal muscle glucose transport, and prolonged hyperglycemia may be a primary mechanism leading to insulin resistance in skeletal muscle of *IRS2*<sup>-/-</sup> mice.

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