LEPTIN AND ADIPOGENIC DIABETES

MEDICAL GRAND ROUNDS
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KONISHIKI

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His research has focused on the physiology and pathophysiology of the pancreatic islets and their roles in normal and abnormal fuel homeostasis.

CASE REPORT

PAST HISTORY

The patient is a 33-year-old Hawaiian-American Sumo wrestler, whose ring name is Konishiki, who came from the island of Hawaii when he was 18 years old. His height was 185 cm and his body weight was 170 kg. He had 9 brothers and sisters. He came to Japan to become a Sumo wrestler. Sumo wrestlers exercise early in the morning, eat breakfast and sleep in the afternoon. In the evening they eat a lot to increase their body weight. Konishiki became bigger and stronger and became a champion. He continued to eat a huge amount of food and his weight rose to 280 kg. He started to diet but to no avail. Because of his enormous weight he injured his knees and lost his championship. His blood chemistry data are not known because the Japanese Sumo Association never reveals data to the press due to the fact that many Sumo wrestlers have diabetes, hyperlipidemia, hypertension and fatty liver and die 15 or 20 years earlier than normal. He married a Japanese woman and got Japanese citzenship. He is one of the most popular Sumo wrestlers in Japan, despite the fact that he is handicapped by knee damage.

FUTURE HISTORY

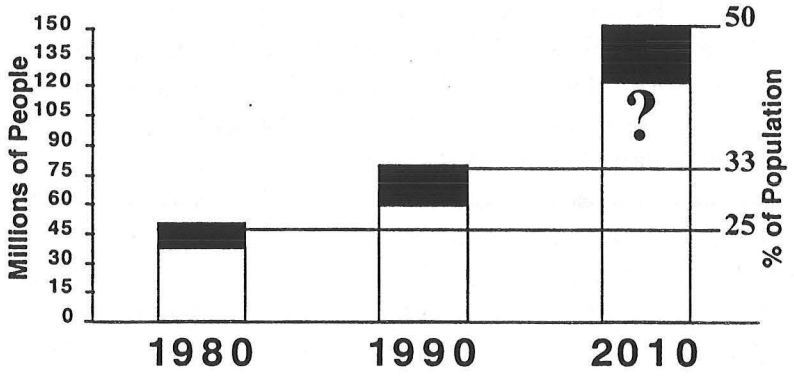
In the year 2001, Konishiki retired from wrestling and gained an additional 15 kg. In 2005, at the age of 41, Konishiki was found to have a fasting glucose of 397 mg/dl, triglycerides of 490 mg/dl and a plasma leptin level of 17 ng/ml. He was found to have background diabetic retinopathy, early diabetic nephropathy, hypertension and evidence of coronary artery disease.

He developed progressively worsening respiratory difficulties with hypoxia, congestive failure, Pickwickian syndrome and intractable severe hyperglycemia. Aggressive weight reduction was attempted. Draconian restriction of food intake was minimally effective and resulted in ketosis and marked hyperuricemia in association with a decline in plasma leptin to <1 ng/ml. He was, therefore, treated with a long-acting leptin analog. Free fatty acids, triglycerides and ketones declined and he lost weight rapidly without ketosis or hyperuricema. His insulin requirement declined from 150 U/d to 20 U/d.

INTRODUCTION

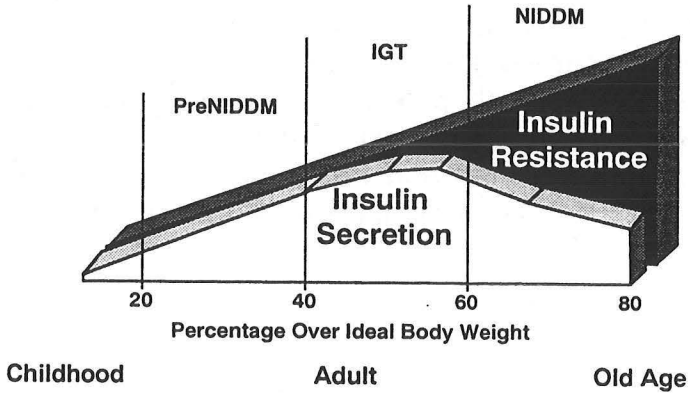
Obesity, the most prevalent of American health problems, afflicts an estimated 45,000,000 persons and the prevalence is increasing (1) (Figure 1). At least 20% will develop noninsulin-dependent diabetes (NIDDM) at some point in their life. The mechanism of the ß-cell failure has been a matter of intense specula-

FIG. 1: Obesity □ and NIDDM ■ in the U.S.



tion for decades. Since insulin resistance precedes by many years the appearance of NIDDM, it has been assumed that in most obese individuals ß-cells somehow "sense" the level of increased insulin production required to compensate for insulin resistance; but at some point ß-cells undergo unexplained "fatigue" and decompensation may occur, a scenario reminiscent of high output cardiac failure (Figure 2). While semantically accurate, the foregoing pathophysiologic description fails to provide a mechanistic explanation of 1) how adipocytes signal the islets to maintain precisely sufficient hyperinsulinemia to meet the increased demand and thereby maintain glucose tolerance within normal limits, or 2) how and why this perfect adaptive relationship of ß-cells to insulin resistance so frequently dissolves, causing adipogenic NIDDM.

FIG. 2: Natural history of Adipogenic NIDDM. IGT = Impaired glucose tolerance. Insulin resistance increases progressively with body weight, but insulin secretion reaches a "ceiling" and then recedes.



ZUCKER DIABETIC FATTY (ZDF) RATS

The answer to these questions requires a model of obesity and diabetes in which the pancreatic islets can be readily obtained for study and in which the entire course of the disease spans weeks to months rather than the years to decades, as in the human version of the disorder. Homozygous ZDF rats *(fa/fa)* are obese because of a Glu 269→ Pro substitution in their leptin receptor (2) (Figure 3). This mutation results in hyperphagia and decreased thermogenesis (3-8). NIDDM appears in virtually all homozygous males between 8-12 weeks of

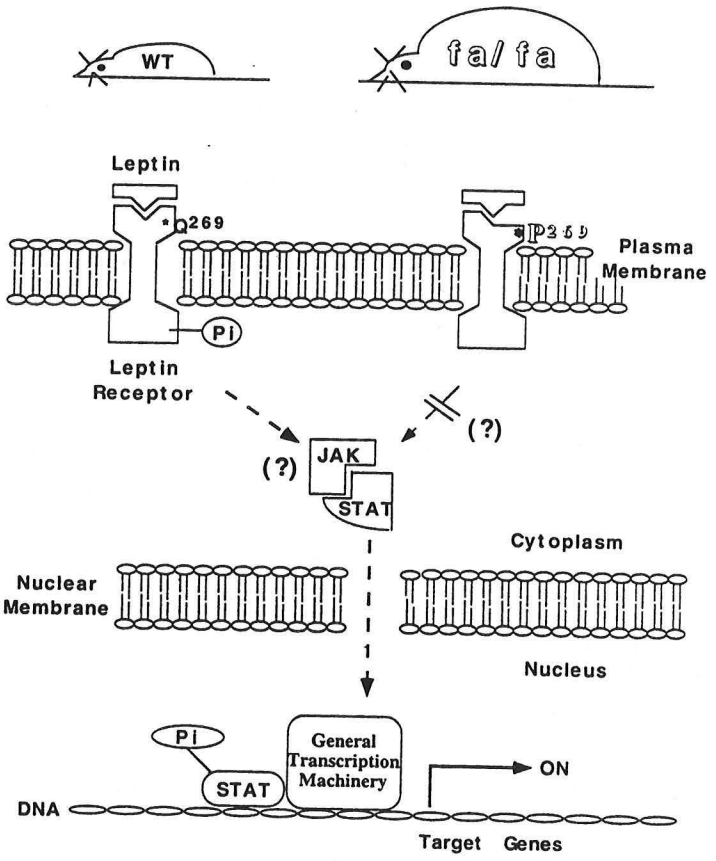


FIG. 3: The leptin receptor (OB-R) mutation in ZDF consists of a Glu 269 → Pro in the extracellular domain. (Courtesy of May-Yun Wang)

age, while most females remain euglycemic with impairment in glucose tolerance. Although this rodent model cannot be assumed to be a facsimile of the human form of the disease, both humans and ZDF rats exhibit antecedent obesity and insulin resistance, followed by a loss of glucose responsiveness of ß-cells, and are thus phenotypically similar. While the proximal cause of obesity in the two species may be different, the consequences of lipid overload on cellular functions appear to be very similar. In this conference we attempt to understand how a point mutation in the leptin receptor causes hyperglycemia.

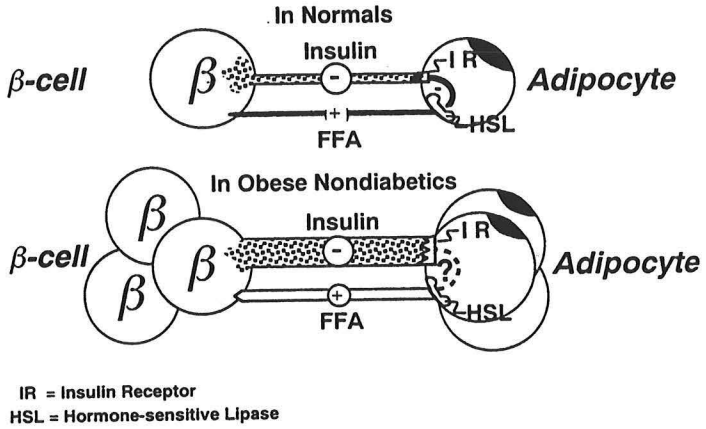
FFA AND PHYSIOLOGY OF INSULIN SECRETION

The primary meal-induced influence upon ß-cell activity is the "enteroinsular axis", a battery of hormonal and neurotransmitted signals originating in the gastrointestine that stimulate insulin release even before the ingested and digested substrates have entered the arterial circulation (9). In young, lean individuals a large oral glucose load may be associated with a flat arterial blood glucose curve because of anticipatory insulin release prior to glucose entry into the circulation. The same is true of amino acids (10). However, glucopenia always has "veto power" over insulin secretion, i.e., it can cancel insulin responses to any insulin secretagogue if the secreted insulin would pose a risk of glucopenia by reducing availability of glucose to the brain (11).

Unlike rises in blood glucose and AA, which are meal-induced events, a rise in FFA normally is the result of food deprivation and/or exercise, circumstances in which a rise in insulin secretion would be undesirable. Insulin is suppressed by the associated glucopenia; the rise in FFA is vital to reduce glucose utilization in muscle. By substituting for glucose, FFA conserve glucose for the brain (12). Thus, despite the well-established stimulatory action of FFA on ß-cells in experimental settings (13,14), in normal real life situations, the glucopenic "veto power" allows FFA to act only as a fuel and not as an insulin secretagogue (Figure 4 - Upper).

In obesity, however, FFA is not fully suppressed during meals, and at that time, when glucose is abundant, it enhances insulin output (Figure 4 - Lower). The antilipolytic action of insulin would normally lower FFA. But in obesity, adipocyte resistance to insulin-mediated antilipolysis prevents the lowering of plasma FFA despite hyperinsulinemia (15). Thus, the tissues are exposed around the clock to inappropriately high FFA levels pouring out of the vastly expanded adipocyte mass and the hyperinsulinemia fails to lower them. This causes a reversal of the "master-servant" relationship between ß-cells and fat cells. Whereas normally the ß-cells are in command of the fat cells, promoting lipogenesis and inhibiting lipolysis in appropriate fashion, in obesity the adipocytes are the "boss", refusing to heed ß-cell commands and actually altering the expression of ß-cell genes through FFA-mediated signals (Figure 4).

FIG. 4: Who's the boss? Upper - The ß-cells. The ß-cells can inhibit the FFA flux from adipocytes by inhibiting HSL. FFA are low except during fasting and exercise, when they have little or no insulin-stimulating activity. Lower - The Adipocytes. In obesity insulin is unable to lower FFA even during meals, when they are potent insulin secretagogues. Despite the resulting hyperinsulinemia, FFA are abnormally elevated.



OBESITY, ß-CELLS AND INSULIN TARGET TISSUES

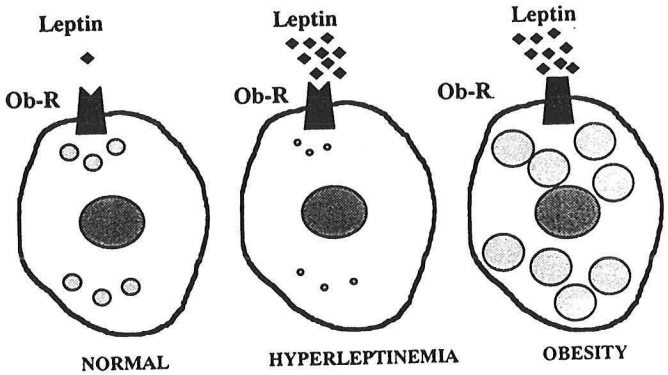
Obesity is a form of substrate overload. The normal subjugation of adipocytes to insulin-mediated commands wanes progressively as fat cells become larger and more numerous; the attenuation of the antilipolytic effect of the meal-induced rise in insulin secretion allows non-fasting FFA levels to remain at or above concentrations appropriate for the fasting state. Tissue levels of triglycerides and fatty acyl CoA become chronically elevated from local and circulating lipid sources (Table 1). In muscle this causes insulin resistance; in islets this increases insulin production to levels that compensate for the insulin resistance to insulin-mediated glucose metabolism by maintaining euglycemia but are unable to normalize lipolysis. We now have evidence that obesity is more than just an overaccumulation of fat in adipocytes -- nonadipocytes also become fat (Figure 5).

Table 2
Triglycerice Content in Islets of 6- and 12-week-old rats

		Islet Triglyceride (ng/islet)			% Change
	n	6 weeks old	n	12 weeks old	12 weeks old
Wistar male	6	0.01 ± 0.002	6	0.02 ± 0.01	100
ZDF *(fa/+)* male	6	0.04 ± 0.01	6	0.18 ± 0.01†	325
ZDF *(fa/fa)* female	6	0.13 ± 0.02*	6	0.28 ± 0.01†	210
ZDF *(fa/fa)* male	6	0.17 ± 0.01*	6	1.00 ± 0.03‡	688

*P<0.01. Wistar and ZDF *(fa/+)* lean vs. ZDF *(fa/fa)* male and female rats at 6 weeks of age; †P<0.01, Wistar vs. ZDF rats; ‡P<0.01, ZDF *(fa/fa)* female vs. ZDF *(fa/fa)* male rats.

FIG. 5: Hypothetical intracellular TG storage sites in non-adipocytes of normal obese and hyperleptin-emic rats.



How FFA induce ß-cell compensaton. The high rate of insulin production in compensated euglcyemic obesity is the result of the following FFA-induced responses in normal islets: 1) a 3-fold increase in low Km in glucose metabolism (Figure 6) which permits increased insulin production without hyperglycemia (Figure 7A); 2) an increase in proinsulin mRNA; and 3) a 4-fold increase in ß-cell replication (Figure 7B). These same 3 effects attributed to FFA *in vivo* can be induced *in vitro* by culturing normal islets in 1 or 2 mM FFA for 7 days (16). The *in vitro* effects of FFA are the same as the changes observed *in vivo* in islets of obese insulin-resistant rats: 1) 3-fold increase in low Km glucose metabolism; 2) an increase in proinsulin mRNA; and 3) a 4-fold increase in the volume fraction of ß-cells. We have proposed that FFA reaching the islets via plasma FFA and TG provide the means by which ß-cells compensate for FFA-induced insulin resistance (16,17).

FIG. 6: Glucose usage in islets from obese Zucker rats compared to lean Wistar rats. A similar 4-fold increase in glucose metabolism can be induced in Wistar rat islets by culturing them for 7 days in 1 and 2 mM FFA, concentrations found in the plasma of obese rats.

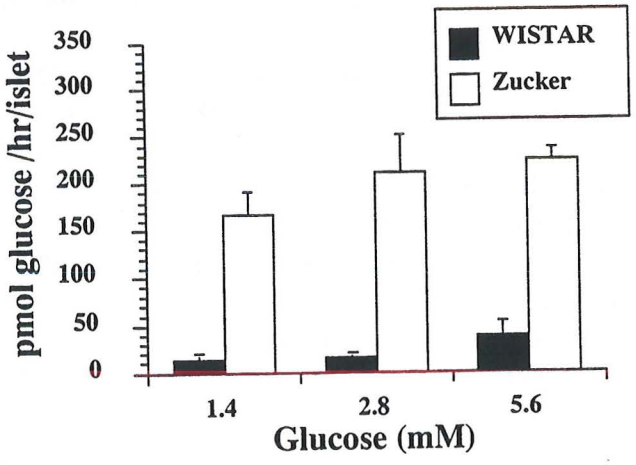
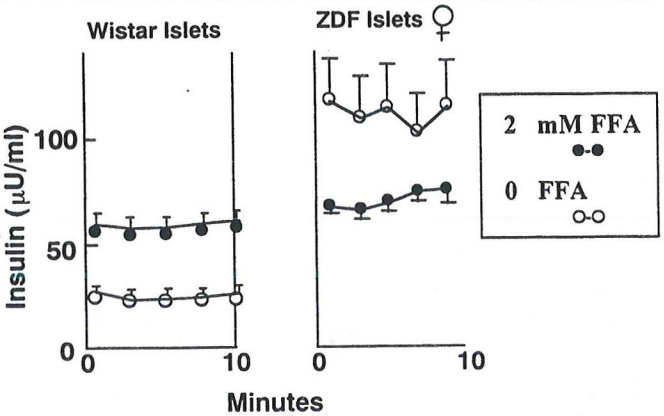
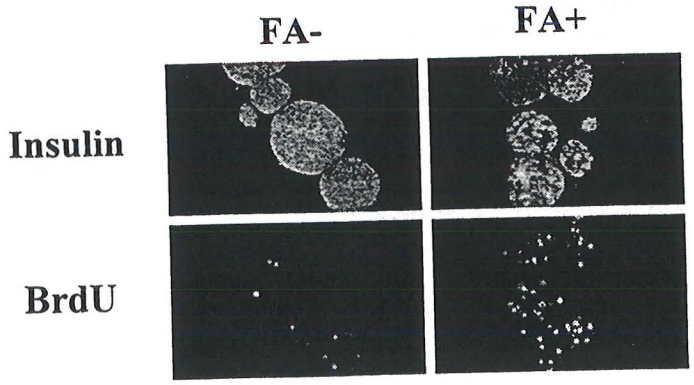


FIG. 7:
A) Comparison of insulin secretion by islets of lean Wistar rats after 7-day culture with either 0 or 2 mM FFA. Islets are then perifused with substimulatory levels of glucose (3 mM). The higher insulin secretion is attributed to the higher rate of glucose metabolism induced by the FFA (cf. Fig. 6).



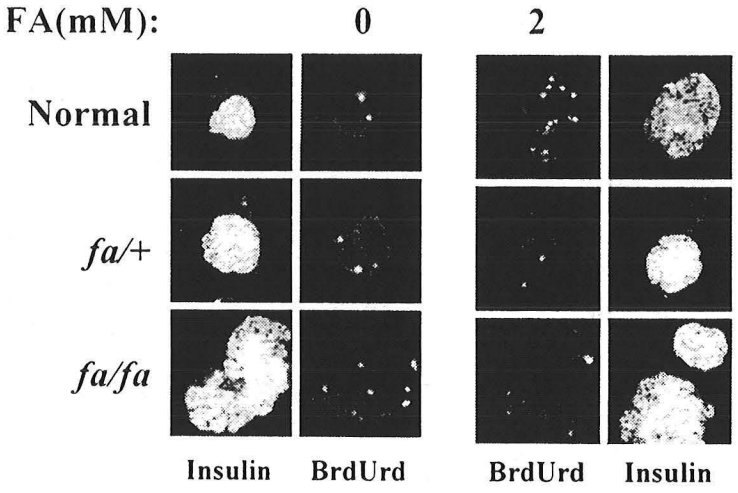
B) BrdU uptake in normal islets cultured with 0 or 2 mM FFA. FFA caused a 4-fold greater uptake by insulin-stained cells (these are contiguous sections). This index of cell replication suggests that FFA cause the 4-fold greater ß-cell volume found in obese ZDF rats.



What FFA do to islets of prediabetic rats. In cultured islets isolated from prediabetic obese animals destined to develop adipogenic diabetes, FFA *suppress paradoxically* every one of the foregoing compensatory responses that had been induced during the phase of compensation (18):

- They reduce the high rate of insulin production.
- They decrease the high rate of low Km glycolysis.
- They decrease proinsulin mRNA.
- They decrease the high rate of ß-cell replication (Figure 8).

FIG. 8:
BrdU uptake by islets of homozygous *(fa/fa)* and heterozygous *(fa/+)* ZDF rats compared with Wistar rats after 7 day culture with either 0 or 2 mM FFA. FAA have no stimulatory effect on islets of rats carrying even a single allele, evidence that a rise in FFA will not stimulate hyperplasia of ß-cells if even a single *fa* allele is present.



In other words, although initially ß-cells have successfully compensated for a given level of insulin resistance, a further increase in insulin resistance may not be accompanied by a parallel further increase in the foregoing compensatory adjustments by ß-cells; rather these adjustments *regress* and hyperglycemia appears.

We now know that this regression is temporally associated with a 10-fold increase in the triglyceride (TG) content of the islets (Figure 9) (19). TG in islets and in tissues all over the body are elevated in all obese rats whether or not they become diabetic, but rise even further as hyperglycemia appears (Figure 9). Lipids in the target tissues of insulin cause the insulin resistance; because TG content in islets is about the same as in the target tissues, insulin secretion rises to a level that maintains euglycemia; thus, the TG content in tissues both increases insulin need and signals the ß-cells as to the level of insulin need and conditions them to meet the need. But this TG-induced ß-cell compensation has a "ceiling". Once TG overload in ß-cells exceeds this ceiling then ß-cell function is paradoxially reduced, while further TG overload in target tissues only increases insulin resistance and insulin need and hyperglycemia ensues.

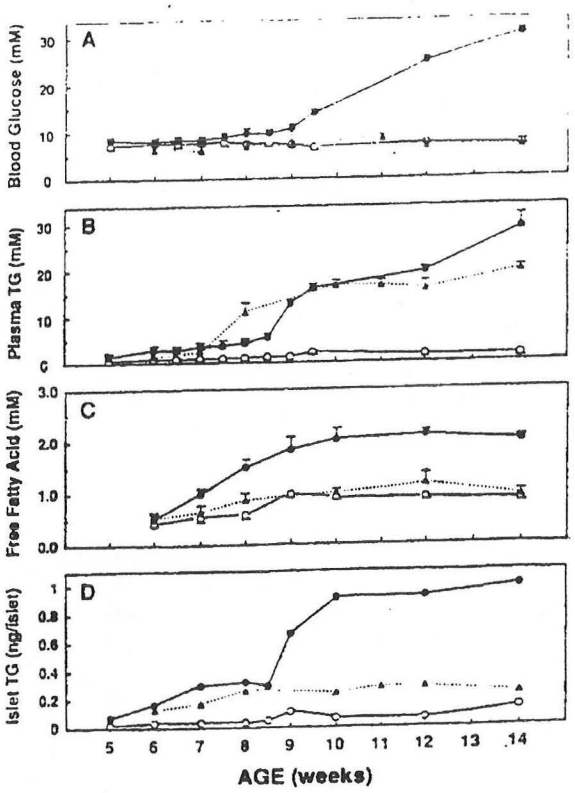
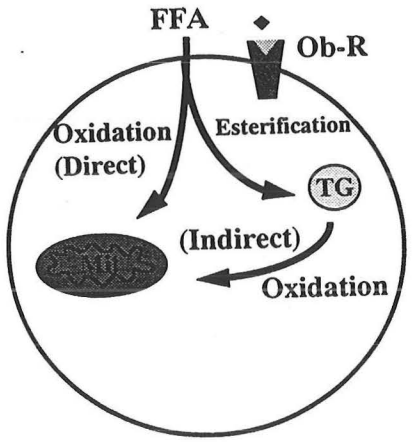


FIG. 9:
Longitudinal studies of blood glucose (Panel A), plasma triglycerides (Panel B), free fatty acid levels (Panel C) and triglyceride content of islets (Panel D). In lean male ZDF rats *(fa/+)* (-O-), obese female ZDF rats *(fa/fa)* that do not develop diabetes (-s-), and obese male ZDF rats *(fa/fa)* (-l-) that develop diabetes between the ages of 8 and 10 weeks.

THE INDIRECT FAA METABOLIC PATHWAY

Plasma FFA are taken up by cells and, according to current belief, are directly used as a fuel. We now have strong evidence, however, for an indirect pathway in which FFA are taken up and reesterified to TG, where they are stored for future purposes (Figure 10) (20).

FIG. 10:
The *indirect pathway* of FFA metabolism in non-adipocytes. As in adipocytes, FFA from plasma is taken up and esterified to TG, rather than directly oxidized. These intracellular stores can provide TG as required for energy and/or signalling. Leptin regulates this pathway, blocking esterification and enhancing oxidation, thereby reducing intracellular fat *(vide infra)*.



How intracellular TG content affects cell function. TG are the storage form of long chain fatty acids (FFA) deposited in adipocytes and hepatocytes for later export to other tissues as a fuel. Storage of TG is not generally recognized to be an integral component of cells other than adipocytes and hepatocytes. However, we have recently measured TG content in various tissues, skeletal muscle, heart, whole pancreas and islets of normal rats. After removal of adipocytes we find in normal rats a tissue TG content that ranges from 3 to 9 ng/g wet weight. In obese rats the tissue TG content is approximately 10 times higher. While contamination of muscle and whole pancreas with adipocytes cannot be excluded, it is extremely unlikely that isolated islet preparations would contain any adipocytes. We have observed a wide range in TG content of islets ranging from 0 in fat-depleted rats made lipopenic by leptin gene therapy, 23 ng/islet in normal rats and 230 ng/islet in obese rats (20,21) (Table 2). Since careful ultrastructural morphology to identify TG-containing bodies has not been completed, one cannot yet know how, or even if, the TG is diffusely distributed or arrayed as fat-containing inclusions, as depicted schematically in Figure 5.

Table 2
Islet TG from Hyperleptinemic Rats

	TG (µg/islet)
Hyperleptinemia	0
Pair Fed	0.014
ß-Gal	0.023

Intuitively, one would guess that putative fat pockets serve vital functions in the biology of the cell irrespective of the ultrastructural anatomy. First, they may provide certain cells with a reservoir of fuel independent of the variable plasma concentrations of FFA. Second, FFA may serve as a second messenger and, in fact, are known to induce expression of a number of genes including insulin and inducible nitric oxide snythase (iNOS) (22).

LESSONS FROM HYPERLEPTINEMIC, LIPOPENIC RATS

Function of intracellular FFA. Evidence that the intracellular fat stores are real and constitute an active source of FFA that plays a vital role in the function of certain has come from the hyperleptinemic rat (20). This model teaches us that these stores are under the control of leptin, i.e., that controls fat content, not only in adipocytes, but in other cells as well. We have produced a syndrome of chronic hyperleptinemia (Figure 11) in normal rats by infusing a recombinant adenovirus containing the leptin cDNA (Adv-CMV-leptin) (20). In these rats leptin is overexpressed ectopically in liver.

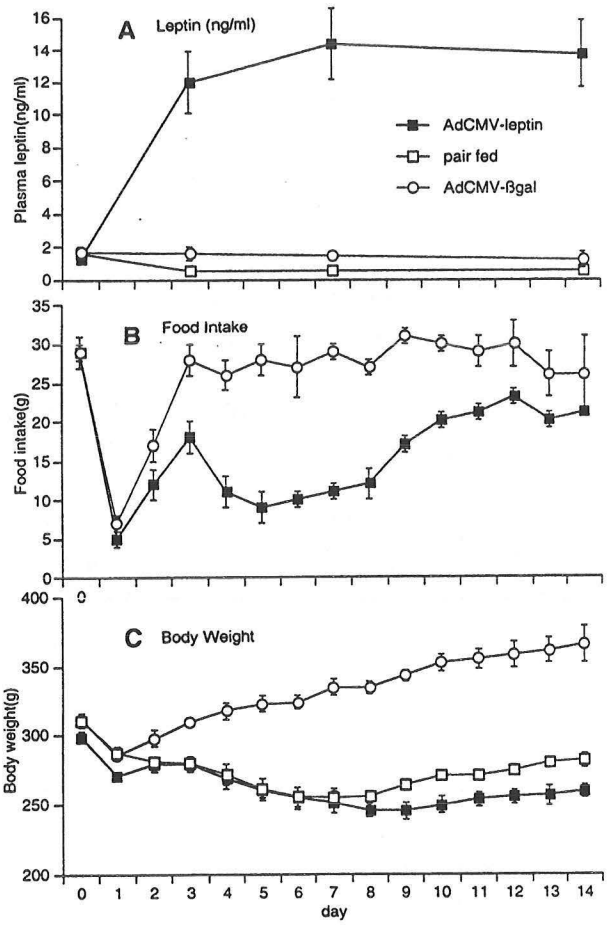


FIG. 11:
Comparison of A) plasma leptin levels; B) food intake; and C) body weight in Wistar rats infused with recombinant adenovirus containing the leptin cDNA (AdCMV-leptin) or adenovirus containing an irrelevant ß-galactosidase cDNA (AdCMV-ßGal) as a control. A third group of Wistar rats were pairfed to the AdCMV-leptin-infused rats.

Leptin is normally expressed only in adipocytes and is currently considered to act only on the hypothalamus. There it reduces food intake by suppressing neuropeptide Y and by increasing thermogenesis via sympathetic nerves (23). After 1 week of hyperleptinemia, all visible body fat disappears and the TG content in tissues declines to unmeasurable levels. At this point insulin levels fall to 25% of normal, but blood glucose drops sharply because of concomitant lipopenia in the tissues' target, these hypoinsulinemic animals are so insulin-

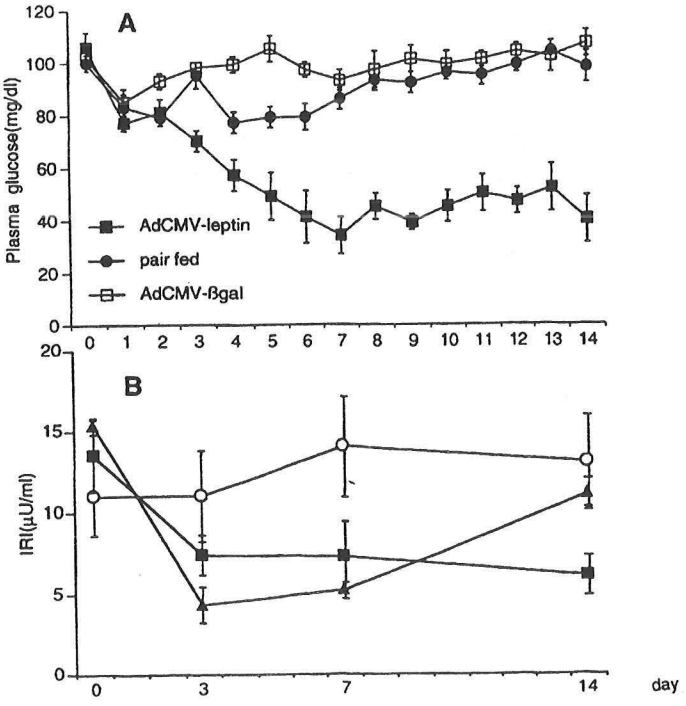


FIG. 12:
A) Plasma glucose levels in AdCMV-leptin; and *B)* plasma insulin levels (IRI) in AdCMV-leptin-infused rats and pairfed and AdCMV-ßGal controls.

sensitive that they become hypoglycemic (Figure 12). If one perfuses the pancreas of hyperleptinemic lipopenic animals, the response to glucose and arginine is completely absent (Figure 13A). In other words, ß-cells devoid of intracellular fat cannot respond to stimuli. Morphologically these ß-cells are perfectly normal in both number and in appearance. When FFA are coperfused, all responses are instantly restored to supranormal levels (Figure 13B). This ß-cell "paralysis" must be due to loss of a FFA signal rather than lack of fuel, since glucose perfusion should have provided ample fuel and yet failed to restore ß-cell function. And the FFA-mediated resurrection of ß-cell function was rapid -- again pointing to a role as a signal rather than as a fuel. When both the intracellular and extracellular sources of TG are depleted by chronic hyperleptinemia, ß-cell function is annulled. Stein et al. have demonstrated previously the same effect in fasted rats deprived of adequate plasma FFA by antilipolytic drugs (24).

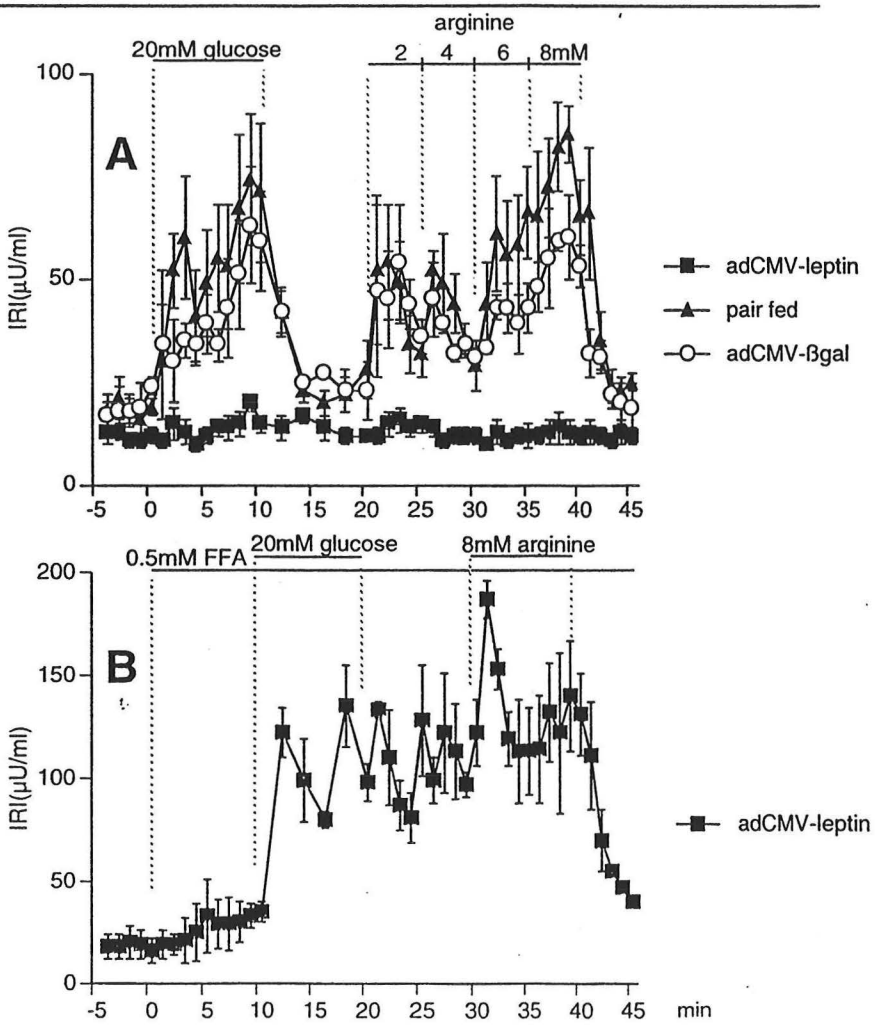
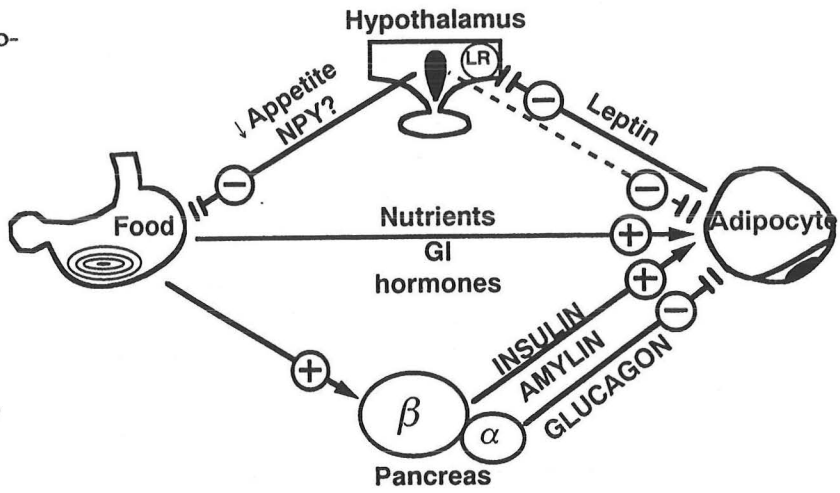


FIG. 13:
A) Comparison of insulin secretion by the perfused pancreas isolated from AdCMV-leptin-infused rats, pairfed controls and AdCMV-ßGal controls. *B)* The coperfusion of 0.5 mM FFA corrects the unresponsiveness of the hyperleptinemic rats, strongly suggesting that FFA or a metabolic derivative is required for the ß-cell response to other nutrients.

Leptin and the indirect pathway. Although current dogma regards the hypothalamus as the only target of leptin (Figure 14), we have compelling evidence that the intracellular TG content in many tissues is directly regulated by leptin.

FIG. 14:
Current concept of leptin pathway for controlling body composition. Leptin is secreted from adipocytes and acts on a long receptor isoform (LR), known as OB-Rb, in the hypothalamus to suppress neuropeptide Y (NPY), an appetite-stimulating peptide. This reduces food intake. Also leptin stimulates nuclei for sympathetic connections to brown fat and thereby increases thermogenesis. A high food intake, either directly or by stimulating insulin secretion, increases leptin secretion.



1. In the hyperleptinemic rat the disappearance of body fat is even more rapid than in total insulin deficiency, and yet, neither FFA nor ketoacids rise in the plasma as in insulin deficiency (Figure 15). This must mean that the FFA are not leaving the cells but are being oxidized intracellularly, i.e., these cells are behaving like brown fat. This must be happening in all cells -- not just adipocytes and most, if not all of these cells have leptin receptors (Table 3).
2. When normal islets are cultured in the presence of leptin, their TG content falls, FFA esterification declines and FFA oxidation rises (Figure 16). Thus, leptin normally regulates the intracellular TG pool by restraining esterification of extracellular FFA and enhancing intracellular lipolysis and FFA oxidation.
3. ZDF rats have a defect in the leptin receptor (OB-R) (Figure 3). Leptin does not lower their elevated TG content (Figure 17). Also, hyperleptinemia induced in ZDF rats by infusing AdCMV-leptin has no effect on body weight (Figure 18), and does not lower the TG content of their islets or any other tissue. Islets isolated from prediabetic ZDF rats have a higher esterification capacity for FFA and a diminished FFA oxidative capacity which is not enhanced by leptin (25) (Figure 17). This is why they accumulate triglycerides at such an abnormally high rate when cultured in FFA (Figure 19) (25). The consequences of too much and too little leptin action are depicted in Figure 20.

Since this mutation causes both the obesity and the diabetes, it strongly implies that TG overload, the only known cellular consequence of the mutation, must be the etiologic factor, causing both the insulin resistance and the ß-cell dysfunction. Leptin action markedly enhances insulin sensitivity to the point of causing hypoglycemia despite hypoinsulinemia (Figure 12), so it is clearly anti-diabetic. It follows that when leptin action is absent because of leptin resistance, as in the ZDF rat, TG overload in muscle causes insulin resistance in peripheral tissues, while TG overload in islets somehow causes ß-cell dysfunction.

FIG. 15:
Comparison of plasma FFA and ß-hydroxybutyrate (ß-OH) and urinary ketones in hyperleptinemic rats and pairfed and AdCMV-ßGal-infused controls. FFA and ß-OH do not rise while body fat disappears and there is no ketonuria, evidence that FFA, stored as TG, are being oxidized intracellularly. Note the rise in all 3 parameters in pairfed controls.

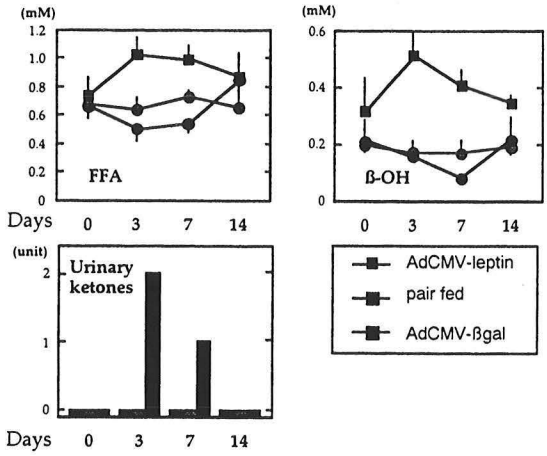


Table 3
Tissue-specific Expression of Leptin Receptors

Isoform a: Testis > Hypothalamus > Adipose tissue > Brain > Heart

Isoform b: Hypothalamus > Brain > Stomach ≈ Pancreas > Heart ∞ Testis > Skeletal muscle > Bone marrow ≈ Small intestine > Thymus ≈ Fat ≈ Mammary gland > Liver > mesent. lymph node ≈ Uterus > Spleen ≈ Periph. lymph node ≈ Lung.

Isoform c: Adipose tissues >> Heart > Testis > Liver ≈ Brain > Kidney ≈ Hypothalamus > Small intestine.

Isoform d: Adipose tissues > Testis >> Heart ≈ Small intestine > Brain > Hypothalamus.

Isoform e: Heart ≥ Adipose tissues > Hypothalamus ≈ Testis ≥ Brain.

Isoform f: Spleen ≈ Liver > Stomach ≈ Lung ≥ Hypothalamus ≈ Kidney > Brain > Thymus

Isoform g: $CD34^+$ stem cells

Isoform h: Human granulocytic erythroleukemia hematopoietic cell line > Murine leukemia hematopoietic cell line > Murine lymphoid T cells > Human myelogenous leukemia hematopoietic cell line ≈ Human lymphoid B cells.

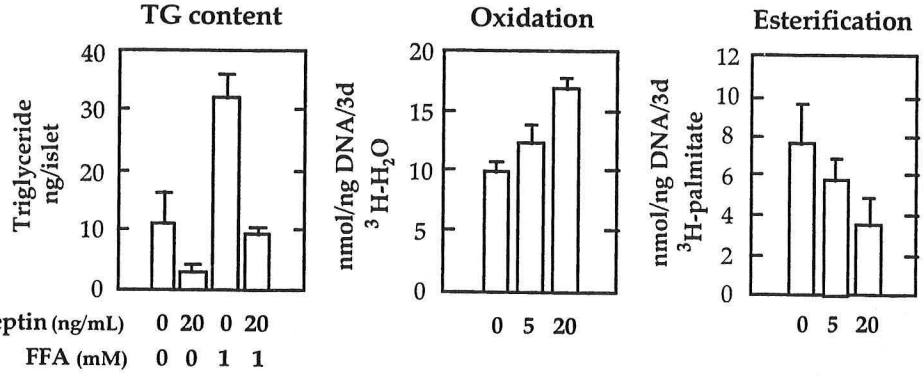


FIG. 16:
Effect of leptin on endogenous triglyceride content of normal rat islets cultured for 3 days and on their TG content when FFA are in the culture medium (left). Leptin effects on the oxidation (center) and esterification of ^{3}H-palmitate (right) account for the effects.

FIG. 17:
Effect of leptin on triglyceride content (upper), ^{3}H-palmitate oxidation (center) and esterification (lower) in islets of lean wild type ZDF *(+/+)* rats and lean heterozygous *(fa/+)* and obese homozygous *(fa/fa)* rats. Note the unresponsiveness to leptin in the latter group.

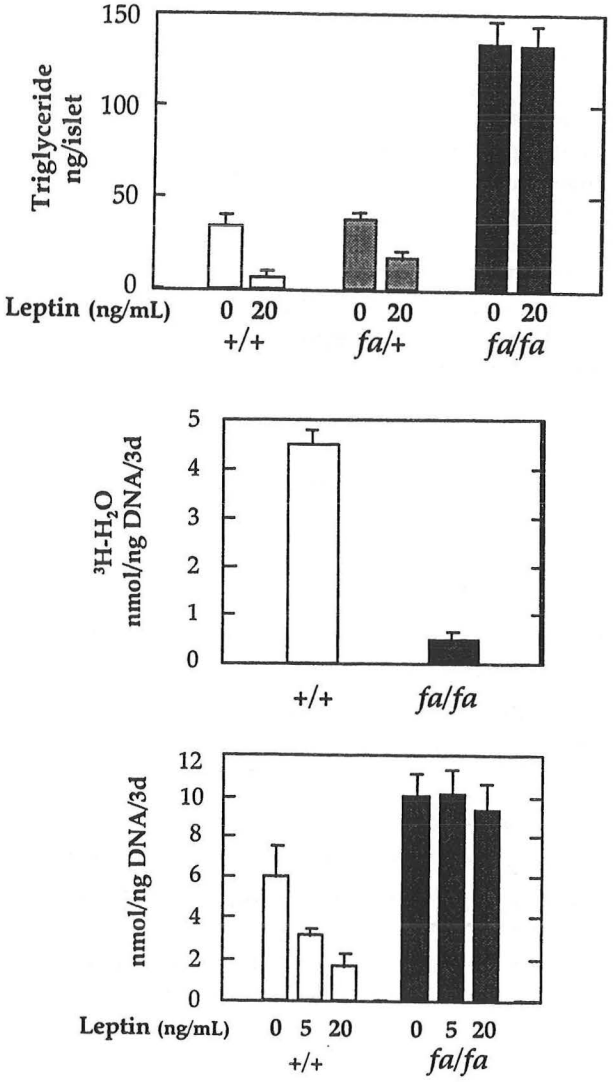


FIG. 18:
Comparison of food intake and body weight before and 14 days after AdCMV-leptin infusion, pairfeeding of intact rats to the hyperleptinemics and after AdCMV-ßGal control infusion in normal Wistar, lean heterozygous and obese homozygous rats.

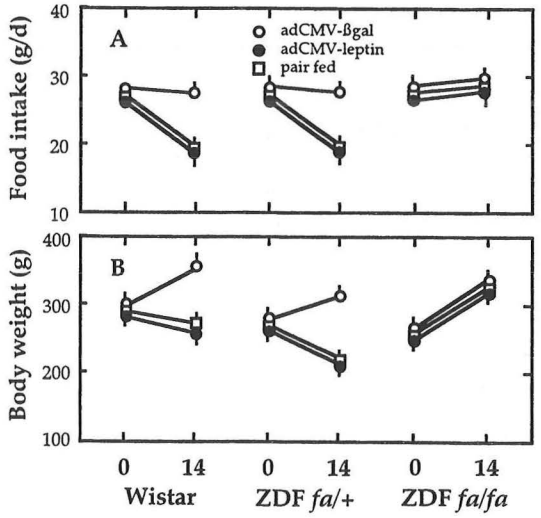
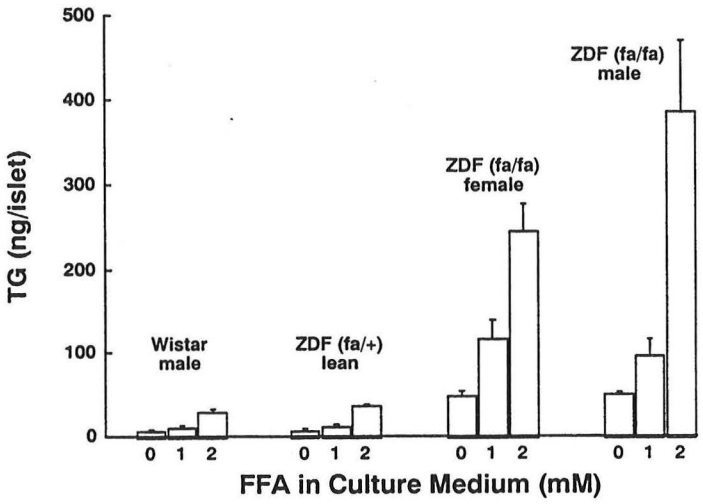


FIG. 19:
Comparison of TG content in islets of Wistar, lean and obese ZDF rats cultured for 7 days in 0, 1 or 2 mM FFA.



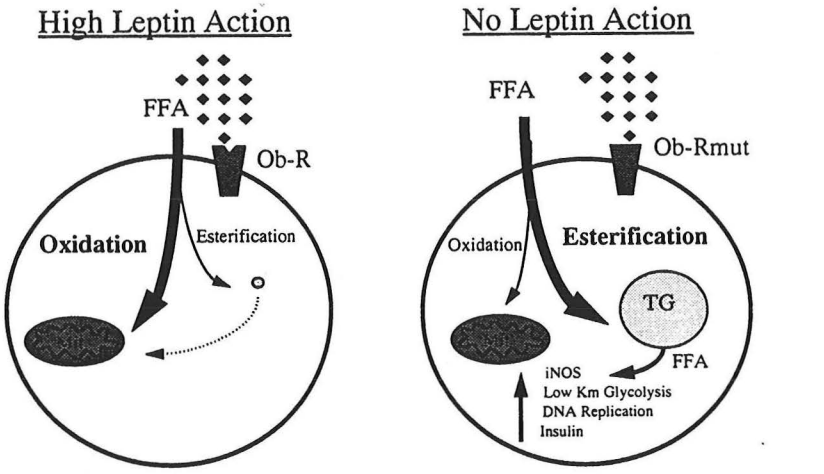


FIG. 20:
Comparison of intracellular FFA metabolism in normal nonadipocytes exposed to hyperleptinemia and cells of ZDF rats in which a mutated leptin receptor (Ob-Rmut) prevents leptin action.

HOW FFA CAUSE DYSFUNCTION AND DEATH OF ß-CELLS

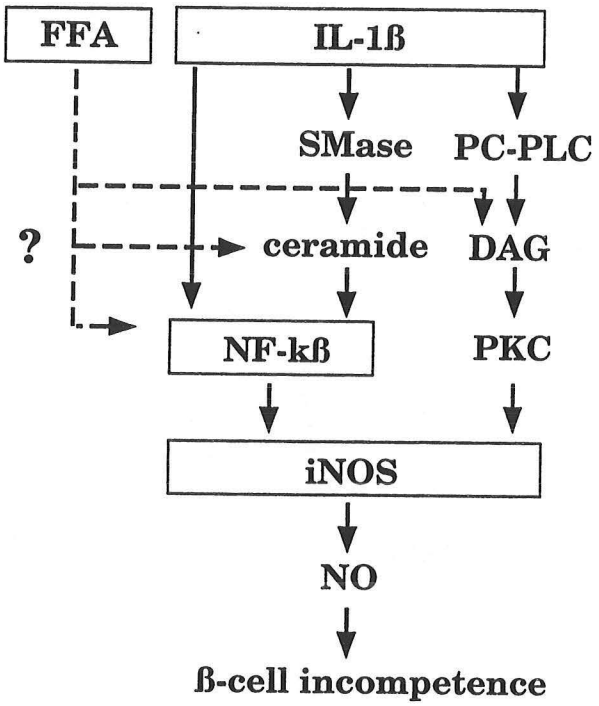
The ZDF diabetes is associated with the following abnormalities of ß-cells:

a) basal hyperinsulinemia (26);
b) loss of glucose-stimulated insulin secretion (26);
c) reduction (~75%) in ß-cell volume fraction (27); and
d) loss of ß-cell GLUT-2 (28).

These changes coincide with the rise in islet TG. How might increased lipids cause these changes? Are they secondary to the glucose-fatty acid cycle (29-31) as currently believed? It seems unlikely that FFA-induced interference in glucose metabolism could cause chronic changes of this magnitude, particularly

the disappearance of 75% of ß-cells. Rather, these islet changes are somewhat reminiscent of very early autoimmune diabetes; before the ß-cell destruction of autoimmune diabetes is complete all of the above-mentioned abnormalities are present (32) -- except the high TG content. Autoimmune ß-cell damage is IL-1ß-induced (33) and is thought to be nitric oxide (NO)-mediated (34), at least in rats. We, therefore, studied the effects of FFA on NO generation in cultured islets from normal and prediabetic rats (Figure 21). We found that: 1) FFA

FIG. 21:
Theoretical scheme in which FFA, like IL-1ß, induce iNOS and cause ß-cell dysfunction and damage similar to that in autoimmune diabetes.



increase NO generation (Figure 22). 2) FFA induce inducible NO synthase (iNOS) (Figure 23). 3) FFA induce iNOS to much higher levels in islets of prediabetic rats than in those from normal rats (35).

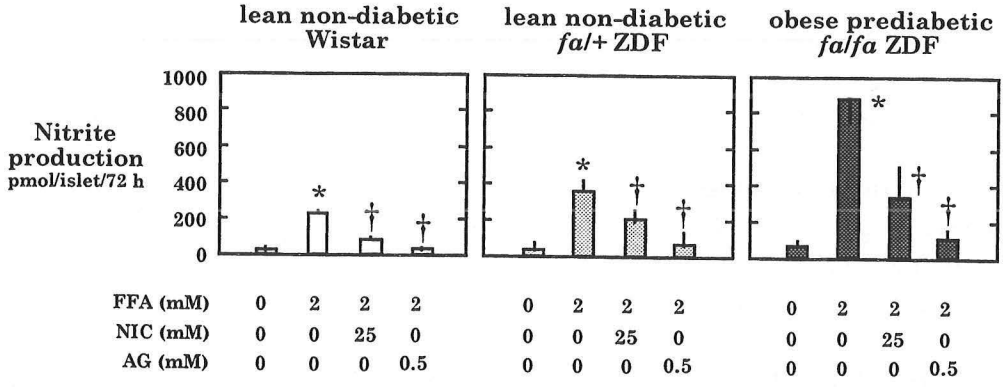


FIG. 22:
Effect of nicotinamide (NIC) and aminoguanidine (AG) on FFA-induced nitrate production in 72 h cultured islets isolated from normal Wistar and *fa/+* and *fa/fa* ZDF rats.

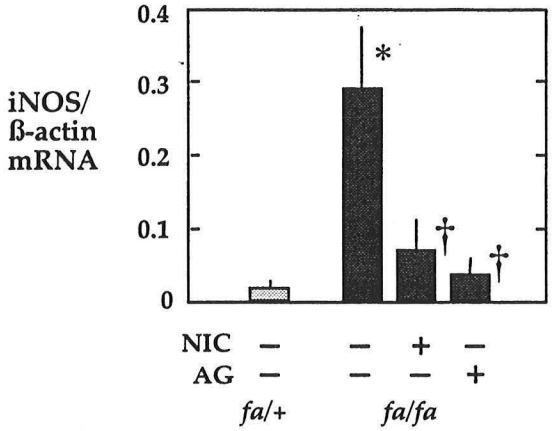


FIG. 23:
Effect of nicotinamide (NIC) and aminoguanidine (AG) on iNOS mRNA expression in islets of obese prediabetic *fa/fa* ZDF rats.

How can a leptin receptor mutation amplify the iNOS-inducing action of FFA? Perhaps the higher TG content of these cells simply adds more endogenous FFA to that entering from the culture medium. Alternatively and more likely, the increase in leptin-mediated oxidation of FFA, which is absent in the leptin receptor-defective prediabetic islets, protects against excessive FFA-mediated induction of iNOS, by reducing ceramide and/or DAG, both of which can induce iNOS (Figure 21) or via mechanisms that have not yet been discovered.

EFFICACY OF ANTI-NO THERAPY

The role of NO in producing ß-cell dysfunction death in adipogenic diabetes is supported by the following evidence:

1. Suppressors of iNOS expression, nicotinamide (NIC) (36) and aminoguanidine (AG) (37) and competitive inhibitors of iNOS, N^E arginine methyl ester (NAME) (38) all reduce FFA-induced NO production and attenuate the FFA-induced loss of ß-cell function *in vitro* (Figures 22, 23). They also restore ß-cell function to near normal (Figure 24; Table 4).
2. Daily injections of NIC or AG in prediabetic ZDF rats prevent the 20-fold increase in iNOS that occurs in islets of sham-treated prediabetic control rats (Figure 23). Both agents also completely prevent all of the NIDDM phenotype (Figure 25): 1) Development of hyperglycemia and abnormal glucose tolerance (Figure 26); 2) Loss of glucose-stimulated insulin secretion; 3) Loss of ß-cell GLUT-2 (Figure 26); 4) Loss of ß-cell volume fraction. In other words adipogenic diabetes is completely delayed or prevented by inhibiting iNOS overexpression.

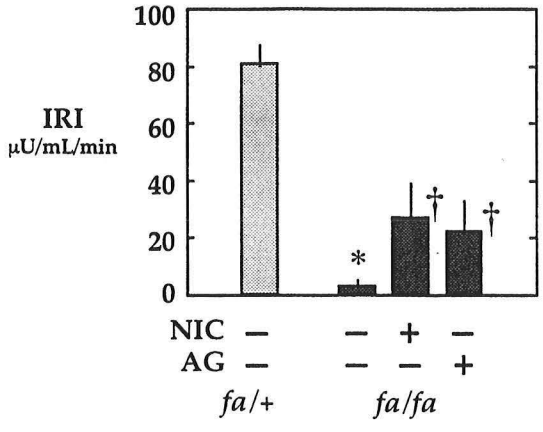


FIG. 24:
Effect of nicotinamide (NIC) and aminoguanidine (AG) on incremental insulin secretion to 20 mM glucose in pancreata isolated from homozygous *(fa/fa)* ZDF rats.

Table 4
Insulin Secretion by Pancreata Isolated from *fa/+* and *fa/fa* ZDF rats treated with NIC or AG

	FFA 0 mM	FFA 2 mM	FFA 2 mM + NIC 25 mM	FFA 2 mM + NAME 1 mM
Wistar				
3 mM glucose	2.30 ± 0.16	6.68 ± 0.32	5.15 ± 0.50	9.05 ± 0.73
23 mM glucose	5.17 ± 1.17	12.56 ± 1.58	13.24 ± 3.04	16.12 ± 1.62
***fa/+* ZDF**				
3 mM glucose	4.37 ± 0.66	2.49 ± 0.22	6.18 ± 0.93	5.10 ± 1.34
23 mM glucose	5.36 ± 1.18	2.69 ± 0.32	7.78 ± 1.84	6.52 ± 1.93
***fa/fa* ZDF**				
3 mM glucose	9.59 ± 0.23	6.66 ± 0.23	8.93 ± 0.48	7.54 ± 0.29
23 mM glucose	10.12 ± 0.22	6.34 ± 0.16	12.14 ± 0.45	11.24 ± 0.72

µU/mL/50 islets/min

FIG. 25:
Effect of nicotinamide (NIC) and aminoguanidine (AG) on plasma glucose and free fatty acids (FFA) in obese prediabetic *fa/fa* ZDF rats.

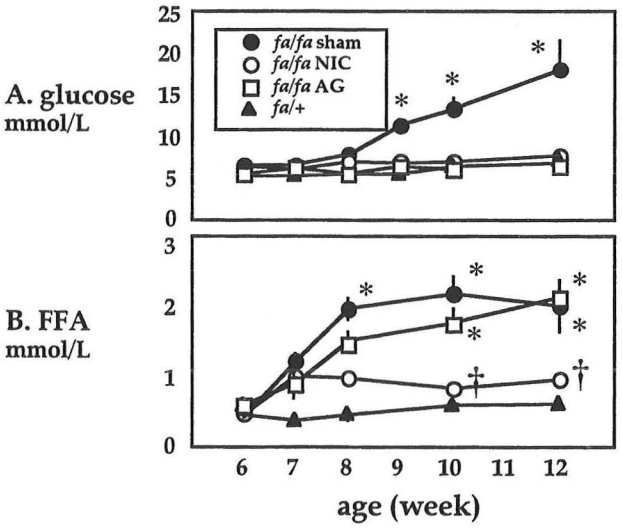
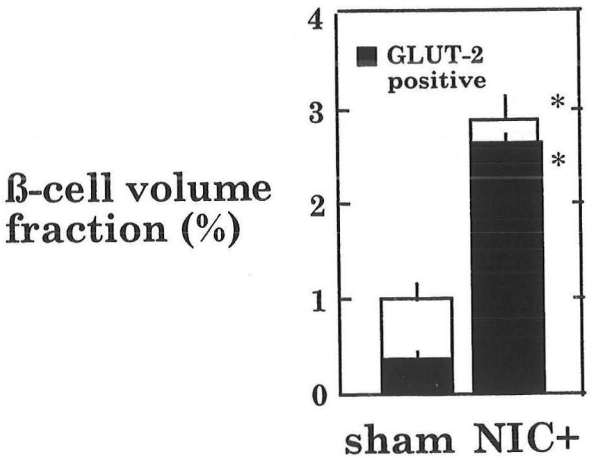


FIG. 26:
Effect of nicotinamide (NIC) on ß-cell volume fraction and GLUT-2 positivity in obese *fa/fa* ZDF rats.



THE CONCLUSIONS AND PRACTICAL IMPLICATIONS OF THIS RESEARCH

1. Identification of the Indirect Pathway of FFA Metabolism. Nonadipocytes are able to store FFA as TG and retrieve them for oxidation or other purposes.
2. Extraneural sites of leptin action. The indirect pathway is regulated by leptin via a direct action.
3. Prevention and Treatment of Adipogenic Diabetes with Antilipolytic and/or Anti-NO drug. Agents that lower plasma FFA and/or reduce TG in islets and in target tissues of insulin and/or reduce islet NO should prevent or attenuate adipogenic diabetes.
4. Fat-free Farm Animals. The rat model of adenovirus-mediated hyperleptinemia demonstrates the feasibility of total depletion of body fat in a small animal. If a similar result would occur in poultry and in larger animals that are used for human consumption, it should be possible to produce low-fat or fat-free meat at a fraction of the cost of injecting recombinant leptin.
5. Treatment of Human Obesity. Although leptin or leptin receptor mutations have not been reported in human obesity, it is clear that obese humans are markedly resistant to their own elevated plasma leptin levels (Figure 27) and thus accumulate fat. It is possible that by inducing more marked hyperleptinemia the resistance would be overcome and obesity would recede. While recombinant leptin injections would undoubtedly be preferable to adenovirally induced hyperleptinemia in the treatment of humans; it is possible that cell-based delivery systems will produce a more active product than the injectible product. If so, this approach would be indicated in severe obesity with life-threatening complications such as cardiopulmonary disease, uncontrolled diabetes and/or hypertension which do not respond to conventional therapy (as predicted in the Case Report). Perhaps oral agents will emerge to produce leptin-like lipopenia while bypassing the resistance to endogenous leptin.
6. Prevention of Autoimmune Diabetes. Speculation: Does the normal islet TG content, together with normal plasma FFA potentiate IL-1ß-mediated iNOS induction and ß-cell apoptosis in autoimmune diabetes? If so, leptin-induced lipopenia might protect ß-cells from autoimmune destruction.

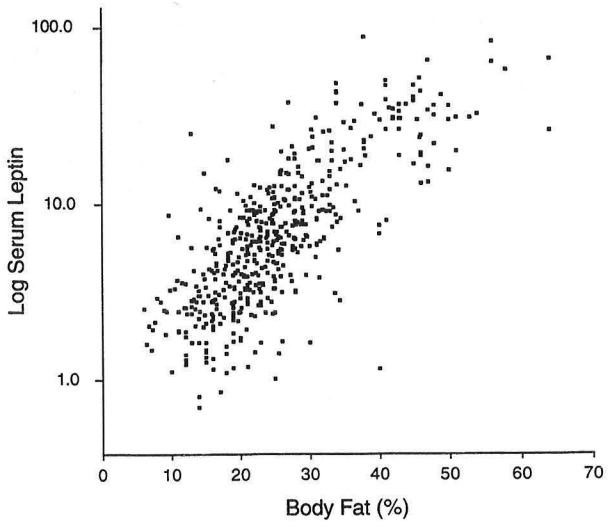


FIG. 27:
Relation between percent body fat and serum leptin in humans.

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