International Journal of Obesity (2002) 26, 897–904
 0307–0565/02 $25.00
www.nature.com/ijo

REVIEW

Impaired ('diabetic') insulin signaling and action occur in fat cells long before glucose intolerance—is insulin resistance initiated in the adipose tissue?

U Smith[1]*

[1]*The Lundberg Laboratory for Diabetes Research, Department of Internal Medicine, Sahlgrenska Academy at Göteborg University, Göteborg, Sweden*

This review postulates and presents recent evidence that insulin resistance is initiated in the adipose tissue and also suggests that the adipose tissue may play a pivotal role in the induction of insulin resistance in the muscles and the liver. Marked impairments in insulin's intracellular signaling cascade are present in fat cells from type 2 diabetic patients, including reduced IRS-1 gene and protein expression, impaired insulin-stimulated PI3-kinase and PKB/Akt activities. In contrast, upstream insulin signaling in skeletal muscle from diabetic subjects only shows modest impairments and PKB/Akt activation *in vivo* by insulin appears normal. However, insulin-stimulated glucose transport and glycogen synthesis are markedly reduced.

Similar marked impairments in insulin signaling, including reduced IRS-1 expression, impaired insulin-stimulated PI3-kinase and PKB/Akt activities are also seen in some (~30%) normoglycemic individuals with genetic predisposition for type 2 diabetes. In addition, GLUT4 expression is markedly reduced in these cells, similar to what is seen in diabetic cells. The individuals with reduced cellular expression of IRS-1 and GLUT4 are also markedly insulin resistant and exhibit several characteristics of the Insulin Resistance Syndrome.

Thus, a 'diabetic' pattern is seen in the fat cells also in normoglycemic subjects and this is associated with a marked insulin resistance *in vivo*. It is proposed that insulin resistance and/or its effectors is initiated in fat cells and that this may secondarily encompass other target tissues for insulin, including the impaired glucose transport in the muscles.
International Journal of Obesity (2002) **26,** 897–904. doi:10.1038/sj.ijo.0802028

Keywords: insulin resistance; type 2 diabetes; IRS-1; IRS-2; GLUT4

Introduction

Estimation of whole-body insulin sensitivity and action with the euglycemic clamp technique is mainly a reflection of the glucose disposal by the muscles (60–70%).[1] The adipose tissue only accounts for ~10% of the insulin-stimulated whole body glucose uptake and the liver for ~30%. Thus, an impaired insulin-stimulated glucose disposal during a euglycemic clamp is mainly due to a reduced glucose uptake by the muscles. This fact has led to the extrapolation that whole body insulin resistance not only occurs in, but also starts in, the muscles. This is an unwarranted extrapolation, which may lead us wrong in the search for pathogenetic mechanisms.

Animal models, both transgenic overexpressing and gene 'knock-outs', have provided us with exciting insights into the phenotypic consequences of specific gene overexpression or ablation. Gene ablation of the important docking proteins IRS-1 and IRS-2 have produced growth-retarded and markedly insulin-resistant (IRS-1)[2] or insulin-resistant and diabetic animals with an impaired insulin secretion (IRS-2).[3] Muscle-specific GLUT4 ablation leads to insulin resistance,[4] but so does adipose-specific GLUT4 gene knock-out, in fact to what appears to be a similar degree.[5] This finding is obviously not congruent with an unimportant role of the adipose tissue for whole-body glucose disposal. Another interesting finding in the animal models is that muscle GLUT4 depletion is associated with a marked increase in

*Correspondence: U Smith, The Lundberg Laboratory for Diabetes Research, Department of Internal Medicine, Sahlgrenska Academy at Göteborg University, S-413 45 Göteborg, Sweden.
E-mail: ulf.smith@medic.gu.se
Received 25 June 2001; revised 12 February 2002; accepted 19 February 2002

glucose uptake by the fat and with an expanded adipose tissue mass.[6] This cross-talk between tissues supports the possibility that insulin resistance may be initiated in one tissue which then is followed by a series of events in other tissues/organs.

This brief overview summarizes our recent findings in human subjects showing that insulin resistance and an impaired insulin effect occur early in the adipose tissue; in fact, long before glucose intolerance develops. It is then speculated that the adipose tissue may initiate and/or be the initial target organ for insulin where insulin resistance develops. Recent data where insulin signaling and action have been studied in the target tissues in man in insulin-resistant states, in particular in type 2 diabetes, will be reviewed. Since virtually nothing is known about insulin signaling downstream of the insulin receptor in human liver, comparisons can only be made between skeletal muscle and adipose cells.

Type 2 diabetes

The effect of insulin, either infused *in vivo* or added *in vitro*, on glucose transport and insulin signaling in skeletal muscle from type 2 diabetic subjects has been recently reviewed.[7] The salient findings are an impaired insulin-stimulated tyrosine phosphorylation of IRS-1, associated with ~50% reduction in PI3-kinase activity. However, the downstream activation of the important serine/threonine kinase PKB/Akt appears to be normal[8] or only impaired in the presence of a supraphysiological insulin concentration added *in vitro*.[9] The impaired tyrosine phosphorylation does not appear to be due to a reduced IRS-1 protein expression, although lower levels have been seen in some cells in gestational diabetes.[10] An increased serine phosphorylation of IRS-1 may reduce the insulin-stimulated tyrosine phosphorylation,[11] but it is currently unknown whether this is the case in type 2 diabetes. Taken together, the data suggest that the activation of PI3-kinase, and presumably the generation of PI3, 4- and PI3, 4, 5 phosphates, is reduced but still sufficient to allow a normal activation of the downstream signaling events. This has led to the conclusion that insulin resistance in skeletal muscle is caused by an impaired activation of effector or signaling molecules downstream of PKB/Akt.[8]

Insulin-stimulated glucose transport is also reduced in skeletal muscle from type 2 diabetic subjects.[12] Surprisingly, however, recent *in vitro* studies have shown that this appears to be mainly caused by a 'glucose toxicity'. Preincubating the tissue biopsies for only 2 h at a high glucose concentration impairs the effect of insulin,[13] while preincubating diabetic muscle strips for 2 h at a physiological glucose concentration normalizes the insulin response.[13] However, it is also possible that the preincubation period overcomes the effect of other circulating antagonists to insulin action such as TNFα, the interleukins and/or free fatty acids (FFA).[14,15] Taken together, currently available data suggest that there are only modest, and obviously not functionally critical, impairments in insulin signaling upstream of PKB/Akt in skeletal muscle from type 2 diabetic subjects. Furthermore, the impaired insulin-stimulated glucose transport appears to be rapidly reversible *in vitro* by preincubating the tissue samples in fresh medium containing a physiological glucose concentration. These findings are also in agreement with the consistent demonstration that both the GLUT4 protein content and mRNA expression are normal in skeletal muscle in type 2 diabetes.[7,16]

The situation is quite different in the adipose tissue. Adipocytes from type 2 diabetic subjects also have a marked reduction in the insulin-stimulated tyrosine phosphorylation of IRS-1. However, this is mainly due to a ~70% reduction in IRS-1 protein expression.[17] Similarly, total PI3-kinase activity is reduced ~70%. In contrast, IRS-2 expression is normal and this molecule also becomes the main docking protein for insulin-stimulated PI3-kinase activation.[17] In agreement with the reduced PI3-kinase activation, the downstream activation of PKB/Akt is also markedly impaired, mainly due to a major reduction in the insulin-stimulated serine phosphorylation.[18] Glucose transport in response to insulin is also reduced in fat cells from type 2 diabetic subjects due to both the impaired insulin signaling as well as a marked reduction (~70–80%) in GLUT4 protein and mRNA expression.[17,19,20]

In contrast to muscle cells as discussed above, preincubating human fat cells for 16 h at physiological (5.6 mmol/l) or high glucose concentrations (16.8 and 25 mmol/l) does not impair the acute stimulatory effect of insulin on glucose uptake (Figure 1) nor does preincubation of diabetic cells at a physiological glucose concentration restore the acute insulin response after 6 h (unpublished observations). This is consistent with the reduced GLUT4 protein expression in adipocytes which probably requires a longer time for reversal.

Table 1 summarizes the salient differences in the upstream insulin-stimulated events in muscle and fat from individuals with type 2 diabetes compared to non-diabetic subjects.

Normoglycemic, insulin-resistant states

Studies with skeletal muscle from non-diabetic relatives to type 2 diabetic subjects have shown that both insulin-stimulated glucose uptake and glycogen synthesis are reduced.[7,20] Some defects in insulin signaling have been reported and they appear to be similar to those seen in type 2 diabetes. These perturbations include a modest reduction in insulin receptor phosphorylation and tyrosine kinase activity,[21] in insulin-stimulated IRS-1 tyrosine phosphorylation[22,23] and PI3-kinase activity.[22–24] However, IRS-1 protein expression appears to be unchanged,[22–24] but a small (~30%) reduction in IRS-1 protein expression was reported in morbidly obese subjects.[25] Insulin-stimulated downstream activation of PKB/Akt appears to be normal or only moderately decreased.[22,23] Cultured skeletal muscle from insulin-sensitive and -resistant subjects showed no impairments

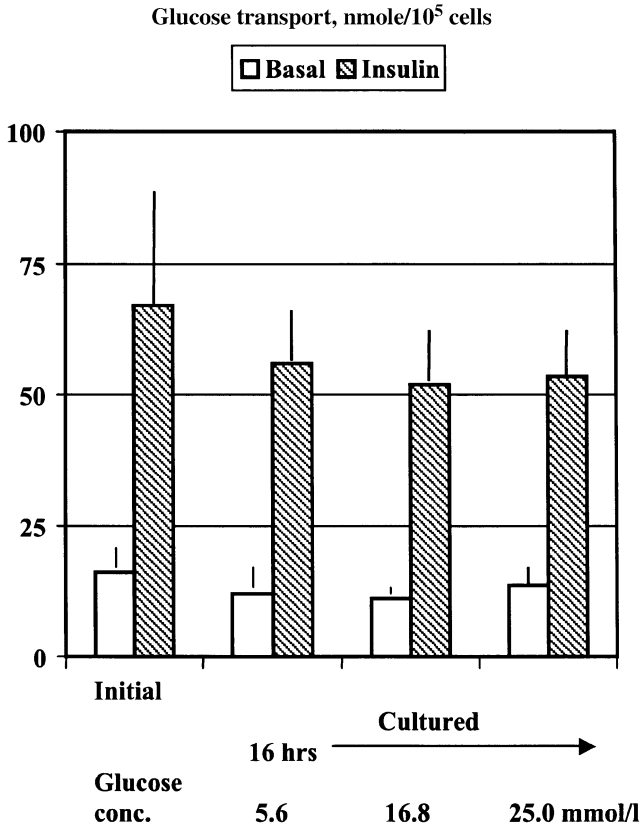

Figure 1 Glucose uptake by explants of human subcutaneous adipose tissue before (initial) or after culture for 16 h at 5.6, 16.7 or 25.0 mM glucose. After the culture period, isolated cells were incubated with 6.9 nM insulin and 0.15 μCi [^{14}C-U] glucose for 60 min to determine glucose uptake. Data are means ± s.e.m. of four seperate experiments.

Table 1 Comparison of the upstream insulin-stimulated signaling events in muscle and fat from individuals with type 2 diabetes compared to healthy subjects

Signaling molecules	*Muscle*	*Adipocytes*
pY-insulin receptor	⇓ ⇒	⇓ ⇒
pY-IRS-1	⇓ ⇓	⇓ ⇓
IRS-1 protein	⇒	⇓ ⇓
PI3-kinase activation	⇓	⇓ ⇓
PKB/Akt activation	⇒ (⇓)	⇓ ⇓
Glucose transport	⇓ ⇓	⇓ ⇓
Glut4 protein/mRNA	⇒	⇓ ⇓

⇒, normal; ⇓ reduced; ⇓ ⇓ markedly reduced (< 50% of normal); pY = phosphotyrosine.

in the ability of insulin to increase the receptor tyrosine kinase activity, IRS-1-associated PI3-kinase activity or serine phosphorylation (ie activation) of PKB/Akt.[26] In contrast, an impaired glucose transport and glycogen production in response to insulin have been documented in cultured skeletal muscle cells from non-diabetic insulin-resistant subjects.[27] Taken together, although insulin-stimulated glucose transport and glycogen synthesis are reduced in skeletal muscle from both diabetic and non-diabetic, insulin-resistant subjects, only modest defects have been found in the intracellular signaling events. These findings are also in agreement with the modest upstream impairments in insulin signaling in type 2 diabetes as discussed above.

In contrast, we have recently found that a cohort of healthy subjects, particularly in those with a marked genetic predisposition for type 2 diabetes (two first-degree relatives with the disease), exhibit similar abnormalities in insulin signaling in the adipocytes as those seen in diabetic cells.[28,29] Thus, IRS-1 expression was reduced ~70% (Figure 2), and insulin-stimulated PI3-kinase activity and PKB/Akt serine phosphorylation and activity were similarly reduced.[29] Interestingly, insulin-stimulated glucose transport and GLUT4 expression were also reduced to a similar extent as in diabetic cells.[29] These abnormalities were seen in ~30% of individuals with a marked genetic predisposition for type 2 diabetes but only in ~5% of the subjects with no known diabetes heredity. We also found similar abnormalities in some morbidly obese subjects (Figure 2).[28] Unfortunately, no information was available on diabetes heredity in this group. However, since diabetes heredity by itself is associated with a higher body weight and obesity,[30–32] as well as an increased weight gain in prospective studies,[31] it is feasible that the obese individuals with low IRS-1 expression in the fat cells also had a genetic predisposition for type 2 diabetes. We found no association between a low IRS-1 expression and the common Arg972 Gly polymorphism of the IRS-1 gene.[28]

Thus, both low IRS-1 and GLUT4 gene and protein expression are seen in fat cells from type 2 diabetic subjects as well as in a group of healthy individuals, mainly those with a marked heredity for type 2 diabetes. The downstream signaling events for insulin are also similarly impaired in these groups. The healthy individuals with these cellular abnormalities are also markedly insulin resistant *in vivo*, have higher fasting insulin and triglyceride levels, thus exhibiting several signs of the Insulin Resistance (or Metabolic) Syndrome.[33] Furthermore, the fact that these individuals were resistant to the ability of insulin to stimulate glucose uptake *in vivo*

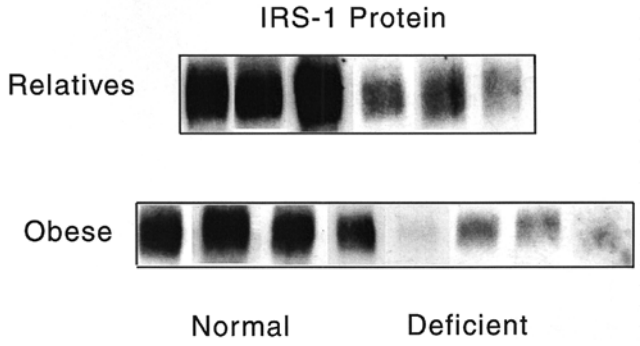

Figure 2 IRS-1 protein expression in fat cells from obese subjects or non-obese healthy relatives to subjects with type 2 diabetes. Data reproduced from Carvalho *et al*[28] by permission.

during a euglycemic clamp shows that muscle uptake was reduced, probably due to an impaired glucose transport.[34]

It is unlikely that the molecular abnormalities seen in the adipose cells are secondary to the insulin resistance and hyperinsulinemia. Although IRS-1 protein can be reduced by prolonged and marked hyperinsulinemia *in vitro*,[35] many obese subjects had both hyperinsulinemia and normal IRS-1 expression. Furthermore, the reduced GLUT4 expression cannot be explained by this possibility.

As discussed above, the major consistent finding in muscle in type 2 diabetes seems to be an impairment (rapidly reversible?) in insulin-stimulated glucose transport and glycogen synthesis, while PKB/Akt activation is normal. Although this does not exclude major abnormalities in other, still undefined, molecular targets of insulin action in muscle like c-Cbl-associated protein (CAP),[36] it is also clear that there are major differences in this regard between fat and muscle. Thus, a low IRS-1 expression in the adipocytes is a biomarker of insulin resistance and propensity for type 2 diabetes.[28]

A key question, then, is why there are these differences in insulin signaling and gene and protein expression between two major target tissues for insulin. Although there are no firm answers to this, one possibility is that the adipose tissue initiates and/or is the initial tissue where insulin resistance develops. This could then lead to a series of events whereby insulin resistance is induced or augmented in muscle and liver.

One possibility is that the reduced IRS-1 and GLUT4 expression in the fat cells by itself leads to a reduced whole-body insulin sensitivity. As discussed above, a reduction in the relatively small glucose uptake by the adipose tissue ($\sim$10%) is unlikely to lead to a marked insulin resistance *in vivo*. However, by the same token, specific GLUT4 deletion in the adipose tissue produced animals with a marked insulin resistance.[5] Interestingly, both the liver and skeletal muscle were insulin resistant *in vivo* while insulin-stimulated glucose uptake was normal in skeletal muscle *in vitro*.[5] This discrepancy suggests that circulating antagonists, possibly induced by a low glucose uptake in the adipose tissue, accounted for the insulin resistance in liver and muscle *in vivo*. Since there were no differences in circulating FFA levels between the wild-type and GLUT4-depleted animals,[5] other possibilities have to be considered. The endocrine function of the adipose tissue provides a possible explanation to this discrepancy through, for instance, an increased production of cytokines like TNFα, IL-6 or resistin. Interestingly, experimental studies in 3T3-L1 cells have shown that chronic exposure to TNFα reduces both IRS-1 and GLUT4 expression.[37] Similarly, we have recently found that IL-6 is capable of producing the same effects (Rotter *et al*, submitted for publication). Thus, low IRS-1 and GLUT4 may be markers of and/or lead to an excessive production of IL-6 and/or TNFα or other cytokines or hormones, which both reduce the expression of these proteins in the adipocytes in an autocrine or paracrine fashion as well as inducing insulin resistance in muscles and, possibly, the liver. In addition, cytokines like TNFα have been found to markedly increase lipolysis and FFA release, at least in part through a reduced perilipin expression[38] and decreased Gi protein expression,[39] further augmenting the impaired cellular insulin signaling and glucose uptake.[15]

Recently, the adipose tissue was found to secrete another peptide, resistin,[40] which may be related to the insulin resistance in obesity. A similar protein, called FIZZ1[41] was previously isolated from inflammatory cells in pulmonary lavage from animals with experimentally induced asthma. However, the overall role of resistin in insulin resistance in man is conjectural. Two recent studies were unable to detect resistin expression in human fat cells[42,43] irrespective of degree of obesity[42] or insulin resistance.[43]

An additional possibility is that low IRS-1 and GLUT4 expression in the fat cells is associated with elevated lipolysis and circulating FFA levels which, in turn, impair insulin action *in vivo*.[15] However, fasting FFA levels are not different in these subjects when compared to carefully matched individuals with a normal expression of these proteins but the ability of insulin to lower the FFA levels is, as expected, impaired.

Although there is much evidence to support an endocrine cross-talk between fat and muscle (and liver?), it is currently unclear how such a mechanism can explain the fact that lipoatrophy is also associated with insulin resistance and diabetes. In one animal model of lipoatrophy, it was found that the insulin resistance was probably due to lack of leptin.[44] Administering leptin to these animals markedly improved insulin sensitivity, possibly due to an increased oxidation of the excessively accumulated lipids in muscle and other tissues.[45] In contrast, in another animal model of total lipoatrophy, leptin was unable to improve the insulin resistance but transplantation of fat led to a marked improvement.[46]

Thus, the adipose tissue not only produces peptides which can elicit insulin resistance but also hormones which can improve insulin resistance such as leptin[45] and adiponectin.[47–49] Circulating adiponectin levels are positively correlated to insulin sensitivity and negatively related to BMI.[48] Furthermore, administration of adiponectin to animal models of insulin resistance and diabetes improves insulin sensitivity.[49] Thus, it is likely that the balance of the production of hormones from the adipose tissue that accentuate (like IL-6 and TNFα) or alleviate (like leptin and adiponectin) insulin resistance, as well as eliciting other effects, is due to several factors including adipose mass, nutritional state and genetic background.

Effects of thiazolidinediones on IRS-1 and IRS-2 expression

Thiazolidinediones (TZD), the novel insulin sensitizers used in the treatment of type 2 diabetes, are ligands for PPARγ which is predominantly expressed in the adipose tissue.[50]

Support for the pivotal role of the adipose tissue for the insulin-sensitizing effect of TZD comes from the recent work of Gavrilova *et al*.[46] These authors found that TZDs lost their beneficial effect on insulin sensitivity in totally lipoathropic mice while the lipid-lowering (probably PPARα) effect remained. Thus, an interesting question for us was to examine whether TZD could restore or increase the expression of IRS-1 in the fat cells. We addressed this by using both differentiated 3T3-L1 adipocytes as well as human adipose tissue in culture with or without the addition of different PPAR-ligands. However, we found no evidence that IRS-1 was a target for TZD but, interestingly, the IRS-2 gene was clearly activated by PPARγ but not PPARα ligands.[51] IRS-2 mRNA was rapidly increased (within 4 h) and remained elevated over the observation period of 48 h. Furthermore, IRS-2 protein was markedly increased.[51] Thus, these data show that TZD increase IRS-2 gene and protein expression and suggest that this may be one mechanism for the insulin sensitizing effect of these drugs. This possibility is further supported by our recent finding that IRS-2 expression was also increased by pioglitazone in cultured human fat cells from type 2 diabetic (and, thus, having low IRS-1 expression) individuals (Figure 3).

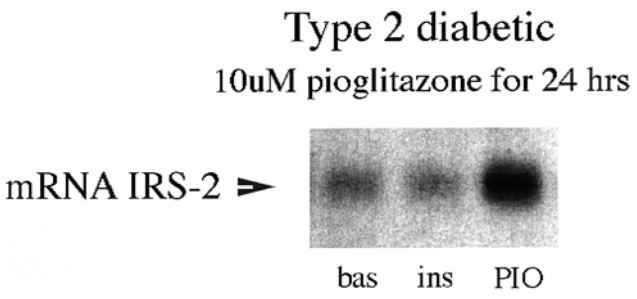

Figure 3 Effect of pioglitazone (10 μM) on IRS-2 mRNA expression in fat cells from a type 2 diabetic individual incubated for 16 h as indicated.

IRS-2 is the main docking protein for PI3-kinase activation in fat cells when IRS-1 is markedly reduced, such as in type 2 diabetes,[17] as discussed above. Similarly, IRS-2 functions as a major docking protein in cells from IRS-1 'knock-out' animals.[52] In addition, IRS-2 appears to be the predominant IRS-molecule expressed in liver and β-cells[3,53] and abnormalities in these organs also appear to be a major cause of the 'type 2' diabetes in IRS-2 'knock-out' animals.[54] We have recently examined cellular IRS-2 levels in *ob*/*ob* animals treated for 6

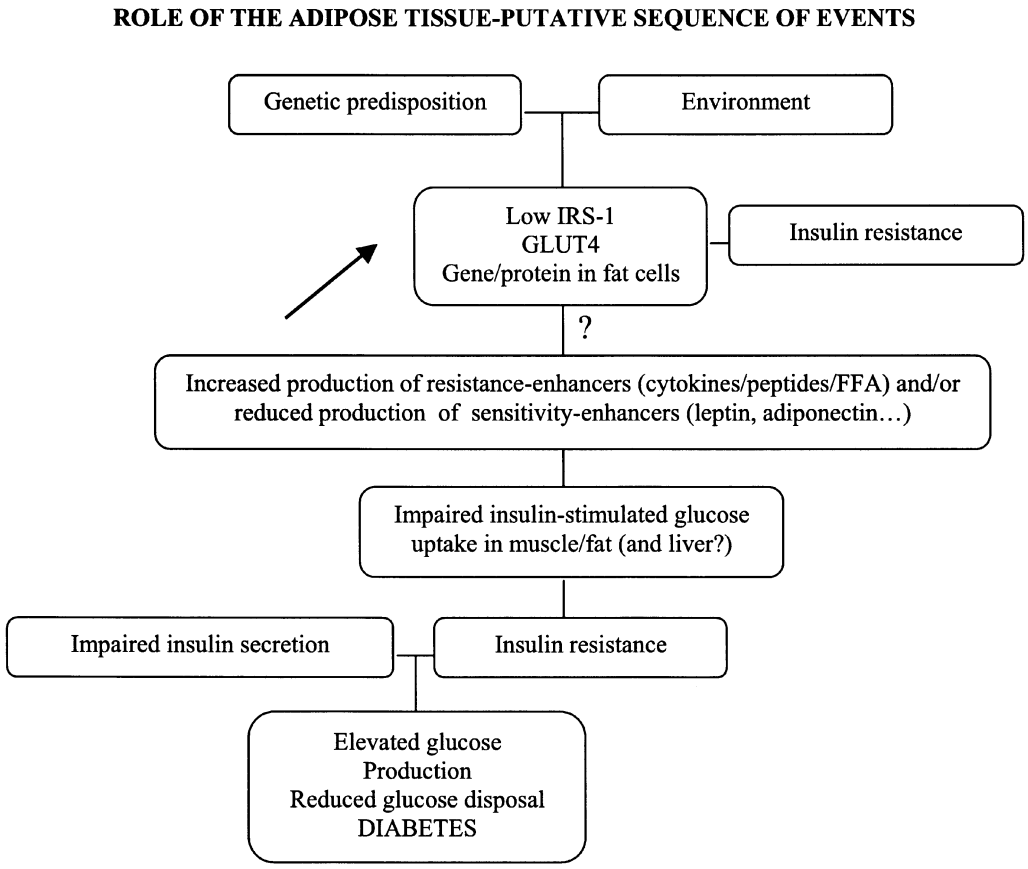

Figure 4 Potential sequence of events whereby the adipose tissue can induce insulin resistance.

days with TZD and also find an increased expression in fat cells (unpublished observation). However, it is currently unclear if TZD also increase IRS-2 expression in muscle, liver and β-cells but this is the subject of an ongoing study.

An increased IRS-2 expression in fat, liver and/or muscle could substitute for the reduced IRS-1 protein and/or the impaired phosphorylation and activation by insulin, leading to an increased insulin sensitivity. Furthermore, a putative increase in IRS-2 expression in β-cells by TZD may be important for both growth and function.[55] However, it would seem an attractive therapeutic possibility to have agents which directly increase IRS-1 expression since this docking protein is the major activator of PI3-kinase in response to insulin in human fat cells and, in contrast to IRS-2, is markedly reduced in adipocytes in insulin-resistant states. We here suggest that the tissue-specific reduction in gene and protein expression of IRS-1 and GLUT4 may play an important role in the development of the whole-body insulin resistance either directly or indirectly by an association with an increased production of cytokines and/or other insulin-antagonistic factors (Figure 4). TZD may alleviate or normalize this effect by increasing IRS-2 expression in fat cells and, possibly, also other target tissues for insulin and the pancreatic β-cells.

Although this review is focused on recent findings relating insulin resistance to an early impaired insulin signaling and action in fat cells through a reduced IRS-1/GLUT4 expression, and the effect of TZD on IRS-1/IRS-2, TZD clearly also elicit other important changes in the adipose tissue. These include the recruitment of new and smaller fat cells through an increased adipogenesis, a process where both IRS-1 and IRS-2 play a critical role,[56] altered expression of genes directly or indirectly related to insulin action,[57] including an inhibition of cytokine release by the fat cells.[57,58] In addition, the ability of TNFα to stimulate lipolysis and FFA release is also antagonized by TZD.[38]

However, insulin is a key regulator of lipolysis and circulating FFA levels *in vivo* and the antilipolytic effect of insulin is mediated through the activation of PI3-kinase.[59] Thus, a reduced IRS-1 expression and insulin-stimulated PI3-kinase activity will also link insulin resistance, as defined by a reduced insulin-stimulated glucose uptake, to an impaired ability of insulin to suppress lipolysis.

Acknowledgements

The studies performed in the author's laboratory were supported by grants from the Swedish Medical Research Council (project B-3506), the Swedish Diabetes Association, the European Community (QLG1-CT-1999-00674), Gullan and Sven-Erik Karlsson, the Sonya Hedenbratt Memorial Fund and the IngaBritt and Arne Lundberg Foundation.

References

1 DeFronzo RA, Bonadonna RC, Ferrannini E. Pathogenesis of NIDDM. A balanced overview. *Diabetes Care* 1992; **15**: 318–368.
2 Araki E, Lipes MA, Patti ME, Bruning JC, Haag B III, Johnson RS, Kahn CR. Alternative pathway of insulin signalling in mice with targeted disruption of the IRS-1 gene. *Nature* 1994; **372**: 186–190.
3 Withers DJ, Gutierrez JS, Towery H, Burks DJ, Ren JM, Previs S, Zhang Y, Bernal D, Pons S, Shulman GI, Bonner-Weir S, White MF. Disruption of IRS-2 causes type 2 diabetes in mice. *Nature* 1998; **391**: 900–904.
4 Zisman A, Peroni OD, Abel ED, Michael MD, Mauvais-Jarvis F, Lowell BB, Wojtaszewski JF, Hirshman MF, Virkamaki A, Goodyear LJ, Kahn CR, Kahn BB. Targeted disruption of the glucose transporter 4 selectively in muscle causes insulin resistance and glucose intolerance. *Nature Med* 2000; **6**: 924–928.
5 Abel ED, Peroni O, Kim JK, Kim YB, Boss O, Hadro E, Minnemann T, Shulman GI, Kahn BB. Adipose-selective targeting of the GLUT4 gene impairs insulin action in muscle and liver. *Nature* 2001; **409**: 729–733.
6 Kim JK, Michael MD, Previs SF, Peroni OD, Mauvais-Jarvis F, Neschen S, Kahn BB, Kahn CR, Shulman GI. Redistribution of substrates to adipose tissue promotes obesity in mice with selective insulin resistance in muscle. *J Clin Invest* 2000; **105**: 1791–1797.
7 Zierath JR, Krook A, Wallberg-Henriksson H. Insulin action and insulin resistance in human skeletal muscle. [In Process Citation]. *Diabetologia* 2000; **43**: 821–835.
8 Kim YB, Nikoulina SE, Ciaraldi TP, Henry RR, Kahn BB. Normal insulin-dependent activation of Akt/protein kinase B, with diminished activation of phosphoinositide 3-kinase, in muscle in type 2 diabetes. [See comments.] *J Clin Invest* 1999; **104**: 733–741.
9 Krook A, Roth RA, Jiang XJ, Zierath JR, Wallberg-Henriksson H. Insulin-stimulated Akt kinase activity is reduced in skeletal muscle from NIDDM subjects. *Diabetes* 1998; **47**: 1281–1286.
10 Wu X, Sallinen K, Anttila L, Makinen M, Luo C, Pollanen P, Erkkola R. Expression of insulin-receptor substrate-1 and -2 in ovaries from women with insulin resistance and from controls. *Fertil Steril* 2000; **74**: 564–572.
11 Rui L, Aguirre V, Kim JK, Shulman GI, Lee A, Corbould A, Dunaif A, White MF. Insulin/IGF-1 and TNF-alpha stimulate phosphorylation of IRS-1 at inhibitory Ser307 via distinct pathways. *J Clin Invest* 2001; **107**: 181–189.
12 Zierath JR, He L, Guma A, Odegoard Wahlstrom E, Klip A, Wallberg-Henriksson H. Insulin action on glucose transport and plasma membrane GLUT4 content in skeletal muscle from patients with NIDDM. *Diabetologia* 1996; **39**: 1180–1189.
13 Zierath JR, Galuska D, Nolte LA, Thorne A, Kristensen JS, Wallberg-Henriksson H. Effects of glycaemia on glucose transport in isolated skeletal muscle from patients with NIDDM: in vitro reversal of muscular insulin resistance. *Diabetologia* 1994; **37**: 270–277.
14 Hotamisligil GS, Murray DL, Choy LN, Spiegelman BM. Tumor necrosis factor alpha inhibits signaling from the insulin receptor. *Proc Natl Acad Sci USA* 1994; **91**: 4854–4858.
15 Dresner A, Laurent D, Marcucci M, Griffin ME, Dufour S, Cline GW, Slezak LA, Andersen DK, Hundal RS, Rothman DL, Petersen KF, Shulman GI. Effects of free fatty acids on glucose transport and IRS-1-associated phosphatidylinositol 3-kinase activity. *J Clin Invest* 1999; **103**: 253–259.
16 Pedersen O, Bak JF, Andersen PH, Lund S, Moller DE, Flier JS, Kahn BB. Evidence against altered expression of GLUT1 or GLUT4 in skeletal muscle of patients with obesity or NIDDM. *Diabetes* 1990; **39**: 865–870.
17 Rondinone CM, Wang LM, Lonnroth P, Wesslau C, Pierce JH, Smith U. Insulin receptor substrate (IRS) 1 is reduced and IRS-2 is the main docking protein for phosphatidylinositol 3-kinase in adipocytes from subjects with non-insulin-dependent diabetes mellitus. *Proc Natl Acad Sci USA* 1997; **94**: 4171–4175.
18 Carvalho E, Eliasson B, Wesslau C, Smith U. Impaired phosphorylation and insulin-stimulated translocation to the plasma membrane of protein kinase B/Akt in adipocytes from type II diabetic subjects. [In Process Citation.] *Diabetologia* 2000; **43**: 1107–1115.

19 Garvey WT, Maianu L, Hancock JA, Golichowski AM, Baron A. Gene expression of GLUT4 in skeletal muscle from insulin-resistant patients with obesity, IGT, GDM, and NIDDM. *Diabetes* 1992; **41**: 465–475.
20 Vaag A, Henriksen JE, Beck-Nielsen H. Decreased insulin activation of glycogen synthase in skeletal muscles in young nonobese Caucasian first-degree relatives of patients with non-insulin-dependent diabetes mellitus. *J Clin Invest* 1992; **89**: 782–788.
21 Handberg A, Vaag A, Vinten J, Beck-Nielsen H. Decreased tyrosine kinase activity in partially purified insulin receptors from muscle of young, non-obese first degree relatives of patients with type 2 (non-insulin-dependent) diabetes mellitus. *Diabetologia* 1993; **36**: 668–674.
22 Pratipanawatr W, Pratipanawatr T, Cusi K, Berria R, Adams JM, Jenkinson CP, Maezono K, DeFronzo RA, Mandarino LJ. 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. *Diabetes* 2001; **50**: 2572–2578.
23 Storgaard H, Song XM, Jensen CB, Madsbad S, Bjornholm M, Vaag A, Zierath JR. Insulin signal transduction in skeletal muscle from glucose-intolerant relatives with type 2 diabetes. *Diabetes* 2001; **50**: 2770–2778.
24 Cusi K, Maezono K, Osman A, Pendergrass M, Patti ME, Pratipanawatr T, DeFronzo RA, Kahn CR, Mandarino LJ. Insulin resistance differentially affects the PI 3-kinase- and MAP kinase-mediated signaling in human muscle. *J Clin Invest* 2000; **105**: 311–320.
25 Goodyear LJ, Giorgino F, Sherman LA, Carey J, Smith RJ Dohm GL. Insulin receptor phosphorylation, insulin receptor substrate-1 phosphorylation, and phosphatidylinositol 3-kinase activity are decreased in intact skeletal muscle strips from obese subjects. *J Clin Invest* 1995; **95**: 2195–2204.
26 Krutzfeldt J, Kausch C, Volk A, Klein HH, Rett K, Haring HU, Stumvoll M. Insulin signaling and action in cultured skeletal muscle cells from lean healthy humans with high and low insulin sensitivity. *Diabetes* 2000; **49**: 992–998.
27 Mott DM, Pratley RE, Bogardus C. Postabsorptive respiratory quotient and insulin-stimulated glucose storage rate in nondiabetic Pima Indians are related to glycogen synthase fractional activity in cultured myoblasts. *J Clin Invest* 1998; **101**: 2251–2256.
28 Carvalho E, Jansson PA, Axelsen M, Eriksson JW, Huang X, Groop L, Rondinone C, Sjostrom L, Smith U. Low cellular IRS 1 gene and protein expression predict insulin resistance and NIDDM. *FASEB J* 1999; **13**: 2173–2178.
29 Carvalho E, Jansson PA, Nagaev I, Wenthzel AM, Smith U. Insulin resistance with low cellular IRS-1 expression is also associated with low GLUT4 expression and impaired insulin-stimulated glucose transport. *FASEB J* 2001; **15**: 1101–1103.
30 Morris RD, Rimm DL, Hartz AJ, Kalkhoff RK, Rimm AA. Obesity and heredity in the etiology of non-insulin-dependent diabetes mellitus in 32,662 adult white women. *Am J Epidemiol* 1989; **130**: 112–121.
31 Lapidus L, Bengtsson C, Lissner L, Smith U. Family history of diabetes in relation to different types of obesity and change of obesity during 12-yr period. Results from prospective population study of women in Goteborg, Sweden. *Diabetes Care* 1992; **15**: 1455–1458.
32 Grill V, Persson G, Carlsson S, Norman A, Alvarsson M, Ostensson CG, Svanstrom L, Efendic S. Family history of diabetes in middle-aged Swedish men is a gender unrelated factor which associates with insulinopenia in newly diagnosed diabetic subjects. *Diabetologia* 1999; **42**: 15–23.
33 Reaven GM. Banting lecture 1988. Role of insulin resistance in human disease. *Diabetes* 1988; **37**: 1595–1607.
34 Cline GW, Petersen KF, Krssak M, Shen J, Hundal RS, Trajanoski Z, Inzucchi S, Dresner A, Rothman DL, Shulman GI. Impaired glucose transport as a cause of decreased insulin-stimulated muscle glycogen synthesis in type 2 diabetes. [See comments.] *New Engl J Med* 1999; **341**: 240–246.
35 Ricort JM, Tanti JF, Van Obberghen E, Le Marchand-Brustel Y. Alterations in insulin signalling pathway induced by prolonged insulin treatment of 3T3-L1 adipocytes. *Diabetologia* 1995; **38**: 1148–1156.
36 Chiang SH, Baumann CA, Kanzaki M, Thurmond DC, Watson RT, Neudauer CL, Macara IG, Pessin JE, Saltiel AR. Insulin-stimulated GLUT4 translocation requires the CAP-dependent activation of TC10. *Nature* 2001; **410**: 944–948.
37 Stephens JM, Lee J, Pilch PF. Tumor necrosis factor-alpha-induced insulin resistance in 3T3-L1 adipocytes is accompanied by a loss of insulin receptor substrate-1 and GLUT4 expression without a loss of insulin receptor-mediated signal transduction. *J Biol Chem* 1997; **272**: 971–976.
38 Souza SC, de Vargas LM, Yamamoto MT, Lien P, Franciosa MD, Moss LG, Greenberg AS. Overexpression of perilipin A and B blocks the ability of tumor necrosis factor alpha to increase lipolysis in 3T3-L1 adipocytes. *J Biol Chem* 1998; **273**: 24665–24669.
39 Gasic S, Tian B, Green A. Tumor necrosis factor alpha stimulates lipolysis in adipocytes by decreasing Gi protein concentrations. *J Biol Chem* 1999; **274**: 6770–6775.
40 Steppan CM, Bailey ST, Bhat S, Brown EJ, Banerjee RR, Wright CM, Patel HR, Ahima RS, Lazar MA. The hormone resistin links obesity to diabetes. *Nature* 2001; **409**: 307–312.
41 Holcomb IN, Kabakoff RC, Chan B, Baker TW, Gurney A, Henzel W, Nelson C, Lowman HB, Wright BD, Skelton NJ, Frantz GD, Tumas DB, Peale FV Jr, Shelton DL, Hebert CC. FIZZ1, a novel cysteine-rich secreted protein associated with pulmonary inflammation, defines a new gene family. *EMBO J* 2000; **19**: 4046–4055.
42 Savage DB, Sewter CP, Klenk ES, Segal DG, Vidal-Puig A, Considine RV, O'Rahilly S. Resistin/Fizz3 expression in relation to obesity and peroxisome proliferator-activated receptor-gamma action in humans. *Diabetes* 2001; **50**: 2199–2202.
43 Nagaev I, Smith U. Insulin resistance and type 2 diabetes are not related to resistin expression in human fat cells or skeletal muscle. *Biochem Biophys Res Commun* 2001; **285**: 561–564.
44 Shimomura I, Hammer RE, Ikemoto S, Brown MS, Goldstein JL. Leptin reverses insulin resistance and diabetes mellitus in mice with congenital lipodystrophy. *Nature* 1999; **401**: 73–76.
45 Unger RH, Orci L. Diseases of liporegulation: new perspective on obesity and related disorders. *FASEB J* 2001; **15**: 312–321.
46 Gavrilova O, Marcus-Samuels B, Graham D, Kim JK, Shulman GI, Castle AL, Vinson C, Eckhaus M, Reitman ML. Surgical implantation of adipose tissue reverses diabetes in lipoatrophic mice. *J Clin Invest* 2000; **105**: 271–278.
47 Maeda N, Takahashi M, Funahashi T, Kihara S, Nishizawa H, Kishida K, Nagaretani H, Matsuda M, Komuro R, Ouchi N, Kuriyama H, Hotta K, Nakamura T, Shimomura I, Matsuzawa Y. PPARgamma ligands increase expression and plasma concentrations of adiponectin, an adipose-derived protein. *Diabetes* 2001; **50**: 2094–2099.
48 Halleux CM, Takahashi M, Delporte ML, Detry R, Funahashi T, Matsuzawa Y, Brichard SM. Secretion of adiponectin and regulation of apM1 gene expression in human visceral adipose tissue. *Biochem Biophys Res Commun* 2001; **288**: 1102–1107.
49 Yamauchi T, Kamon J, Waki H, Terauchi Y, Kubota N, Hara K, Mori Y, Ide T, Murakami K, Tsuboyama-Kasaoka N, Ezaki O, Akanuma Y, Gavrilova O, Vinson C, Reitman ML, Kagechika H, Shudo K, Yoda M, Nakano Y, Tobe K, Nagai R, Kimura S, Tomita M, Froguel P, Kadowaki T. The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. *Nature Med* 2001; **7**: 941–946.
50 Olefsky JM. Treatment of insulin resistance with peroxisome proliferator-activated receptor gamma agonists. *J Clin Invest* 2000; **106**: 467–472.
51 Smith U, Gogg S, Johansson A, Olausson T, Rotter V, Svalstedt B. Thiazolidinediones (PPARgamma agonists) but not PPARalpha agonists increase IRS-2 gene expression in 3T3-L1 and human adipocytes. *FASEB J* 2001; **15**: 215–220.

52 Patti ME, Sun XJ, Bruening JC, Araki E, Lipes MA, White MF, Kahn CR. 4PS/insulin receptor substrate (IRS)-2 is the alternative substrate of the insulin receptor in IRS-1-deficient mice. *J Biol Chem* 1995; **270**: 24670–24673.
53 Shimomura I, Matsuda M, Hammer RE, Bashmakov Y, Brown MS, Goldstein JL. Decreased IRS-2 and increased SREBP-1c lead to mixed insulin resistance and sensitivity in livers of lipodystrophic and *ob*/*ob* mice. *Mol Cell* 2000; **6**: 77–86.
54 Kubota N, Tobe K, Terauchi Y, Eto K, Yamauchi T, Suzuki R, Tsubamoto Y, Komeda K, Nakano R, Miki H, Satoh S, Sekihara H, Sciacchitano S, Lesniak M, Aizawa S, Nagai R, Kimura S, Akanuma Y, Taylor SI, Kadowaki T. Disruption of insulin receptor substrate 2 causes type 2 diabetes because of liver insulin resistance and lack of compensatory beta-cell hyperplasia. *Diabetes* 2000; **49**: 1880–1889.
55 Withers DJ, Burks DJ, Towery HH, Altamuro SL, Flint CL, White MF. Irs-2 coordinates Igf-1 receptor-mediated beta-cell development and peripheral insulin signalling. *Nature Genet* 1999; **23**: 32–40.
56 Miki H, Yamauchi T, Suzuki R, Komeda K, Tsuchida A, Kubota N, Terauchi Y, Kamon J, Kaburagi Y, Matsui J, Akanuma Y, Nagai R, Kimura S, Tobe K, Kadowaki T. Essential role of insulin receptor substrate 1 (IRS-1) and IRS-2 in adipocyte differentiation. *Mol Cell Biol* 2001; **21**: 2521–2532.
57 Rosen ED, Spiegelman BM. PPARgamma: a nuclear regulator of metabolism, differentiation, and cell growth. *J Biol Chem* 2001; **276**: 37731–37734.
58 Peraldi P, Xu M, Spiegelman BM. Thiazolidinediones block tumor necrosis factor-alpha-induced inhibition of insulin signaling. *J Clin Invest* 1997; **100**: 1863–1869.
59 Rahn T, Ridderstrale M, Tornqvist H, Manganiello V, Fredrikson G, Belfrage P, Degerman E. Essential role of phosphatidylinositol 3-kinase in insulin-induced activation and phosphorylation of the cGMP-inhibited cAMP phosphodiesterase in rat adipocytes. Studies using the selective inhibitor wortmannin. *FEBS Lett* 1994; **350**: 314–318.