Tissue-Specific Regulation of Glucose Transporters in Different Forms of Obesity

(43421)

BARBARA B. KAHN*[,1] AND OLUF PEDERSEN[†]

Diabetes Unit, Department of Medicine, Beth Israel Hospital and Harvard Medical School, Boston, Massachusetts 02215 and Steno Diabetes Center,[†] Niels Steensensvej 2, DK-2820 Gentofte, Denmark*

Obesity is a state of insulin-resistant glucose uptake *in vivo*. This insulin resistance and the resultant hyperinsulinemia appear to play a major role in the morbidity of obesity. Furthermore, insulin resistance is a key feature in the link between obesity and diabetes. Our recent studies have investigated the potential role of glucose transporters in the pathogenesis of this insulin-resistant glucose uptake. Glucose transport in highly insulin-responsive tissues is mediated by GLUT1, the Hep G2/brain glucose transporter that is also present in non-insulin-responsive tissues, and GLUT4, the adipose-muscle glucose transporter that is present primarily in highly insulin-responsive tissues (1, 2). The present manuscript discusses recent work from our laboratory demonstrating two key points: (i) there is tissue-specific regulation of these glucose transporters, so that in insulin-resistant states, transporter expression is much more affected in adipose cells than in muscle; and (ii) changes in insulin-stimulated glucose transport may be due primarily to alterations in the subcellular distribution or function of glucose transporters, rather than to alterations in their levels of expression.

More than a decade ago, the translocation hypothesis was proposed by Wardzala and Cushman and independently by Kono and colleagues as the major mechanism by which insulin stimulates glucose transport in adipose cells and muscle (3). In these tissues, insulin binds to its receptor, thereby generating a series of signals which result in the translocation of transporters from an intracellular pool associated with specific membrane vesicles to the plasma membrane. There they bind, fuse, and promote glucose entry into the cell. If insulin dissociates from its receptor, transporters are reendocytosed within the cell. In fact, recent data suggest that transporters may be constantly recycling between the plasma membrane and an intracellular pool and that insulin stimulation favors the exocytotic state.

In altered metabolic states, the abundance of glucose transporters in the intracellular pool may be changed, thereby affecting the number translocated to the plasma membrane in response to insulin (4). In addition, the activity of the transporters in these states may also be altered, so that they may transport a small, a moderate, or a larger amount of glucose (2–4). The levels of glucose transport and transporters are affected in both dietary-induced and genetic obesity in rats and in human obesity and diabetes.

Dietary Regulation of Glucose Transporters in Adipose Cells

When rats are fed a high calorie diet, in which the same proportions of carbohydrate, protein and fat are maintained as in standard chow, basal glucose transport more than doubles and the absolute insulin-stimulated rate is maintained, although the increment above the basal level is decreased (5). When rats are fed a *high fat* diet with the same number of calories as the *ad libitum* fed control group, basal glucose transport increases and insulin stimulated transport is severely diminished. Although these rats do not weigh more than the controls, they are obese based on percentage of body fat and fat cell size. When rats are placed on calorie restriction to 70% of the calories consumed by *ad libitum*-fed controls, basal transport decreases and insulin-stimulated transport is maintained. Hence, dietary manipulations have dramatic effects on glucose transport in the adipose cell. Can these results be explained by alterations in the expression of glucose transporter isoforms?

We determined the level of GLUT1 and GLUT4 transporters by immunoblotting a total membrane preparation from adipose cells from these rats with specific GLUT1 and GLUT4 antisera. Levels of GLUT1 are not altered by obesity or calorie restriction. However, levels of GLUT4 are decreased by high fat feeding (5). In fact, adipose cells from rats fed this high

[1] To whom requests for reprints should be addressed at Diabetes Unit, Beth Israel Hospital, 330 Brookline Avenue, Boston, MA 02215.

P.S.E.B.M. 1992, Vol 200: 214–217

0037-9727/92/2002-0214$3.00/0

fat diet have markedly reduced membrane protein. When this factor is taken into account, the number of GLUT4 transporters per cell is even more severely reduced. It has been hypothesized that GLUT1 may be responsible for basal glucose transport in adipose cells and GLUT4 for insulin-stimulated transport. However, from these studies, it appears that the increases in basal glucose transport in the two obese groups cannot be explained by changes in GLUT1, but the decrease in insulin-stimulated glucose transport with high fat feeding can be explained by a decrease in GLUT4.

Glucose Transporters in Adipose Cells of Genetically Obese Zucker Rats

How generalizable are these findings to other types of obesity, especially since these high fat-fed rats are *not* markedly hyperinsulinemic (5) in contrast to obese humans? To answer this question, we investigated changes in the glucose transport system in genetically obese Zucker *fa/fa* rats. The Zucker rat is a model of endogenous hyperinsulinemia and insulin-resistant glucose uptake *in vivo*. We studied rats at 5, 10, and 20 weeks of age to determine changes in transporter expression over the evolution of obesity with increasing insulin resistance. As an indication of glucose transport in adipose cells, we used conversion of glucose to lipids because adipose cells from 20-week-old rats were so large and fragile that we could not maintain adequate cell density for a glucose transport assay. However, this lipogenesis assay has been evaluated under many conditions and shown to reflect primarily glucose transport.

As others have shown, young obese Zucker rats have increased basal glucose transport and markedly hyperresponsive insulin-stimulated glucose transport. As these rats age, they become more obese and basal rates increase dramatically to exceed insulin-stimulated rates in the lean controls. However, unlike rats fed a high fat diet, in obese Zucker rats, the insulin-stimulated glucose transport also increases. Even as the lean rat ages, basal and insulin-stimulated transport increase. Expression of glucose transporters was examined in these same cells from lean and obese Zucker rats. At all ages, GLUT1 levels were elevated in obese rats and levels changed very little with age. In contrast, GLUT4 levels increased in 5- and 10-week-old obese rats, but decreased in 20-week-old obese rats. How do these changes compare with glucose transport rates?

In the 20-week-old rats, both basal glucose transport and GLUT1 transporters increased with obesity. In 20-week-old obese rats, insulin-stimulated transport increased, but GLUT4 transporters decreased. There are several possible explanations for this discrepancy. One is that the translocation or intrinsic activity of GLUT4 may be altered in adipose cells in this model of obesity. Another is that GLUT1 may contribute to insulin-stimulated glucose transport in adipose cells from these obese rats.

Glucose Transporter Expression in Skeletal Muscle of Zucker Rats

Before pursuing the mechanism for these changes, it is important to determine whether the same effects are evident in skeletal muscle, since muscle is the major tissue responsible for insulin-mediated glucose uptake *in vivo*. Preliminary Northern blotting of GLUT4 mRNA levels in hind limb muscle from these same lean and obese Zucker rats shows that at all ages, the levels of GLUT4 mRNA in muscle are similar in lean and obese rats in contrast to the dramatic changes in adipose cells. Immunoblotting of glucose transporters in skeletal muscle of 20-week-old Zucker rats shows that GLUT4 protein levels are unchanged with obesity or diabetes. How can the *in vivo* insulin-resistant glucose uptake in these rats be explained, especially since several studies indicate a glucose transport defect in muscle from obese Zucker rats?

These studies have examined the overall level of expression of transporters within muscle, which is unaltered. This implies that there must be a functional defect either in the translocation of transporters to the plasma membrane, in their fusion with the plasma membrane and exposure to extracellular milieu, or in their activity once in the plasma membrane (6). A recent abstract (7) indicates that the translocation of transporters is deranged in muscle of obese Zucker rats and this defect may be the major mechanism for insulin-resistant glucose uptake in this model. However, in other insulin-resistant states, the translocation of glucose transporters is intact (unpublished data). We hypothesize that there are several potential mechanisms for insulin-resistant glucose uptake in muscle in other metabolic states. It may be due to decreased intrinsic activity of the transporters, so that each transporter is transporting less glucose. Alternatively, transporter vesicles may be associated with the plasma membrane, for example via docking proteins, but not actually fused. A third possibility is that transporters may be somewhat cryptic in deep invaginations of the plasma membrane and may not be exposed to the extracellular milieu.

Regulation of Glucose Transporters in Human Obesity and Diabetes

We have investigated the expression of GLUT4 and GLUT1 in skeletal muscle of insulin-resistant humans. First, we examined transporter expression in lean and obese nondiabetic subjects and lean and obese subjects with non-insulin-dependent diabetes mellitus (NIDDM) (8). Groups were matched for age and both obese groups had moderately increased body mass indices. Subjects were receiving a spectrum of treatments and showed a range of blood glucose control. GLUT4

mRNA levels were determined by slot blots of RNA from vastus lateralis thigh muscle. The level of GLUT4 mRNA is similar in lean and obese controls and in lean and obese NIDDM subjects, even when the diabetic subjects are stratified as untreated versus treated or as those with poor blood glucose control versus those with more adequate control. The same is true for the GLUT4 protein. Furthermore, we find no correlation between GLUT4 protein levels and important metabolic factors, including age, sex, body mass index, diabetes duration, hemoglobin A1c, fasting plasma insulin, or mode of antidiabetic therapy (8).

It is possible that the hyperinsulinemia in obese and NIDDM subjects may maintain glucose transporter levels. Therefore, we might be more likely to see changes in transporter expression in type 1 diabetics. So in the second study (9), we examined 10 control and 20 insulin-dependent diabetic subjects. These subjects were, for the most part, younger and all were nonobese. Duration of diabetes ranged from 1 to 24 years. Subjects received regular (short-acting) insulin before each meal and NPH (intermediate-acting) insulin at bedtime.

Blood glucose control was mediocre in this group, with almost a 2-fold increased hemoglobin A1c. Fasting plasma insulin levels, however, were similar to control. The GLUT1 and GLUT4 mRNA levels in skeletal muscle from these subjects were not different between groups. The same was true for the GLUT4 protein, and its levels did not correlate with specific physiological factors. Several other groups have now found similar results in human muscle. However, in adipose cells, one group showed a decrease in GLUT4 with obesity (10) and two groups showed a decrease with NIDDM (10, 11). So in human obesity and diabetes mellitus, as in rat models, there is tissue-specific regulation of GLUT4. Transporter expression is much more affected in adipose cells than in muscle. However, changes in the level of expression of these transporters do not simply explain changes in either basal or insulin-stimulated glucose transport. Importantly, in human obesity, NIDDM or insulin-dependent diabetes mellitus, alterations in the expression of GLUT4 in skeletal muscle do not appear to explain *in vivo* insulin-resistant glucose uptake.

Effects of Exercise on Glucose Transporter Expression in Human Skeletal Muscle

Even if the level of GLUT4 is not decreased in a group of insulin-resistant subjects, it is still possible that increasing the level of GLUT4 expression in muscle could increase insulin responsiveness. Thus, we investigated whether therapeutic interventions would modulate GLUT4 levels in human skeletal muscle. Since several studies in rats recently showed increased GLUT4 expression in response to chronic exercise training (12), we examined the effects of exercise training in humans. Recruitment and training of subjects and physiological studies were carried out by Dariush Elahi in the Gerontology Division, Beth Israel Hospital, and William Evans and Virginia Hughes at Tufts University. We studied 12 middle-aged subjects with impaired glucose tolerance. All subjects underwent exercise training on a bicycle at 50–75% VO2 max, three times a week for 3 months. Importantly, all studies were performed 96 hr after the last bout of exercise to eliminate any residual effect of acute exercise. Evaluation included oral glucose tolerance tests, euglycemic clamp studies, and vastus lateralis muscle biopsies for determination of GLUT4 levels.

In nine of the 12 subjects, GLUT4 levels increased significantly with chronic exercise training. Quantitation of results from all 12 subjects showed that mean levels of GLUT4 increased 60% with training. This increase in GLUT4 levels was associated with modest improvement in oral glucose tolerance tests and in glucose disposal measured by euglycemic clamp. Our data provide a potential mechanism for the recent observation that increased physical activity is associated with decreased incidence of NIDDM, even among obese people (13, 14). It is clear that it is possible to modulate GLUT4 levels in human skeletal muscle, and longer intervention studies will explore whether enhanced GLUT4 expression could potentially play a role in preventing the decompensation from the insulin resistance of obesity to frank diabetes.

Conclusions and Future Directions

Several mechanisms may be important in the alterations in insulin-stimulated glucose uptake in peripheral tissues. Different forms of obesity may be associated with increased or decreased expression of transporters in adipose cells, but minimal change in muscle. Our studies in muscle will now focus on whether there are changes in the subcellular distribution of GLUT4 or in various functional aspects, including GLUT4 translocation in response to insulin, fusion with the plasma membrane and exposure to the extracellular milieu, affinity for glucose (a K_m change), or intrinsic activity (a V_{max} effect).

Although initial studies to define such functional effects have not detected biochemical alterations in the transporter, it is possible that these functional defects may be due to factors extrinsic to the transporter, such as changes in the vesicles with which they are associated. These studies open new avenues for exploring these functional defects. Understanding such defects could lead to new therapeutic approaches to prevent the decompensation from the insulin-resistant state of obesity to frank diabetes.

These studies were supported by NIH grant DK43051 to Dr. Kahn. Dr. Kahn is the recipient of a CAPPS Scholar Award from

Harvard University. Dr. Pedersen is supported by a grant from the Danish Research Council. The authors are grateful for the technical assistance of Alan Rosen.

1. Bell GI, Kayano T, Buse JB, Burant CF, Takeda J, Lin D, Fukumoto H, Seino S. Molecular biology of mammalian glucose transporters. Diabetes Care **13**:198–208, 1990.
2. Kahn BB, Flier JS. Regulation of glucose-transporter gene expression in vitro and in vivo. Diabetes Care **13**:548–564, 1990.
3. Simpson IA, Cushman SW. Hormonal regulation of mammalian glucose transport. Annu Rev Biochem **55**:1059–1089, 1989.
4. Kahn BB, Cushman SW. Subcellular translocation of glucose transporters: Role in insulin action and its perturbation in altered metabolic states. Diabetes Metab Rev **1**:203–227, 1985.
5. Pedersen O, Kahn CR, Flier JS, Kahn BB. High fat feeding causes insulin resistance and a marked decrease in the expression of glucose transporters (Glut4) in fat cells of rats. Endocrinology **129**:771–777, 1991.
6. Kahn BB. Alterations in glucose transporter expression and function in diabetes. J Cell Biochem **48**:122–128, 1992.
7. Horton ED, King PA, Hirshman NF, Horton ES. Failure of insulin to stimulate WF transporter translocation in skeletal muscle from the obese (fa/fa) Zucker rat. Diabetes **39**:83A, 1990.
8. Pederson 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 **39**:865–870, 1990.
9. Kahn BB, Rosen AS, Bak JF, Andersen PH, Lund S, Pedersen O. Expression of GLUT1 and GLUT4 glucose transporters in skeletal muscle of humans with insulin-dependent diabetes mellitus: Regulatory effects of metabolic factors. J Clin Endocrinol Metab **74**:1101–1109, 1992.
10. Garvey WT, Maianu L, Huecksteadt TP, Birnbaum MJ, Molina JM, Ciaraldi TP. Pretranslational suppression of a glucose transporter protein causes insulin resistance in adipocytes from patients with non-insulin-dependent diabetes mellitus and obesity. J Clin Invest **87**:1072–1081, 1991.
11. Sinha MK, Raineri-Maldonado C, Buchanan C, Pories WJ, Carter-Su C, Pilch PF, Caro JO. Adipose tissue glucose transporters in NIDDM. Diabetes **40**:472–477, 1991.
12. Ploug T, Stallknecht BM, Pedersen O, Kahn BB, Ohkuwa T, Vinten J, Galbo H. The effect of endurance training on glucose transport capacity and expression of glucose transporters in rat skeletal muscle. Am J Physiol **259**:E778–E786, 1990.
13. Helmrich SP, Ragland DR, Leung RW, Paffenbarger RS Jr. Physical activity and reduced occurrence of non-insulin-dependent diabetes mellitus. N Engl J Med **325**:147–152, 1991.
14. Manson JE, Rimm EB, Stampfer MJ, Colditz GA, Willett WC, Krolewski AS, Rosner B, Hennekens CH, Speizer FE. Physical activity and incidence of non-insulin-dependent diabetes mellitus in women. Lancet **338**:774–778, 1991.