Lipotoxicity in the Pathogenesis of Obesity-Dependent NIDDM

Genetic and Clinical Implications

Roger H. Unger

We review evidence that increased tissue levels of fatty acyl CoA cause the β-cell abnormalities of nondiabetic obesity and ultimately result in obesity-dependent diabetes. Nondiabetic obesity in Zucker rats is characterized by hypersecretion of insulin at normal fasting and subfasting glucose concentrations. This is a result of β-cell hyperplasia and increased low $K_m$ glucose usage and oxidation. These abnormalities, the hyperinsulinemia, the hyperplasia of β-cells, i.e., its in vitro equivalent, enhanced bromodeoxyuridine incorporation, and the increased low $K_m$ glucose usage can be induced by culturing normal islets with 2 mmol/l free fatty acids (FFAs). Once obese Zucker diabetic fatty rats become diabetic, glucose-stimulated insulin secretion (GSIS) is absent and β-cell GLUT2 reduced. Islet triglyceride (TG) content is increased 10-fold, probably reflecting increased FFA delivery (plasma FFA levels >1.5 mmol/l) beginning about 2 weeks before the onset of diabetes. These β-cell abnormalities, GSIS loss, GLUT2 loss, and TG accumulation, are prevented by reducing plasma FFAs by caloric restriction and by nicotinamide injection. The loss of GSIS and the accumulation of TGs, but not the GLUT2 loss, can be induced in vitro in normal islets cultured in a 2 mmol/l FFA-containing medium, but prediabetic islets seem far more vulnerable to FFA-induced functional impairment and TG accumulation. It is proposed that in uncomplicated obesity, increased lipid availability (FFA levels <1.5 mmol/l) induces both hyperinsulinemia and insulin resistance in parallel fashion, thereby maintaining normoglycemia. A further increase in substrate overload impairs β-cell compensation for insulin resistance and hyperglycemia appears. Diabetes 44:863–870, 1995

he U.S. faces a health problem of alarming proportions as a consequence of a progressive increase in the incidence of obesity. As of 1990, 30% of Americans were obese, up from 25% in 1980 (1). Given the well-established linkages between obesity and age and non-insulin-dependent diabetes mellitus (NIDDM) (2), it follows that the prevalence of overt NIDDM in the U.S. must be increasing in proportion to the population’s expanding girth and advancing age. Recently estimated to be approaching 7% (3), the true prevalence of NIDDM may be even higher. The morbidity and spiraling costs generated by this disease make a more complete understanding of its pathogenesis a goal of the highest priority.

Fundamental to such understanding is the issue of the pathogenic role of insulin resistance in obesity and NIDDM (4,5). Epidemiological evidence that obesity exerts a dose effect on the incidence of NIDDM raises the possibility that the insulin resistance of obesity is sufficient in and of itself to cause the disease. In Japan, where obesity is rare, the prevalence of NIDDM has been estimated to be 1% (6), yet at least 30% of massively obese Sumo wrestlers develop NIDDM in later life (H. Hirose, personal communication). Similarly, among Americans weighing >40% of ideal body weight, the prevalence of fasting hyperglycemia and abnormal oral glucose tolerance approaches 50% (7). On the other hand, 50% of insulin-resistant individuals never develop overt NIDDM. This suggests that insulin resistance by itself is not sufficient to cause NIDDM and that a second defect is required (4,5,8–10). Because the onset of overt NIDDM is invariably accompanied by a loss of glucose-stimulated insulin secretion (GSIS) (11–16) and, at least in rodents, by a reduction in β-cell GLUT2 (9,10,13–16), glucose incompetence of β-cells has been viewed as the second defect (14). If it is, the familial clustering of NIDDM (17) would imply that β-cell glucose incompetence, like insulin resistance, is inherited.

If we accept this premise, we must next determine the relationship of the glucose incompetence to the insulin resistance. Are they the result of two independent but coinciding primary defects, one intrinsic to β-cells, the other extrinsically expressed in insulin’s target tissues; or is one defect primary and the other secondary to it; or are both defects secondary to a single primary abnormality? Herein
we review evidence that in obesity-dependent NIDDM the insulin resistance of target tissues and the glucose incompetence of β-cells are both caused by a single abnormality extrinsic to β-cells. We examine the idea that the extrinsic abnormality is an increase in the delivery of free fatty acids (FFAs) to tissues. This includes the target tissues of insulin and the islets of Langerhans.

ADIPOCYTE-β-CELL RELATIONSHIPS IN NORMOGLYCEMIC OBESITY

Physiological versus pathophysiological hyperlipidemia. Normally FFAs provide an alternative fuel to glucose to spare glucose for cerebral requirements and during prolonged fasting to conserve body proteins that would otherwise be used as a source of gluconeogenic substrate. This glucose-sparing action of FFAs thus prolongs survival during starvation (18). (FFAs, which can stimulate insulin secretion in the fed state [19–21], do not do so in glucopenic conditions such as fasting. During prolonged fasting the β-cells normally become refractory to nonglucose secretagogues, as part of the defense against hypoglycemia.) Conversely, in the fed state the powerful antilipolytic action of insulin suppresses FFA levels, thereby permitting ingested glucose to be metabolized without FFA-induced hindrance.

In obesity plasma FFAs are not fully suppressed by feeding (22,23), either because of insensitivity of adipocytes to the antilipolytic action of insulin (23) or because of the increase in adipocyte mass (24) or both. An elevation of fatty acyl CoA in muscle could cause resistance to the glucoregulatory action of insulin (25–27), as reviewed by McGarry (28,29). Despite insulin resistance, glucose tolerance remains normal because β-cells somehow sense the level of insulin secretion required for full compensation. One possible explanation for the perfect match between insulin secretion and insulin resistance is that both are caused by the same abnormality. High tissue levels of FFAs qualify as such an abnormality. First, plasma levels of FFAs are elevated in obesity (22–24,30–32). Second, chronic exposure of cultured islets to FFAs causes basal hypersecretion of insulin (33). Third, FFA levels are high in obesity because they are not fully suppressed by the hyperinsulinemia they induce (23,34), i.e., there is attenuation of the feedback between β-cells and adipocytes that normally would prevent the coexistence of high insulin and high FFA levels. This hypothesis is depicted in Fig. 1A.

β-cells in normoglycemic obesity. Zucker fatty rats provide an ideal model in which to study the mechanism of β-cell hypersecretion in obesity. Like obese humans, these rats exhibit high basal insulin secretion, exaggerated insulin responses (9), and relative hyperlipidemia (35). When perfused with glucose in concentrations at or below the normal fasting range, the isolated pancreases of nondiabetic obese rats secrete 10 times as much insulin as those of lean controls (13,35). In normal islets insulin secretion is turned off when glucose is perfused at concentrations <4.2 mmol/l; this is not the case in islets of obese rats, which secrete as much insulin at 1.4 mmol/l glucose as normal islets secrete at 5.6 mmol/l glucose (35) (Fig. 2).

To determine if this hypersecretion is the result of β-cell hyperplasia, we compared the β-cell volume fraction and islet DNA content of obese and lean groups. The volume fraction of β-cells in pancreases of the obese rats was 3.9 times normal and their DNA content per islet was 3.5 times normal (35). However, this degree of hyperplasia could not fully explain the increase in basal insulin secretion, which was 10 times normal, nor could it account for the functional data in Fig. 2, indicating that in obese rats insulin secretion is not fully suppressed during glucopenia. Given the well-accepted relationships between glucose metabolism and insulin secretion (36–38), one might expect an increase in low Kₘ glucose metabolism in islets that hypersecrete insulin during glucopenia. In fact, we observed an eightfold greater rate of glucose oxidation and usage in the islets from obese rats (35) (Table 1). When glucose concentration was lowered from 5.6 to 1.4 mmol/l, glucose utilization declined by 65% in normal islets, compared with only 11% in islets

![Diagram](https://example.com/diagram.png)

**FIG. 1.** The lipotoxic hypothesis. A: In obese rats without diabetes there is a relative increase in plasma FFA levels (>0.5 <1.5 mmol/l) and presumably in the tissue levels of fatty acyl CoA (FACoA). This hyperlipidemia may be a consequence of the expanded adipocyte mass or of insensitivity of adipocytes to the antilipolytic action of insulin or both. The presumed increase in tissue FFA levels throughout the body interferes with normal glucose metabolism at multiple levels. In target tissues of insulin, such as muscle, this interference is referred to as insulin resistance. In islets, the increased FFA levels stimulate insulin secretion and induce β-cell proliferation and expansion of low Kₘ glucose metabolism, which increases basal insulin secretion and potentiates insulin responses to all stimuli. Since FFA-induced changes in tissues are proportional to the levels of FFAs, both insulin resistance and the insulin hypersecretion are perfectly matched and glucose tolerance remains normal. B: In obese rats that became diabetic, FFAs rose to still higher levels (>1.5 mmol/l) and "marbleization" of tissues occurs as FACoA is increasingly esterified to TGs. In muscle this intensifies insulin resistance, while the islets, which have already responded fully to more moderate FFA overload, are incapable of further increases in insulin secretion to match insulin resistance. The FFA overload interferes with glucose metabolism and impairs the capacity of β-cells to respond to postprandial hyperglycemia. Hyperinsulinemia at this point no longer matches the increases in insulin resistance and NIDDM begins.

![Graph](https://example.com/graph.png)

**FIG. 2.** Insulin secretion by isolated pancreases (mean ± SE) of obese Zucker (□, n = 6) or lean Wistar rats (○, n = 5) during perfusion with glucose in concentrations (5.6 and 4.2 mmol/l) or subfasting (2.8 and 1.4 mmol/l) ranges. Note the leftward shift of the glucose-insulin dose-effect curve (35). IRI, immunoreactive insulin.
TABLE 1
Glucose usage (5-[3H]glucose→3H2O) and glucose oxidation ([U-14C]glucose→14CO2) at subfasting and fasting glucose concentrations

<table>
<thead>
<tr>
<th>Glucose usage (pmol·h⁻¹·islet⁻¹)</th>
<th>Glucose oxidation (pmol·h⁻¹·islet⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.4 mmol/l</td>
</tr>
<tr>
<td>Wistar n=6</td>
<td>19.5 ± 4.6</td>
</tr>
<tr>
<td>Zucker n=6</td>
<td>155.4 ± 25.4</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data are means ± SE. From Milburn et al. (35).

From obese rats. This combination of enhanced low $K_m$ glucose metabolism and β-cell hyperplasia appears to explain the hypersecretion of insulin at fasting and glucopenic concentrations of glucose by islets of obese Zucker rat.

Can increased FFA levels induce the β-cell phenotype of normoglycemic obesity? If insulin hypersecretion resulting from β-cell hyperplasia and increased low $K_m$ glucose metabolism in islets is caused by high FFA levels, these abnormalities should be inducible in normal rat islets by exposing them to comparable concentrations of FFAs. Zhou and Grill (33) previously reported a fourfold increase in insulin released at $3\text{ mmol}\text{ glucose}$ from normal rat islets cultured for $48\text{ h}$ in FFAs. We repeated their study and observed a doubling of insulin secretion in normal islets perfused with $3\text{ mmol}\text{ glucose}$ after $7\text{ days}$ in culture in the presence of $2\text{ mmol\text{ FFA}}$ and $2\%$ bovine serum albumin (39).

We also noted an FFA concentration–dependent increase in glucose usage at glucose concentrations of $2.8$ and $5.6\text{ mmol}\text{ glucose}$ in islets cultured in $1\text{ and }2\text{ mmol}\text{ FFAs}$ (35) (Table 2). Finally, in normal islets cultured in $2\text{ mmol}\text{ FFAs}$, we found an increase in bromodeoxyuridine (BrdU) incorporations from $2.3 \pm 0.3 \text{ per islet}$ in controls to $7.4 \pm 1.1 \text{ per islet}$, evidence that FFA stimulates DNA replication (35) (Fig. 3). Thus, long-chain FFAs can induce in normal islets the same functional, metabolic, and morphometric abnormalities that occur in β-cells of obese rats.

ADIPOCYTE–B-CELL RELATIONSHIPS IN OBESITY-DEPENDENT DIABETES

β-cells in obesity-dependent diabetes. Before the onset of diabetes, which begins in Zucker diabetic fatty (ZDF) rats between 7 and 10 weeks of age (40), the β-cells of obese prediabetic male ZDF rats are functionally and morphologically indistinguishable from those of nonprediabetic obese female littermates. Basal insulin secretion is high (Table 3, 6-week-old rats), GLUT2 is present in ~100% of β-cells, and the β-cell mass is enlarged. However, at the onset of NIDDM <50% of β-cells are GLUT2-positive (41), and GSIS has virtually disappeared (9,13) (Table 3, 12-week-old rats). As the diabetes progresses, <20% of β-cells remain GLUT2-positive and GLUT2 mRNA declines to ~25% of normal (13).

Similar observations have been made in all models of rodent NIDDM thus far studied, including the GK rat (15), the db/db mouse (14), and the Chinese hamster (42).

Role of GLUT2 loss in glucose incompetence of β-cells. The remarkable correlation between the reduction in GLUT2-positive β-cells and glucose incompetence (13) seemed to suggest that a defect in glucose transport in β-cells was the underlying cause of the functional impairment (9). But subsequent evidence of defects distal to GLUT2 (43,44) has cast doubt on the role of impaired β-cell glucose transport in the loss of GSIS, the report of a transport-defective GLUT2 mutation in a patient with NIDDM (45) notwithstanding. Ironically, however, there are reasons to suspect that GLUT2 in β-cells may nevertheless be required for glucose responsiveness, albeit through functions other than glucose transport. First, expression of GLUT2 antisense mRNA in β-cells results in NIDDM in transgenic mice (46). Second, GLUT2 expression confers glucose responsiveness in GLUT2-deficient glucose-unresponsive insulin-secreting cell lines (47,48), but transfer of a GLUT2 cDNA into GLUT2-deficient insulinoma cells is associated with enhanced glucokinase activity (48). Consistent with this evidence of the functional versatility of GLUT2 is the fact that transfection of insulin-secreting AT-20 cells to express either GLUT2 or GLUT1 results in a similar enhancement of glucose transport and yet confers glucose responsiveness only to the GLUT2-expressing cells (49). These findings suggest that GLUT2 may serve as the proximal anchor of a signaling pathway for high $K_m$ glucose metabolism (47–49).

Lipotoxicity as cause of glucose incompetence and GLUT2 loss. The original assumption that loss of GLUT2 was the cause rather than the consequence of the associated metabolic abnormalities of diabetes (9) was based on the fact that normalization of glycemia did not prevent the loss (41). But Thorens et al. (14) subsequently found that GLUT2 disappeared from normal β-cells when transplanted into streptozotocin-induced diabetic mice and, conversely, that GLUT2 reappeared on GLUT2-deficient β-cells of diabetic db/db mice when transplanted to nondiabetic recipients (14).

TABLE 2
FFA effects on glucose usage by cultured islets

<table>
<thead>
<tr>
<th>FFA (nmol/l)</th>
<th>n</th>
<th>Glucose usage (pmol·h⁻¹·islet⁻¹)</th>
<th>Glucose oxidation (pmol·h⁻¹·islet⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2.8 mmol/l</td>
<td>5.6 mmol/l</td>
</tr>
<tr>
<td>0</td>
<td>3</td>
<td>11.42 ± 1.26</td>
<td>16.86 ± 3.53</td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>23.28 ± 1.74</td>
<td>35.81 ± 2.84</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>32.28 ± 1.74</td>
<td>46.80 ± 1.52</td>
</tr>
</tbody>
</table>

Data are means ± SE. From Milburn et al. (35).
LIPOTOXICITY OF OBESITY-DEPENDENT NIDDM

FIG. 3. Effect of FFA on BrdU incorporation by islet cells. Islets isolated from 6-week-old Wistar rats were cultured for 7 days with (FA+) or without (FA-) a 2 mmol/l palmitate-oleate mixture. Adjacent sections were stained with either anti-insulin (upper panels) or anti-BrdU antiserum (lower panels) to determine if BrdU incorporation is in β-cells. From Milburn et al. (35).

This proved conclusively that loss of β-cell GLUT2 required a metabolic abnormality that was present in diabetic mice. Since hyperglycemia had already been excluded as the metabolic cause of glucose incompetence (50) and of GLUT2 loss in β-cells (41,51), a lipid abnormality was suspected, particularly since acute inhibitory effects of FFAs on GSIS (52,53), glucokinase (54), phosphofructokinase (55), glucose oxidation (52,53), and glucose-stimulated insulin biosynthesis (35) had previously been established. We therefore measured plasma FFA and triglyceride (TG) levels longitudinally in obese male ZDF rats before and after the onset of hyperglycemia, using nonobese male and obese nonprediabetic female ZDF littermates as controls (39) (Fig. 1). Plasma TG levels were increased in both prediabetic and nonprediabetic obese groups (Fig. 1B). Plasma FFA levels were high only in the prediabetic rats (Fig. 1C). The hyperlipidemia began at 7 weeks of age, 2 weeks before the onset of diabetes, and peaked at 2 mmol/l at 9 weeks, at which time hyperglycemia first appeared. Every hyperglycemic rat had an FFA level above 1.5 mmol/l (Fig. 5) despite a high basal

TABLE 3
Insulin levels during pancreas perfusion at 5.6 mmol/l glucose (baseline) and at 20 mmol/l glucose (incremental), β-cell volume fractions and percentage of GLUT2-positive β-cells in 6- and 12-week-old Wistar and Zucker rats

<table>
<thead>
<tr>
<th>Animals</th>
<th>Immunoreactive insulin (μU/ml)</th>
<th>β-cell volume fraction (%)</th>
<th>β-cell GLUT2 (%)</th>
<th>Islet TG (μg/islet)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Incremental</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6-week-old Wistar</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>male</td>
<td>6.9 ± 0.9</td>
<td>168.8 ± 14.4</td>
<td>0.43 ± 0.1</td>
<td>~100</td>
</tr>
<tr>
<td>ZDF (fa/+) male</td>
<td>11.8 ± 1.6</td>
<td>169.6 ± 15.4</td>
<td>0.40 ± 0.07</td>
<td>96 ± 0.5</td>
</tr>
<tr>
<td>ZDF (fa/aa) female</td>
<td>27.5 ± 2.5</td>
<td>207 ± 34.2</td>
<td>1.01 ± 0.17</td>
<td>98.5 ± 1.6</td>
</tr>
<tr>
<td>ZDF (fa/aa) male</td>
<td>23.6 ± 1.9</td>
<td>226 ± 35</td>
<td>1.32 ± 0.34</td>
<td>100.3 ± 2.1</td>
</tr>
<tr>
<td>12-week-old Wistar</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>male</td>
<td>3.4 ± 0.3</td>
<td>231.8 ± 12.7</td>
<td>0.45 ± 0.09</td>
<td>~100</td>
</tr>
<tr>
<td>ZDF (fa/+) male</td>
<td>9.6 ± 1.6</td>
<td>304.3 ± 15.4</td>
<td>0.45 ± 0.14</td>
<td>98.5 ± 2.5</td>
</tr>
<tr>
<td>ZDF (fa/aa) female</td>
<td>119.2 ± 12.1</td>
<td>138.3 ± 13.2</td>
<td>1.52 ± 0.34</td>
<td>98.5 ± 1.6</td>
</tr>
<tr>
<td>ZDF (fa/aa) male</td>
<td>145.5 ± 12.2</td>
<td>4.7 ± 23.4</td>
<td>1.78 ± 0.35</td>
<td>25.1 ± 6.4</td>
</tr>
</tbody>
</table>

Data are means ± SE. From Ohneda et al. (58).
insulin secretion (Table 3, 12-week-old rats). While the timing and the magnitude of the FFA elevations were consistent with a role in the pathogenesis of the β-cell malfunction, increased tissue levels of fatty acyl CoA would be required to impair β-cell function. If, in fact, islet fatty acyl CoA is high, the glycerol 3-phosphate generated by glucose metabolism would provide a substrate for TG formation. We therefore measured the islet TG content of the three groups from 5 to 14 weeks of age as an index of substrate overload. Compared with lean controls islet TG content was increased in islets of both prediabetic and nonprediabetic obese rats beginning at 6 weeks of age. However, at the onset of overt diabetes at 9 weeks of age the TG content of islets in the prediabetic group had risen to 0.6 μg/islet, more than twice that of obese nonprediabetic controls and 10 times that of lean controls (Fig. 4D). At present we consider the high TG content of diabetic islets to be the passive consequence of chronically elevated fatty acyl CoA in islet cells, rather than a cause of the β-cell abnormalities. (TG formation and lipolysis in islets is probably not rapid enough to create a quantitative significant diversion of glycerol 3-phosphate from its postulated role in glucose signaling [56,57].)

Chronic excesses of fat in islets could, however, have late sequela. Late in the course of diabetes in ZDF rats, the increased β-cell volume noted at onset declines by almost 75% and fibrosis appears (58); conceivably the chronically high fat content in these islets plays a role in the cellular depletion and associated fibrosis analogous to steatonecrosis in liver.

**Effects of reducing FFAs on NIDDM β-cell phenotype.** If increased islet FFA levels do in fact cause the β-cell changes of NIDDM, these abnormalities should be preventable by measures that reduce the substrate overload. Two such maneuvers, caloric restriction by pair-feeding to lean littermates (58) and nicotinamide treatment (M. Ohneda, Y. Nagasawa, Y. Lee, J. Milburn, R.H.U., unpublished data), reduce plasma FFA levels to <1.5 mmol/l and prevent the accumulation of islet TGs. They also prevent the hyperglycemia (Fig. 6), the basal hyperinsulinemia, the loss of GLUT2, and the glucose incompetence (58) (Table 3). However, the fact that these interventions have actions other than reduction of plasma and tissue FFA levels makes it impossible to ascertain the mechanism of these dramatic effects.

**Can FFAs induce the β-cell phenotype in nondiabetic islets?** High concentrations of free fatty acids are known to impair β-cell function. Sako and Grill (52) observed a 50% reduction in GSIS by the perfused pancreas of normal rats after 48 h of a TG emulsion infusion, while Elks (53) noted a more substantial loss of GSIS in perifused islets exposed to 1 mmol/l palmitate. However, Zhou and Grill (33) observed a reduction of only 30–50% in GSIS in islets isolated from normal rats and cultured for 48 h in the presence of long-chain FFAs. Clearly, in vitro suppression of GSIS by FFAs is less complete than that which occurs at the onset of NIDDM in intact ZDF rats.

To determine if this difference reflected a greater vulnerability of islets from ZDF rats to the lipotoxic effects of high FFAs, we compared their effects on islets isolated from prediabetic obese male ZDF rats, from nonprediabetic obese female ZDF rats, and from normal Wistar rats. All islets were cultured for 7 days in 2 mmol/l FFAs and 2% bovine serum albumin. Evidence of qualitative and quantitative differences in FFA effects on the different groups were observed (H. Hirose, J. Milburn, Y. Nagasawa, J.H. Johnson, R.H.U., unpublished observations). In normal islets from Wistar rats, 2 mmoV/l FFAs lowered the insulin response to 23 mmol/l glucose by 5%, compared with 91 and 98% in islets from lean ZDF and obese prediabetic rats, respectively. Interestingly, the accumula-
tion of TGs was more than 10 times higher in islets from obese rats than in control islets during 7 days of exposure to 2 mmol/l FFA (H. Hirose, J. Milburn, Y. Nagasawa, J.H. Johnson, R.H.U., unpublished observations). As mentioned, we interpret this greater accumulation of TGs as reflective of higher tissue levels of fatty acyl CoA, which inhibit glycolysis (54,65) and glucose oxidation (25) and thus cause the glucose incompetence. It is not yet clear if the greater accumulation of TGs is the result of increased FFA uptake and acylation, decreased fatty acid oxidation, decreased lipolysis, or combinations thereof.

**FFAs and GLUT2 loss.** Thus far it has not been possible to induce a clear-cut loss of immunostainable GLUT2 in β-cells of either Wistar or ZDF lean rats or ZDF prediabetic rats cultured in 2 mmol/l FFA for up to 3 weeks (H. Hirose, unpublished findings). These negative results could mean that loss of GLUT2 in vivo in NIDDM is caused by factors other than or in addition to hyperlipidaemia. Alternatively, they could reflect the extraordinarily slow rate of GLUT2 degradation reported in cultured islets (59).

**GENETIC SPECULATIONS RELATING TO THE LIPOTOXIC HYPOTHESIS**

The preliminary evidence of quantitative and qualitative differences in the inducibility of β-cell abnormalities in islets of genetically distinct groups of rats by identical concentrations of FFA raises the possibility of a genetic predisposition to fatty acid overload in tissues exposed to high FFA levels. Of the mutations thus far studied in the search for NIDDM genes (45,60,61), only two are directly relevant to lipid metabolism (62,63). Certain apolipoprotein polymorphisms increase the risk of NIDDM in a Chinese-American cohort (62), while linkage between maximal insulin action and \( FABP_p \), the intestinal fatty acid–binding protein gene, has been reported in Pima Indians (63).

A most significant advance in obesity research has been the cloning of the \( ob \) gene (64), believed to encode a secreted peptide expressed in adipocytes that inhibits food intake and/or regulates energy expenditure. Mice with a nonsense mutation of this gene or with a failure to express it develop massive obesity and NIDDM. Parabiotic experiments (65) suggest that the \( db \) gene of mice (equivalent to the \( fa \) gene in rats [66]) encodes the receptor for the \( ob \) peptide, in which case a defect in the \( db \) gene could cause the obesity. If the associated hyperlipidaemia causes both the insulin resistance and the β-cell abnormalities, the entire syndrome could be ascribed to a single gene.

In support of this monogenic model is the fact that restriction of food intake in prediabetic obese rats by pair feeding them with lean littermates reduces the hyperlipidemia and prevents the hyperglycemia and the entire β-cell phenotype of NIDDM (58). However, the weight reduction thereby achieved comes at the expense of lean body mass and does not prevent subcutaneous and intra-abdominal obesity. This fact, together with preliminary evidence of increased vulnerability of islets of ZDF rats to high FFA levels in vitro (H. Hirose, J. Milburn, Y. Nagasawa, J.H. Johnson, R.H.U., unpublished observations), requires an abnormality in lipid metabolism that would account for both the reduced antilipolytic action of insulin and the excessive formation of fat, not only in adipocytes, but also in islets and in the target tissues of insulin. Unless an abnormality in fat storage (67) can be caused by mutation of the \( ob \) or \( db \) genes, at least one other gene defect must be postulated. The genes that encode the proteins involved in FFA uptake (63) and transport, mitochondrial and peroxisomal FFA oxidation, esterification of FFA, and lipolysis warrant consideration as possible candidates.

In liver cells, for example, fatty acid overload is prevented by FFA-induced peroxisomal proliferation, which provides extramitochondrial β-oxidation when mitochondria are operating at maximal capacity (68). This induction is associated with increased \([\text{3H}]\) thymidine incorporation and hyperplasia (68–70), not unlike that observed in islets (35). M.J. McPhaul and J. Milburn (personal communication) have suggested that peroxisomal proliferation may be relevant to islet lipotoxicity and account for the in vivo hyperplasia of β-cells in islets of obese rats and the increase in BrdU incorporation induced in vitro in normal islets by 2 mmol/l FFAs. A genetic defect at either this or another locus involved in the prevention of intracellular FFA overload might well increase the vulnerability of β-cells to hyperlipidaemia.

**CLINICAL IMPLICATIONS OF THE LIPOTOXICITY HYPOTHESIS FOR HUMAN OBESITY**

While it remains to be determined if the lipotoxicity hypothesis applies to the human form of obesity-dependent NIDDM, there is no a priori reason why human islets would be immune to the effects of high tissue FFA levels presumed to occur in obesity. The relationship between the prevalence of diabetes and the severity of the obesity (7), together with the fact that caloric restriction, which reduces plasma FFA levels, is so effective in managing hyperglycemia (71), are consistent with this. The poor adherence of most obese patients to such dietary regimens makes it important to determine if antilipolytic agents will be useful in the prevention of NIDDM in obese patients who are not able to lose weight. Several compounds with antilipolytic activity have been reported to be effective in reducing hyperglycemia (72,73), but their effects on islet function have not been characterized.
This work was supported by National Institutes of Health Grants DK-02700 and 1-P01-DK42582 and Veterans Administrations Research Support Grant 549–8000.

We wish to thank Drs. J. Denis McGarry, Daniel W. Foster, Christopher B. Newgard, and Helen Hobbs for their constructive criticisms of this manuscript and Teresa Auyert for outstanding secretarial work.

ACKNOWLEDGMENTS

REFERENCES

28. Dukes ID, McIntyre MS, Mertz RJ, Phillips LR, Roe MW, Spencer B,


