CHARACTERIZATION OF INSULIN SENSITIVITY AND SIGNALING IN ADIPOCYTES FROM CONGENIC RATS

Emilia Guirguis1,2, Karin Berger1, Holger Luthman3, Eva Degerman1

Key words: Niddm1f, Niddm1i, lipogenesis, protein kinase B, adipocyte, congenic strain

SUMMARY

Genetic analysis of the diabetic Goto-Kakizaki rat has identified a major diabetes quantitative trait locus (Niddm1). Congenic strains generated for two different subloci, Niddm1f and Niddm1i, show defects in insulin action and secretion, respectively. In this work, we demonstrated that insulin-induced lipogenesis and phosphorylation of protein kinase B are reduced in adipocytes from Niddm1f rats as compared to adipocytes from control F344 rats. On the other hand, tyrosine phosphorylation of insulin receptor substrate 1 and serine phosphorylation of acetyl coenzyme A carboxylase were similar in both strains. In contrast to the results on Niddm1f adipocytes, insulin-induced lipogenesis in adipocytes from Niddm1i rats was increased. In summary, adipocytes from Niddm1f rats show insulin resistance, whereas adipocytes from Niddm1i rats show improved insulin action when comparing to F344 rats. Defect phosphorylation of protein kinase B most likely contributes to insulin resistance in Niddm1f adipocytes.

INTRODUCTION

Type 2 diabetes (T2D) is a heterogeneous metabolic dysfunction, influenced by environmental and genetic factors and characterized by insulin resistance in peripheral tissues as well as impaired insulin secretion from pancreatic β-cells (1,2). The Goto-Kakizaki (GK) rat, which was obtained by inbreeding of individuals selected for hyperglycemia in successive generations, is a well-established model for spontaneous type 2-like diabetes (3-5). Nonobese GK rats have defects in both insulin action and secretion and also demonstrate late complications related to diabetes (3-5). It has been shown that the major diabetes quantitative trait locus (Niddm1), which segregates in crosses between GK rats and normoglycemic F344 rats, encodes at least two different diabetes susceptibility genes, Sorcs and Tcf7l2 (6,7). With the aim to identify genes involved in the pathophysiology of T2D, congenic strains for several Niddm1 subloci, such as Niddm1f and Niddm1i, have been generated by transfer of GK alleles onto the genome of F344 rats (8,9). Whereas both Niddm1f and Niddm1i rats show postprandial
hyperglycemia (8,10), primary defects in Niddm1f and Niddm1i rats appear to be in insulin action (10) and insulin secretion (9,11), respectively. In this work we compared insulin-induced lipogenesis from Niddm1f, Niddm1i and normoglycemic F344 rats at different ages. Furthermore, we compared activation of insulin signaling components in Niddm1f and F344 rats.

MATERIALS AND METHODS

Rat breeding

All strains were maintained by sister-brother mating. The non-diabetic F334/DuCrl2Swe rats were originally purchased from Charles River Laboratories (Wilmington, MA). Diabetic GK/Swe rats were originally from Kyoto University. Transfer of the GK alleles onto the genome of F344 rats by repeated back crossing (10 generations) established the homozygous congenic strains Niddm1f and Niddm1i. Rats were maintained at a constant temperature and humidity in a 12-h light/dark cycle with free access to standard laboratory chow and water. Lund University Ethics Committee approved all experiments.

General experiment design

Experiments on male Niddm1f, Niddm1i and F344 rats were performed at 35 and 40-45 days of age. Animals from each strain designated for a single experiment including all time points were in most cases litter mates. For adipocyte preparation at each time point, 2-5 rats (depending on age) from each strain were pooled. The weight of age-matched animals did not vary significantly either between the strains or between the litter mates.

Preparation of adipocytes

Adipocytes were isolated from epididymal fat pads of 35- to 45-day-old male rats as described previously (11,12).

Lipogenesis assay

Lipogenesis was measured as previously described (13). One milliliter aliquots of 2% (v/v) adipocytes in Krebs-Ringer-HEPES (KRH) buffer with low glucose (0.55 mM) and 3.5% BSA were added to vials containing 0.4 mCi D-[6-3H]-glucose (Amersham) and incubated as indicated in Result section for 30 min at 37 ºC. Reactions were stopped with a toluol-based scintillation liquid containing 0.3 g/L POPOP (1,4-bis[5-phenyl-2-oxazolyl]benzene, 2,2’-p-phenylenebis[5-phenylloxazole]) and 5 g/L PPO (2,5-diphenyl oxazole). Incorporation of D-[6-3H]-glucose into cellular lipids was measured by scintillation counting.

Preparation of adipocyte homogenates

One milliliter aliquots of 10% (v/v) adipocytes in KRH were incubated as indicated in Result section for 30 min at 37 ºC. Cells were then homogenized in 1 mL of homogenization buffer (50 mM TES, 2 mM EGTA, 1 mM EDTA, 250 mM sucrose, 40 mM phenylphosphate, 5 mM NaF, 1 mM dithioerythriol, 1 mM phenylmethylsulphonylfluoride, 0.05 mM sodium orthovanadate, 10 mg/mL antipain, 10 mg/mL leupeptin and 1 mg/mL pepstatin A, pH 7.4) and centrifuged at 15500 xg for 5 min at 4 ºC. The solidified fat was removed and the infranatant was collected.

SDS-PAGE and western blot analysis

Samples were mixed with Laemmli sample buffer and subjected to polyacrylamide gel electrophoresis (PAGE) (9% acrylamide) and western blot analysis. After the electrotransfer of proteins onto a polyvinylidene difluoride membrane (Millipore), membranes were blocked for 30 min with 5% milk in TBS-T buffer (50 mM Tris pH 7.5, 150 mM NaCl and 0.1% (w/v) Tween-20), and incubated overnight with anti-P-Ser473 protein kinase B (PKB) antibodies (Biosource), anti-PKB beta antibodies (raised in rabbits against the peptide RYDSLGSLELDQRTHC and affinity purified), anti-Acetyl Coenzyme A Carboxylate (ACC) antibodies (Cell Signaling), anti-pACC (p-Ser79) antibodies (Cell Signaling) and anti-pIRS-1 (p-Tyr612) antibodies (Cell Signaling).
washing in TBS-T buffer, membranes were incubated with anti-rabbit antibodies linked with horseradish peroxidase (Biosource), washed again and developed with the 1:1 solution of peroxide and Luminol (Pierce). The chemoluminescent signal was analyzed and quantified using the Fuji LAS 1000 Plus system (Fuji Photo Film, Tokyo, Japan).

Data analysis

Data are expressed as mean values ± SEM of the indicated number of experiments. Statistical significance (*P<0.05, **P<0.01, ***P<0.001) was evaluated with the use of unpaired Student’s t test and is marked with asterisks in figures.

RESULTS

Adipocytes from Niddm1f rats show reduced insulin-induced lipogenesis

The Niddm1 substrains, Niddm1f and Niddm1i, were established as described in Materials and Methods (Fig. 1). Both substrains displayed postprandial hyperglycemia (8,10). Adipocytes from Niddm1f rats showed reduced insulin-induced lipogenesis as compared to adipocytes from F344 (control) rats. Thus, insulin-induced lipogenesis in adipocytes from 35-day-old Niddm1f rats (Fig. 2A) was decreased by 21% for 0.3 nM insulin, 25% for 1 nM insulin and 29% for 3 nM insulin as compared to adipocytes from F344 rats. We then compared insulin-induced lipogenesis in 40- to 45-day-old Niddm1f and F344

![Figure 1.](image1.png)

**Figure 1.** Map of congenic strains N1F and N1I showing the extent of GK genotype (hatched) on an otherwise genome wide F344 genotype.

![Figure 2.](image2.png)

**Figure 2.** Insulin-induced lipogenesis is decreased in Niddm1f rats as compared to F344 rats. Adipocytes from age-matched Niddm1f and F344 rats were stimulated with 0.1, 0.3, 1 or 3 nM insulin for 30 min. Lipogenesis was measured and expressed as means; n=6 and n=7 for 35 (A) and 40-45 (B) day-old animals, respectively; *significantly different from non-treated (control) adipocytes.
rats (Fig. 2B). The lowering of insulin-induced lipogenesis was the same as compared to 35-day-old rats. Regarding non-stimulated (basal) lipogenesis, no differences were detected between the different strains. Thus, the Niddm1f rats showed reduction in insulin-mediated lipogenesis already at the age of 35 days and no further deterioration was observed in 40- to 45-day-old rats.

Having observed a significant decrease in insulin-induced lipogenesis in adipocytes from Niddm1f as compared to F344 rats, we next investigated the possible mechanisms that could be involved in the development of insulin resistance in Niddm1f adipocytes. Hence, we studied tyrosine phosphorylation of insulin receptor substrate 1 (IRS-1) and serine phosphorylation of protein kinase B (PKB), the two key insulin signaling events. As shown in Figure 3A, phosphorylation of PKB on Ser 473 induced by 1 nM insulin was decreased by almost 40% in adipocytes from Niddm1f as compared to F344 rats. Basal PKB phosphorylation, measured in non-treated adipocytes was not altered. Insulin-induced tyrosine phosphorylation of IRS-1 did not differ significantly in adipocytes from the two strains (Fig. 3B). Also, the ability of insulin to phosphorylate the key lipogenic enzyme, acetyl coenzyme A carboxylase (ACC), at Ser79 did not differ in adipocytes from the two strains (data not shown).

**Insulin-induced phosphorylation of protein kinase B is decreased in adipocytes from Niddm1f as compared to F344 rats**

As shown in Figure 3A, phosphorylation of PKB on Ser 473 induced by 1 nM insulin was decreased by almost 40% in adipocytes from Niddm1f as compared to F344 rats. Basal PKB phosphorylation, measured in non-treated adipocytes was not altered. Insulin-induced tyrosine phosphorylation of IRS-1 did not differ significantly in adipocytes from the two strains (Fig. 3B). Also, the ability of insulin to phosphorylate the key lipogenic enzyme, acetyl coenzyme A carboxylase (ACC), at Ser79 did not differ in adipocytes from the two strains (data not shown).

**Insulin-induced lipogenesis is increased in adipocytes from Niddm1i as compared to F344 rats**

As shown in Figure 4, insulin-induced lipogenesis in adipocytes was significantly increased when comparing 35-day-old Niddm1i rats with age-matched control F344 rats. Thus, in adipocytes from Niddm1i rats, lipogenesis increased by 38%, 30% and 25% as compared to control rats in the presence of 0.3, 1 and 3 nM insulin, respectively. These data indicate that adipocytes from Niddm1i rats are hypersensitive to insulin. This hypersensitivity could be detected at an early age (35 days) and was maintained at approximately the same level in adipocytes from older animals (40- to 45-day-old; data not shown).
DISCUSSION

In this work, we demonstrated that adipocytes from the Niddm1f congenic strain showed reduced insulin-induced lipogenesis in 35-and 40- to 45-day-old rats as compared to age-matched F344 rats. Previous studies on adipocytes from 60-day-old Niddm1f rats have shown similar results with regard to maximal insulin-induced lipogenesis, which was decreased as compared to F344 rats (8). On the other hand, in contrast to our results, basal lipogenesis was also decreased in adipocytes obtained from those rats (8). However, the fact that no effect on basal lipogenesis was observed in the present study could be explained by younger rats. We also studied basal and insulin-induced lipogenesis in adipocytes from Niddm1i rats. As previously described, the phenotype of these rats indicates defects in insulin secretion rather than in insulin action (9,11). Therefore, we were not surprised to find increased insulin-induced lipogenesis in adipocytes from this strain as compared to F344 rats. Possibly, increased insulin-induced lipogenesis can be explained by the reduced ability of these rats to secrete insulin, leading to up-regulation of insulin signaling networks. However, in a previous study on adipocytes from 60-day-old Niddm1i rats, an increased basal and reduced insulin-induced maximal lipogenesis was observed (8). It is possible that aggravation of diabetes with age might counteract compensatory up-regulation of insulin sensitivity observed in our study of 35-day-old animals.

Insulin-induced phosphorylation of PKB is believed to be important in mediating a number of insulin metabolic effects (15). Thus, the finding that insulin induced phosphorylation of PKB was reduced in adipocytes from Niddm1f rats as compared to F344 rats might explain the mechanism whereby adipocytes from Niddm1f rats become insulin resistant. Interestingly, reduced insulin-induced phosphorylation of PKB appears to be mediated downstream of IRS-1 (16) since insulin-induced tyrosine phosphorylation of IRS-1 was the same in adipocytes from Niddm1f and F344 rats. Furthermore, no difference in insulin induced phosphorylation of the key lipogenic enzyme ACC on serine 79 was observed when comparing adipocytes from the two strains. Therefore, the exact signaling events and target proteins that are dysregulated as a result of reduced insulin-induced PKB phosphorylation and cause a decrease in insulin-stimulated lipogenesis need to be further explored.

In conclusion, insulin sensitivity is reduced in Niddm1f adipocytes and increased in Niddm1i adipocytes from 35- to 45-day-old rats. Furthermore, reduced PKB phosphorylation appears to be a key mediator of insulin resistance development in Niddm1f adipocytes.

Acknowledgments

We thank Eva Ohlson for excellent technical assistance. This work was supported by the Swedish Research Council Project 3362 and by a grant from Alice Wallenberg foundation. Grants were obtained from the following foundations: Swedish Diabetes Association; Novo Nordisk foundations, Denmark; The Swedish Society of Medicine, Dr. P. Håkansson, Albert Pählsson, Fredrik och Ingrid Thuring, Åke Wiberg, Lars Hierta, Åhlén, Tore Nilsson and Magnus Bergvall.
REFERENCES