Altered β-Cell Distribution of pdx-1 and GLUT-2 After a Short-Term Challenge With a High-Fat Diet in C57BL/6J Mice

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Mechanisms involved in the islet adaptation to insulin resistance were examined in mice of the C57BL/6J strain challenged with a high-fat (58%) diet for 8 weeks. Basal hyperglycemia commenced after 1 week, whereas hyperinsulinemia evolved after 8 weeks. Glucose elimination after an intravenous glucose challenge (1 g/kg) was significantly delayed after 1, 4, and 8 weeks on the high-fat diet compared with normal-diet–fed mice. This result was associated with unchanged insulin responses. However, glucose-stimulated insulin secretion from isolated islets was increased in a compensatory fashion at all glucose levels over a wide range (3.3–22 mmol/l) after 8 weeks on the high-fat diet, whereas no compensatory hypersecretion of insulin was evident after 1 or 4 weeks, except at 22 mmol/l glucose. Immunohistochemistry revealed that the islet architecture of insulin and glucagon cells remained intact in islets from mice fed a high-fat diet. However, the nuclear translocation of the homeobox transcription factor, pdx-1, and the plasma membrane translocation of GLUT2 were both impaired in high-fat–fed animals after 1 week. In contrast, the expression of the full-length leptin receptor (ObRb) was not affected by high-fat feeding. The study thus shows that 8 weeks are required for the development of a compensatory hypersecretion of insulin after high-fat feeding in mice, and even then the in vivo insulin secretion is insufficient to normalize impaired glucose tolerance. The early-onset islet dysfunction is accompanied by impaired β-cell trafficking of two factors, pdx-1 and GLUT-2, which are involved in β-cell proliferation and glucose recognition. The mechanisms compromising this β-cell trafficking remain to be established. ***Diabetes*** **51 (Suppl. 1):S138–S143, 2002**

Insulin resistance is associated with islet adaptation ensuring adequate hyperinsulinemia to maintain normoglycemia. However, if the islet adaptation is impaired, hyperinsulinemia is inadequate and metabolic perturbations such as hyperglycemia might ensue (1). A model to study the mechanisms of impaired islet adaptation in insulin resistance is the high-fat feeding of C57BL/6J mice, which are especially susceptible to high-fat treatment with regard to the development of glucose intolerance compared with other strains (2,3). We have previously shown that this model is associated with obesity, hyperglycemia, hyperinsulinemia, and hyperlipidemia (4); increased insulin mRNA expression in islets (5); and exaggerated insulin response to challenges with β-cell secretagogues (6,7). In contrast, the early islet changes after feeding these mice a high-fat diet are less well studied. Therefore, we have explored whether the development of glucose intolerance provoked by short-term feeding of a high-fat diet in C57BL/6J mice is associated with changes in insulin secretion, islet architecture of insulin and glucagon cells, and islet localization of factors thought to be of particular relevance for β-cell adaptation to insulin resistance. One such factor is the homeobox transcription factor pdx-1, which is normally expressed in β-cells and is associated with normal proliferation of pancreatic cells during development (8–10). Its expression and nuclear translation might therefore be of importance for accurate proliferative response under conditions of increased insulin requirement, such as insulin resistance. Another factor is the glucose transporter 2 (GLUT2), which is essential for glucose recognition and the transcription of which is reduced in several animal models of diabetes (11–14). Finally, we also examined the expression of the functional full-length leptin receptor (ObRb) because β-cells express this receptor (15), and circulating leptin is increased in high-fat–fed mice (4,16,17).

RESEARCH DESIGN AND METHODS

Experiments. All animal experiments were approved by the local ethics committee and followed the guidelines for experiments in animals (European Economic Community Council Directive 86/609/EEC). Four-week-old female C57BL/6J mice obtained from the Bomholtgaard Breeding and Research Center, Denmark, were used for this study. Animals were divided into two groups, receiving either standard rodent food or a high-fat diet (diet nos. D12310 and D12309, respectively; Research Diets, New Brunswick, NJ). On a caloric base, the high-fat diet consisted of 16.4% protein, 25.6% carbohydrates, and 58.0% fat, which corresponds to an energy content of 23.6 kJ/g. The normal diet consisted of 25.8% protein, 62.8% carbohydrates, and 11.4% fat

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AUC, area under the curve; HBSS, Hank's balanced salts solution; IVGTT, intravenous glucose tolerance test; ObRb, full-length leptin receptor; PBS, phosphate-buffered saline.

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TABLE 1
Antibodies used in this study

Antigen	Host	Dilution	Source
Insulin	Guinea pig	1:1,280	Chemicon International
Glucagon	Rabbit	1:10,240	Chemicon International
pdx-1	Rabbit	1:400	Rab1858.5; gift from Palle Serup, Hagedorn Research Institute, Novo Nordisk, Copenhagen, Denmark
ObRb	Rabbit	1:100	Alpha Diagnostic, San Antonio, TX
GLUT2	Rabbit	1:200	Chemicon International

(12.6 kJ/g). The animals were kept under a standard 12 h:12 h light-dark cycle and given food and water ad libitum. After 1, 4, and 8 weeks, blood was drawn in awake mice from the intra-orbital bulbar plexus for the measurement of basal insulin and glucose levels. After centrifugation, plasma was stored at −20°C until further analysis. After 1, 4, and 8 weeks of challenge with high-fat or control diet, the mice were also anesthetized with an intraperitoneal injection of midazolam (Dormicum, 0.14 mg/mouse; Hoffman-La-Roche, Basel) and a combination of fluanison (0.9 mg/mouse) and fentanyl (0.02 mg/mouse) (Hypnorm; Janssen, Beerse, Belgium). Thereafter, a blood sample was taken from the retrobulbar intra-orbital capillary plexus in a heparinized pipette, after which D-glucose (1 g/kg; British Drug Houses, Poole, U.K.) was injected rapidly intravenously. The volume load was 10 μl/g body wt. New blood samples were taken after 1, 5, 20, 50, and 75 min. The samples were taken in heparinized tubes. After immediate centrifugation at 4°C, plasma was separated and stored at −20°C until analysis. At these time points after the start of the respective diets, islets were also isolated for in vitro studies. In brief, after a midline laparotomy, the common bile duct was cannulated and ligated at the papilla of Vater. The pancreas was filled with 3 ml ice-cold Hank's balanced salts solution (HBSS) supplemented with 0.4 mg/ml collagenase P (Boehringer Mannheim, Mannheim, Germany) before removal and incubation at 37°C for 19 min. After washing three times in HBSS, the islets were handpicked under a stereomicroscope. The isolated islets were then cultured for 48 h in an RPMI medium supplemented with 2.06 mmol/l L-glutamine (Life Technologies, Täby, Sweden), 10% fetal bovine serum, 100 units/ml penicillin, and 0.5 mg/ml streptomycin (all from Kebo Laboratory, Spånga, Sweden) at 37°C in 5% CO_2–95% air. After the overnight incubation, islets were washed in a HEPES buffer containing 125 mmol/l NaCl, 5.9 mmol/l KCl, 1.28 mmol/l $CaCl_2$, 1.2 mmol/l $MgCl_2$, 25 mmol/l HEPES, 3.3 mmol/l glucose, and 0.1% human serum albumin (pH 7.36) and preincubated in the same medium for 60 min. Thereafter, three islets were incubated in 100 μl of the medium for 60 min at 37°C in the presence of glucose at varying concentrations. After incubation, aliquots of 25 μl in duplicate were collected and stored at −20°C until analysis of insulin content. Finally, at 1, 4, and 8 weeks, pancreases were also dissected and fixed in Stefanini's fixative at 4°C overnight. After rinsing in phosphate-buffered saline (PBS), pancreases were cryoprotected by immersion in 20% sucrose in PBS overnight at 4°C, sectioned on a cryostat at 10 μm, and used for immunohistochemical analysis.

Analyses. Insulin was determined by double antibody radioimmunoassay using guinea pig anti–rat insulin, mono-^{125}I-human insulin, and, as standard, rat insulin (Linco Research, St. Charles, MO). Glucose was determined by the glucose oxidase method.

Immunohistochemistry. Pancreatic sections were separately incubated with primary antibodies specific for pdx-1 and GLUT2 (Chemicon International, Temecula, CA). In double-immunofluorescence experiments, antisera against insulin and glucagon were used simultaneously. For information on antisera and dilutions, see Table 1. Primary antibodies were diluted in blocking buffer (10 mmol/l phosphate buffer, pH 7.2; PBS containing 0.25% bovine serum albumin and 0.25% triton-X), incubated at 4°C overnight, and rinsed three times for 5 min in PBS. Sections were incubated with fluorochrome-conjugated secondary antibodies (goat anti–guinea pig Ig-Alexa 546 and goat anti–rabbit Ig-Alexa 488, both 1:300 in blocking buffer) for 45 min at room temperature. After rinsing in PBS as before, sections were mounted in PBS:glycerol (1:1) and viewed in an Olympus BX 51 microscope (LRI, Lund, Sweden). Images of the specimen were taken with a digital camera.

Calculations and statistics. From the intravenous glucose tolerance test (IVGTT), the total area under the curve (AUC) for both glucose and insulin concentrations was calculated individually with the trapezoid rule for each animal. Furthermore, the acute insulin response, which is the arithmetic mean of the suprabasal 1- and 5-min insulin value, was used for assessment of insulin secretion. The results were compared group-wise. Statistical differences were computed with the software StatView from the SAS Institute (Cary, NC). Comparisons for differences between high-fat–fed and normal diet–fed mice were performed by the Student's two-tailed unpaired t test with Bonferroni's post hoc analysis. For all comparisons, a difference of $P < 0.05$ was considered significant.

RESULTS

Body weight and basal glucose and insulin. The high-fat diet induced a larger increase in body weight than the normal control diet, with the total weight change after 8 weeks being 48% in high-fat–fed mice compared with 17% in control mice ($P < 0.001$; Table 2). The high-fat group also showed a higher variation in body weight (coefficient of variation, 11 vs. 4.5%). The high-fat diet induced significant hyperglycemia after 1 week of treatment, which remained throughout the 8-week study period, whereas hyperinsulinemia evolved after 8 weeks of the high-fat diet (Table 2).

IVGTT. Glucose elimination after the intravenous glucose challenge was delayed in high-fat–fed mice compared with mice fed a normal diet after 1, 4, and 8 weeks, as judged by the high $AUC_{glucose}$ (Table 3). In contrast, the insulin

TABLE 2
Body weight and basal fed plasma glucose and insulin levels in female C57BL/6J mice after treatment with a high-fat or normal diet for 1, 4, and 8 weeks

	Start	1 week	4 weeks	8 weeks
Body weight (g)				
High-fat diet	18.5 ± 0.1 (42)	19.4 ± 0.1 (41)*	23.3 ± 0.4 (25)*	27.9 ± 0.8 (16)*
Normal diet	18.6 ± 0.1 (40)	18.6 ± 0.1 (39)	20.7 ± 0.2 (22)	21.9 ± 0.3 (14)
Glucose (mmol/l)				
High-fat diet	5.7 ± 0.2 (42)	7.8 ± 0.2 (23)*	6.7 ± 0.3 (17)*	9.4 ± 0.6 (10)*
Normal diet	5.5 ± 0.1 (40)	5.2 ± 0.2 (25)	5.4 ± 0.2 (16)	6.3 ± 0.2 (8)
Insulin (pmol/l)				
High-fat diet	106 ± 13 (38)	71 ± 6 (24)	122 ± 19 (18)	308 ± 59 (10)*
Normal diet	92 ± 13 (36)	87 ± 9 (22)	151 ± 13 (16)	100 ± 14 (8)

Data are means ± SE (n). All analyses were performed as described under RESEARCH DESIGN AND METHODS. *$P < 0.05$, determined by the Student's t test.

TABLE 3
$AUC_{insulin}$, acute insulin response, and $AUC_{glucose}$ during an IVGTT (1 g glucose/kg intravenously) in C57BL/6J mice after feeding on a high-fat or normal diet for 1, 4, and 8 weeks

IVGTT	1 week	4 weeks	8 weeks
$AUC_{insulin}$ (nmol/l × 75 min)			
High-fat diet	17.2 ± 1.3	29.4 ± 7.4	54.4 ± 17.7
Normal diet	17.1 ± 2.4	37.0 ± 3.9	30.1 ± 10.2
Acute insulin response (pmol/l)			
High-fat diet	518 ± 84	328 ± 93	755 ± 134
Normal diet	501 ± 72	378 ± 109	547 ± 98
$AUC_{glucose}$ (mmol/l × 75 min)			
High-fat diet	1,013 ± 35*	1,193 ± 27	1,429 ± 19
Normal diet	877 ± 30	906 ± 62	998 ± 46

Data are means ± SE. AUC is the suprabasal area under the curve for insulin and glucose, and acute insulin response is the suprabasal 1- and 5-min insulin response. At all time points, $n = 6$, except for 1 week ($n = 12$). Student's t test, *$P < 0.05$, †$P < 0.001$, determined by the Student's t test.

response to the intravenous glucose was not significantly different between the groups at any time point.

Islet studies. Insulin secretion over a wide range of glucose concentrations was similar in islets from controls and high-fat–fed mice after 1 and 4 weeks of treatment, with the exception of enhanced insulin secretion in islets from high-fat mice at 22 mmol/l glucose (786 ± 49 vs. 287 ± 51 pmol/l/three islets at 1 week [$P < 0.001$] and 777 ± 99 vs. 473 ± 86 pmol/l/three islets at 4 weeks [$P = 0.003$]). In contrast, after 8 weeks, insulin secretion was enhanced at all glucose concentrations in islets from high-fat–fed mice compared with that in islets from control mice (Fig. 1).

Immunocytochemistry. Double immunofluorescence showed that the relative distribution of insulin- and glucagon-containing cells was not changed in animals receiving a high-fat diet (Figs. 2*A* and *B*). Similarly, the ObRb immunoreactivity was not altered (Fig. 2*C* and *D*). In contrast, the immunocytochemical appearance of islet pdx-1 and GLUT2 differed between the two groups. Thus, in control mice, staining with an antiserum specific for pdx-1 showed immunoreactivity in both the cytoplasm and the nucleus of β-cells, and in most islets, nuclear staining was more prominent than cytoplasmic staining. In contrast, pancreases from high-fat–fed mice displayed pdx-1 immunoreactivity mostly in the cytoplasm, and only weak nuclear staining was observed (Fig. 3*A* and *B*). In mice fed a normal diet, GLUT2 was localized to the majority of islet cells, and the immunoreactivity was confined to the cell membrane. The staining intensity was decreased in sections from animals that had received a high-fat diet, and in these islets, accumulation of immunoreactive material was located over the cytoplasm (Fig. 3*C* and *D*, arrows). This difference in pdx-1 and GLUT2 immunoreactivity between the groups occurred after 4 and 8 weeks of the high-fat diet, but not after 1 week.

DISCUSSION

This study monitored short-term islet effects of a high-fat diet in C57BL/6J mice to increase our knowledge on early islet adaptation to high-fat feeding–induced insulin resistance. This mouse strain has been shown to be susceptible to this diet and develops glucose intolerance more readily than other strains (3,16). The long-term effect of a high-fat intake on glucose metabolism in this mouse strain has been established in several studies (2–7,16), but so far, no early and sequential data are available that show after what time period these effects begin to become manifest. In this study, we related the islet morphology to changes in glucose tolerance and insulin secretion during the first 8 weeks of high-fat feeding in mice. We found that impairment of glucose elimination occurred after 1 week on the high-fat diet. The impaired glucose elimination was associated with insulin responses that were not potentiated, i.e., the insulin resistance was not accompanied by a compensatory increase in insulin secretion, suggesting that the high-fat diet had compromised islet adaptation. Interestingly, adaptation was evident in vitro after 8 weeks when glucose-stimulated insulin secretion was exaggerated. This shows that a considerable time (2 months) is required for a detectable islet compensation. However, in spite of this increased glucose-stimulated insulin secretion in vitro, no such augmentation was evident in vivo, suggesting that integrative actions in vivo opposed primary islet adaptation.

In association with the development of insulin resistance and glucose intolerance, we found compromised β-cell translocation of the homeobox transcription factor pdx-1 after the high-fat diet. pdx-1 is known to be associated with normal proliferation of pancreatic cells during development, and it has been shown that targeted disruption of the pdx-1 gene results in pancreatic agenesis, whereas heterozygous pdx-1–deleted mice develop glucose intolerance in adulthood (18). This would suggest an important role of this transcription factor with regard to β-cell function. We found that the inability of the islets to adapt to the insulin resistance after a high-fat diet was associated with compromised translocation of pdx-1 to the nucleus in that pdx-1 was localized mainly in the cytoplasm in high-fat animals, whereas in control mice, both a nuclear and cytoplasmic localization was observed. pdx-1

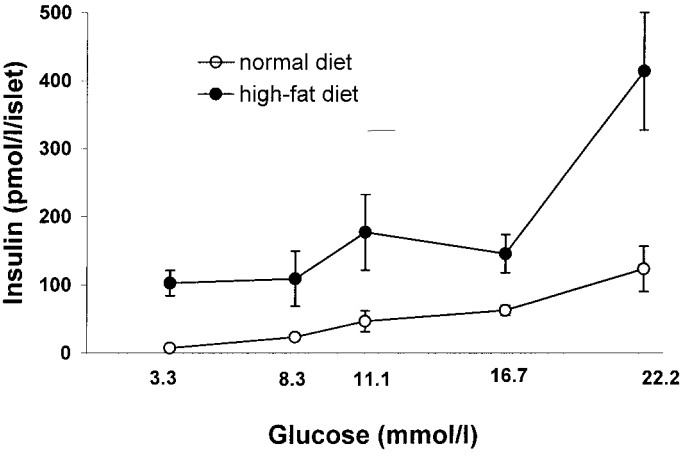

FIG. 1. Glucose-stimulated insulin secretion during a 60-min incubation of islets isolated from C57BL/6J mice after 8 weeks of a high-fat diet or control diet. Results are expressed as means ± SE for each group and concentration ($n = 12–24$). Insulin secretion is enhanced in high-fat islets at all glucose concentrations tested ($P < 0.05$).

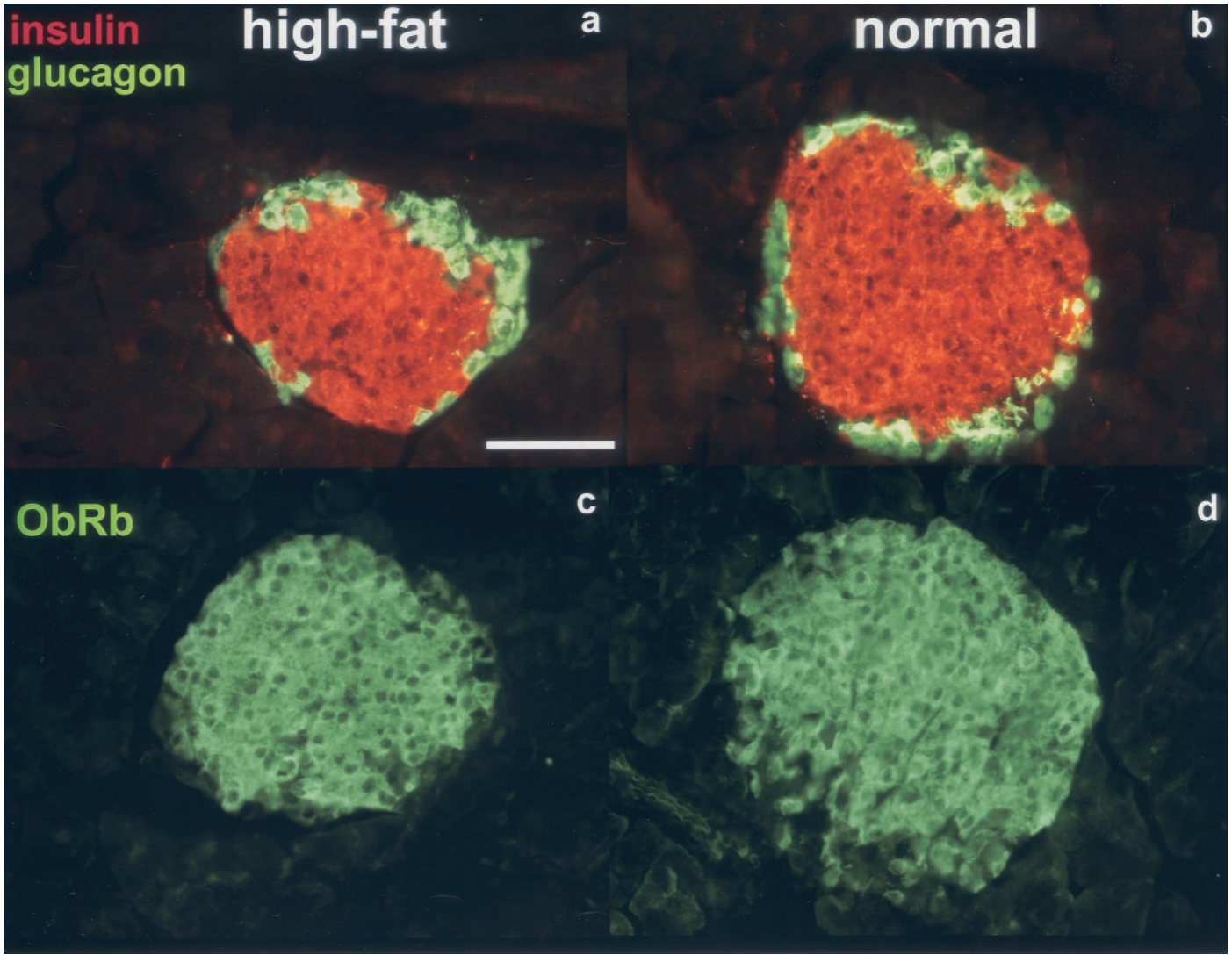

FIG. 2. Immunofluorescence staining of pancreatic sections from high-fat–fed and control mice after 8 weeks. *A* and *B*: Results of a double-staining experiment for insulin and glucagon. Insulin (red staining) is contained in the core zone of the islet, while glucagon (green staining) is found in the mantle zone. The cytoarchitecture of the islet does not seem to be disturbed in response to dietary treatment. ObRb staining is shown in high-fat diet (*C*) and controls (*D*). No apparent difference in staining intensity or localization could be found. Scale bar = 200 μm.

is normally translocated into the nucleus and binds to the insulin gene, which leads to the transcription of insulin mRNA; in fact, together with E47 and β2/neuroD, pdx-1 is recognized as a main effector of insulin promoter transactivation (19,20). The mechanism of the compromised nuclear translocation of pdx-1 in high-fat–fed mice is not known. Activation of pdx-1 seems to be induced by nutrient stimulation (21) and, as shown by Macfarlane et al. (22), high glucose induces nuclear translocation of pdx-1 in rat islets by a mechanism governed by SAPK2. Furthermore, pdx-1 expression has been shown to be impaired in a patient with persistent hyperinsulinemic hypoglycemia of infancy (23).

We also found that the translocation of GLUT2 to the plasma membrane was compromised in mice fed the high-fat diet, which would perturb the islets' capacity to adequately respond to glucose. This result is analogous to reduced expression of GLUT2 in several animal models of diabetes (11–14). Interestingly, GLUT2 expression in islets is known to be regulated by pdx-1, and therefore, the impaired translocation of these two factors might have a common mechanism (24). Finally, we also studied the immunolocalization of ObRb in islets from diet-treated mice. Islet β-cells express this functional form of the ObRb (15), and circulating leptin is increased in the mice fed a high-fat diet (16,17). The hyperleptinemia may be a consequence of the obesity due to increased expression in triglyceride-storing adipocytes but may also be a sign of leptin resistance. Leptin has been demonstrated to inhibit insulin secretion in vitro (15,25,26). However, in vivo studies in diabetic models have shown that leptin treatment improves insulin secretion in *ob/ob* mice (27), and overexpression of the leptin receptor in a model homozygous for a mutation of the ObRb, the Zucker diabetic rat, restores GLUT2 expression and insulin secretion (28). By using an ObRb-specific antibody, we observed immunoreactivity for this receptor in the β-cells of both normal and high-fat islets with no apparent difference. This finding would suggest unaltered islet ObRb expression in this model. It would therefore be of interest to also examine

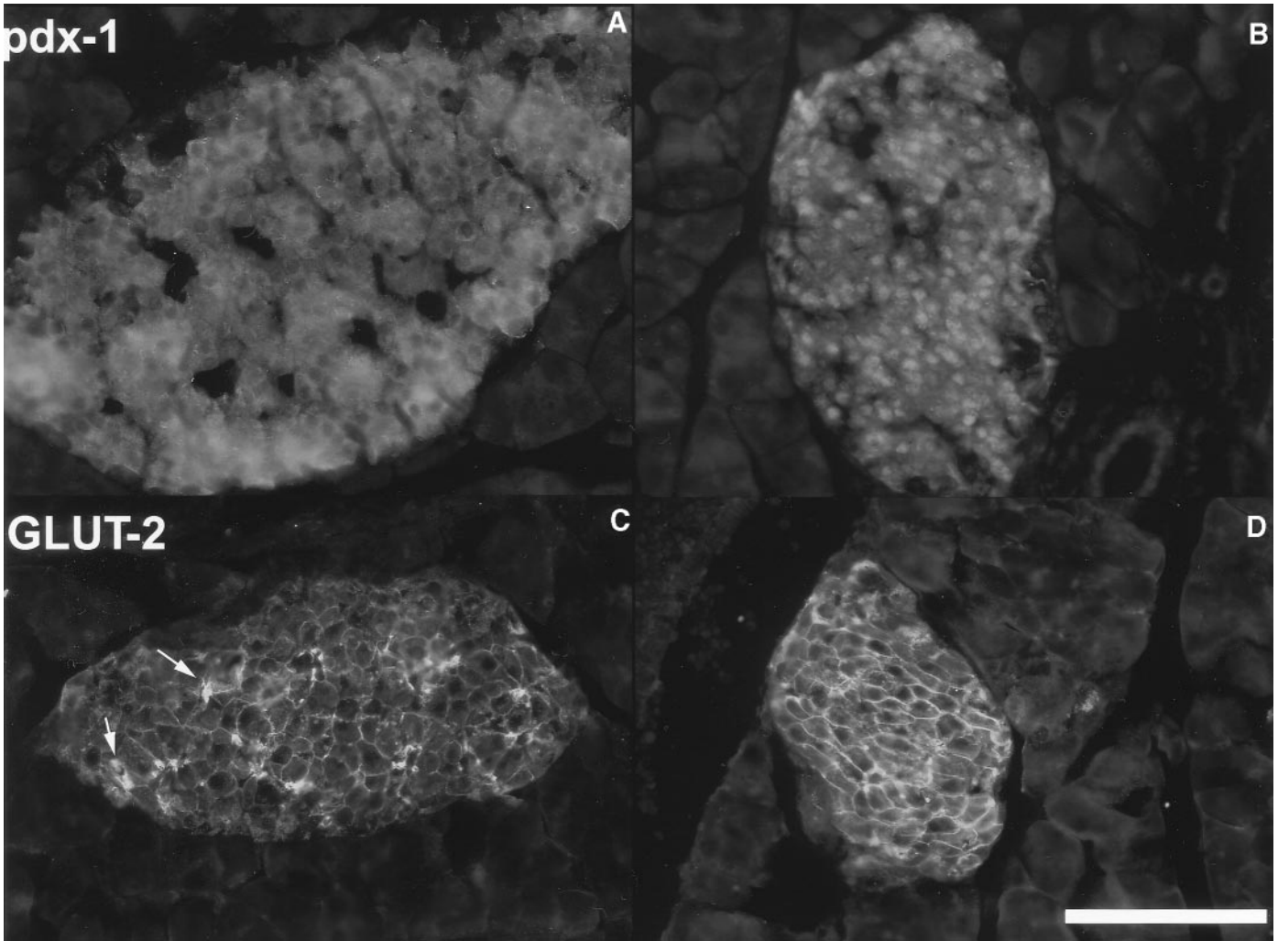

FIG. 3. Immunofluorescence staining of pancreatic sections from high-fat–fed and control mice after 8 weeks. *A* and *B*: Staining for pdx-1. Immunoreactivity for pdx-1 was found mainly in the cytoplasm of cells in the core zone of the islets. Nuclear staining was very weak in these specimens. In contrast, in controls, nuclear pdx-1 staining in some of the islets was markedly enhanced, with less intense staining of the cytoplasm. *C* and *D*: GLUT2 immunoreactivity. Membranous GLUT2 staining was much weaker in high-fat islets (*C*) than in controls (*D*). Furthermore, an accumulation of immunoreactive material could be found in the core of high-fat islets (arrows). Scale bar = 200 μm.

the effect of leptin in this animal model of glucose intolerance.

In conclusion, an 8-week high-fat diet challenge to glucose-intolerant prone C57BL/6J mice results in impaired glucose tolerance and insufficient compensation in insulin secretion. This is accompanied by compromised translocation of pdx-1 to the nucleus and GLUT-2 to the plasma membrane but unaltered expression of the ObRb. Our results therefore indicate that high-fat feeding impairs β-cell trafficking of factors of importance both for β-cell glucose recognition and insulin gene expression.

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REFERENCES

1. Porte D, Kahn SE: The key role of islet dysfunction in type II diabetes mellitus. *Clin Invest Med* 18:247–254, 1995
2. Surwit RS, Kuhn CM, Cochrane C, McCubbin JA, Feinglos MN: Diet-induced type II diabetes in C57BL/6J mice. *Diabetes* 37:1163–1167, 1988
3. Surwit RS, Feinglos MN, Rodin J, Sutherland A, Petro AE, Opara EC, Kuhn CM, Rebuffe-Scrive M: Differential effects of fat and sucrose on the development of obesity and diabetes in C57BL/6J and A/J mice. *Metabolism* 44:645–651, 1995
4. Ahrén B, Scheurink AJW: Marked hyperleptinemia after high-fat diet associated with severe glucose intolerance in mice. *Eur J Endocrinol* 139:461–467, 1998
5. Mulder H, Mårtensson H, Sundler F, Ahrén B: Differential changes in islet amyloid polypeptide (amylin) and insulin mRNA expression after high-fat diet-induced insulin resistance in C57BL/6J mice. *Metabolism* 49:1518–1522, 2000
6. Simonsson E, Ahrén B: Potentiated β-cell response to non-glucose stimuli in insulin-resistant C57BL/6J mice. *Eur J Pharmacol* 350:243–250, 1998
7. Ahrén B, Sauerberg P, Thomsen C: Increased insulin secretion and normalisation of glucose tolerance by cholinergic agonism in high-fat fed C57BL/6J mice. *Am J Physiol* 277:E93–E102, 1999
8. Ahlgren U, Jonsson J, Jonsson L, Simu K, Edlund H: Beta-cell-specific inactivation of the mouse Ipf1/Pdx1 gene results in loss of the beta-cell phenotype and maturity onset diabetes. *Genes Dev* 12:1763–1768, 1998
9. Ohlsson H, Thor S, Edlund T: Novel insulin promoter- and enhancer-

binding proteins that discriminate between pancreatic alpha- and beta-cells. *Mol Endocrinol* 5:897–904, 1991
10. Serup P, Petersen V, Pedersen EE, Edlund H, Leonard J, Petersen JS, Larsson LI, Madsen OD: The homeodomain protein IPF-1/STF-1 is expressed in a subset of islet cells and promotes rat insulin 1 gene expression dependent on an intact E1 helix-loop-helix factor binding site. *Biochem J* 310:997–1003, 1995
11. Bonny C, Roduit R, Gremlich S, Nicod P, Thorens B, Waeber G: The loss of GLUT2 expression in the pancreatic beta-cells of diabetic db/db mice is associated with an impaired DNA-binding activity of islet-specific transacting factors. *Mol Cell Endocrinol* 135:59–65, 1997
12. Jörns A, Tiedge M, Sickel E, Lenzen S: Loss of GLUT2 glucose transporter expression in pancreatic beta cells from diabetic Chinese hamsters. *Virch Arch* 428:177–185, 1996
13. Jörns A, Klempnauer J: Insulin and GLUT2 glucose transporter immunoreactivity in B-cells of whole pancreas isografts and allografts in the streptozotocin-diabetic rat. *Exp Clin Endocrinol Diabetes* 2:103–106, 1995
14. Orci L, Ravazzola M, Baetens D, Inman L, Amherdt M, Peterson RG, Newgard CB, Johnson JH, Unger RH: Evidence that down-regulation of beta-cell glucose transporters in non-insulin-dependent diabetes may be the cause of diabetic hyperglycemia. *Proc Natl Acad Sci U S A* 87:9953–9957, 1990
15. Emilsson V, Liu YL, Cawthorne MA, Morton NM, Davenport M: Expression of the functional leptin receptor mRNA in pancreatic islets and direct inhibitory action of leptin on insulin secretion. *Diabetes* 46:313–316, 1997
16. Ahrén B: Plasma leptin and insulin in C57BI/6J mice on a high-fat diet: relation to subsequent changes in body weight. *Acta Physiol Scand* 165:233–240, 1999
17. Ahrén B, Månsson S, Gingerich RL, Havel PJ: Regulation of plasma leptin in mice: influence of age, high-fat diet, and fasting. *Am J Physiol* 273:R113–R120, 1997
18. Jonsson J, Carlsson JL, Edlund T, Edlund H: Insulin-promoter-factor 1 is required for pancreas development in mice. *Nature* 371:606–609, 1994
19. Cordle SR, Henderson E, Masuoka H, Weil PA, Stein R: Pancreatic beta-cell-type-specific transcription of the insulin gene is mediated by basic helix-loop-helix DNA-binding proteins. *Mol Cell Biol* 11:1734–1738, 1991
20. Naya FJ, Huang HP, Qiu Y, Mutoh H, DeMayo FJ, Leiter AB, Tsai MJ: Diabetes, defective pancreatic morphogenesis, and abnormal enteroendocrine differentiation in BETA2/neuroD-deficient mice. *Genes Dev* 11:2323–2334, 1997
21. Petersen HV, Peshavaria M, Pedersen AA, Philippe J, Stein R, Madsen OD, Serup P: Glucose stimulates the activation domain potential of the PDX-1 homeodomain transcription factor. *FEBS Lett* 431:362–366, 1998
22. Macfarlane WM, McKinnon CM, Felton-Edkins ZA, Cragg H, James RF, Docherty K: Glucose stimulates translocation of the homeodomain transcription factor PDX1 from the cytoplasm to the nucleus in pancreatic beta-cells. *J Biol Chem* 274:1011–1016, 1999
23. MacFarlane WM, Chapman JC, Shepherd RM, Hashmi MN, Kamimura N, Cosgrove KE, O'Brien RE, Barnes PD, Hart AW, Docherty HM, Lindley KJ, Aynsley-Green A, James RF, Docherty K, Dunne MJ: Engineering a glucose-responsive human insulin-secreting cell line from islets of Langerhans isolated from a patient with persistent hyperinsulinemic hypoglycemia of infancy. *J Biol Chem* 274:34059–34066, 1999
24. Waeber G, Thompson N, Nicod P, Bonny C: Transcriptional activation of the GLUT2 gene by the IPF-1/STF-1/IDX-1 homeobox factor. *Mol Endocrinol* 19:1327–1334, 1996
25. Ahrén B, Havel PJ: Leptin inhibits insulin secretion induced by cellular cAMP in a pancreatic B cell line (INS-1 cells). *Am J Physiol* 277:R959–R966, 1999
26. Kieffer TJ, Habener JF: The adipoinsular axis: effects of leptin on pancreatic beta-cells. *Am J Physiol* 278:E1–E14, 2000
27. Khan A, Narongoda S, Ahrén B, Holm C, Sundler F, Efendic S: Long-term leptin treatment of ob/ob mice improves glucose-induced insulin secretion. *Int J Obes Relat Metab Disord* 6:816–821, 2001
28. Wang MY, Koyama K, Shimabukuro M, Mangelsdorf D, Newgard CB, Unger RH: Overexpression of leptin receptors in pancreatic islets of Zucker diabetic rats restores GLUT-2, glucokinase, and glucose-stimulated insulin secretion. *Proc Natl Acad Sci U S A* 95:11921–11926, 1998