Insulin resistance with low cellular IRS-1 expression is also associated with low GLUT4 expression and impaired insulin-stimulated glucose transport

EUGÉNIA CARVALHO, PER-ANDERS JANSSON, IVAN NAGAEV, ANNE-MARIE WENTHZEL, AND ULF SMITH

The Lundberg Laboratory for Diabetes Research, Department of Internal Medicine, Sahlgrenska University Hospital, Göteborg, Sweden

SPECIFIC AIMS

Low expression of the key docking protein insulin receptor substrate-1 (IRS-1) has been found in fat cells from type 2 diabetic subjects as well as a cohort of non-diabetic but insulin-resistant individuals. In this study, we examined the in vivo phenotypes as well as the insulin signaling and action in cells from healthy subjects characterized as having normal or low (≤50% of normal) IRS-1 expression.

PRINCIPAL FINDINGS

1. Phenotypic characterizations

Subjects with low IRS-1 were slightly older than the group with normal IRS-1 expression. They had significantly larger fat cells and waist/hip circumference ratio (WHR), which suggests an abdominal fat distribution. Because of a predominance of males in the low IRS-1 group (18/20), these individuals were also compared with the male controls only. However, the differences in WHR and fat cell size still differed significantly. A clear overrepresentation of individuals with a known genetic predisposition for diabetes in the low IRS-1 group (14/20; 70%) was apparent, whereas 6/20 (30%) had no known genetic predisposition.

2. Insulin signaling and protein expression

As shown in Fig. 1, the low IRS-1 group had ~65% lower IRS-1 protein expression than the control group (p<0.001). However, the expression of other examined intracellular proteins, such as p85, IRS-2, PkB/Akt, and MAP kinase, did not differ between the groups.

The reduced IRS-1 expression was also associated with a marked impairment in insulin-stimulated PI3-kinase activity in total antiphosphotyrosine immunoprecipitates (Fig. 1). The average reduction in PI3-kinase activity in four deficient versus nondeficient cells was ~70%. Thus, low IRS-1 expression is also associated with low cellular insulin-stimulated PI3-kinase activity.

We also examined the consequences of the low PI3-kinase activity on the downstream signaling in response to insulin. PkB/Akt serine phosphorylation was reduced ~60%–70% (Fig. 1).

3. Glucose transport and GLUT4 expression

Maximally insulin-stimulated glucose transport was significantly lower (~60% reduction) in IRS-1-deficient cells (Fig. 2), whereas basal (nonstimulated) glucose uptake was similar. This impairment in activation of glucose transport by insulin could be due to the impairment in insulin signaling (Fig. 1) or to a reduced GLUT4 protein expression. This condition was examined by adding 1 μM okadaic acid, a PP1 and 2A threonine/serine phosphatase inhibitor that exerts a full insulin-like effect which is PI3-kinase independent, on glucose transport in human fat cells. Okadaic acid improved but did not restore the insulin-stimulated glucose transport. This finding suggests that GLUT4 protein expression may be reduced, and Fig. 2 shows that low cellular IRS-1 expression is associated with ~60% reduction in GLUT4 expression.

4. Gene expression of IRS-1 and GLUT4

Real-time RT-PCR was used to assay mRNA levels for IRS-1 and GLUT4 because large tissue samples were generally not obtained in these nonobese individuals. For comparison, we also assayed the gene expression in fat cells from five type 2 diabetic subjects.

Both IRS-1 and GLUT4 gene expression levels were significantly lower in diabetic cells when compared with the control group (~50% and ~60% reduction, respectively; P<0.02 and P<0.005). The mRNA levels were reduced ~40% for GLUT4 (P<0.05) in the IRS-1 deficient cells when compared with nondiabetic cells.

1 To read the full text of this article, go to http://www.fasebj.org/cgi/doi/10.1096/fj.00-0435 to cite this article, use (February 6, 2001) FASEB J. 10.1096/fj.00–0435fje

2 Correspondence: The Lundberg Laboratory for Diabetes Research, Department of Internal Medicine, Sahlgrenska University Hospital, S-413 45 Göteborg, Sweden. E-mail: ulf.smith@medic.gu.se
IRS-1 mRNA was reduced ~20% in the deficient cells but this finding was not statistically significant because of large interindividual variations in both groups.

CONCLUSIONS

The present study is an extension of our previous investigation, in which we found that low cellular IRS-1 expression, defined as ≤50% of normal, identifies individuals with insulin resistance and various markers of the insulin resistance syndrome in healthy, nonobese subjects. Enlarging the study to include about twice the number of nonobese subjects confirmed our previous result that a low cellular IRS-1 expression occurs approximately two- to threefold more frequently in individuals with a genetic predisposition for type 2 diabetes. Furthermore, this finding is associated with a larger waist/hip circumference ratio, which is a well-established marker of insulin resistance and propensity for type 2 diabetes, also in this nonobese group.

The novel salient findings of the present study are that low IRS-1 expression is associated with a marked impairment in downstream insulin signaling, including PI3-kinase activation; serine phosphorylation of PKB/Akt and maximally insulin-stimulated glucose transport is impaired; and GLUT4 protein expression is also markedly reduced. In fact, all these perturbations are similar, both in terms of extent and proteins involved, to what is seen in fat cells from subjects with manifest type 2 diabetes. From this point of view, it appears that healthy individuals with a low IRS-1 expression also exhibit an adipose tissue with diabetic traits in terms of insulin signaling and response. This concept needs to be extended by characterizing other aspects of adipose tissue metabolism and function such as the lipolytic response and the antilipolytic effect of insulin.

The concept that the adipose tissue already exhibits several diabetic traits in these healthy subjects is indeed astonishing. Similar to diabetic cells, PKB/Akt serine phosphorylation was impaired even in the presence of a supramaximal insulin concentration. Whether this finding is due to the expression of a truncated PKB/Akt lacking the serine phosphorylation site and/or to the ~70% reduction in insulin-stimulated PI3-kinase activity remains to be established. However, this trait appears to be a consistent finding with fat cells from different insulin-resistant states.

A low IRS-1 expression was also associated with a low GLUT4 expression and a reduced insulin-stimulated glucose transport. Okadaic acid, which activates glucose transport and PKB/Akt in human fat cells to a similar extent as insulin but in a PI3-kinase independent manner, did not restore the glucose transport to normal. These data suggest that low GLUT 4 expression was the major reason for the impaired insulin-stimulated glucose transport in these cells. Furthermore, the data suggest the possibility of a coordinate regulation of IRS-1 and GLUT4. The low mRNA expression for both IRS-1 and GLUT4 in the diabetic cells when compared with the control group suggests that this expression occurs at the level of gene transcription. Similarly, mRNA for GLUT4 was reduced significantly in IRS-1-deficient cells, whereas the difference in IRS-1 expression was not significant due to large interindividual variations in both groups.

Figure 1. Comparisons between IRS-1-deficient and normal cells. Top left: Scanned values of IRS-1 protein expression in normal (n=13) versus deficient (n=8) cells. ***P < 0.001. Bottom: Scanned values of antiphosphotyrosine-associated PI3-kinase activity in normal and deficient cells following preincubation for 10 min with 6.9 nM insulin. Top right: Scanned values of PKB/Akt serine phosphorylation in normal (n=9) and deficient cells (n=12). ***P < 0.001.

Figure 2. Top: Glucose transport in normal and deficient cells following incubation with 6.9 nM insulin for 60 min. GLUT4 protein expression (middle) and scanned values of normal (n=12) and deficient (n=9) cells (bottom). *P < 0.05, **P < 0.01.
variations in both groups. One possibility is that deficient cells have an elevated production of TNF-α, which has been reported to lower both IRS-1 and GLUT4 expression. This and other possibilities are the focus of ongoing studies in our laboratory.

A key question raised by the present data is whether the reduced IRS-1 and GLUT4 expression in the adipose tissue could be causally related to the insulin resistance and type 2 diabetes or whether it is a marker of a common pathogenetic mechanism(s). Support for the first possibility is the recent observation that adipose-specific GLUT4 gene disruption in mice is associated with a marked insulin resistance. Furthermore, total IRS-1 gene disruption in mice leads to a marked insulin resistance. No current animal model shows tissue-specific IRS-1 gene knockout to indicate the relative role of the adipose tissue versus muscle and liver for the insulin resistance.

The present study has shown that a low IRS-1 expression is associated with a marked impairment in insulin-stimulated PI3-kinase activity and downstream insulin signaling. Furthermore, GLUT4 protein expression and insulin-stimulated glucose transport are also reduced, which suggests the possibility of a coordinate regulation of IRS-1 and GLUT4, probably at the level of gene transcription. Whether these perturbations are causally related to insulin resistance or whether they reflect a common pathogenetic mechanism(s) remains to be established. However, the abnormalities seen in this group of healthy subjects are very similar to those seen in cells from type 2 diabetic subjects. Thus, it appears that diabetic traits can be present in the adipose tissue long before type 2 diabetes develops.

**Figure 3.** Schematic diagram of the hypothetical sequence of events whereby low IRS-1 expression in fat cells is associated with insulin resistance and type 2 diabetes. Genetic predisposition for type 2 diabetes (plus additional environmental factors?) is associated with large fat cells, high waist/hip ratio and an impaired transcription of IRS-1/GLUT4, as well as low cellular protein expression levels. This dichotomy leads to insulin resistance directly or indirectly via an increased production of cytokines or other hormones/peptides.