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GLYCOPROTEIN REDUCTIONS IN THE ADIPOCYTE CYTOPLASMIC MEMBRANE FROM OBESE ZUCKER RATS

Ronald A. Makula and Virginia Goekjian

Department of Biochemistry
University of Georgia
Athens, Georgia 30602

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Summary: The adipocyte cytoplasmic membranes from lean and obese Zucker rats were analyzed. A reduction in the galactose-containing glycoproteins was demonstrated from adipocyte cytoplasmic membranes of obese rats. A compensatory increase was observed in several membrane proteins which did not contain carbohydrate. This reduction was observed in obese rats at 5 and 16 weeks old.

Several lines of evidence have indicated that the plasma membrane insulin receptor is glycoprotein in nature. This evidence includes the inhibition of functional receptor formation by tunicamycin, an inhibitor of protein N-glycosylation (1-3), and the influence of glycosidases and lectins on the insulin-receptor interaction (4,5). The level of insulin receptors of target cells has been suggested as an important factor in the lowered sensitivity of cells to insulin (insulin-resistance) in certain pathological states (1). Several studies have suggested that obesity (6), diet (7), or diabetes (8) can modify plasma membrane glycoprotein composition. The Zucker rat, a genetic model of obesity, demonstrates insulin-resistance in the later stages of obesity. The adipose tissue of this model of obesity was analyzed for changes in plasma membrane composition. A reduction in the adipocyte membrane glycoprotein composition of the obese Zucker rat as compared to lean animals is described.

MATERIALS AND METHODS

The Zucker strain of obese rats (Fa/fa) was used in comparisons to their lean littermates. The rats were fed rat chow and water ad libitum. Both male or female pairs were sacrificed at 4 to 5 months of age unless specified otherwise.

Adipose cells from perirenal and either ovarian or epididymal fat pads were isolated according to Lewis et al. (9).

Isolated adipocytes were diluted 1:4 in 10mM Tris-HCl, 1mM EDTA, and 0.25M sucrose, pH 7.2 (Buffer A) and homogenized by 10-12 hand-driven strokes

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of a Teflon pestle in a glass homogenizer. Homogenates were centrifuged at 3000 x g for 30 min and the pellets were used for isolation of cytoplasmic membranes. For mitochondrial preparations, the supernatant solution was centrifuged at 17,400 x g for 30 min followed by 100,000 x g centrifugation for 60 min to obtain a microsomal membrane fraction. All pellets (except the microsomal fraction) were washed by resuspending in Buffer A and re-centrifugation at 18,000 x g. Pellets were resuspended in Buffer A and layered on discontinuous sucrose gradients prepared as follows: 10 ml of 37% (wt/wt) sucrose, 15 ml of 33% (wt/wt) sucrose, and 10 ml of 24% (wt/wt) sucrose. Gradients were spun 16-18 hours at 132,000 x g in a Type SW 28 swinging bucket rotor. Cytoplasmic membranes banded at the top of the 33% sucrose layer (density sucrose g/cm^3 = 1.1414).

An endoplasmic reticulum marker enzyme, NADPH-cytochrome c reductase was assayed according to Phillips and Langdon (10). The mitochondrial enzyme, succinic dehydrogenase, was assayed according to Pennington (11). Reagents for 5' nucleotidase was purchased from Sigma Chemical Co. and the assay done according to Lewis et. al. (9).

SDS-polyacrylamide gels were used with 7.5% acrylamide running gel and a 4.5% acrylamide stacking gel according to the method of O'Farrell (12).

Adipocyte plasma membranes (usually 1 mg membrane protein) from lean and obese rats were labelled by two different methods: 1) the galactose-containing glycoproteins were labelled by the method of Gahmberg and Hakomori (13) with ^{3}H-borohydride following treatment of the membranes with neuraminidase and galactose oxidase; and 2) reductive methylation with formaldehyde and ^{3}H-borohydride (14) was used to label total membrane proteins. The membranes were analyzed by SDS gel electrophoresis and tritium pattern of the proteins visualized by fluorography according to Bonner and Laskey (15).

RESULTS

Cytoplasmic membranes of rat adipocytes were prepared as described. Enzymatic markers and electron microscopic analysis were used to determine the purity of the membrane fractions. Less than 2% contamination of plasma membranes with mitochondria was found using succinic dehydrogenase as a mitochondrial enzyme marker and less than 5% contamination by endoplasmic recticulum was found by analysis of cytochrome c reductase. Electron microscopic analysis also demonstrated that the cytoplasmic membrane fraction did not contain any intact mitochondria.

The protein profile of the cytoplasmic membranes from adipocytes of lean and obese Zucker rats obtained by 7.5% SDS-polyacrylamide gel electrophoresis is shown in Figure 1. Several major peptide bands are evident and are essentially the same bands observed in other studies (16,17). A reduction in three of the major peptide bands (A, B, and C) from adipocyte cell membranes of obese rats was demonstrated when compared to the adipocyte cell membranes from lean

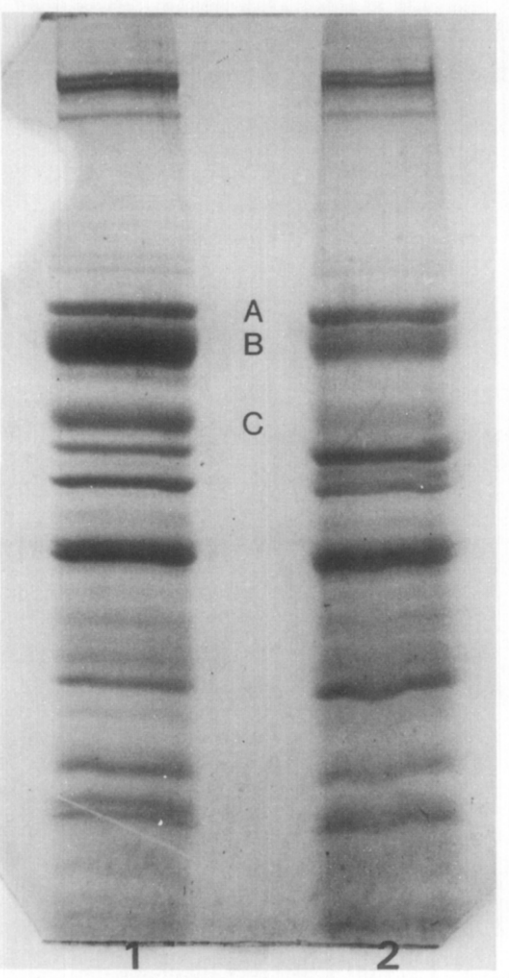


Figure 1. SDS-polyacrylamide gel electrophoresis of adipocyte cytoplasmic membranes (100 µg protein) from lean (lane 1) and obese (lane 2) rats. A, B, and C are glycoproteins with apparent molecular weights of 116,000-, 94,000-, and 78,000-daltons, respectively. Proteins stained with coomassie blue.

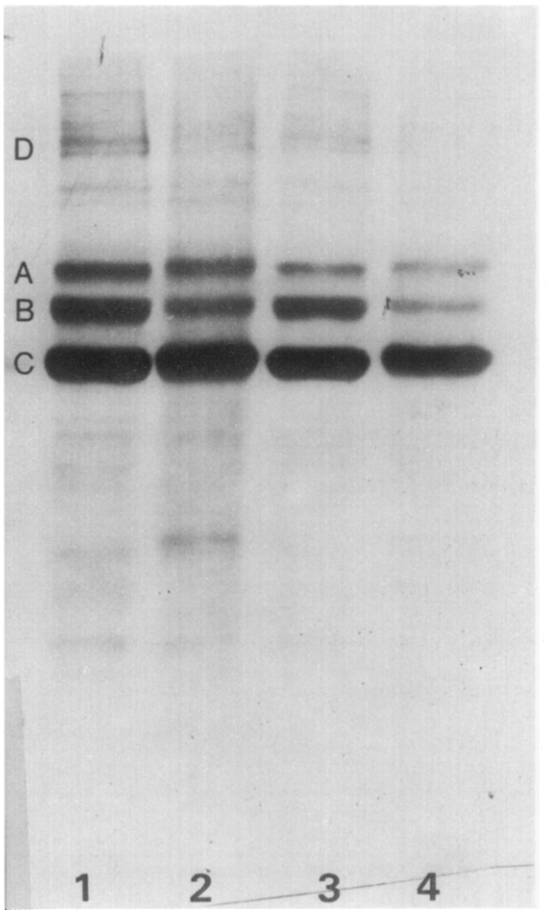
littermates. These peptides represent the major glycoprotein species, as determined by the periodic-Schiff procedure for carbohydrate. Molecular weight standards indicated that peptides A, B, and C correspond to glycoproteins with apparent molecular weights of 116,000-, 94,000-, and 78,000-daltons, respectively. These glycoproteins have also been described by other investigators (16, 17). These glycoproteins were not found in either the mitochondrial or microsomal membrane fractions.

The observed decrease in the three major glycoprotein species was extended by radiolabelling the galactose residues of the glycoproteins with ^{3}H-potassium

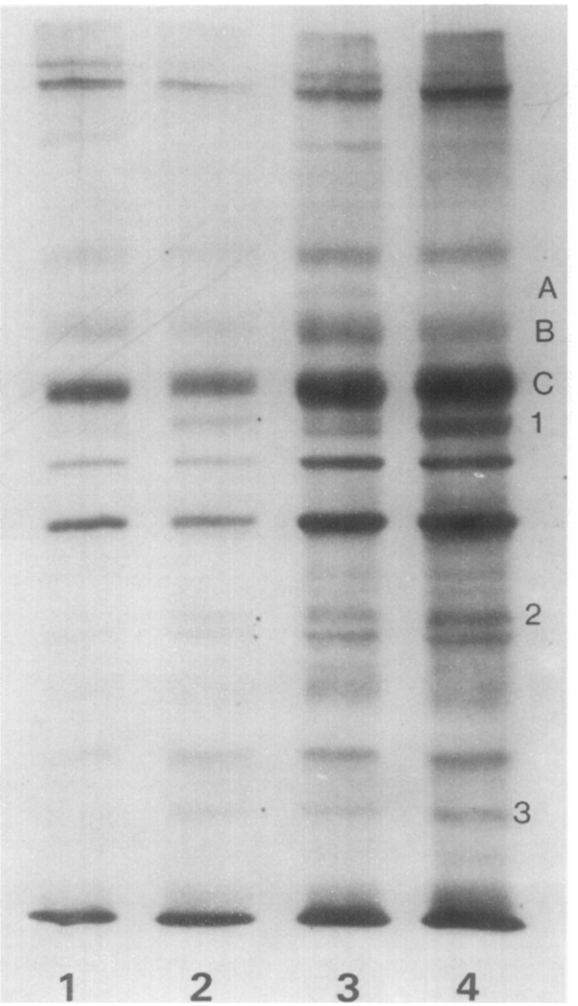
borohydride after incubation of the adipocyte cytoplasmic membranes from lean and obese animals with neuraminidase followed by galactose oxidase. Equal amounts of adipocyte cytoplasmic membrane protein were analyzed by SDS-polyacrylamide gel electrophoresis and the tritium pattern of the galactose-containing glycoproteins visualized by fluorography (Fig. 2). No labelled proteins were observed in controls in which the galactose oxidase step was omitted. The three glycoproteins (A, B, and C) were reduced in adipocyte cytoplasmic membranes from obese animals (lanes 2 and 4) compared to the membranes from lean animals (lanes 1 and 3). Several glycoproteins, approximately 200,000-daltons, were also demonstrated by the ^{3}H-borohydride labelling method (lane 1, D). These glycoproteins were also reduced in the cytoplasmic membrane protein profile derived from obese rats (lane 2). Analysis of the fluorographs by densitometry followed by quantitation of the resulting profiles from three separate experiments demonstrated a reduction of 35%, 60%, and 42% for glycoproteins A, B, and C, respectively.

Reductive methylation was used to label the ε-amino groups of the lysine residues of the membrane proteins to determine which peptide species were increasing in the adipocyte cytoplasmic membranes of the obese rat to compensate for the observed decrease in the glycoproteins. Analysis of the adipocyte cytoplasmic membrane proteins by SDS-polyacrylamide gel electrophoresis followed by fluorography (Fig. 3) demonstrated an increase in a peptide doublet (1) with molecular weight of approximately 70,000-daltons, and slight increases in proteins with approximate molecular weights of 45,000-daltons (2) and 30,000-daltons (3) in the membranes from obese rats. These proteins are not labelled by the neuraminidase-galactose oxidase-^{3}H-borohydride procedure.

The lean and obese rats used in most of these experiments were 16 to 20 weeks old. Young rats (5 weeks old) were also analyzed to determine if the glycoprotein decrease observed in older rats also occurred at an early age. Analysis of the adipocyte cytoplasmic membranes demonstrated that a decrease in glycoproteins had already occurred in obese rats at 5 weeks of age.



(2)



(3)

Figure 2. SDS-polyacrylamide gel electrophoresis of adipocyte cytoplasmic membranes from lean (lane 1, 54 µg; lane 3, 32 µg) and obese (lane 2, 54 µg; lane 3, 32 µg) rats. A, B, and C are the same as in Fig. 1. D represents glycoproteins with an apparent molecular weight of 200,000-daltons. [^{3}H]-Labelled glycoproteins visualized by fluorography.

Figure 3. SDS-polyacrylamide gel electrophoresis of adipocyte cytoplasmic membranes labelled with [^{3}H]-borohydride after reductive methylation from lean (lane 1, 28 µg; lane 3, 56 µg) and obese (lane 2, 28 µg; lane 4, 56 µg) rats. A, B, and C are the same as in Fig. 1. Proteins which have increased are labelled 1, 2, and 3. See text for details. Proteins visualized by fluorography.

DISCUSSION

The results presented in this study have demonstrated a reduction in galactose-containing glycoproteins of adipocyte cytoplasmic membranes of obese Zucker rats when compared to membranes from lean littermates. This decrease may reflect an inability of adipocytes from obese animals to synthesize glycoproteins

in general or some other alteration such as an increase in the catabolic rate of the glycoproteins. Increased levels of serum glycosidases have been described in patients with diabetes mellitus (18).

A reduction in the glycoprotein content of rat liver plasma membranes from streptozotocin-induced diabetic rats has been demonstrated (8) as well as changes in lectin-binding characteristics of liver-plasma membranes in ob/ob and db/db mice (6). The results from this latter study led the authors to conclude that a generalized defect in glycoprotein biochemistry might exist in the obese state. Other studies have shown increases in galactose groups in hepatocyte and kidney glomerular basement membranes and in intestinal microvillous membrane proteins (19). The results suggested changes in cell membrane structure and function found in the chronic diabetic occur as a result of alterations in glycoprotein biosynthesis.

Further studies are required to define the mechanism responsible for the reduced levels of glycoproteins in the adipocyte cytoplasmic membranes of the genetically obese Zucker rat. Whether or not this generalized alteration in glycoprotein content is responsible for the insulin-resistance observed in the latter stages of obesity is unknown at this time.

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