

# Lipotoxicity of $\beta$ -Cells in Obesity and in Other Causes of Fatty Acid Spillover

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**A recently identified function of leptin is to protect nonadipose tissues from the nonoxidative metabolic products of long-chain fatty acids (FAs) during periods of overnutrition by increasing the  $\beta$ -oxidative metabolism of surplus FAs and reducing lipogenesis. When this protective system fails, harmful products of nonoxidative metabolism such as ceramide increase in nonadipose tissues, including the pancreatic islets and heart, and cause nitric oxide-mediated lipotoxicity and lipoapoptosis. The triacylglycerol content in nonadipocytes provides a useful index of overall nonoxidative metabolism. In normal animal tissue, triacylglycerol is maintained within a narrow range; even when the caloric intake is excessive, compensatory FA-induced upregulation of oxidation prevents overaccumulation. However, if leptin is deficient or if leptin receptors (Ob-R) are nonfunctional, this autoregulatory system does not operate, and triacylglycerol content rises in nonadipose tissues. This provides a source of excess FAs that enter potentially toxic pathways of nonoxidative metabolism leading to apoptosis of certain tissues. FA overload in skeletal muscle causes insulin resistance; in myocardium, it impairs cardiac function; and in pancreatic islets, it causes  $\beta$ -cell dysfunction, apoptosis, and diabetes. All abnormalities in these tissues can be blocked by troglitazone, an inhibitor of FA accumulation. *Diabetes* 50 (Suppl. 1):S118-S121, 2001**

**T**he evolution of the adipocyte may well constitute one of the most transforming events in phylogenetic development. As proposed in the "thrifty gene" hypothesis of Neel (1), the ability to pre-load calories and to store them in the form of triacylglycerol for use during famine and/or exertion improved survival for many species. Recent work from this lab (2) suggests that the adipocytes have an additional function, namely, to protect

nonadipocytes, which have only a limited capacity to store triacylglycerol, from excessive accumulation of lipids and their entry into nonoxidative metabolic pathways. This protection requires the permissive action of the adipocyte hormone leptin (3), which, as will be explained below, reduces lipogenesis and increases oxidation in nonadipocytes during overnutrition, dissipating the unneeded energy of the fatty acid (FA) excess as heat (4). This limits FA entry into potentially toxic nonoxidative metabolic pathways (5).

## LIPOTOXIC DISEASES

Lipotoxicity refers to the tissue disease that may occur when FA spillover in excess of the oxidative needs of those tissues enhances metabolic flux into harmful pathways of nonoxidative metabolism. Although lipotoxicity is not currently recognized as a clinical entity in obese humans, human disorders of FA metabolism exhibit clinical syndromes similar to those of the obese rodent. It seems reasonable, therefore, to group them under the rubric of lipotoxic diseases. The disorders in which lipotoxicity due to FA overflow is suspected include congenital generalized lipodystrophy (CGL), obesity due to mutations in the leptin or leptin receptor genes, diet-induced obesity, and aging (Table 1).

The toxic consequences of lipid overload will depend on the magnitude and duration of the imbalance between FA influx and FA oxidation in a given tissue. Congenital disorders would obviously be the most severe and have the longest duration. In CGL, for example, the absence of adipocytes not only removes the normal storage depot for FAs but also eliminates the main source of leptin, on which regulation of the oxidative capacity of nonadipose tissues depends (4). Consequently, the ability of such patients to tolerate a positive caloric balance is minimal, and severe lipotoxicity appears early in life.

In rats with obesity resulting from mutations in leptin or in the leptin receptor gene (Ob-R) (6,7), evidence of lipid overload in nonadipose tissues also appears early in life (2); the hyperphagia resulting from loss of leptin's hypothalamic actions assures a positive caloric balance, whereas lack of peripheral leptin action on nonadipocytes results in lipotoxicity (8).

In diet-induced obesity, by contrast, nonadipose tissues are protected by hyperleptinemia during the initial phase of the FA excess; later, leptin resistance of undetermined etiology develops and seems to be responsible for a slow overaccumulation of lipids and lipotoxicity.

Finally, old age is accompanied by profound leptin resistance (9,10), which may explain the increase in total body fat, the elevated lipid content of nonadipose tissues, and the reduction in lean body mass in elderly patients.

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Received for publication 27 April 2000 and accepted 29 August 2000.

This article is based on a presentation at a symposium. The symposium and the publication of this article were made possible by an unrestricted educational grant from Les Laboratoires Servier.

ACO, acyl CoA oxidase; CGL, congenital generalized lipodystrophy; CPT-1, carnitine palmitoyl transferase 1; FA, fatty acid; iNOS, inducible nitric oxide synthase; NO, nitric oxide; PPAR, peroxisome proliferator-activated receptor; SPT, serine palmitoyl transferase.

TABLE 1  
Disorders of intracellular homeostasis of FA (steatosis)

Condition	Severity	Obesity
Generalized lipodystrophy	Extreme	No
Leptin deficiency	Severe	Yes
Leptin unresponsiveness	Severe	Yes
Diet-induced obesity	Moderate	Yes
Aging	Moderate	Often

### LIPOTOXIC DIABETES

Although lipid overload in skeletal muscle, pancreatic islets, myocardium, and liver are common in all of the foregoing clinical states, causing insulin resistance (11–13), type 2 diabetes (14), cardiac dysfunction (15), and hepatic steatosis (16), respectively, this review focuses on lipotoxic disease of islets (type 2 diabetes). The availability of a rat model of obesity and diabetes secondary to a loss-of-function mutation in the leptin receptor (Ob-R) (6,7) has permitted the elucidation of the pathogenesis of one form of obesity-related diabetes. In lean wild-type ZDF rats with normal Ob-R, nonadipose tissues are protected from overaccumulation of lipids, even during the feeding of a diet in which 60% of the calories are derived from fat. After 8 weeks of such a diet, adipocyte fat, measured by magnetic resonance spectrophotometry, increased almost 150 times, but triacylglycerol levels in pancreatic islets and other nonadipose tissues rose no more than threefold. Because at high concentrations, free fatty acids probably enter nonadipocytes and adipocytes at approximately the same rates (17), the relatively normal triacylglycerol content of the nonadipose tissues implies that the excess FAs were oxidized. This speaks for a well-regulated system of FA metabolism that protects tissues from the deleterious consequences of excessive nonoxidative metabolism.

This homeostatic system for FAs requires biologically active leptin and responsive leptin receptors because whenever leptin is deficient or its receptor nonfunctional, FA homeostasis in nonadipose tissues is profoundly deranged, causing serious dysfunction therein (2). In the leptin-unresponsive obese ZDF rat, for example, nonadipose tissues accumulate large quantities of triacylglycerol, even on a diet containing only 6% fat. The islets in this disorder have been carefully studied from the preobese period (before 5 weeks of age), through the obese prediabetic phase (from 6 to 10 weeks of age), to the diabetic phase (at age 10–12 weeks) (18). In the preobese phase of the disease, the islets are perfectly normal functionally and morphologically. Thereafter, there is a gradual increase in their triacylglycerol content, reaching a peak of ~50 times that of normal at 12–14 weeks of age. In the obese prediabetic phase at 7–9 weeks of age, they are hyperplastic, with the  $\beta$ -cell mass having risen fourfold; this is associated with hyperinsulinemia sufficient to compensate for the accompanying insulin resistance. However, by 10–12 weeks, as the triglyceride content reaches its peak,  $\beta$ -cell function wanes and diabetes begins.  $\beta$ -Cells begin to disappear until the  $\beta$ -cell mass is reduced to its original size before obesity (18). This leaves the animals with an insulin-secreting capacity far below that required to compensate for the insulin resistance.

These islets are functionally deranged, lacking GLUT2 and the normal response to glucose. Morphologically, the  $\beta$ -cells

appear disorganized and interspersed with abundant fibrous tissue, which might be categorized as “cirrhosis” of the islets (19). About 85% of the mitochondria of  $\beta$ -cells are severely altered and are difficult to recognize as such. Although rare, apoptotic changes can be noted in the  $\beta$ -cell nuclei (19).

Studies in these leptin-unresponsive rats have made it clear that leptin action is required for protection of  $\beta$ -cells from overaccumulation of triacylglycerol (steatosis), loss of normal  $\beta$ -cell function (lipotoxicity), and disappearance of  $\beta$ -cells (lipoapoptosis).

### MECHANISMS OF LIPOTOXICITY

Compared with lean wild-type ZDF controls (+/+) and normal Wistar rats, the islets of obese *fa/fa* ZDF rats have a markedly increased lipogenic capacity (20). When cultured in either  $^{14}$ C-labeled glucose or  $^{14}$ C-labeled palmitate, their islets incorporate the label into triacylglycerol at a much higher rate. This is attributed to a profound alteration in the relative expression of the transcription factors that control the enzymes of lipogenesis and FA oxidation—peroxisome proliferator-activated receptors (PPARs) expressed in the *fa/fa* islets. The mRNA levels of PPAR- $\alpha$  and the transcription factor for carnitine palmitoyl transferase 1 (CPT-1) and acyl CoA oxidase (ACO) (the major enzymes of FA oxidation) are low, as are the mRNA levels of the enzymes themselves. But, the mRNA level of PPAR- $\gamma$ , the transcription factor for lipogenic enzymes, is elevated, as is the expression of acetyl CoA carboxylase (ACC), fatty acid synthase (FAS), and glycerol phosphate acyl transferase (GPAT), enzymes of lipogenesis. Moreover, there is evidence of impaired FA homeostasis; whereas in normal islets cultured in FA a substantial increase in PPAR- $\alpha$ , CPT-1, and ACO expression is induced, no such FA-induced upregulation occurs in *fa/fa* islets (21) (Fig. 1). Islets of *fa/fa* rats lack this autoregulatory capability and therefore are vulnerable to lipid overload whenever the caloric intake exceeds caloric need. These conclusions have been further strengthened by transgenic experiments using adenoviral technology in which the wild-type Ob-Rb was overexpressed in the islets of *fa/fa* rats with abnormal endogenous Ob-R (22). In such islets, leptin effectively lowered their triacylglycerol content, upregulated the expression of PPAR- $\alpha$  and the oxidative enzymes, and prevented the FA-induced apoptosis and the functional and histologic abnormalities (22,23). This proved that leptin is essential for the protective FA homeostasis of the islets during lipid overload.

What remained unclear, however, was the mechanism by which lipid overload damaged the islets. There was reason to doubt that the triglycerides themselves were responsible for the 15-fold increase in DNA laddering (5), an index of apoptosis, in these islets. More likely, the triacylglycerol was inert but did provide a mirror of the magnitude of nonoxidative FA metabolism in the islets and an intracellular source of FA. Immunologically induced apoptosis of  $\beta$ -cells has been attributed to the increase in ceramide derived from sphingomyelin breakdown (24), but the role of ceramide in FA-induced apoptosis was unexplored. FA-induced apoptosis, quantified by DNA laddering, is twice that of normal prediabetic *fa/fa* islets (5,25); when such islets are cultured in the presence of either [ $^3$ H]serine or [ $^3$ H]palmitate, the appearance of [ $^3$ H]ceramide is markedly increased over normal +/+ islets (Fig. 2). Both the ceramide increase and the severe apoptosis are blocked by inhibitors of de novo ceramide synthesis

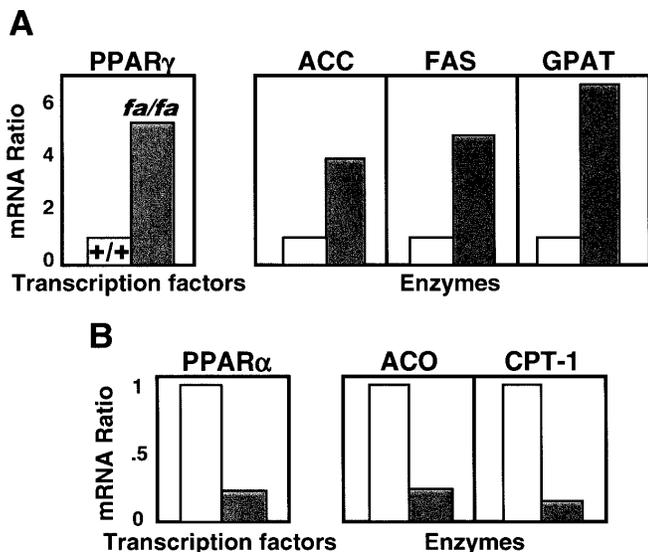


FIG. 1. Semiquantification of mRNA of transcription factors and enzymes of fatty acid synthesis and oxidation to determine the mechanism of the increased lipogenic capacity. **A:** Fatty acid synthesis. There is a marked increase in mRNA of the anabolic transcription factor PPAR- $\gamma$  and the enzymes of FA synthesis that it upregulates—acetyl CoA carboxylase (ACC), fatty acid synthase (FAS), and glycerol phosphate acyl transferase (GPAT)—in islets isolated from obese ZDF rats (*fa/fa*). **B:** Fatty acid oxidation. There is a profound reduction in expression of the catabolic transcription factor PPAR- $\alpha$  and the two enzymes of fatty acid  $\beta$ -oxidation that it upregulates: the peroxisomal enzyme ACO and the mitochondrial enzyme CPT-1. Reprinted with permission (21).

(25). The condensation of serine and palmitate, the first step in de novo ceramide formation, is catalyzed by the enzyme serine palmitoyl transferase (SPT) (26); SPT mRNA is markedly increased in *fa/fa* islets compared with +/+ islets (25). Moreover, when the normal Ob-R is overexpressed in the *fa/fa* islets, SPT expression decreases, as does DNA laddering (Fig. 3). Finally, FA-induced apoptosis is profoundly inhibited by adding to the culture medium a potent inhibitor of ceramide formation—fumonisins-B<sub>1</sub>.

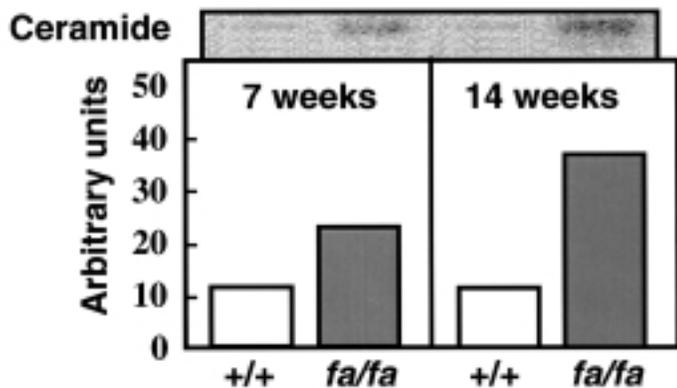


FIG. 2. [<sup>3</sup>H]ceramide formation from [<sup>3</sup>H]serine in normal (+/+) and obese (*fa/fa*) rats at 7 and 14 weeks of age (prediabetic and diabetic). At both prediabetic and diabetic ages, formation of ceramide from serine is increased. These findings suggest that ceramide is probably formed de novo from the excess FA rather than from sphingomyelin breakdown, as is thought to occur in autoimmune  $\beta$ -cell destruction. Reprinted with permission (26).

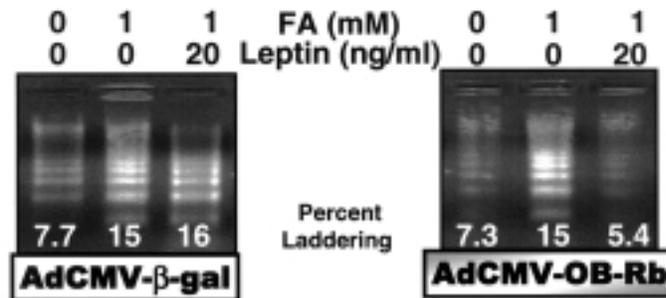


FIG. 3. Effect of transgenic expression of normal leptin receptors on prevention of FA-induced apoptosis. In normal islets (not shown), DNA laddering, an index of apoptosis, is below 1%. In fat-laden islets of prediabetic ZDF rats, laddering is >7% and rises to 15% in the presence of FAs. Leptin has no effect on this. If leptin receptors (OB-Rb) are expressed, leptin prevents the FA-induced increase in laddering. AdCMV, recombinant adenovirus with cytomegalovirus promoter, containing either the cDNA of OB-Rb or, as a control,  $\beta$ -galactosidase. Reprinted with permission (5).

Ceramide can induce apoptosis by activating NF $\kappa$ B (27), which upregulates the expression of inducible nitric oxide synthase (iNOS). The increased formation of nitric oxide (NO) forms peroxynitrite, which is probably an important cause of the apoptosis (28). Compared with normal islets, *fa/fa* islets exhibit a far greater increase in iNOS mRNA and in NO formation when cultured in 1 mM FA; the iNOS inhibitors, nicotinamide and aminoguanidine, both reduce NO formation under these conditions in vitro (5) and prevent the  $\beta$ -cell apoptosis and diabetes in vivo (29). Nevertheless, there are other possible lipotoxic pathways that have not yet been explored (Fig. 4).

These studies suggest important new strategies to prevent lipoapoptosis through interventions at multiple levels: 1) reducing FA in islets, 2) blocking ceramide formation, 3) inhibiting iNOS, and 4) restoring leptin action. At present, only the first and third options are available for therapy in humans. Clinical studies in prediabetic ZDF rats have demonstrated that of the changes described earlier—the overaccumulation of triacylglycerol, the loss of function, and

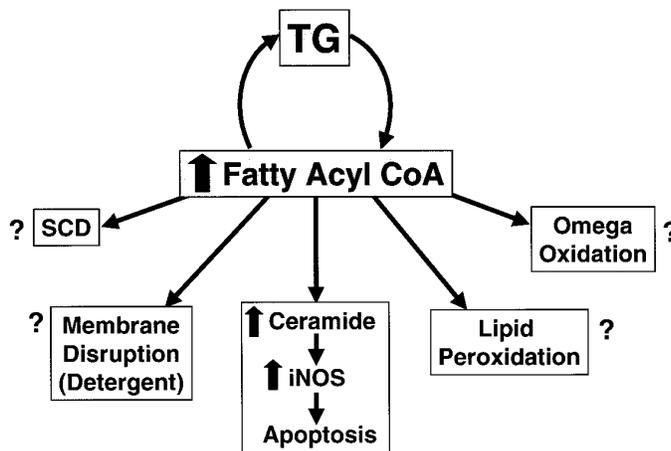


FIG. 4. Possible lipotoxic pathways through which a surplus of unoxidized long-chain fatty acids might impair function and/or cause death of nonadipose cells. SCD, stearoyl-CoA desaturase; TG, triacylglycerol.

the loss of  $\beta$ -cells through lipoapoptosis—all can be prevented by treatment with a thiazolidinedione, troglitazone, if begun in the prediabetic stage of the disorder (19). Despite the lack of leptin action in these rats, troglitazone treatment appears to prevent the spillover of lipids from adipose tissues into nonadipocytes, i.e., it increases adiposity but reduces ectopic lipid deposition. The reduction of FA influx into  $\beta$ -cells lowers ceramide content and inhibits apoptosis. It completely prevents the severe mitochondrial alterations that otherwise occur (19). Similar clinical results can be obtained by treating prediabetic obese ZDF rats with the iNOS inhibitors nicotinamide or aminoguanidine (29).

These results prove that an ectopic lipid overload can cause dysfunction and ultimately the destruction of  $\beta$ -cells and that the resulting diabetes is preventable by pharmacological agents that are currently available. Prevention of lipotoxic heart disease with troglitazone has also been observed (26).

Given the increase in both obesity and aging in the American population, a progressive increase in clinical lipotoxicity is predictable. If so, early recognition of the disorder will be required if the serious late complications of lipotoxicity are to be prevented.

#### ACKNOWLEDGMENTS

This work was supported by the Department of Veterans Affairs Institutional Support, the National Institutes of Health (DK02700-37), the National Institutes of Health/Juvenile Diabetes Foundation Diabetes Interdisciplinary Research Program, and the Novo-Nordisk Corporation.

We thank Susan Kennedy for outstanding secretarial work and Kay McCorkle for excellent assistance with the illustrations.

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