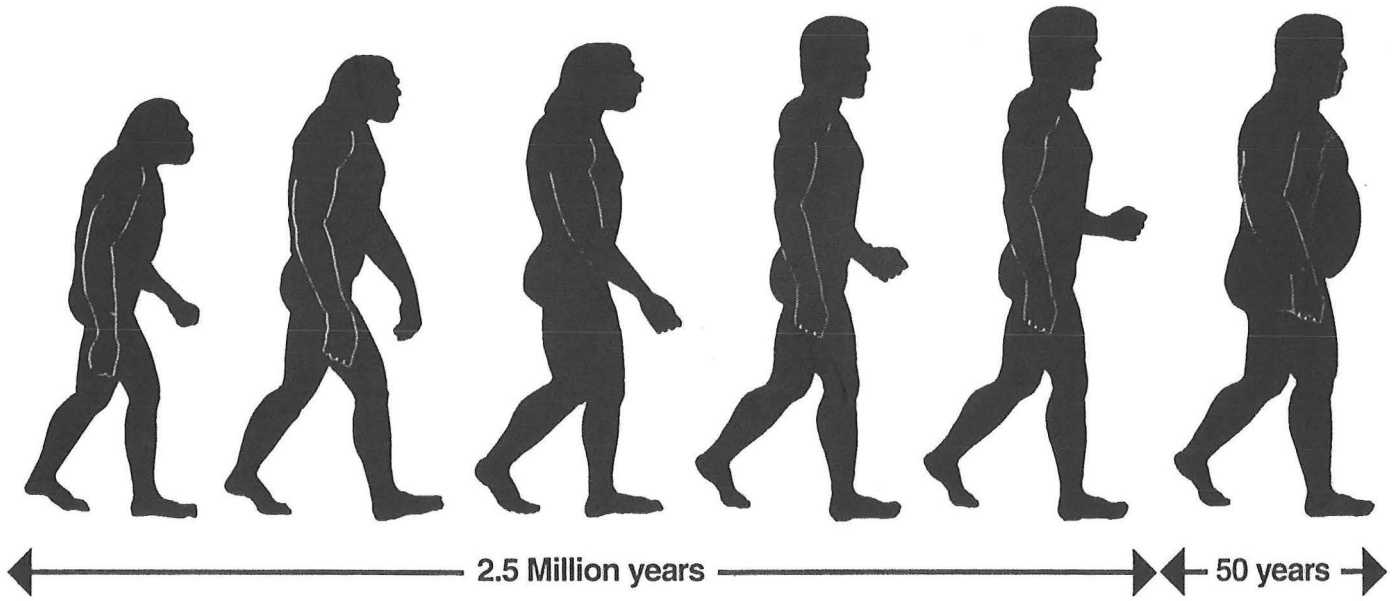
MEDICAL GRAND ROUNDS

September 30, 1999

Lipotoxic Disorders: America's impending clinical crisis



Roger H. Unger, M.D.

This is to acknowledge that Roger H. Unger, M.D., has disclosed no financial interests or other relationships with commercial concerns related directly or indirectly to this program.

Dr. Unger is Director of the Touchstone Center for Diabetes Research and holds the Touchstone-West Distinguished Chair in Diabetes Research. He is a Professor of Internal Medicine at UT Southwestern and was Senior Medical Investigator at Dallas Veterans Affairs Medical Center.

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

THE MAGNITUDE OF THE PROBLEM (Figure 1):

The United States is in the throes of a historically unprecedented pandemic of excess weight that now affects more than 50% of our population **(Fig. 1).** For the first time in history, normal individuals with a body mass index below 25 have become a minority on our continent. Despite the widespread recognition that obesity is very common, neither the gravity nor the mechanism of the problem is fully appreciated.

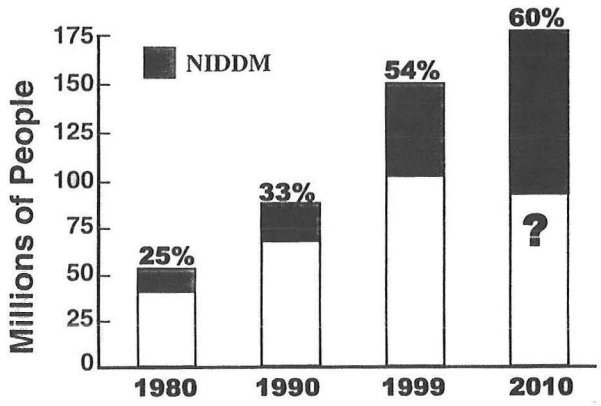


Figure 1 – The number and % of Americans with a body mass index in excess of 25. The densely shaded area gives the approximate proportion of overweight individuals with non-insulin dependent diabetes mellitus. The values on top of each bar indicate the % of overweight individuals in the U. S.

THE GRAVITY OF THE PROBLEM (Table 1):

While obesity itself profoundly lowers the quality of life for its sufferers, the morbidity, mortality and health cost consequences of the disorder are the result of so-called "complications" of obesity involving tissues other than adipocytes **(Table 1).** Current statistics place the overall incidence of such complications at no more than 20%, but this ignores the probability that the increase in the duration and/or severity of the obesity will raise the incidence of complications above current levels. Now that juvenile-onset obesity has risen to > 30% in the U.S. we expect the incidence of complications to be higher in the coming generation of obese American adults than it is in the present one. Inasmuch as ~ 50% of Pima Indians, a tribe with juvenile-onset obesity, develop complications such as diabetes (~ 70% of the 55-64 year-old age group have diabetes) (1), a 50% incidence would be a reasonable expectation in juvenile onset obesity.

TABLE 1 OBESITY-RELATED "COMPLICATIONS"	
1. Cancers of colon	4. Gallbladder Disease
2. Vascular Diseases	5. ↑Severity of Autoimmune Disease
a. Coronary artery disease	6. Impaired Respiratory Function (Pickwickian Syndrome)
b. Cardiac syndrome X	7. Pancreatitis
c. Cardiomyopathy	8. *Liver Disease*
d. Hypertension	9. *Insulin Resistance*
e. Cerebrovascular disease	10. *Type II Diabetes*
3. Carpal Tunnel Syndrome	11. Sarcopenia of Aging

MECHANISM OF THE PROBLEM

The mechanism of the complications of obesity has not been determined. As an explanatory link between the increase in adipose tissue and the dysfunction and damage to non-adipose tissues, we have hypothesized ectopic overaccumulation of triglycerides (TG) and other lipids overflowing into tissues that have only a very limited storage capacity for TG. In contrast to the tight regulatory control over the translocation of cholesterol across plasma membranes (1), FA can "flip-flop" across the lipid bilayer of plasma membranes and enter cells without the help of transporter proteins (2), particularly when plasma FFA concentrations are elevated, as they are in obesity (3). Some cells can rid themselves of excess FA; hepatocytes can secrete FA as VLDL and myocytes can dispose of excess FA by increased muscular exercise, but most other nonadipose tissues have no means of divesting themselves of unwanted FA other than through increased β-oxidation in mitochondria and peroxisomes and possibly omega-oxidation in microsomes. These tissues must therefore rely entirely upon a putative homeostatic system to prevent overaccumulation of lipids by increasing the rate of FA oxidation and dissipating the energy produced in the form of heat.

We hypothesize: 1) that such a homeostatic system normally exists in all cells; 2) *that leptin is the regulator of this system*; 3) *that failure of this protective system of intracellular FA homeostasis is the cause of certain complications of obesity; 4) that the complications of obesity* ***(Table 1)*** *and of other conditions* ***(Table 2)*** *are the result of lipid spillover into nonadipocytes (steatosis)*

TABLE 2 - DISORDERS OF INTRACELLULAR OF HOMEOSTASIS OF FA (STEATOSIS)

CONDITION	SEVERITY	OBESITY
GENERALIZED LIPODYSTROPHY	++++++	NO
LEPTIN DEFICIENCY	+++	YES
LEPTIN UNRESPONSIVENESS	+++	YES
DIET-INDUCED OBESITY (DIO)	+	YES
AGING	+	OFTEN

THE EVOLUTIONARY HISTORY OF ADIPOCYTES AND OBESITY

To comprehend fully the teleology of this putative homeostatic system for nonadipocytes, it is necessary to consider the functions and evolutionary impact of the adipocyte (4).

Life before adipocytes: Before the adipocyte had evolved, cellular life must have been confined to a "caloric prison", i.e., any separation in time or distance of a cell from its fuel supply would result in rapidly fatal "starvation". Consequently, in that era FA were precious and were used only as building blocks for phospholipids of cell membranes and intracellular signals, and not as fuel. A small stockpile of FA was almost certainly present in each cell, probably in the form of triglycerides (TG) **(Fig. 2),** but its purpose was to provide an FA reserve for maintenance of existing cell membranes, formation of new phospholipid bilayers during replication, and maintenance of phospholipid signals, rather than to serve as a fuel. It may be assumed that such cells were incapable of storing the much larger quantities of TG that would have been required to permit the use of FA as a fuel, since overaccumulation of TG in most nonadipocytes leads to their dysfunction and demise (5), as does TG depletion (6, 7) **(Fig. 2).**

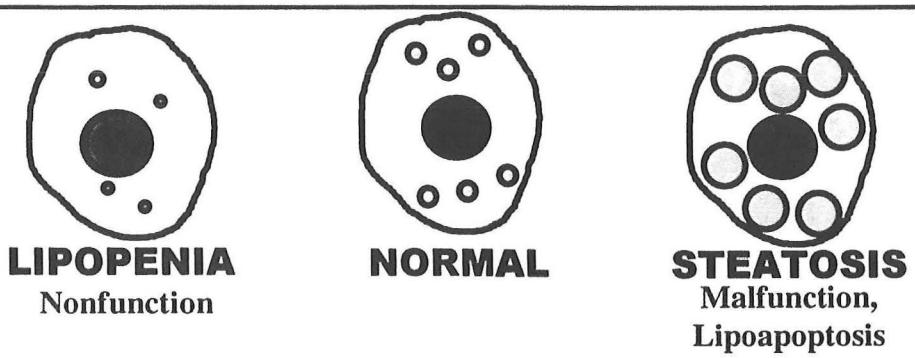


Figure 2 – Depiction of the concept of intracellular FA homeostasis. It is likely that all living cells have a small stockpile of TG represented by the circles in the cytoplasm (center panel). If this stockpile is depleted (lipopenia), cellular function may be impaired; if the storage capacity for TG is exceeded (steatosis), the cells may malfunction and undergo lipoapoptosis. We hypothesize that the normal TG stockpile of nonadipocytes is intended, not to serve as a fuel, but rather to provide FA building blocks for the phospholipids of cell membranes and cell signals.

The advent of the adipocyte: The advent of the adipocyte may well have been the single most transforming event in all of evolution, because it permitted the storage of almost unlimited quantities of the most efficient fuel, FA, and their retrieval according to need **(Table 3).** In the short term, this allowed the food-gathering process to be compressed into discrete meals; in the longer term, it permitted periodic preloading of calories to produce temporary obesity as a means of coping with future famine and/or marked increases in caloric expenditure. Thus, the desert rat could become obese during the rainy season, when food is plentiful, and survive the long desert summer, when food is scarce. Polynesians could preload calories and become temporarily obese prior to rowing 2,000 miles across the Pacific in outrigger canoes to reach Hawaii. For the first time, life forms could engage in activities other than simple food-gathering and procreation, moving more freely around the planet to pursue their particular destinies (8).

TABLE 3: SOME ADIPOCYTE FUNCTIONS
1. Storage of excess calories as TG.
2. Redistribution of stored FA on demand.
3. Protection of nonadipocytes from FA overload (putative).

Of the adipocyte functions listed in **Table 3**, FA storage and FA redistribution are obvious. The third function, protection of nonadipocytes from overaccumulation of TG during caloric overloading or antisteatosis, has recently been proposed by our group (9). We hypothesize that adipocytes secrete a substance that regulates intracellular FA homeostasis in nonadipose tissues, thereby preventing lipotoxicity. That substance is leptin.

THE EVIDENCE THAT LEPTIN REGULATES FA HOMEOSTASIS

1) Leptin rises promptly **(Fig. 3B inset)** upon caloric overloading (60% fat diet) and increases progressively (**Fig. 3B)** in parallel with the rise in TG content of adipocytes **(Fig. 3A).** They are very tightly correlated **(Fig. 3C).**

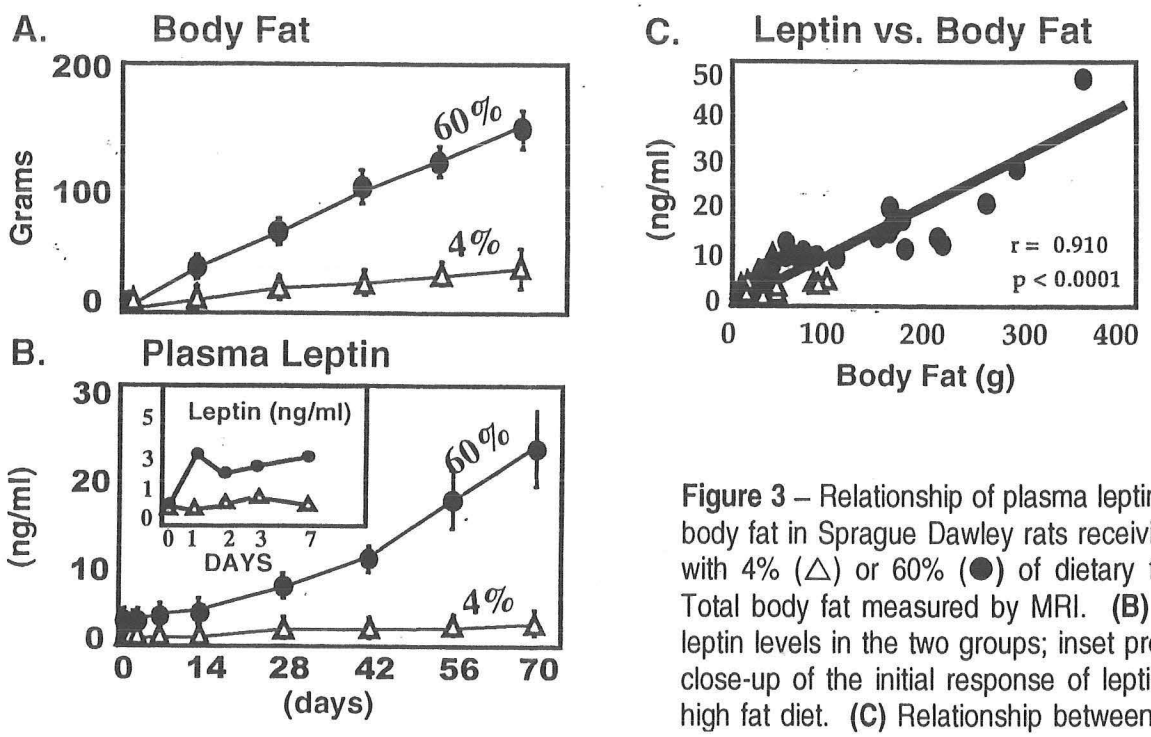


Figure 3 – Relationship of plasma leptin to total body fat in Sprague Dawley rats receiving diets with 4% (△) or 60% (●) of dietary fat. **(A)** Total body fat measured by MRI. **(B)** Plasma leptin levels in the two groups; inset provides a close-up of the initial response of leptin to the high fat diet. **(C)** Relationship between plasma leptin and body fat. The prompt onset of the rise in leptin (inset) and the tight correlation of plasma leptin with body fat are more consistent with a functional role of leptin as an antisteatotic hormone designed to keep TG inside adipocytes than with leptin resistance.

2) In rats with intact leptin and leptin receptor (OB-R) genes, adipocyte TG may rise 150-fold due to caloric overload, but nonadipocyte TG contents remain normal **(Fig. 4).**

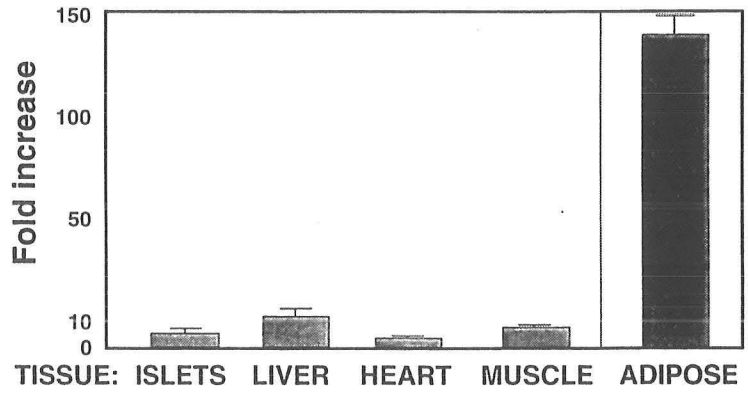


Figure 4 – TG accumulation in tissues of normal Sprague Dawley rats on a 60% fat diet. Adipose tissue TG content, determined by MRI, increases ~ 150-fold, but TG content in nonadipose tissue rises only slightly. This is consistent with a homeostatic system that regulates TG content in nonadipose tissue.

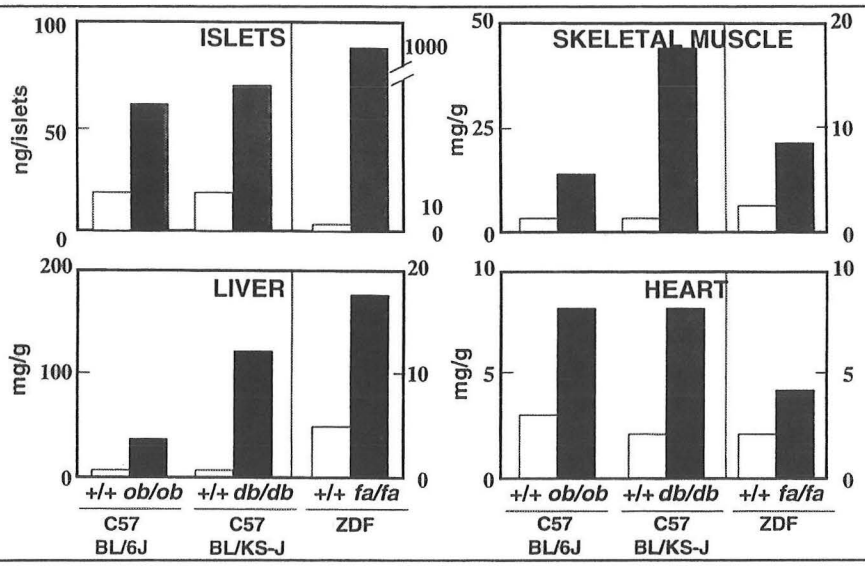


Figure 5 - TG accumulation in nonadipose tissues of rodents with loss-of-function mutations in leptin (*ob/ob* mice), or leptin receptors (*db/db* mice and *fa/fa* rats). These animals received a normal laboratory diet containing 6% fat. Nevertheless, their tissues accumulated far more fat than normal rats did on a 60% fat intake **(Figure 4)**. This suggests that leptin action is required to prevent steatosis.

3) In animals with loss-of-function mutations in the leptin gene or in the leptin receptor (OB-R) gene, protection of nonadipose tissues against TG overload is absent and TG content of nonadipose tissue increases in parallel with adipocyte fat **(Fig. 5).** This loss of antisteatotic protection is attributed to reduced expression of mitochondrial and peroxisomal enzymes of FA oxidation and their transcription factor, peroxisome proliferator-activated receptor-α (PPARα) (10,11) **(Fig. 5).**

4) The TG overload in the nonadipose tissues of OB-R defective rats and the underexpression of enzymes of FA oxidation is reversed by transgenic expression of either normal OB-Rb gene or of PPARα genes **(Fig. 6).**

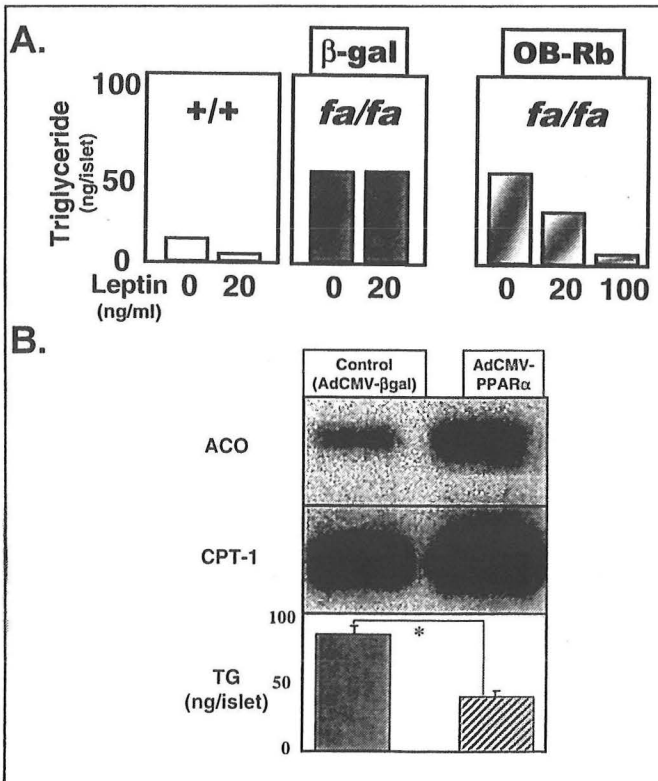


Figure 6 - **(A)** Restoration of the lipopenic effect of leptin in pancreatic islets of wild-type ZDF rats (+/+) **(left panel),** and in two groups of fat-laden islets isolated from obese diabetic ZDF rats (*fa/fa*) **(middle panel)**. In one group of islets from the *fa/fa* rats, the wild-type leptin receptor OB-Rb was overexpressed, while in the other group of islets, β-galactosidase was overexpressed as a control. TG declined in leptin-treated OB-Rb-overexpressing islets of *fa/fa* rats, proving that leptin has lipopenic activity in tissues that express a normal leptin receptor.

(B) PPARα overexpression in the fat-laden islets of obese diabetic ZDF rats (*fa/fa*) **(upper panels)** causes upregulation of expression of the enzymes of oxidation, ACO and CPT-1 and a reduction in TG content **(lower panel)**. Overexpression of PPARα in islets expressing normal OB-Rb upregulates the oxidative enzymes that lower TG.

TESTING THE FATTY ACID OVERLOAD HYPOTHESIS IN RODENTS WITH CONGENITAL OBESITY:

Natural history of lipid abnormalities in ZDF (fa/fa) rats: Since non-insulin diabetes dependent mellitus (NIDDM) is the most common complication of human obesity, occurring in up to 70% of some obese human populations (1), the pancreatic islets seemed an appropriate nonadipose tissue in which to test the FA overload hypothesis. NIDDM is even more common in the rodent models of congenital obesity, occurring in 100% of male Zucker Diabetic fatty rats (ZDF-drt), between the ages of 9 and 14 weeks. As shown in **Figure 7**, plasma levels of FFA and TG increase dramatically two weeks before the onset of hyperglycemia (12), perfusing tissues with a huge excess of FA. This is accompanied by a progressive rise in TG content in tissues such as islets (**Fig. 7D**). These changes precede the onset of hyperglycemia **(Fig. 7A).**

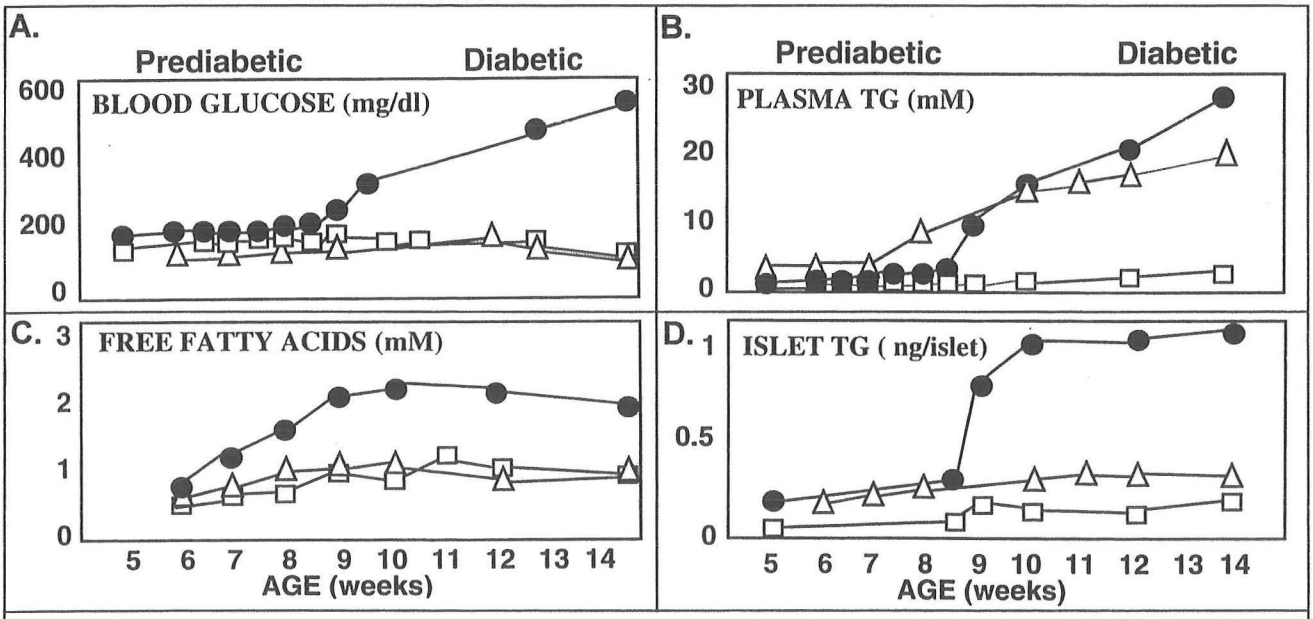


Figure 7 – (A) Longitudinal studies of plasma glucose, **(B)** plasma TG levels, **(C)** FFA levels, and **(D)** TG content of islets in lean male ZDF (*fa/+*) (□); obese female ZDF rats (*fa/fa*) (△), which do not develop diabetes; and obese male ZDF rats (*fa/fa*) (●), which develop diabetes between the ages of 9 and 14 weeks.

Natural history of islet TG content and β-cell morphology: **Figure 8** demonstrates the relationship of islet TG to the islet morphology and the clinical course of the disorder. In the preobese state, the islets and the TG content are perfectly normal. In the obese, prediabetic stage there is a 10-fold increase in TG content, in association with a 4-fold increase in the β-cell mass and in insulin production, which keeps pace with rising insulin requirement (13, 14). As the obesity increases still further, islet TG rises to 50-100 x normal; at this point, a 75% reduction in the β-cell mass and in insulin production has occurred, i.e., the remarkable hyperplasia of β-cells and increased insulin-producing capacity that had characterized the compensated prediabetic phase of obesity has completely disappeared and with it, compensation for the insulin resistance **(Fig. 7)**. Thus, the progressive increase in TG content of the islets is associated with a biphasic pattern of β-cell

morphology: an initial hyperplasia beginning with the onset of obesity, followed by a loss of β-cells that reduces the hyperplastic β-cell mass to its preobese prehyperplastic size. Now, β-cells are incapable of meeting the ever-increasing insulin requirement imposed by the progressively increasing insulin resistance. Diabetes consequently appears (13, 14).

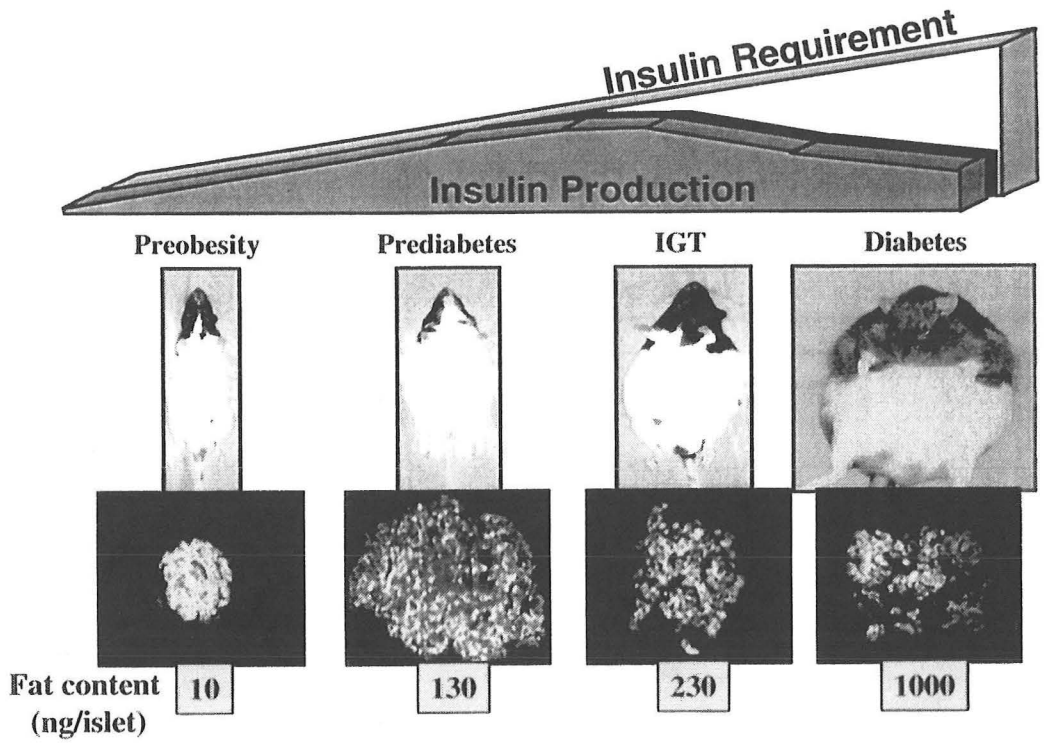


Figure 8 – Natural history of a steatotic complication of obesity, NIDDM. As these ZDF rats become increasingly obese, islet TG increases 10-fold; the β-cells undergo a 4-fold hyperplastic response. This enables them to raise insulin production to meet the increase in insulin requirements caused by parallel TG deposition in the target tissues of insulin **(see Fig. 23).** Later, however, as obesity becomes more severe, islet TG rises to ~ 100 times normal. Now β-cells undergo apoptosis until their mass has regressed to the original size prior to obesity. In other words, all the gain in β-cell mass is lost. The remaining β-cells cannot maintain the high level of insulin production that had compensated for the insulin resistance. The result is diabetes.

New questions arising:

1. **How do the islets accumulate so much TG?**
2. **Do FA cause the β-cell hyperplasia of obesity? If so, how?**
3. **Do FA cause the β-cell apoptosis that results in diabetes?**
4. **How do excess FA disable and destroy certain cells?**

Question 1 – How do the islets accumulate so much TG?

a. Is it entirely seconday to hyperlipidemia? The high plasma FFA and TG levels in obese animals increases the substrate for TG formation. To determine if substrate excess alone is the cause of the increased TG content, we cultured normal islets from wild-type ZDF rats (+/+) and from prediabetic ZDF (*fa/fa*) rats in 0, 1 or 2 mM of a 2:1 oleate:palmitate mixture of FA. Four days later, we measured the TG content of the islets. While normal islets did accumulate small amounts

of TG at high FA concentrations, proving that elevated circulating lipids do increase TG accumulation in normal islets, TG in the islets of *fa/fa* rats was 19x greater, evidence of an intrinsically greater propensity to form TG **(Fig. 9A)** (15).

b. Is it secondary to increased lipogenic capacity of tissues? When *fa/fa* islets were cultured in the presence of either ^{14}C-labeled glucose or ^{14}C-labeled palmitate, the rate of ^{14}C-TG formation far exceeded that of +/+ control islets **(Fig. 9B)** (15). There is also a substantial reduction in ^{14}C-palmitate oxidation (15).

Conclusion: *There is an increased lipogenic capacity in the islets of the obese rodents. Thus, while the increased availability of lipogenic substrate is obviously important, it is the inherent increase in the capacity to esterify FA and to synthesize new FA from glucose that accounts for the massive overaccumulation of TG in islets of these obese ZDF rats.*

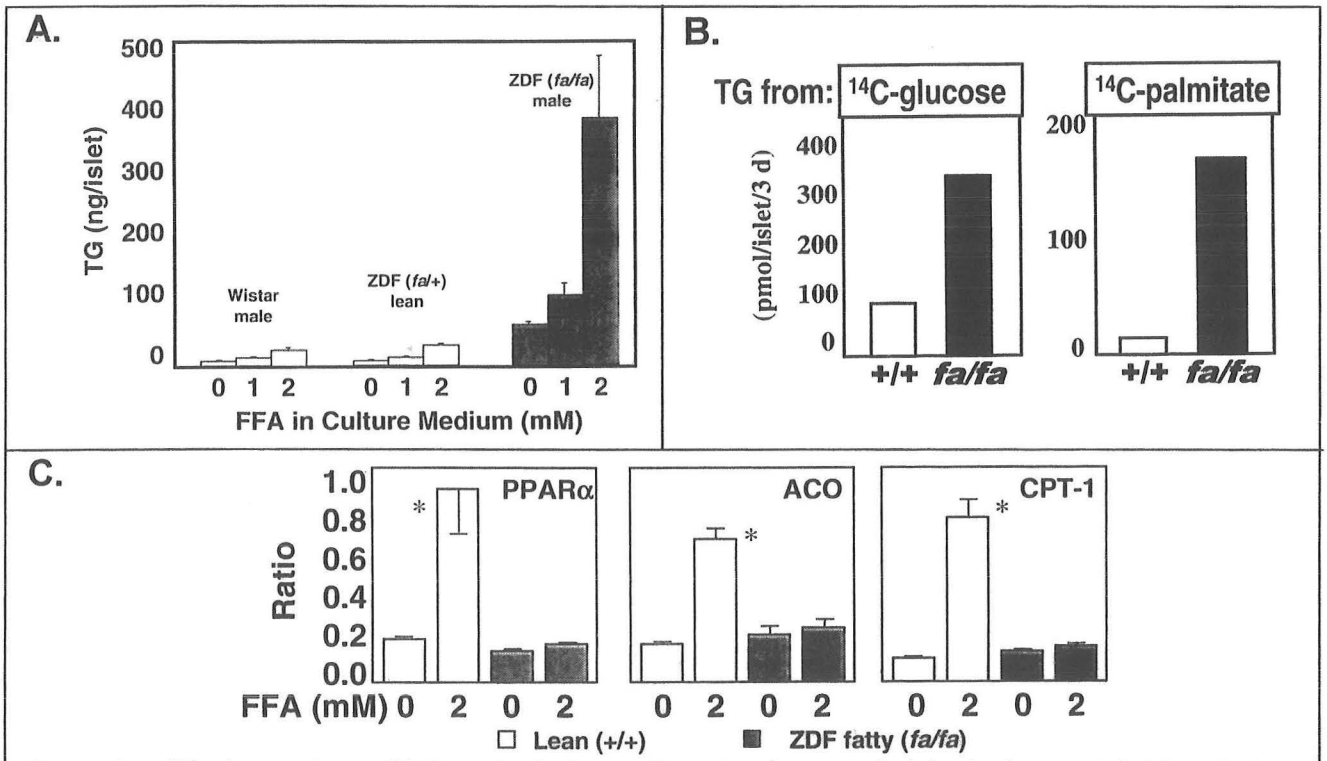


Figure 9 – (A). Comparison of TG content of nonadipocytes (pancreatic islets) of normal (+/+) and obese (*fa/fa*) rats after 4 days in 0, 1, or 2 mM FA (2:1/oleate:palmitate). TG in islets of obese ZDF rats (*fa/fa*) is 19-fold greater, evidence of a marked increase in their esterification capacity. **(B).** ^{14}C-triglyceride formation from ^{14}C-glucose or ^{14}C-palmitate in islets of +/+ and *fa/fa* ZDF rats, i. e., increased *de novo* lipogenesis from glucose and increased esterification. **(C).** FA increases PPARα, ACO and CPT-1 in +/+ but not *fa/fa* islets.

Conclusion*: The fa/fa islets have a far greater capacity to produce triglycerides, both de novo from glucose and via esterification from palmitate.*

c. What causes the increase in lipogenic capacity? To elucidate the reason for the increased lipogenic capacity, we compared the expression of lipogenic enzymes in the islets of *fa/fa* and +/+ rats. As demonstrated in **Figure 10A**, there was a marked increase in the expression of lipogenic enzymes, including acetyl CoA carboxylase (ACC), fatty acid synthase (FAS) and glycerol-

phosphate acyl transferase (GPAT), and their transcription factor, peroxisome proliferator-activated receptor (PPAR)-γ (16). This PPAR-γ excess may be a proximal cause of the increased lipogenic capacity. Interestingly, there was a reciprocal reduction in the mRNA of PPARα, the transcription factor for the enzymes of fatty acid oxidation of those enzymes, acyl CoA oxidase (ACO), a peroxisomal enzyme, and carnitine palmitoyl transferase-1 (CPT-1), a mitochondrial enzyme **(Fig. 10B)** (17). Thus, in a sense, the islets of *fa/fa* rats were "masquerading" as white adipocytes with high levels of mRNA of PPARγ and lipogenic enzymes, while the mRNAs of the putative protective system, PPARα and the enzymes of oxidation were underexpressed. In normal islets increased exposure to FA upregulates PPARα and the enzymes of oxidation (ACO, CPT-1); in *fa/fa* islets FA have no such effects **(Fig.11).** Thus the self-correcting homeostatic system triggered in normal islets by FA excess was inoperative in *fa/fa* islets.

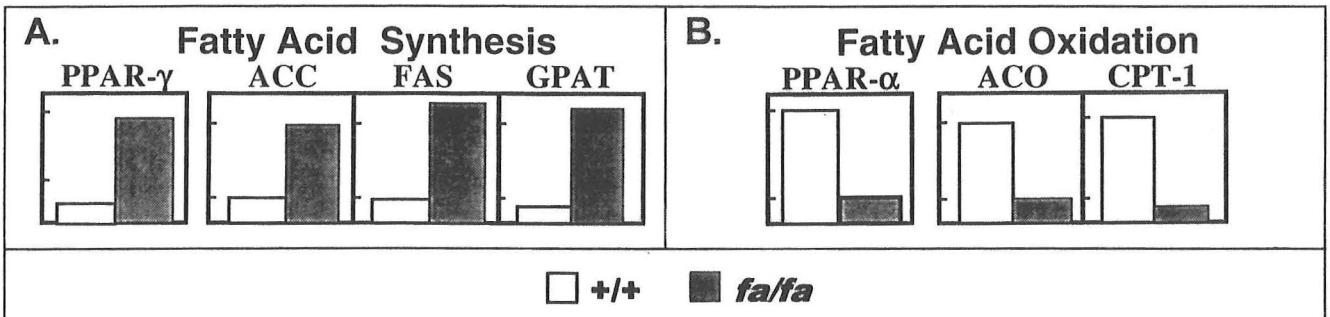


Figure 10 – *Semiquantification of mRNA of transcription factors and enzymes involved in fatty acid synthesis and oxidation to determine the mechanism of the increased lipogenic capacity depicted in Figure 10.* ***(A)*** *Fatty acid synthesis:* There is a marked increase in mRNA of the anabolic transcription factor, PPARγ, and the enzymes of fatty acid synthesis that it upregulates, acetyl CoA carboxylase (ACC), fatty acid synthetase (FAS), and glycerol phosphate acetyl 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, PPARα, and two enzymes of fatty acid β-oxidation that it upregulates, the peroxisomal enzyme acyl CoA oxidase (ACO), and the mitochondrial enzyme carnitine palmitoyl tranferase-1 (CPT-1).

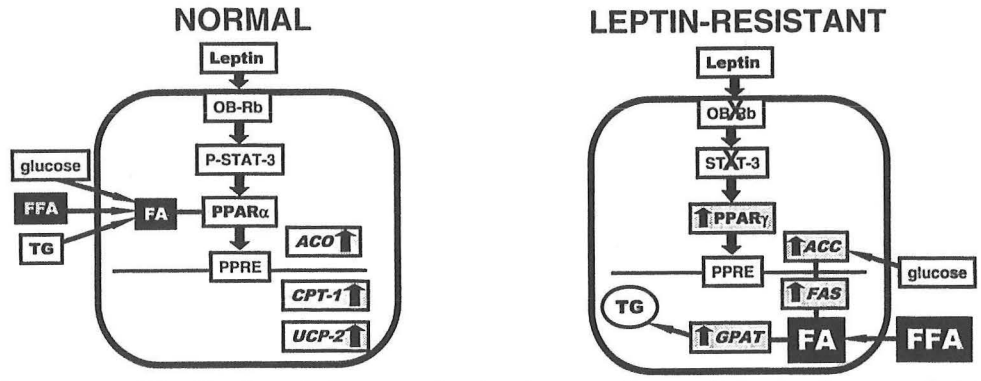


Figure 11 - How excess FA stimulate their own oxidation in normal nonadipocytes (pancreatic islets). FA are ligands for PPAR-α. When FA influx in normal +/+ islets exceeds metabolic needs, there is an increase in the mRNA of PPAR-α, the transcription factor for enzymes of oxidation, ACO and CPT-1. The mRNA of these enzymes rise, implying that oxidative capacity for FA increases to oxidize the unneeded excess. These responses are completely lacking in *fa/fa* islets, which lack functional leptin receptors. The findings imply that leptin action is permissively required for normal intracellular FA homeostasis.

Steral Regulatory Element-Binding Protein-1 (SREP-1) The discovery of a novel transcriptional regulator of enzymes of lipid synthesis in the laboratories of Brown and Goldstein in Dallas (18) and Spiegelman in Boston (19) raised the possibility that the increased lipogenicity in *fa/fa* rats might be the result of overexpression of SREBP-1/ADD-1. As shown in **Figure 12A**, SREBP-1 mRNA was increased in liver of leptin-unresponsive *fa/fa* rats and was reduced in *fa/fa* rats with adenoviral overexpression of OB-Rb *in vivo*. This implies that leptin influences its expression. There was also a dramatic reduction in plasma TG levels **(Fig. 12D)**, together with liver TG **(12C)** and SREBP-1.

Conclusion: *Alterations in important transcription factors that control the expression of enzymes of FA synthesis and oxidation may be the cause of the increased lipogenic capacity. This expression phenotype of lipogenic enzymes and their transcription factors and the reduced expression of oxidative enzymes and their transcription factors appear to be proximal causes of the overaccumulation of TG in the islets of fa/fa rats* ***(Figure 12).***

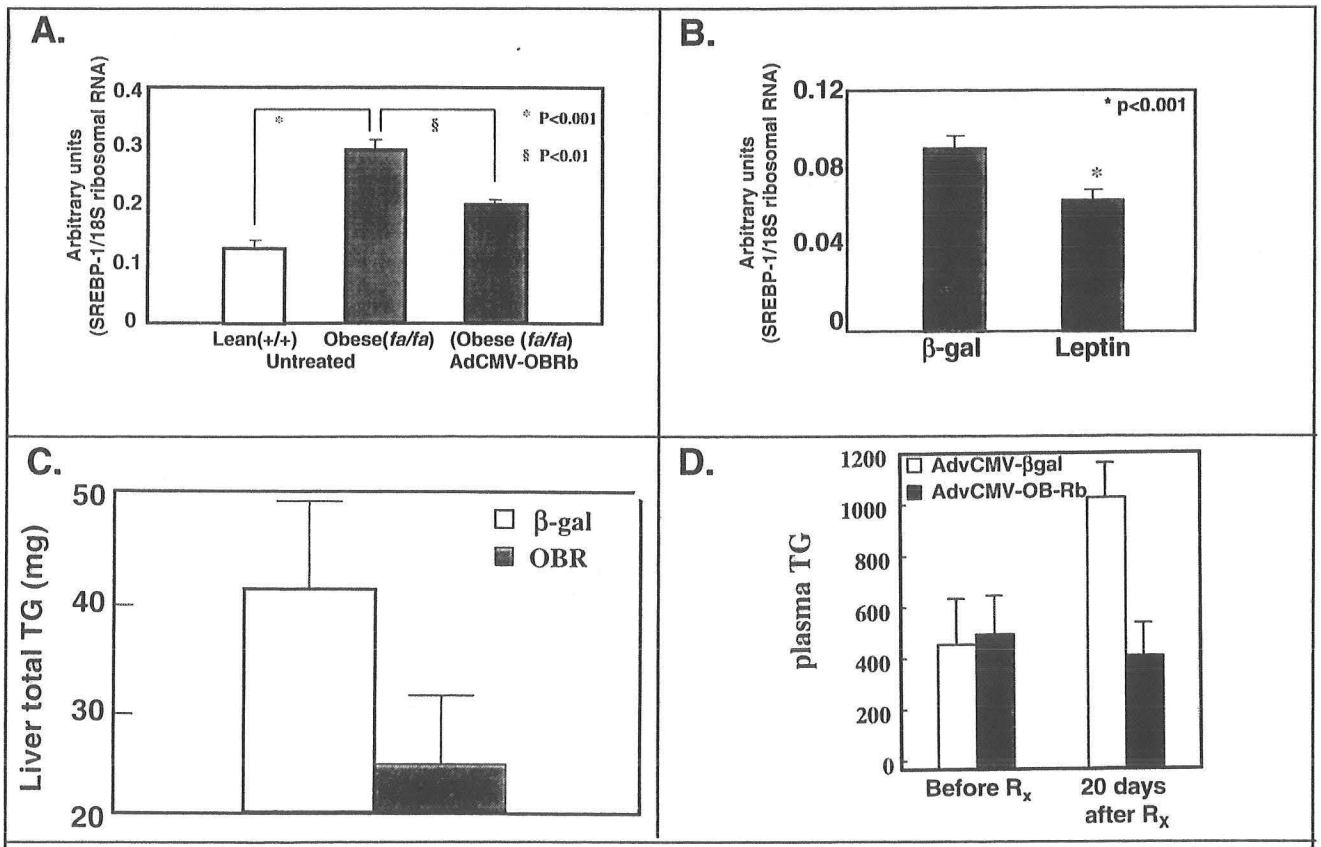


Figure 12 – (A). The SREBP-1/18S ribosomal RNA ratio in lean (*+/+*) and obese (*fa/fa*) ZDF rats and in obese *fa/fa* rats overexpressing normal OB-Rb. SREB-1 is significantly increased in the obese rats and is reduced by OB-Rb overexpression. **(B).** Comparison of the mRNA ratio of SREBP-1 to 18S ribosomal RNA in livers of normal rats treated with an adenovirus-leptin construct (AdCMV-leptin) to produce hyperleptinemia, or with an adenovirus-β-gal (AdCMV-β-gal) construct as a control. There is a small but statistically significant reduction in the ratio as determined by Northern blotting. **(C).** Hepatic TG content of livers of obese ZDF (*fa/fa*) rats with transgenic expression of the normal leptin receptor (OB-Rb) or β-galactosidase. **(D).** Plasma TG content in the same animals.

Question 2 – Do FA cause the β-cell hyperplasia of obesity? If so, how?

To determine the relationship of FA to hyperplasia of β-cells in prediabetic obese rats, we isolated islets from normal rats and cultured them in 1 or 2 mM FA, which is within the range of plasma FFA levels in prediabetic animals. As shown in **Figure 13A**, in normal islets cultured in FA BrdU incorporation increased 4-fold (22). This demonstrates that the elevated FFA levels observed in prediabetic animals could be the direct cause of increased β-cell replication and hyperplasia. Because DAG has been implicated in mitogenic signaling (23), we measured DAG content in prediabetic and diabetic *fa/fa* ZDF islets **(Fig. 13B).** The significantly higher values in prediabetic *fa/fa* ZDF islets suggest that FA-mediated increase in DAG might be involved in the hyperplastic phase of FA excess. TGFβ is also increased (Zhou, Y-T., unpublished).

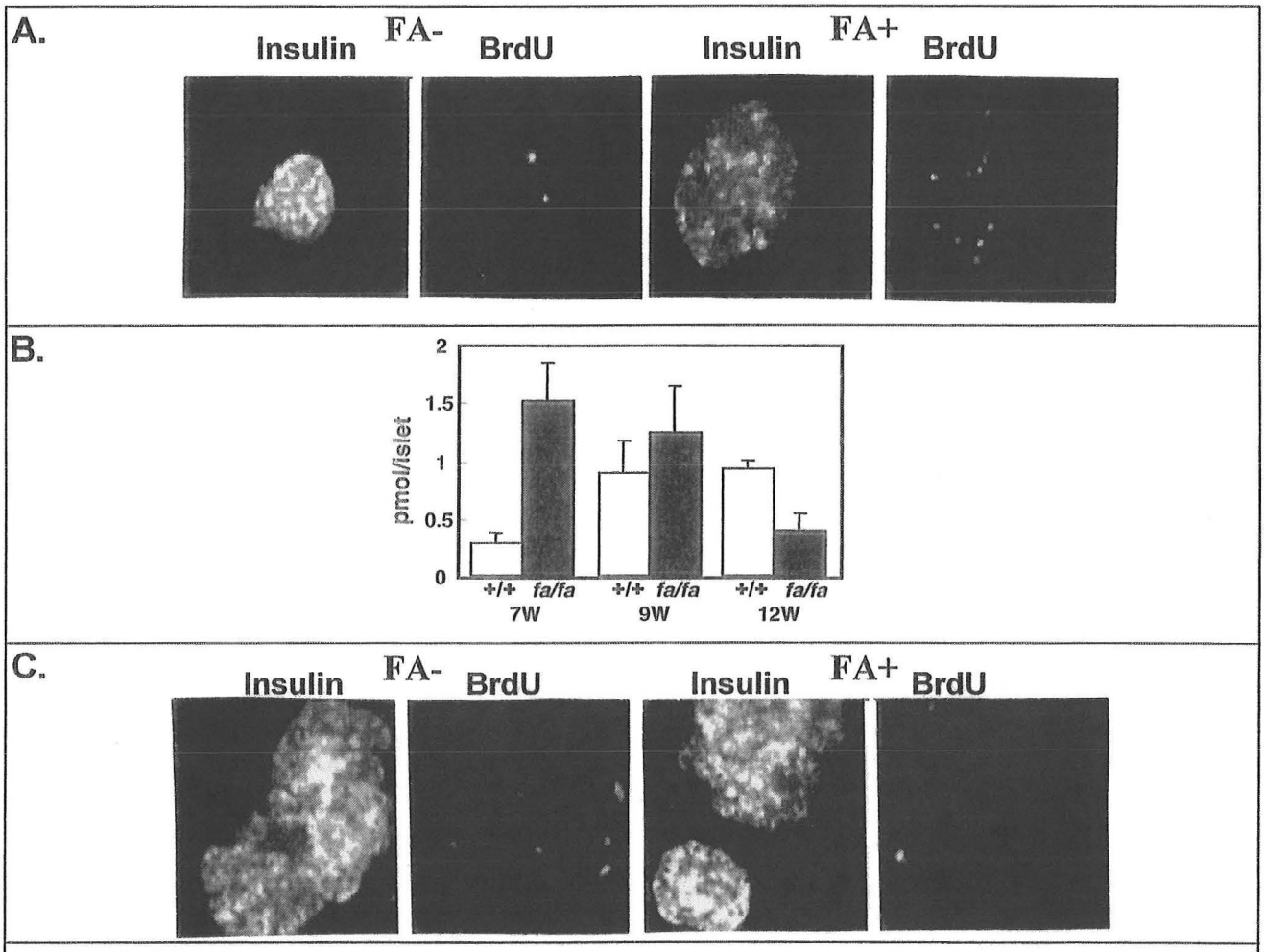


Figure 13 – (A) BrdU uptake, an index of cell replication, in islets isolated from normal rats and cultured in 0 or 1mM FA. There is a 4-fold increase replication in FA-cultured β-cells, identified by the insulin stain. The results imply that the FA induced hyperplasia **(see Figure 8). (B)** Diacylglycerol (DAG) content in islets of obese (*fa/fa*) and lean (+/+) ZDF rats at 7 weeks of age (prediabetic) and 14 weeks of age (diabetic). The significantly higher levels in the obese animals raise the possibility that DAG is involved in the increased replication and hyperplasia of β-cells during the compensated prediabetic phase of obesity. **(C)** In fat-laden hyperplastic islets isolated from obese *fa/fa* ZDF rats FA reduces in BrdU uptake by β-cells. This may be a factor in the loss of β-cell mass and compensatory function that results in diabetes **(see Figure 8)**

NOTE: The mitogenic effect of FA may have other important implications. Epidemiologic evidence links certain neoplasms, in particular cancer of the colon, to the fat content of the diet (Table 1 - #1). Conceivably chronic exposure of normal epithelium in the colon to an increase in fatty acids may be a factor in the malignant transformation of genetically susceptible cells.

Question 3 – Do FA cause the β-cell apoptosis that results in diabetes?

DNA laddering, a useful index of apoptosis is markedly increased by exposure of *fa/fa* islets, but not normal islets, to FA **(Fig. 14).** When leptin-unresponsive *fa/fa* islets are made responsive to leptin by transgenic overexpression of a normal leptin receptor, leptin now blocks the apoptotic action of FA **(Fig. 14).**

Conclusion: *These experiments demonstrate that FA causes β-cell apoptosis and that the action of leptin protects against lipoapoptosis.*

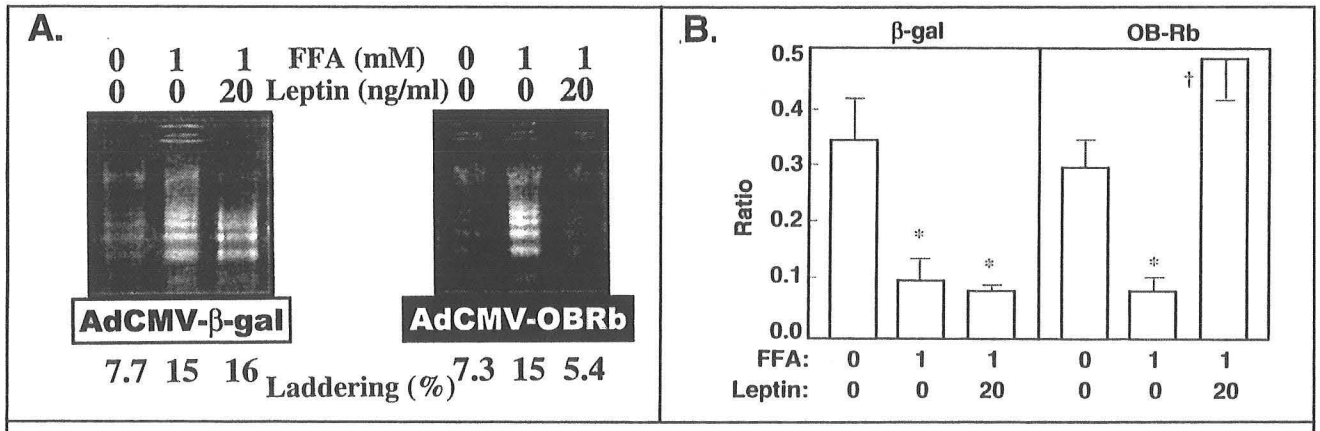


Figure 14 – (A) Effect of transgenic expression of normal leptin receptors on prevention of FA-induced apoptosis. In normal islets (not shown) DNA laddering, an index of apoptosis is below 1%. In fat-ladden islets of prediabetic ZDF rats laddering is over 7% and rises to 15% in the presence of FA. Leptin has no effect on this. If leptin receptors (OB-Rb) are expressed, leptin prevents the FA-induced increase in laddering. **(B)** Lowering by FA of Bcl_2 protein expression in islets of normal lean (+/+) and obese (*fa/fa*) ZDF rats. Leptin blocks the lowering effect in islets from +/+ but not *fa/fa* rats. But when *fa/fa* islets are transgenically altered to express the normal leptin receptor (OB-Rb), leptin blocks Bcl_2 lowering. Since Bcl_2 is an antiapoptotic factor, this may account for the antiapoptotic action of leptin shown in panel **A.**

Conclusion: *These experiments demonstrate that FA causes β-cell apoptosis and that the action of leptin protects against lipoapoptosis.*

Question 4 –How do excess FA disable and destroy certain cells?

1) Through steatosis:

a) When intracellular FA exceed the oxidative requirements and accumulate in nonadipocytes, unutilized FA enter the esterification pathway to TG, accelerated by the increased expression of

lipogenic enzymes as a consequence of the lack of leptin action **(Fig. 15).** The TG themselves are probably inert and, therefore, quite harmless in themselves; however, they can be hydrolyzed, providing yet another source of fatty acyl CoA elevation within the cell, which could be directly toxic or undergo omega-oxidation to carboxylic acids, a process promoted by aspirin and alcohol (see below). No studies of this mechanism are known to exist.

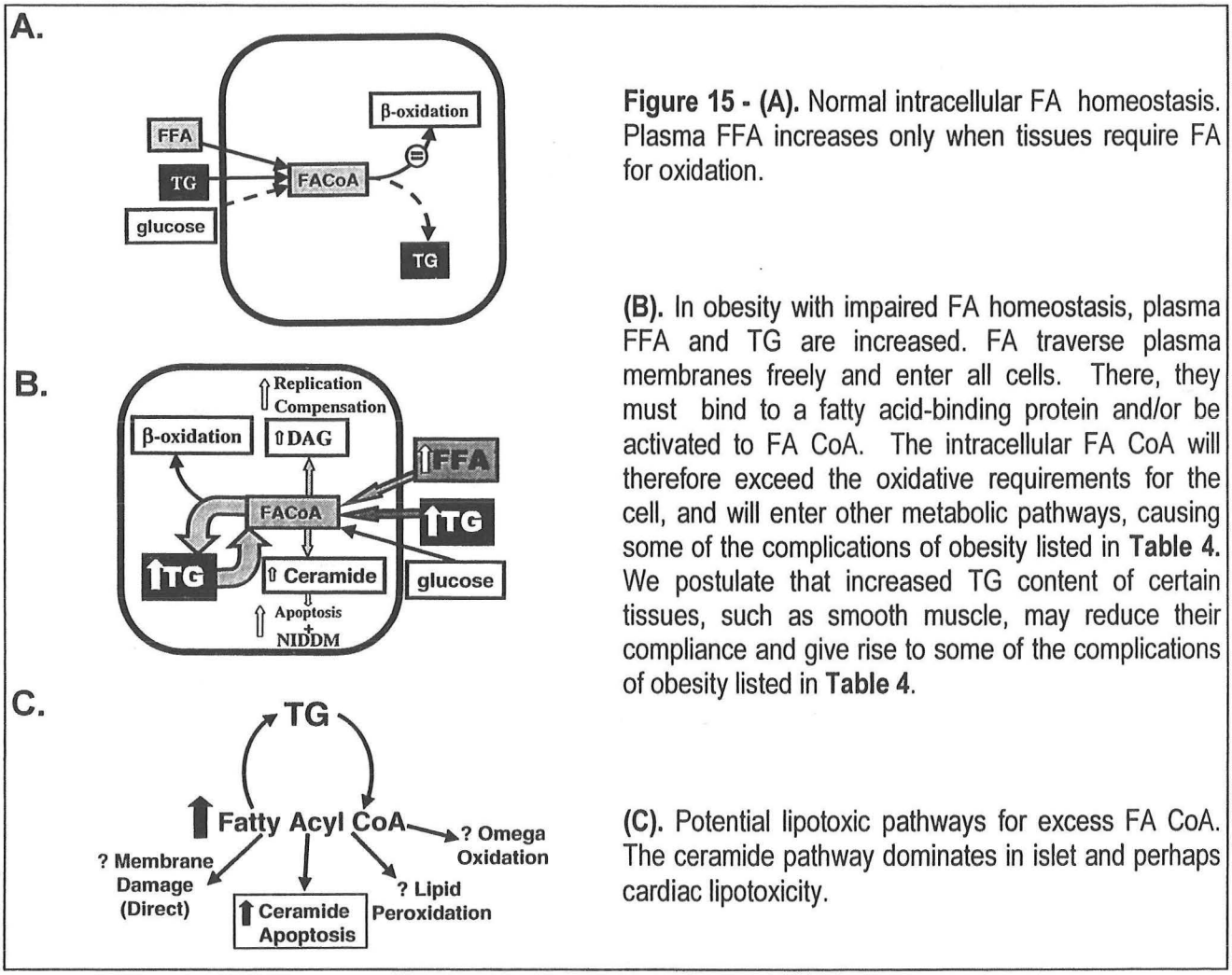


Figure 15 - (A). Normal intracellular FA homeostasis. Plasma FFA increases only when tissues require FA for oxidation.

(B). In obesity with impaired FA homeostasis, plasma FFA and TG are increased. FA traverse plasma membranes freely and enter all cells. There, they must bind to a fatty acid-binding protein and/or be activated to FA CoA. The intracellular FA CoA will therefore exceed the oxidative requirements for the cell, and will enter other metabolic pathways, causing some of the complications of obesity listed in **Table 4.** We postulate that increased TG content of certain tissues, such as smooth muscle, may reduce their compliance and give rise to some of the complications of obesity listed in **Table 4**.

(C). Potential lipotoxic pathways for excess FA CoA. The ceramide pathway dominates in islet and perhaps cardiac lipotoxicity.

b) Displacement of cell contents by large TG droplets: If TG droplets reached a size that significantly displaced cytoplasmic components and/or mechanically impaired certain cell functions, such as the pliability of contracting smooth muscle cells in arterioles or contractile organs that require pliability for optimal function, (e.g. gallbladder), or O_2 diffusion in alveoli, disease might then result **(cf Table 1, #2 a, b, c, d, e, #4, #6).** The potential diseases include hypertension, gallbladder disease, cardiac syndrome X, and Pickwickian syndrome. [*Remarkably, leptin reverses respiratory depression and elevated $PaCO_2$ in ob/ob mice (25)*].

c) Increased fibrogenicity: Finally, TG excess in tissues may be fibrogenic, eliciting a "cirrhotic" response in fat-laden islets of Langerhans **(Fig. 16)** and myocardium **(Figure 24D)**, similar to the familiar hepatic cirrhosis that may follow hepatic steatosis. The mechanism is not known, but TGFβ, which is high in islets of *fa/fa* rats, is suspect (Zhou,Y-T. unpublished).

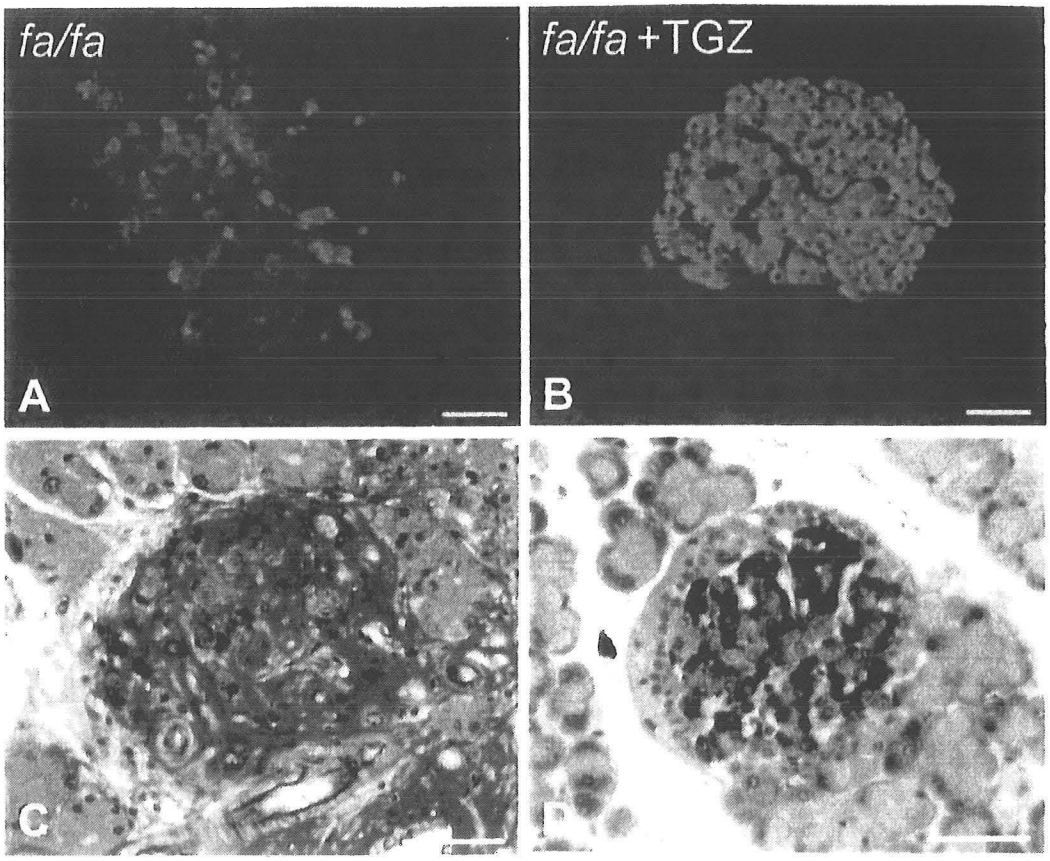


Figure 16 – **(A)** Comparison of pancreatic islets of an obese *fa/fa* ZDF rat **(A, C)** and control islets **(B, D)** stained for insulin **(A, B)** or fibrous tissue (trichrome) **(C, D)**. The remarkable increase in fibrous tissue may be a sequel of the steatosis. The control islet is from a *fa/fa* rat treated with an antisteatotic agent, troglitazone (TGZ) (Courtesy of L. Orci, Univ. of Geneva).

2) Apoptosis in *fa/fa* obesity:

a. Through ceramide-mediated apoptosis: FA-induced apoptosis ("lipoapoptosis") appears to be the major mechanism of β-cell destruction in obese ZDF rats. As unoxidized fatty acyl CoA accumulates in nonadipocytes, it enters the pathway of *de novo* ceramide synthesis **(Figure 17A).** Ceramide rises as a consequence of increased FA availability and reaches much higher levels in *fa/fa* islets than in +/+ islets **(Fig. 17B).** This exaggerated incorporation of FA into ceramide is explained by the much higher tissue level of serine palmitoyl transferase (SPT), **(Fig. 17C),** the enzyme that condenses serine and palmitoyl CoA to form dihydrosphingosine, the first reaction in ceramide synthesis **(Fig. 17A).** Ceramide contains 2 molecules of long-chain FA and, in contrast to cytokine-induced ceramide formation, which is probably derived from sphingomyelin (26), the FA-induced increase in ceramide comes from *de novo* formation of ceramide through condensation of serine and palmitic acid (27) **(Fig. 17D).** Ceramide activates NFκB (27), which upregulates the expression of inducible nitric oxide synthase (iNOS), thereby increasing nitric oxide (NO) production (29) **(Fig. 18A).** The formation of peroxynitrate and perhaps other free radicals is considered the proximal cause of the apoptotic cascade that is unleashed (30). The pathway shown in **Figure 18A** is validated by the experiments depicted in **Figure 18B.**

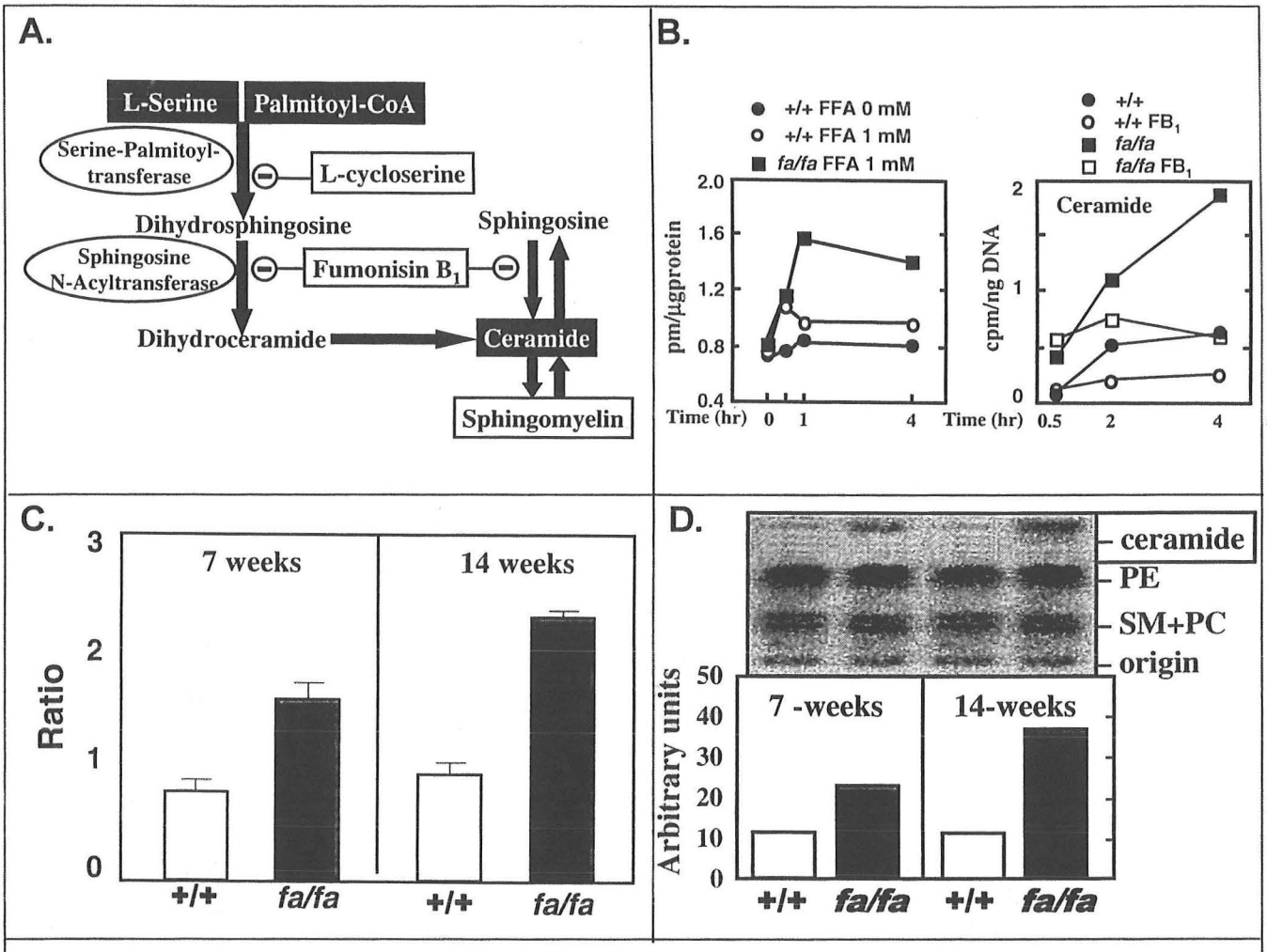


Figure 17 – The role of *de novo* ceramide synthesis in lipoapoptosis. **(A)** The pathways of ceramide formation. *De novo* ceramide formation from condensation of L-serine and palmitoyl CoA catalyzed by serine palmitoyl transferase and the classic pathway of ceramide formation from sphingomyelin by spingomyelinase. **(B) Left panel** - Ceramide levels in islets from normal (+/+) and obese (*fa/fa*) ZDF rats cultured in 0 or 1 mM FA. **Right panel** - Effect of fumonisin B_1 (FB_1), a blocker of ceramide formation on [^{3}H]-palmitate appearance as [^{3}H]-ceramide. *De novo* synthesis of ceramide can be blocked by **(C)** Comparison of serine palmitoyl transferase (SPT) expression in normal islets (+/+) and islets of obese ZDF rats (*fa/fa*) at 7 weeks and 14 weeks of age (prediabetic and diabetic). SPT mRNA is markedly increased in *fa/fa* rat islets. **D)** [^{3}H]-ceramide formation from [^{3}H]-serine in normal (+/+) and obese (*fa/fa*) at 7 weeks and 14 weeks of age (prediabetic and diabetic). At both prediabetic and diabetic ages, formation of ceramide from serine is greatly 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 β-cell destruction.

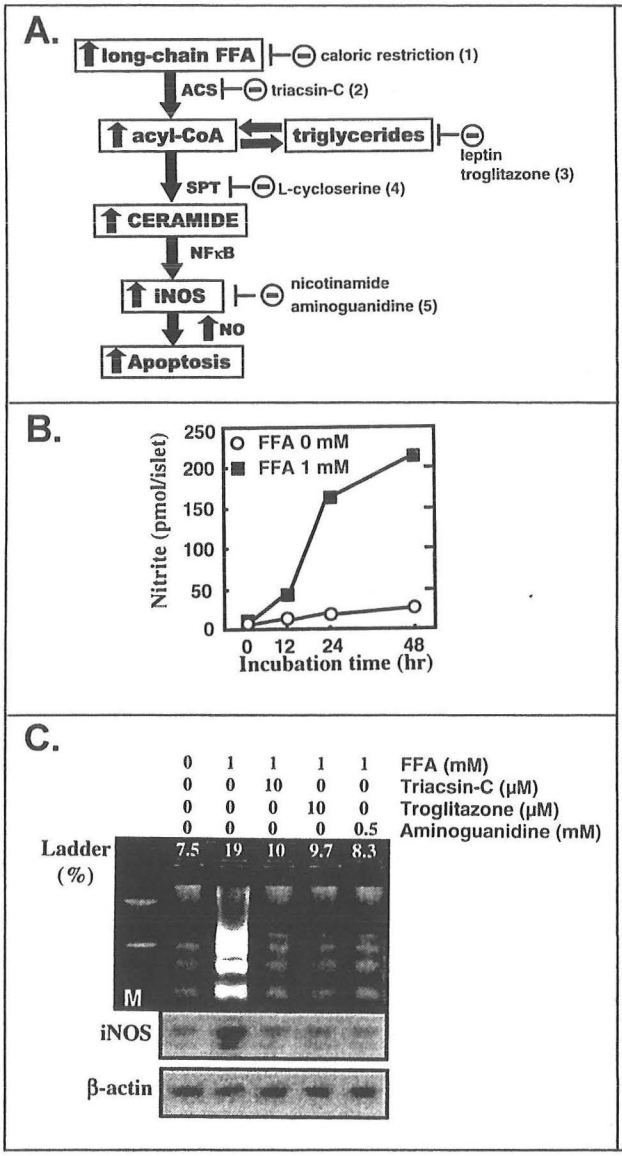


Figure 18 (A). The apoptotic cascade and loss of hyperplastic β-cells can be blocked at any one of the multiple sites both *in vitro* and *in vivo*. Caloric restriction **(#1)** will lower plasma FFA and thereby reduce islet lipid content by limiting FA substrate; this prevents all pathogenic changes (31). One can also block β-cell apoptosis *in vitro* by using an inhibitor of fatty acyl CoA synthase, triacsin-C **(#2),** which, prevents FA from entering the metabolic pathway (27, 32). Troglitazone **(#3),** which increases lipogenesis in white adipocytes *reduces* TG content in islets and other nonadipose tissues. This blocks the apoptotic changes in β-cells (33, 34), including severe alterations of mitochondria **(Fig. 19A),** loss of 75-82% of β–cells **(Fig. 20),** and the severe disruption of the architecture of the islets with extensive fibrosis **(Fig. 16)** (34). A similar, but less complete, protective effect can be obtained more distally by treating islets with L-cycloserine **(#4),** a competitive inhibitor of serine palmitoyl transferase (27, 32). **(B).** Effect of FA on NO production and **(C)** DNA laddering. Note that the striking apoptogenic effect of FA is blocked at level (2) by the ACS inhibitor, triacsin-C, at level (3) by the antisteatotic troglitizone and at level (6) by aminoguanidine (AG). Still, further downstream one can block apoptosis by inhibiting iNOS with nicotinamide or aminoguanidine **(Fig. 21, #5).** These two agents prevent the FA-induced increase in NO formation both *in vitro* **(Fig. 18B)** and *in vivo* **(Figure 21).** (35).

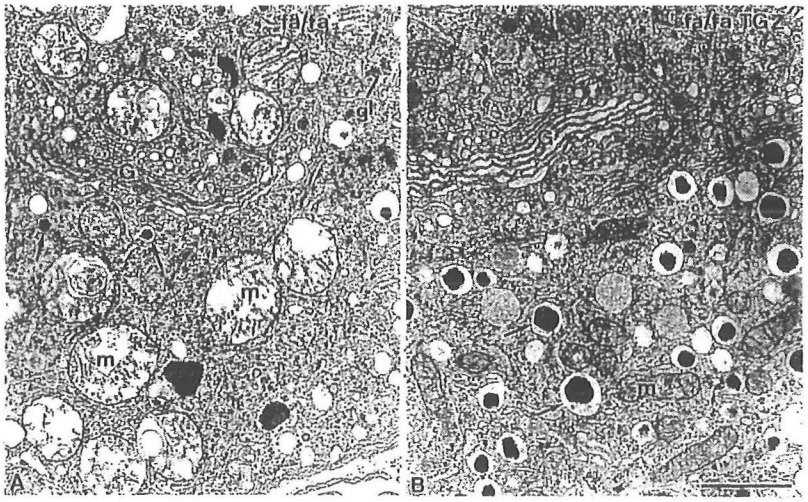


Figure 19 – (A) Thin section of an insulin cell from an untreated ZDF *fa/fa* rat, showing alterations of mitochondria (m) and reduction in the number of dense core secretory granules; areas of dense particles representing glycogen (gl) can also be detected with high frequency. **(B)** Thin section of a β-cell from a troglitazone-treated ZDF *fa/fa* rat. Both mitochondria and dense core secretory granules appear normal in aspect and number. G = Golgi complex (Courtesy of L. Orci, Univ. of Geneva).

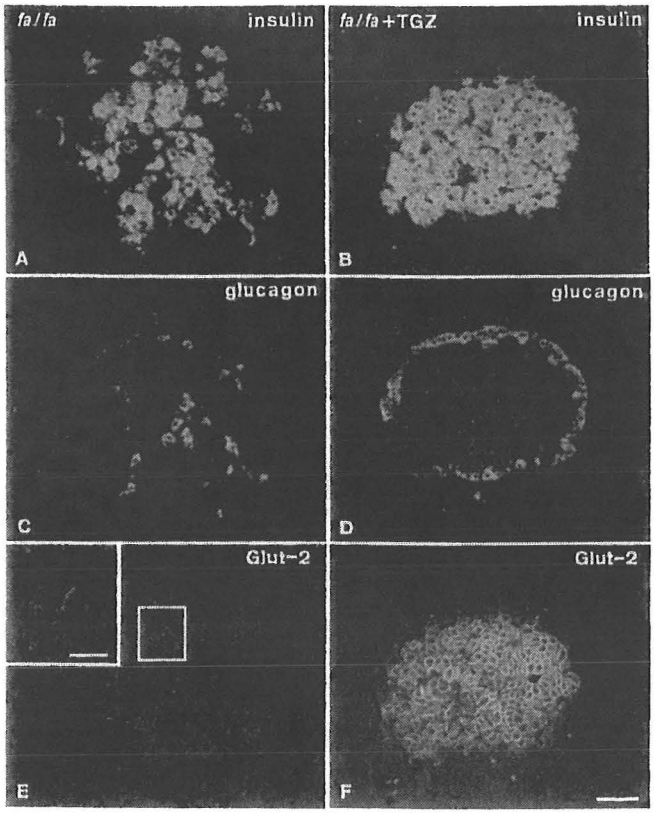


Figure 20 - Immunofluorescent staining for insulin **(A and B)**, glucagon **(C and D)** and glucose transporter 2 (GLUT-2) **(E and F)** in consecutive sections of an islet of an untreated ZDF rat **(A, C, E)** and one of a troglitazone-treated **(B, D, F)** ZDF rats. Compared with the treated condition, the untreated islet shows a reduced number of dispersed insulin-staining cells **(A)**, glucagon-staining cells that have been displaced from their characteristic peripheral location in the islet by the topographical changes **(C)**, and a marked reduction in the GLUT-2 fluorescence in β-cells **(E)**. The inset in **(E)** enhances the staining in a few cells by confocal microscopy. The bars represent 50 mm; in the insert, 20 mm (Courtesy of L. Orci, Univ. of Geneva).

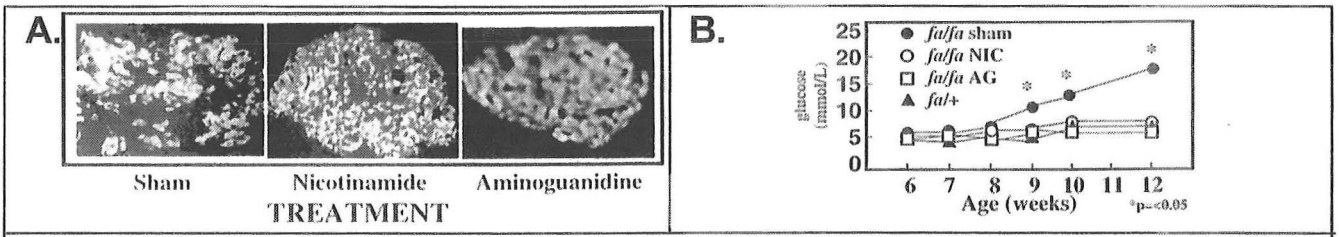


Figure 21 – **(A)** Effects of iNOS inhibitors on β-cells of *fa/fa* ZDF prediabetic rats given sham treatment or treatment with nicotinamide or aminoguanidine for 7 weeks. **(B)** Effect of nicotinamide or aminoguanidine treatment on blood glucose of the *fa/fa* ZDF prediabetic rats. β-cell function is preserved and diabetes prevented (35).

The apoptotic pathway is influenced by differences in the expression of 3 or more genes that have profound direct or indirect effects on the process. First, expression of serine palmitoyl transferase is substantially higher in *fa/fa* islets than in wild-type controls (32) **(Fig. 17C),** thus, providing more enzyme to catalyze the increased fatty acyl CoA substrate into ceramide formation. Second, in normal islets FA reduces the expression of both mRNA and the protein (36) of the antiapoptotic factor Bcl_2 (37), and this reduction is much greater in the *fa/fa* islets (36) **(Fig. 22),** because normal leptin action to reduce FA-induced Bcl_2 down-regulation is absent. Third, the normal paucity of manganese superoxide dismutase in β-cells renders them more vulnerable than other tissues to any apoptotic factor (38). Thus, the vulnerability of β-cells to the apoptotic effect of FA is a convergence of at least 3 factors: the increase in FA availability in nonadipose tissue, the increase in SPT (the first enzyme of *de novo* ceramide synthesis) and a decrease in Bcl_2 (a protector against apoptosis).

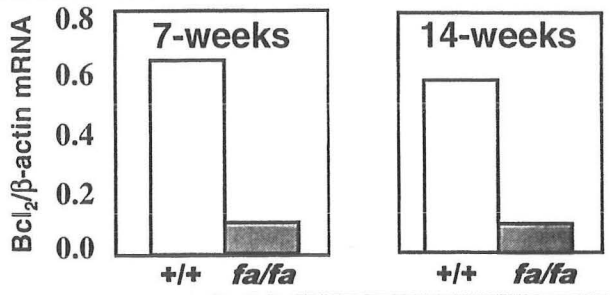


Figure 22 - mRNA of the antiapoptotic factor Bcl_2 in pancreatic islets of obese *fa/fa* ZDF rats and lean +/+ controls at 7 and 14 weeks of age. At both time points, Bcl_2 expression is much lower, implying that the *fa/fa* lack the normal protection by Bcl_2. FA inhibits Bcl_2 expression in both normal and abnormal islets and leptin reverses this inhibition in normal but not in islets with defective leptin receptors. **(Figure 13B).**

b. ***Increased dicarboxylic acid formation through increased omega oxidation:*** A theoretically lipotoxic pathway not yet studied involves omega oxidation of excess FA in microsomes to produce dicarboxylic acid (DCA). Incorporation of DCA into phospholipid membranes would result in leaky plasma membranes and would be cytolethal.

c. ***Lipid peroxidation***

POSSIBLE LIPOTOXICITY IN OTHER TISSUES (TABLE 4)

TABLE 4: OBESITY-RELATED "COMPLICATIONS" OR DISEASES ATTRIBUTABLE OR POTENTIALLY ATTRIBUTABLE TO FA SPILLOVER INTO NONADIPOSE TISSUES

DISEASE CONSEQUENCE	PATHOGENESIS
✱1. Cancers of the colon	Mitogenic effect of excess dietary FA, perhaps DAG-mediated
2. Vascular Disease	
✱a. Coronary artery disease	↑TG and atheromatous deposits in coronary arteries
✱b. Cardiac syndrome X	↑TG deposits in coronary arteries
✱c. Cardiomyopathy	↑TG deposits in myocardium → ↑ceramide → lipoapoptosis
✱d. Hypertension	↑TG in arterioles causing reduced compliance
✱e. Cerebrovascular disease	↑TG in arterioles causing reduced compliance
✱3. Carpal Tunnel Syndrome	↑TG deposition in the tunnel
✱4. Gallbladder Disease	↑TG deposition reduces contractility favoring stone formation
✱5. ↑Severity of Autoimmune Disease	↑Cytokine-induced ceramide formation from FA excess
✱6. Impaired Respiratory Function (Pickwicklan Syndrome)	↑ TG in lungs reducing compliance and diffusion
✱7. Pancreatitis	↑ TG and ↑ ceramide formation → lipoapoptosis
✱8. *Liver Disease*	↑ TG and ↑ceramide → steatonecrosis
9. *Insulin Resistance*	↑ TG with FA inhibtion of glucose metabolism ↑
10. *Type II Diabetes*	↑ TG and ↑ceramide formation → lipoapoptosis
✱11. Sarcopenia of Aging	↑ TG and ↑ceramide formation

✱ Theoretical only (not supported by *in vivo* evidence)
Italics (considerable factual support)

1). Skeletal Muscle (Insulin Resistance) (Table 4, #9): Insulin resistance precedes the diabetes of obesity and has been recognized for several decades to accompany all forms of obesity (39). A link between insulin resistance and long chain fatty acids has been recognized since the early 1960's, when Sir Phillip Randle proposed the glucose-fatty acid cycle, in which long chain FA compete with glucose for oxidation, thereby attenuating insulin-stimulated glucose utilization (40). The many subsequent studies have been reviewed by McGarry in a landmark Science paper in 1992 (41). While the glucose-fatty acid cycle is almost certainly relevant in the short time frame of acute experiments, in the context of chronic life-long obesity it is likely that other factors also contribute. It seems increasingly clear that it is their intracellular lipid content that make cells resistant to insulin-stimulated glucose utilization, rather than the extracellular FFA level, which changes from moment to moment. This is particularly true in skeletal muscle. In **Figure 23A**, the increased lipid content in certain skeletal muscle fibers of obese *fa/fa* rats with marked insulin resistance is vividly demonstrated by Oil red O staining. **Figure 23C** shows the relationship of TG content of skeletal muscle to the steady state level of endogenous insulin required to maintain fasting normoglycemia (42). Clearly, the two are tightly related. The early treatment of these animals with troglitazone prevents the accumulation of TG **(Fig. 23A)** and sensitizes them to insulin action **(Fig. 23B)**.

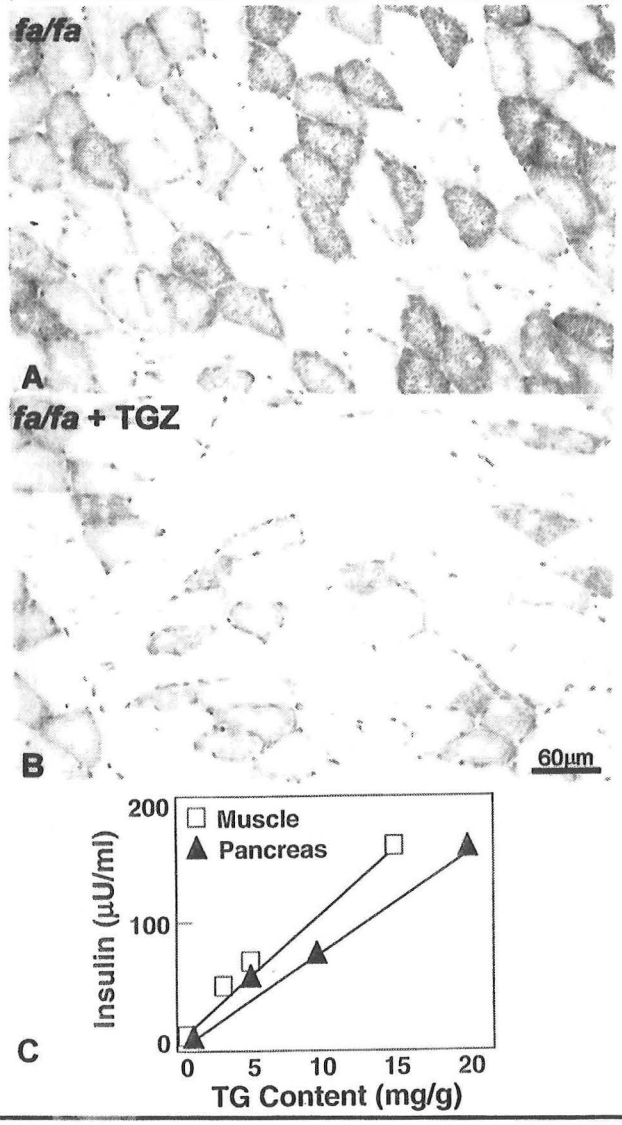


Figure 23 – Relationship between intracellular TG content in skeletal muscle and insulin resistance. **(A)** Oil red O staining of quadriceps muscle of obese (*fa/fa*) ZDF rats at 12 weeks of age showing intense staining of fat droplets in some fiber groups. **(B)** Identical obese (*fa/fa*) ZDF rats that had been treated with troglitazone (TGZ) beginning at the age of 6 weeks. Note the remarkable ability of TGZ treatment to prevent ectopic accumulation of TG in skeletal muscle (Courtesy L. Orci, Univ. of Geneva). **(C)** Relationship between TG content of skeletal muscle and pancreas (x axis) and the fasting insulin level in normoglycemic obese ZDF rats. The latter indicates the level of endogenous insulin required to maintain fasting normoglycemia in rats with varying levels of tissue TG content. Note that the pancreatic and muscle TG content are relatively parallel. Since FA are known to cause β-cell hyperplasia and to increase insulin production (22), the pancreatic TG content may explain how β-cells secrete insulin proportionately to the FA-induced resistance for which it compensates --- at least temporarily.

While intramyocyte fat accumulation may well provide a continuing source of FA that competes with glucose as a substrate, other mechanisms may also be involved in insulin resistance. For example, it has been suggested that NO may be a factor in TNFα-induced insulin resistance (43). Although not yet studied in muscle, FA could increase nitric oxide production in muscle via the same ceramide pathway as in islets **(Fig. 17A).**

Moreover, the chronic apoptogenic effects of increased ceramide formation in muscle could over time cause sarcopenia with a significant reduction in the muscle mass over the years. These negative effects of obesity on muscle will result in weakness, which, by limiting muscular work, further reduces FA oxidation and increases TG and lipoapoptotic muscle. A vicious cycle is thereby created that reduces lean body mass as individuals age and predisposes them to injury and inactivity.

2). Heart (Table 4, # 2 a, b, c):

Cardiac steatosis occurs in the ZDF *fa/fa* rat and in *ob/ob* and *db/db* mice **(Fig. 5).** In *fa/fa* rats lacking antisteatotic protection, TG accumulate rapidly in the heart, as the animals become increasingly obese. In the former rats, TG content and ceramide content in the heart is increased **(Fig. 24A).** By 14 weeks of age, evidence of severe cardiac apoptosis is present in *fa/fa* ZDF rats, reflected by a marked increase in DNA laddering **(Fig. 24B).** At this point, left ventricular end-diastolic diameter has increased significantly **(Fig. 24C).** An increase in fibrous tissue in the myocardium is suggested by the sharp rise in hydroxyproline to protein ratio **(Fig. 24D).**

The functional impact of increased intra-myocyte TG on contractility is unknown, but the extent of myocyte apoptosis and the increasing secondary fibrosis with which it is associated, could certainly be a major factor in the reduction of function.

An etiologic relationship between the steatosis and the reduced contractility is strongly indicated by the fact that the antisteatotic drug, troglitazone, which reduces cardiac TG, prevents both cardiac apoptosis and the impairment of cardiac function **(Fig. 24).** It is likely that the increase in myocardial ceramide content occurred via the same *de novo* pathway described for pancreatic β-cells **(Fig. 17A).** Since ceramide increases iNOS and NO formation, iNOS inhibitors might be therapeutically useful in preventing the consequences of cardiac steatosis.

Thus, if there is a human counterpart of this obesity-related heart disease condition, as scattered reports in generalized lipodystrophy, an exaggerated model of FA spillover, suggest (44-46), there are multiple agents that should be effective in preventing it or arresting it. However, cardiac steatosis in obese humans has never been studied and at present its existence is unproven.

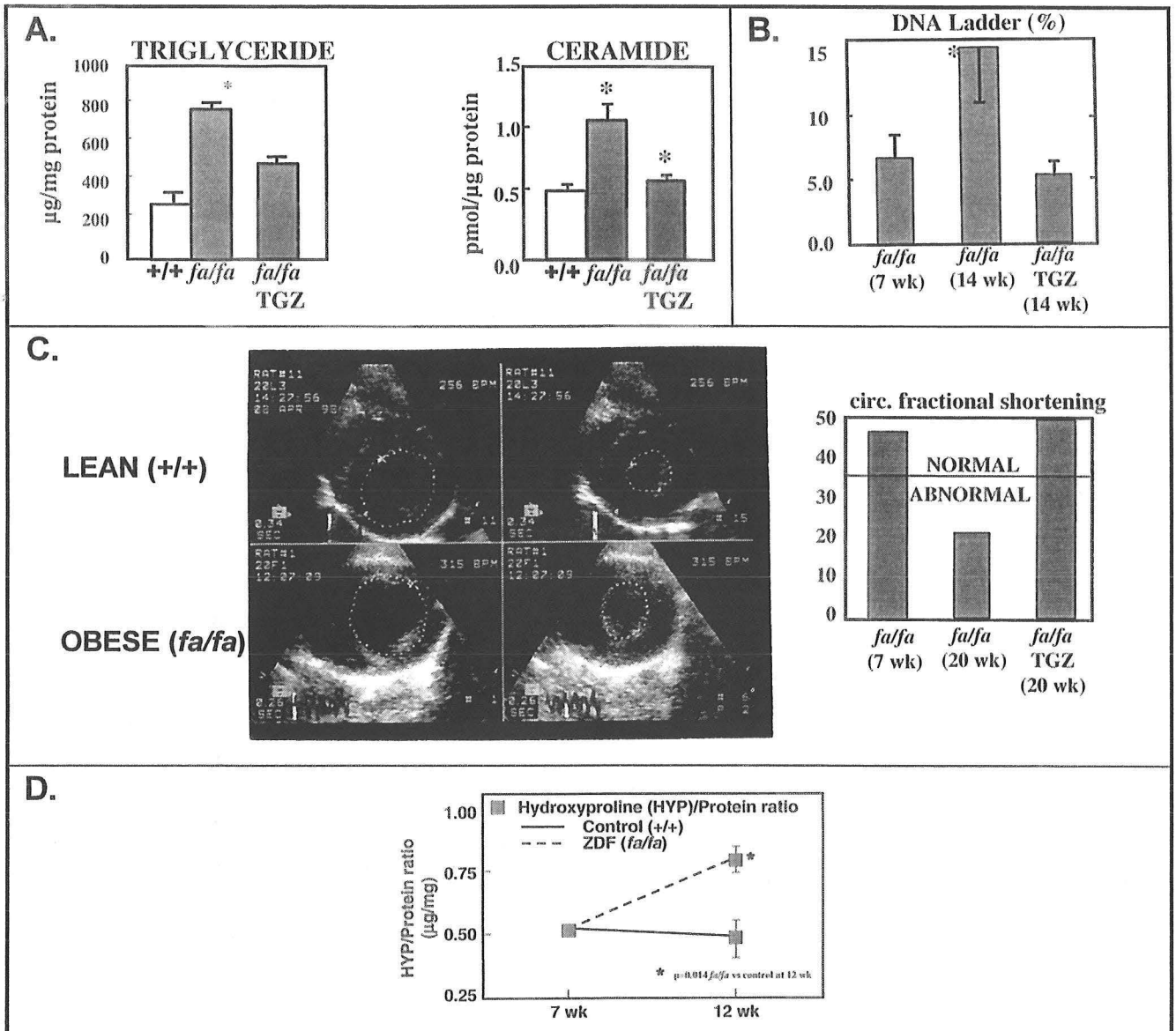
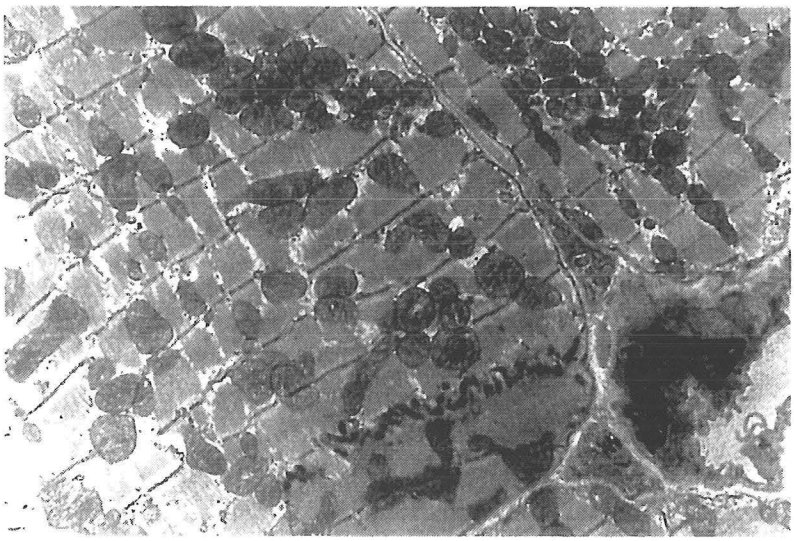


Figure 24 – (A) Cardiac triacylglycerol (TG) and ceramide content in 7-week old (prediabetic) and 14-week old (diabetic) ZDF (*fa/fa*) rats and wild-type controls (+/+). There is a statistically significant increase in both TG and ceramide content at both age points. Troglitazone (TGZ) treatment for 6 weeks significantly reduces the TG and ceramide content towards normal. * $p < 0.01$. **(B).** DNA laddering, an index of apoptosis is increased in heart of obese *fa/fa* ZDF rats at age 14 weeks. The increase is entirely blocked by treatment of the rats with the antisteatotic agent, troglitazone (TGZ), beginning at 7 weeks of age. **(C). Left panel** - Echocardiographic images from a normal rat lean (+/+) rat at age 20 weeks and an obese *fa/fa* age-matched ZDF rat. The dotted lines represent the cavity area for end-diastole **(left)** and end-systole **(right).** Both the end-diastolic and end-systolic areas are much larger in the *fa/fa* rat, indicating a dilated ventricle with poor systolic contraction. **Right panel** - Quantification of circumferential fractional shortening, showing functional loss in 20 week old *fa/fa* prevented by treatment with the antisteatotic drug, troglitazone (TGZ). **(D)** Comparison of the hydroxyproline to protein ratio, an index of fibrous tissue formation in the myocardium of obese *fa/fa* ZDF rats and lean +/+ controls. **(E).** Electonmicroscopic comparisons of myocardium of 20 week old normal **(left)** and obese (*fa/fa*) **(right)** ZDF rats showing dramatic increase in fat droplets **(next page).**

Myocardium from lean +/+ ZDF rats



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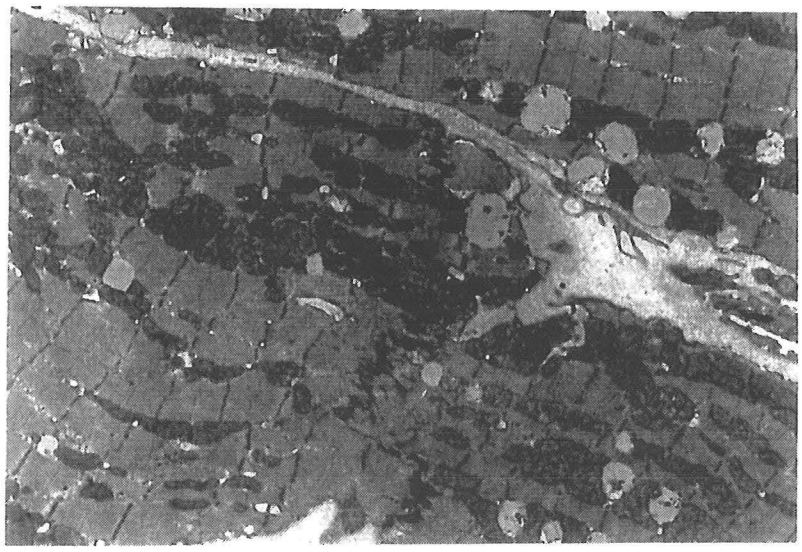
Myocardium from obese *fa/fa* ZDF rats



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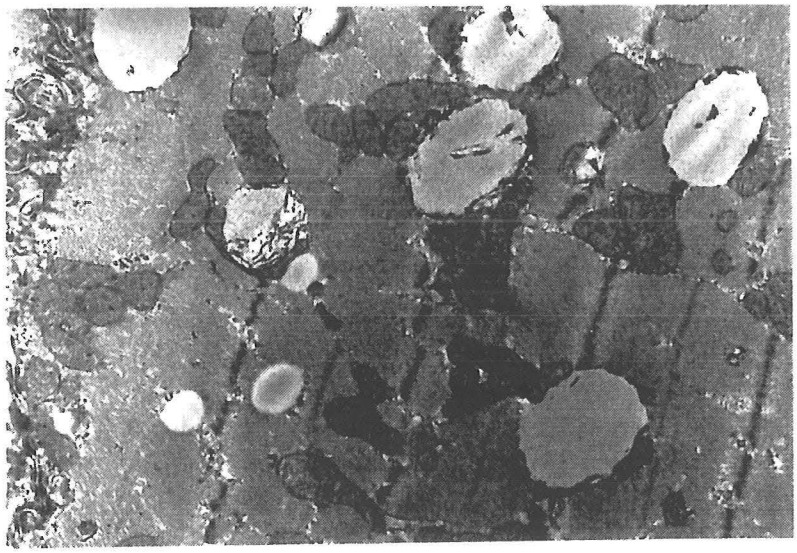
Closeup of obese myocardium



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3) Increased Autoimmunity: There is reason to suspect overlap between the pathways of FA-induced apoptosis and autoimmune apoptosis **(Table 1, #5)**. **Figure 25A** shows the overlap between cytokine-induced autoimmune destruction of β-cells (Type 1 diabetes), in which all cells are killed, and FA-induced lipotoxic destruction of β-cells, in which the loss of β-cells is limited to fat-laden cells. **Figure 25B** shows that FA can potentiate cytokine-mediated destruction of islets, raising the possibility that antisteatotic agents might reduce susceptibility to autoimmune disorders.

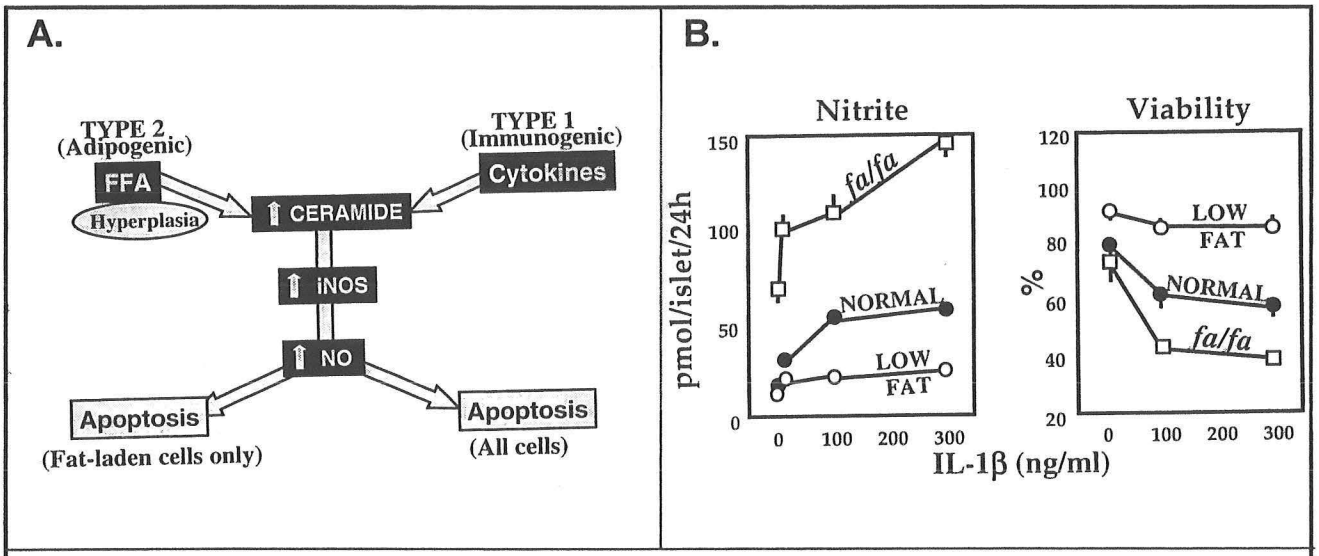


Figure 25 – ***(A)*** *Depiction of the "final common pathway" in Type II adipogenic diabetes and Type I immunogenic diabetes.* In both forms of diabetes, ceramide has been implicated as the signal that increases inducible nitric oxide synthase (iNOS) upregulation and thus increases nitric oxide (NO) formation.The ceramide increase in adipogenic diabetes is believed to arise from *de novo* synthesis from the high FA content (27, 32), whereas in immunogenic diabetes the increase is believed to be the consequence of increased breakdown of sphingomyelin catalyzed by sphingomyelinase (29, **Fig. 17**). (B) Evidence of interplay between islet lipid content and sensitivity to the cytotoxic effects of interleukin (IL)-1β. NO production, quantified as nitrite, is minimal when TG content is low and maximal when high, and loss of viability is minimal when TG is low and maximal when it is high.

DIET-INDUCED OBESITY (DIO)

Loss of protection against steatosis in DIO: Obese *fa/fa* ZDF rats are born without antisteatotic protection. Their congenital hyperphagia results in marked obesity and their lack of antisteatotic protection causes early overaccumulation of TG in nonadipocytes. This is in sharp contrast to DIO, in which hyperphagia and obesity may begin at any age and in which the intact leptin-regulated homeostatic system easily protects nonadipocytes from steatosis. Can DIO cause steatosis? We postulate that the protective system was designed to prevent steatosis during transient overeating, as depicted in **Figures 26A.** But midway in the current century, a radical and historically unprecedented change in this phasic pattern of caloric storage and caloric expenditure took place in the U.S **(Fig. 26B).** Instead of transient dietary excess as a survival device, an insurance against inevitable future need, the intake of unneeded calories became a life-style and the future need indefinitely postponed. Transient obesity was therefore replaced by constant and progressive

obesity **(Fig. 26B).** Even so, the associated hyperleptinemia protected nonadipocytes from TG overload.

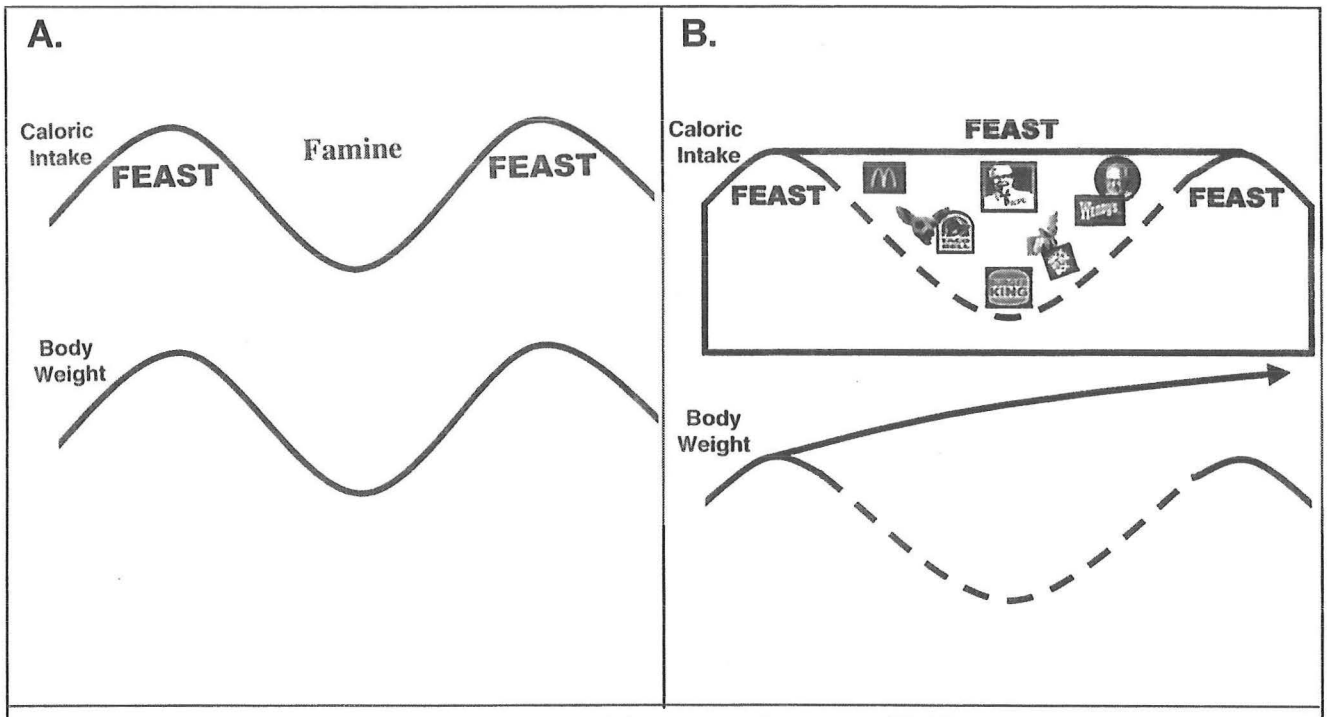


Figure 26 – (A). The pattern of caloric intake and fat storage in nature. **(B)** The pattern of caloric intake and body weight that has become so prevalent in the U.S.

Nevertheless, after decades of dietary excess, the protective capacity against steatosis may be exceeded. At this point, TG increases in nonadipose tissue and, we suppose, complications qualitatively similar to those of congenital obesity in rodents can arise. They could be the consequence of simple overloading of the antisteatotic system, of post-receptor leptin resistance or of aging or combinations thereof **(Table 5).**

TABLE 5: POSSIBLE EXPLANATIONS FOR LATE STEATOTIC COMPLICATIONS OF DIO:
1. SYSTEM OVERLOAD
2. POST-RECEPTOR LEPTIN RESISTANCE
3. AGING

1) System overloading: As depicted in **Fig. 26,** the role of the adipocyte was to serve as a temporary repository for excess calories preloaded for subsequent retrieval and use. It is proposed that continuous overeating and progressively increasing obesity simply overwhelms the capacity of a perfectly normal leptin-regulated system of FA homeostasis **(Figure 26).**

2) Post-receptor leptin resistance: A second explanation for steatotic complications of DIO is that a post-receptor leptin resistance develops. For example, SOCS-3 (Suppressor of Cytokine Signaling-3) (47) has been found to be increased in the adipocytes of rats with DIO **(Fig. 27)**; a

similar increase of SOCS-3 in nonadipose tissue could make these cells leptin-resistant and unresponsive and this would favor steatotic complications. Other candidate leptin resistance factors, such as SHP-2 (48) and PIAS have not yet been studied in DIO.

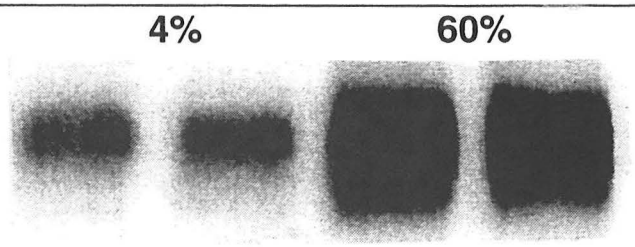


Figure 27 – Immunoblot for SOCS-3 in adipocytes of normal rats on a 4% or 60% fat diet. The latter were massively obese **(Figure 4).**

3) Aging: Leptin responsiveness declines dramatically with age. Since obesity takes many years to develop, much of the resistance to leptin may be age-related, thus explaining why complications of DIO usually occur late in life. We have observed dramatic loss of sensitivity to leptin in aging rats. In young rats adenovirally-induced hyperleptinemia dramatically depletes body fat within 7 days (44, 45); in old rats, the same level of hyperleptinemia has little effect on body weight **(Fig. 28).** Thus aging, with or without obesity, favors the ectopic deposition of TG. Many of the abnormalities ascribed to old age may, in fact, be related to this loss of leptin sensitivity and the resulting steatosis. The cause of age-related leptin resistance has not yet been determined.

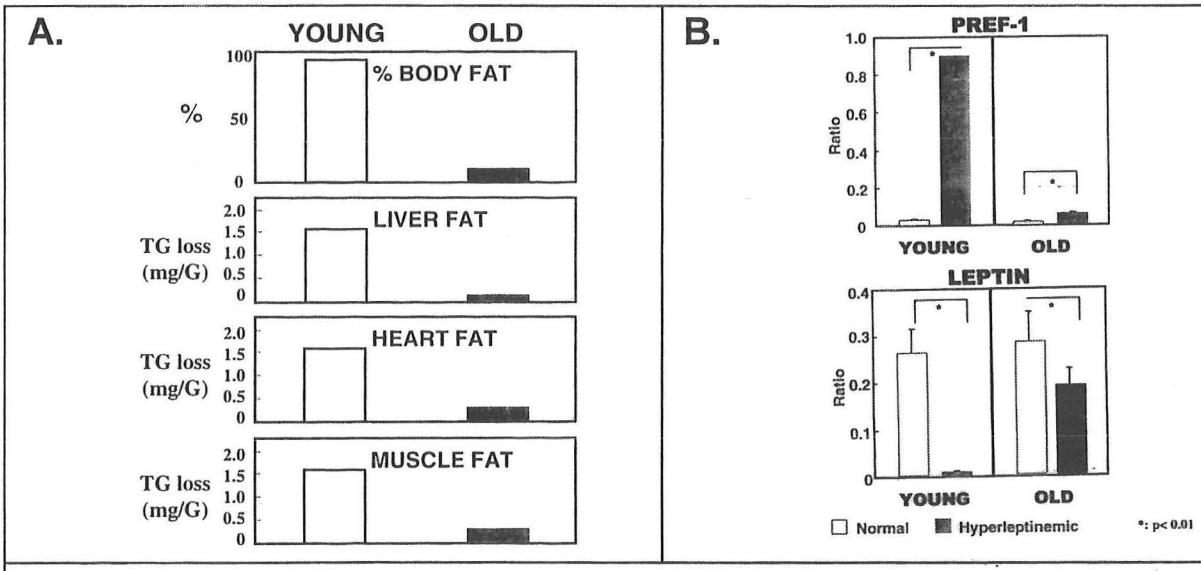


Figure 28 – Comparison of the effect of adenovirus-mediated hyperleptinemia on young (8-week-old) and elderly (72-week-old) normal ZDF rats (+/+). **(A) Upper panel** – effect of hyperleptinemia on total body fat measured by MRI **(lower panel).** Directly measured TG content of liver and heart muscle in hyperleptinemic rats. The results reveal a dramatic loss of the responses to leptin with aging. **(B)** Comparison of 2 indices of leptin action on adipocytes following adenovirus-induced hyperleptinemia in the young and old rats. In young rats, the disappearance of body fat is accompanied by expression of PREF-1, a preadipocyte marker, as TG disappear from adipose tissue **(Fig. 29).** While PREF-1 also increases significantly in the adipocytes of the old rats, the increase is minute compared to that observed in the young rats. Additionally, in young rats, the ectopic hyperleptinemia induced by adenoviral gene transfer virtually wipes out the expression of leptin mRNA in the disappearing adipocytes. In the old rats, this autosuppressive effect, although still statistically significant, is markedly attenuated.

NOTE: These findings point strongly to age-related resistance of adipocytes and nonadipocytes to the lipopenic actions of leptin. They go a long way in helping us to understand the phenotype of aging and its relationship to lipid content.

TREATMENT OF OBESITY:

1) Caloric restriction: Existing anti-obesity treatments and prophylaxis are, for complex reasons, ineffective and are often complicated by undesirable side effects **(Table 6)**, including ketosis, hyperuricemia and loss of lean body mass as well as fat. Moreover, caloric restriction exposes nonadipose tissues to a potentially lipotoxic milieu by flooding them with FA mobilized from adipocytes at a time when protection from hyperleptinemia ends as plasma leptin falls during caloric restriction. The relapse rate following weight reduction by caloric restriction is extremely high. Despite these drawbacks, it remains the mainstay of current treatment options.

Table 6. Drawbacks of Caloric Restriction for Obesity

1. **Usually ineffective**
2. **↑ protein loss**
3. **Lipotoxicity (↑ FA and ↓ leptin)**
4. **Ketosis**
5. **Hyperuricemia**
6. **Very high relapse rate**

2) Alternative anti-obesity approach: A novel approach to the treatment of obesity is suggested by the effect of adenovirally-induced hyperleptinemia in normal rats. Within one week, all body fat disappears without any ketonemia or ketonuria or loss of lean body mass. Adipocytes are transformed from fat-storing white adipocytes into fat-burning cells resembling brown adipocytes (9, 48) **(Fig. 29).** The energy is presumably dissipated as heat, as indicated by the upregulation of uncoupling proteins-1 and 2 (UCP-1 and 2). Most interestingly, the fat-depleted adipocytes lose aP_2, a marker of mature adipocytes and they acquire the preadipocyte marker, PREF-1 **(Fig. 29).** The lipogenic enzymes and their transcription factor, PPARγ, disappear in these cells and are replaced by enzymes of fatty acid oxidation and their transcription factor, PPARα **(Fig. 29).** In other words, the phenotype of white adipocytes is lost and in its place is the phenotype of a preadipocyte. Since lipogenic enzymes are no longer expressed, the dedifferentiated adipocytes redifferentiate very slowly into mature adipocytes, as shown in **Figure 30.** Recovery of body fat after severe hyperleptinemia did not occur over a 9-month follow-up period. Since adenovirally induced hyperleptinemia is not at present feasible for human DIO, an agent which produces the phenotypic alterations induced by leptin would be a potentially therapeutic agent.

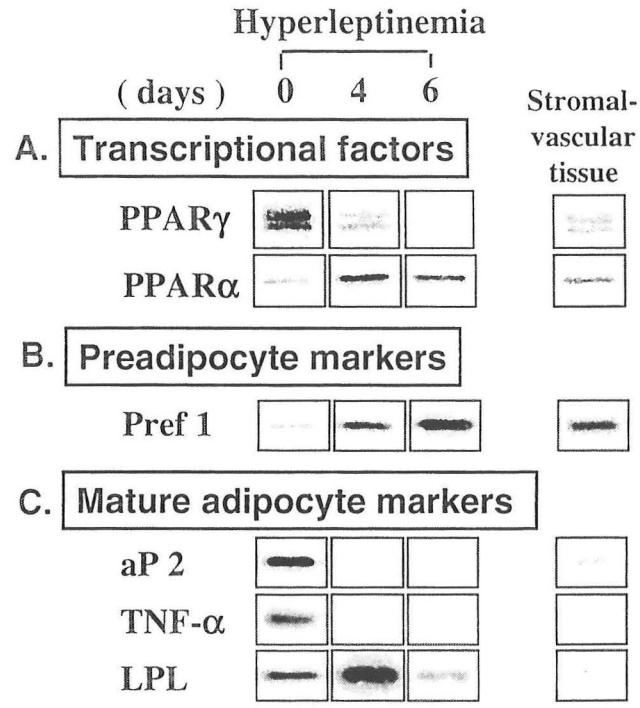
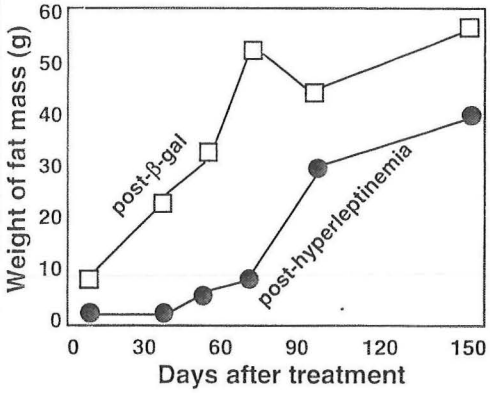


Figure 29 - Effect of hyperleptinemia induced by adenoviral-mediated gene transfer on the expression phenotype of disappearing fat tissue of normal rats. Disappearance of TG is complete by the 7th day, leaving only stromal vascular (SV) tissue. **(A)** Immunoblots quantifying PPARγ, the anabolic transcription factor for enzymes of FA synthesis, normally expressed at high levels in adipocytes, and PPARα, the catabolic transcription factor that upregulates enzymes of FA oxidation. Note that the expression phenotype has changed from that of a fat-storing adipocyte at 0 time (before hyperleptinemia) to that of a "fat-burning" cell after 6 days of hyperleptinemia. **(B)** mRNA markers of preadipocytes (PREF-1) and **(C)** of mature adipocytes (aP_2, TNFα and lipoprotein lipase). aP_2 and TNFα virtually disappear from mature adipocytes as they "dedifferentiate". mRNA levels were semiquantified by RT-PCR.



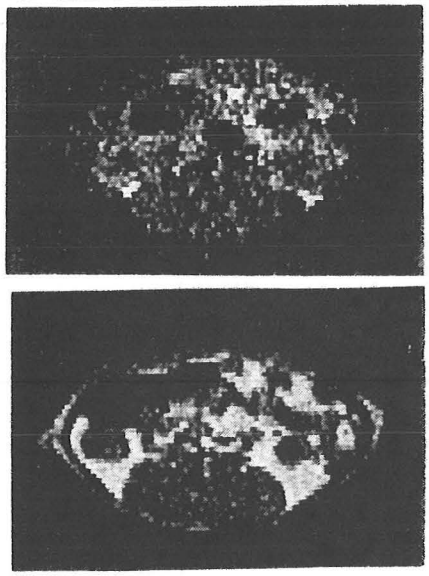


Figure 30 (A). Body fat after virus-induced hyperleptinemia. Regain of body fat in post-hyperleptinemic rats lags behind the controls that received virus-βgal and were diet-matched to virus-leptin group.The controls ate less than the post-hyperleptinemics. **(B).** MRI of a post-hyperleptinemic rat **(upper)** and a post-βgal rat **(lower)** ~5months after treatment. Leptin levels were similar in both groups after about 30 days.

One such agent has been tested. It is derived from a Chinese herbal remedy, Tripterygium Wilfordii Hook F (TWH) and is called triptolide (46). It upregulates PPARα and increases lipolysis in adipocytes, but its effectiveness in reducing in body fat is still under study.

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Former Post-Doctoral Fellows on this project:

Joe Milburn, MD
Hirosi Hirose, MD
Kazumori Koyama, MD

Current Post-Doctoral Fellows:

Moritake Higa, MD
Tetsuyama Kokuma, MD
Wentong Pan

Faculty:

Young Lee, PhD
Yan-Ting Zhou, MD, PhD
May-Yun Wang, PhD
Zhou-Wei Wang, MD

Collaborators:

Lelio Orci, MD
Chris Newgard, PhD
Paul Grayburn, MD

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