INADEQUACY OF FAT CELLS

Mechanisms and Management of America's Major Health Problem

Roger H. Unger, M.D.

March 16, 2007

INTERNAL MEDICINE GRAND ROUNDS
UNIVERSITY OF TEXAS SOUTHWESTERN MEDICAL CENTER AT DALLAS

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THE AMAZING ADIPOCYTES

Adipocytes are unique in the following respects. 1) They are specifically designed to prolong survival during famine by storing surplus calories as triacylglycerol (TAG) and redistributing their free fatty acids (FFA) systemically when needed (Neel, 1962). 2) They constitute the largest endocrine gland with over 30 adipocyte specific secretory products thus far identified (Figure 1). 3) They are dispersed throughout the entire body. 4) They serve to cushion and to insulate. 5) In addition to systemic distribution of FFA, adipocytes maintain symbiotic partnerships with adjacent cells, particularly high-maintenance contractile cells such as the skeletal myocytes, to provide local fueling (Figure 2). 6) Adipocytes have an enormous capacity for hypertrophy and hyperplasia with the potential to expand to more than 2-3 times the weight of the lean body mass, far beyond any other tissue (cf. cover). 7) They protect nonadipocytes from the metabolic burden of ectopic lipid overload by sensing perturbations in caloric balance, by signaling hypothalamic centers that control feeding behavior (Elmquist *et al*, 2005), and by increasing lipo-oxidation in nonadipose tissues (Shimabukuro *et al*, 1997) (Figure 3) through adipocytokines such as leptin (Zhang *et al*, 1994) and adiponectin (Scherer, 1995). 8) Failure of adipocytes to protect against ectopic lipid deposition, irrespective of the reason, will be referred to as "fat cell insufficiency", and is proposed in this Medical Grand Rounds to be the cause of America's most prevalent life-shortening morbidity, the metabolic syndrome.

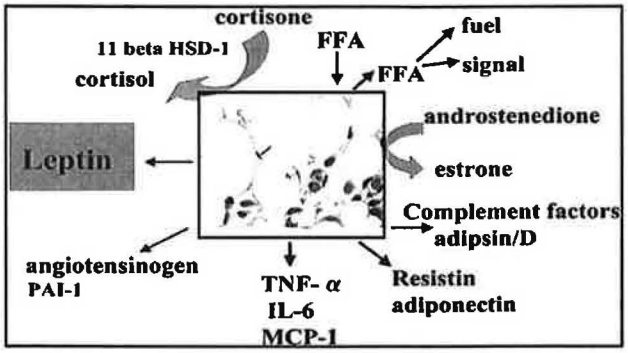


Figure 1. Some of the hormones and other secreted factors of adipocytes. From Flier, J.S. (2004). Cell *116*: 337-350.

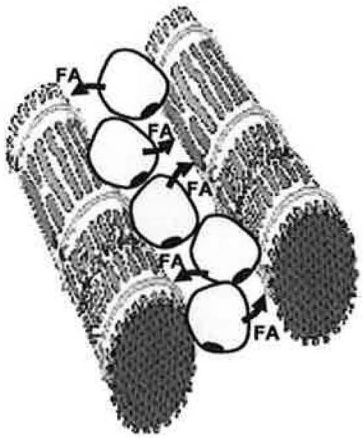


Figure 2. Hypothetical symbiotic relationship between adipocytes and skeletal myocytes. Local adipocytes supply fatty acids (FA) for limited local muscular activities without the need for systemic mobilization of FA release from remote adipocytes. The level of muscular activity would determine the relative abundance of the two cell types. Chronic inactivity, for example, would result in adipocyte hypertrophy and myocyte sarcopenia, i.e., flabby marbleized muscles, while habitual exercise would reduce adipocyte fat and produce lean muscle.

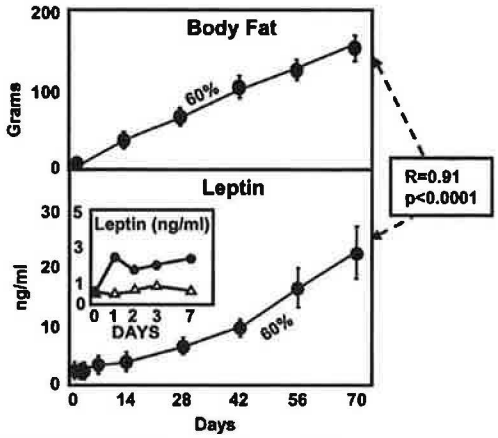


Figure 3. Parallelism between expanding fat mass and plasma leptin levels beginning within 24 hours after start of a 60% fat diet. Overnutrition is the only known physiologic cause of hyperleptinemia.

DEFINITION OF FAT CELL INADEQUACY

"Fat cell inadequacy" is defined as an inability of available adipocyte space to contain the unutilized lipids present in the body. This mismatch may be due to subnormal adipocyte storage capacity, to an excessive caloric intake that exceeds the normal adipocyte capacity, to subnormal caloric utilization or to combinations thereof. The clinical expression of fat cell inadequacy, we believe, is the metabolic syndrome.

ROLE OF ADIPOCYTES AND LEPTIN IN NATURAL SELECTION

The cyclic nature of nutrient availability on the earth made it essential for evolving species to adapt to seasonal and episodic periods of caloric scarcity. All cells have the capacity to store modest amounts of fuel in the form of TAG for emergency use, but their tolerance for lipids is limited by a relatively low threshold for lipid-induced injury. Clearly, extended survival of complex organisms on this planet required the evolution of an independent, high capacity compartment specialized to stockpile unutilized fuels ingested during nutrient abundance and to redistribute them to all tissues when needed (Neel, 1963). At the same time, it was vital that the stockpiled fuels be confined to the high capacity compartment with minimal spillover into organs with a low fuel storage capability (Unger, 2003).

The adipocytes meet these specifications to a remarkable degree. Meal-stimulated insulin secretion promotes the upregulation of lipogenic enzymes in liver and adipocytes, while providing both organs with lipogenic substrate via enhanced glucose and fatty acid (FA) uptake and metabolism. The glucose-derived hepatic lipids reach the adipocytes via very low density lipoprotein (VLDL) secretion, and,together with lipids entering from dietary fat, contribute the FA to be stored in adipocytes as TAG. The adipocytes, in turn, provide protection of nonadipocytes from lipid overload through their remarkable storage capacity and through their adipocytokines, such as leptin (Zhang *el al*, 1994) and adiponectin (Scherer *et al*, 1995; Ahima *et al*, 2006; Matsuzawa, 2005).

THE PHYSIOLOGICAL ROLE OF LEPTIN

The physiologic role of leptin, originally thought to be an anti-obesity hormone, has been controversial. One way to discern the physiologic role of a hormone is by its response to real-life homeostatic perturbations. The only real-life perturbation known to stimulate leptin hypersecretion is overnutrition (Figure 3). During overnutrition leptin levels rise in lock-step with adipocyte expansion, which proves that leptin is not an anti-obesity hormone. It also suggests that the hyperleptinemia of obesity must confer some important survival function during overnutritional expansion of the fat mass. We currently believe that one such function is to protect non-adipose tissues from ectopic lipid deposition (Figure 4).

Hyperleptinemia promotes compartmentalization of surplus calories, 1) by limiting the level of overnutrition by inhibiting orexigenic factors and enhancing anorexic factors in the hypothalamus (Figure 4) (Elmquist *et al*, 2005; Schwartz *et al*, 2000), and 2) by minimizing lipid spillover into peripheral nonadipose tissues by increasing oxidization of ectopically deposited FA through adipocytokines such as leptin working via mechanisms depicted in Figure 4.

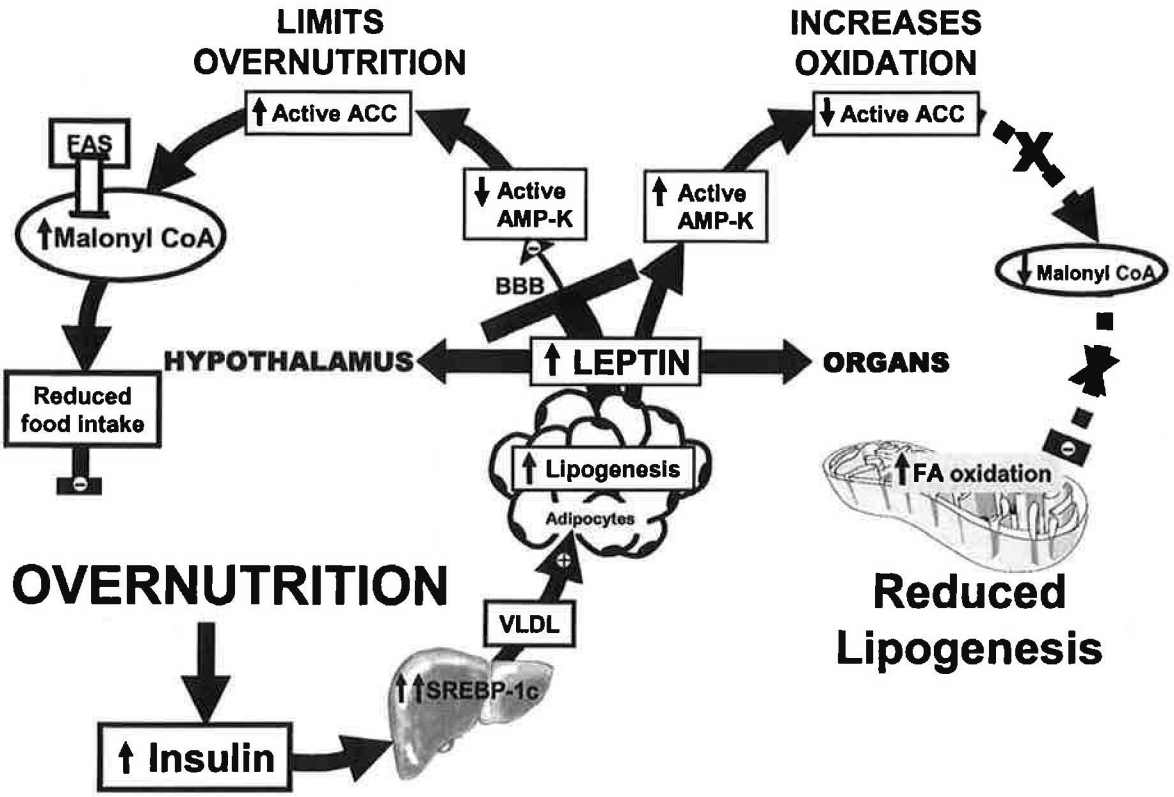


Figure 4. Protection against ectopic lipid overload 1) through stockpiling in an expanded adipocyte mass, 2) through leptin-mediated action on hypothalamus centers to limit overnutrition and 3) on peripheral tissues to increase oxidation of fatty acids overflow. Note that hyperleptinemia inactivates AMP-activated protein kinase (AMPK) in hypothalamus and activates it in peripheral nonadipose tissues. The result in the latter is to lower malonyl CoA and thus remove its inhibition of carnitine palmitoyl transferase-1 (CPT-1), thereby increasing mitochondrial fatty acid (FA) oxidation. In the hypothalamus AMPK inactivation increases malonyl CoA and somehow inhibits appetite (Hu, *et al*, 2003) BBB=blood-brain barrier, FAS= fatty acid synthetase, C-75, a potent FAS inhibitor.

When normal individuals begin to expand their adipocyte mass, initially the surplus calories can be accommodated in the fat cell mass. However, plasma FFA and TAG concentrations rise as obesity progresses. When they exceed the upper limit of normal, nonadipose cells are exposed to nonspecific, unregulated "flip-flop" diffusion of FA across their plasma membranes (Figure 5) (Hamilton, 2003). By contrast, at low FFA concentrations, as after a meal, FA transport into cells is mediated by insulin-regulated translocation of fatty acids transporters (FAT), also known as CD36, from the rough endoplasmic reticulum to the plasma membrane in target tissues of insulin, such as liver, muscle, pancreatic islets and heart (Hajri and Abumrad, 2002). This is analogous to insulin-mediated translocation of glucose transporter, GLUT4, and glucose uptake. The unregulated FA uptake at high plasma FFA concentrations is believed to play a major role in the ectopic deposition of lipids in obesity.

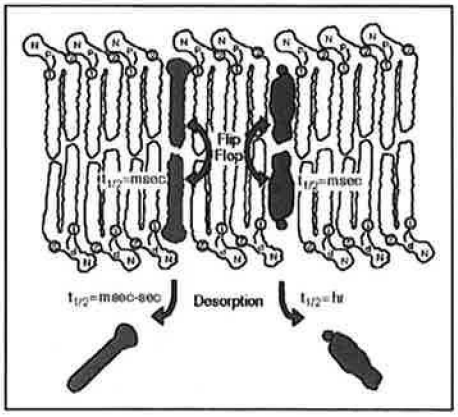


Figure 5. Schematic diagram of transmembrane movement (flip-flop) and desorption of fatty acids (blue) and cholesterol (red) Flip-flop is the reorientation of the molecule and its polar moiety from one aqueous interface to the opposite interface. A phosphatidylcholine bilayer is illustrated, with N representing the choline group; P the phosphate group, and 1 and 2 the sn-1 and sn-2 carbonyl groups. *From:* Hamilton: Curr Opin Lipidol, Volume 14(3).June 2003.263-271

The lipo-oxidative action of leptin on the peripheral non-adipose tissues is mediated both by direct effects mediated by the local leptin receptor (Lepr) and by indirect hypothalamic effects (Park *et al*, 2006). The former include activation of AMP kinase, an energy sensor (Hardie *et al*, 2006), which, by inactivating acetyl CoA carboxylase (ACC), lowers malonyl CoA synthesis; this disinhibits mitochondrial FA oxidation, and blocks FA synthesis (McGarry *et al*, 1977) (Figure 6). It also inhibits SREB-1c expression, which reduces the enzymes of lipogenesis and upregulates those of FA oxidation (Horton *et al*, 2002; Kakuma *et al*, 2000). Finally, it enhances mitochondrial biogenesis by upregulation of peroxisome proliferator-activated receptor-γ coactivator 1α (PGC-1α). Surprisingly, in contrast to its activating effect in peripheral tissues, leptin inactivates AMPK in the hypothalamus (Kahn *et al*, 2000). The integration of hypothalamic and peripheral actions is depicted in Figure 4C (Unger, 2004).

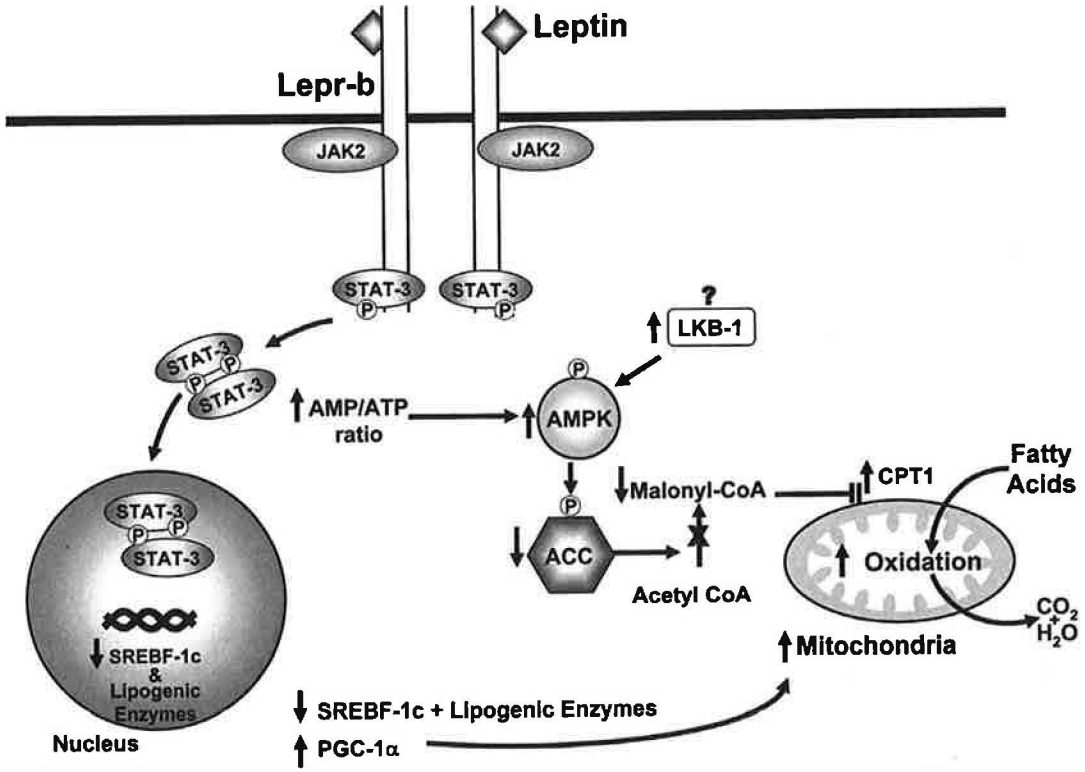


Figure 6. Depiction of hyperleptinemia-induced actions that reduce intracellular lipid content via direct local action on organs by enhancing the activity of AMP-K via unknown actions. Transcription of leptin actions are probably mediated largely through STAT-3 phosphorylation which upregulates PGC-1α, an inducer of mitochondrial biogenesis and uncoupling proteins (UCP)-1 and -2. Downregulation of SREBP-1c and its lipogenic targetenzymes completes the lipopenic actions.

When leptin action is congenitally absent, as in rodents with loss-of-function mutations in their Lepr, the resulting combination of hyperphagia, enhanced lipogenesis and reduced lipo-oxidation leads to ectopic lipid deposition (Figure 7). This causes functional aberrations in the more vulnerable organs and lipoapoptosis of their cells, with ultimate organ failure known as "lipotoxicity" (Lee *et al*, 1994). Both the high TAG content and the organ dysfunction in these organs are improved by organ-specific transgenic expression of a normal Lepr-b.

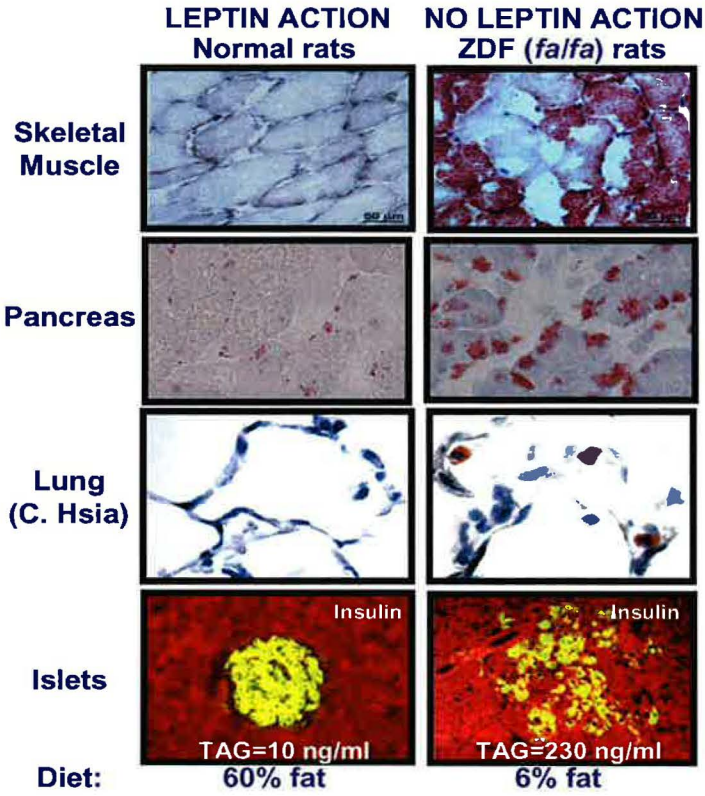


Figure 7. Comparison of oil red O stain of various tissues in normal rats on a 60% fat diet and ZDF obese *fa/fa* rats with unresponsive leptin receptors on a 6% fat diet. The islets were stained for insulin and isolated islets were assayed for TAG.

THE CAUSE OF METABOLIC SYNDROME

Is Insulin Resistance the Cause? Insulin resistance has been proposed as an underlying cause of metabolic syndrome, which is often called "insulin resistance syndrome" (Reaven, 2005) (Figure 8). However, most insulin resistance is now thought to be secondary to the lipid overload in liver and skeletal muscle, rather than to any preexisting abnormality (Samuel *et al*, 2004; Petersen and Shulman, 2002). This raises the possibility that, far from being the proximal cause of disease, the insulin resistance associated with the metabolic syndrome is a manifestation of the ectopic lipid deposition. If so, insulin resistance could be a compensatory mechanism that reduces insulin-stimulated glucose and FA uptake in the lipid-laden tissues and thereby prevents further ectopic lipid deposition (Unger, 2003).

Is Obesity the Cause? It is also widely believed that obesity is an underlying cause of the metabolic syndrome (Kahn *et al*, 2006) (Figure 8). However, evidence presented below suggests that obesity, like insulin resistance, may be protective, at least initially, against the metabolic syndrome. However, the gradually rising FFA levels that accompany obesity will ultimately augment unregulated ectopic influx of FA into peripheral tissues via the flip-flop mechanism.

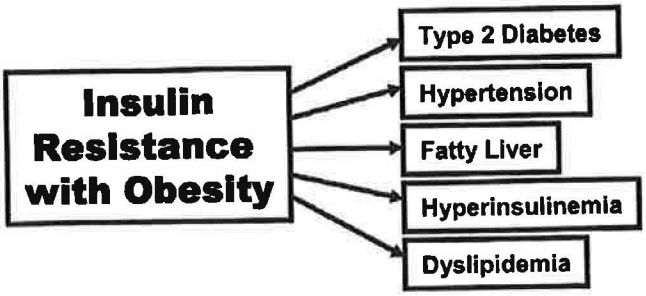


Figure 8. Classic concept of the pathway to metabolic syndrome.

Experiments in overfed rodents indicate that obesity does not directly cause the metabolic syndrome, but rather that it delays it (Figure 9A-E). This was demonstrated in aP2-Lepr-b transgenic mice in which adipocytes cannot expand in size or number, despite a 60% fat diet that causes severe obesity in wild-type controls. If obese *db/db* mice with nonfunctioning Lepr are crossed with aP2-Lepr-transgenic mice, in which obesity cannot occur despite overfeeding, nonobese offspring with the combined lean aP2-Lepr-*b-db/db* genotype develop diabetes 3-4 weeks before the obese *wild type +/+-db/db mice*.

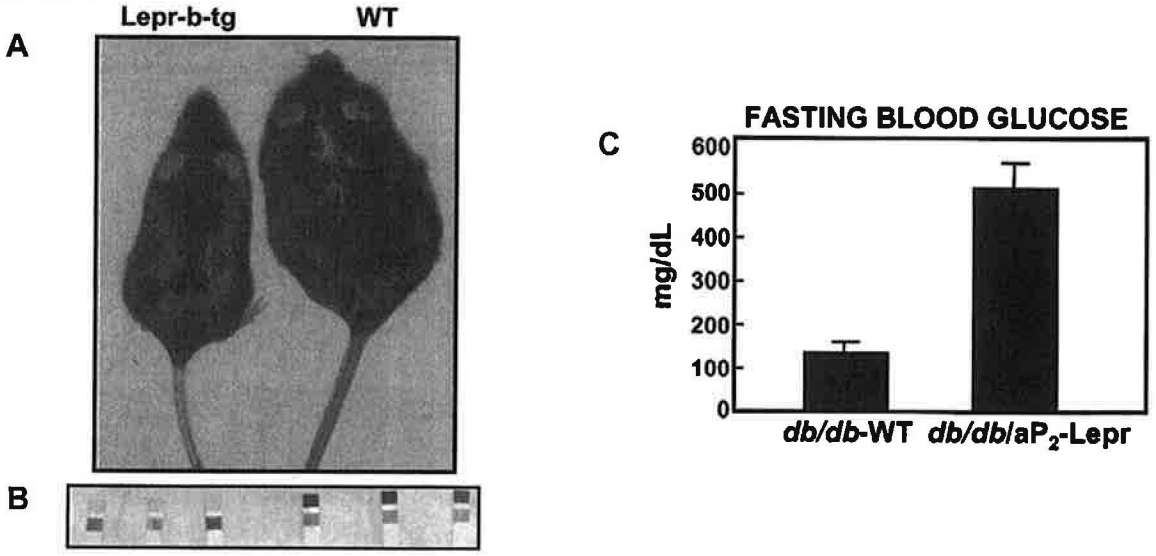


Figure 9. Obesity delays the onset of the diabetes/metabolic syndrome. A. The appearance of Lepr-b-transgenic mice after 10 weeks on a 60% fat diet compared to wild-type (WT) controls. Food intake was identical. B. Urine glucose strips reveal glycosuria in the lean mice but not in the obese mice. C. Fasting blood glucose levels, showing onset of severe hypoglycemia in the lean mice while obese mice are still prediabetic.

They also develop more severe ectopic lipid deposition and organ pathology, such as myocardial hypertrophy (Figure 10) and echocardiographic abnormalities. In other words, obesity delays the metabolic syndrome until plasma FFA reach a critical "flip-flop" level of ~1.5 mM (Lee *et al*, 1994) (Figure 11). Thus, the obesity and the metabolic syndrome are etiologically independent and both are the consequences of overnutrition.

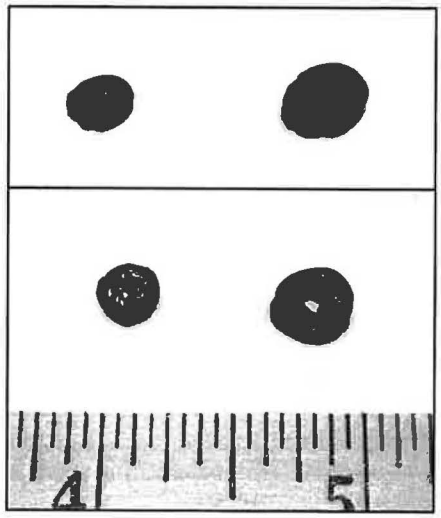


Figure 10. Gross appearance of the heart of a 10-month-old normal mouse (left) and an aP2-Lepr-b transgenic mouse (right) fed a 60% fat diet for 8 months showing hypertrophy and deletion in the latter.

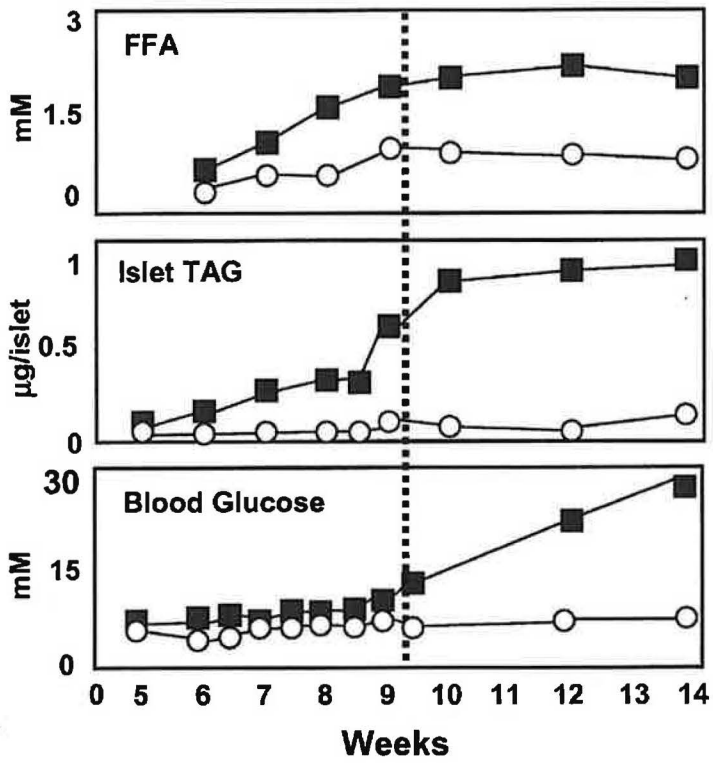


Figure 11. Plasma FFA profile of obese (■) ZDF rats relative to the islet TAG content and blood glucose levels. Note that as FFA levels exceed 1.5 mM, islet TAG content rises precipitously and glucose levels enter the diabetic range. (○)

Is Ectopic Lipid Deposition the Cause? Ectopic lipid deposition has been proposed as a cause of the metabolic syndrome (Unger, 2003). This was based largely on findings in a congenitally unleptinized rodent model, the Zucker Diabetic Fatty (ZDF) rat, in which the features of metabolic syndrome develop. However, the etiologic role of this lipid overload in the destruction of β-cells and other organs involved in the metabolic syndrome has been questioned on 2 counts.

One reason is because the pathogenic consequences of ectopic deposition of lipids in nonadipose organs vary so widely from organ to organ (Figure 12). For example, liver has a very high level of tolerance to ectopic fat deposition, while b-cells have low tolerance; but this may be because liver can secrete much of its lipid burden as VLDL, while b-cells cannot and are already maximally hyperplastic before their destruction begins. Similarly, lipid-laden skeletal muscle reveals little evidence of sarcopenia, while cardiomyocytes are very vulnerable to lipid overload; but this may reflect the high capacity of skeletal myocytes to replace themselves, whereas cardiomyocytes are terminal cells and cannot be replaced.

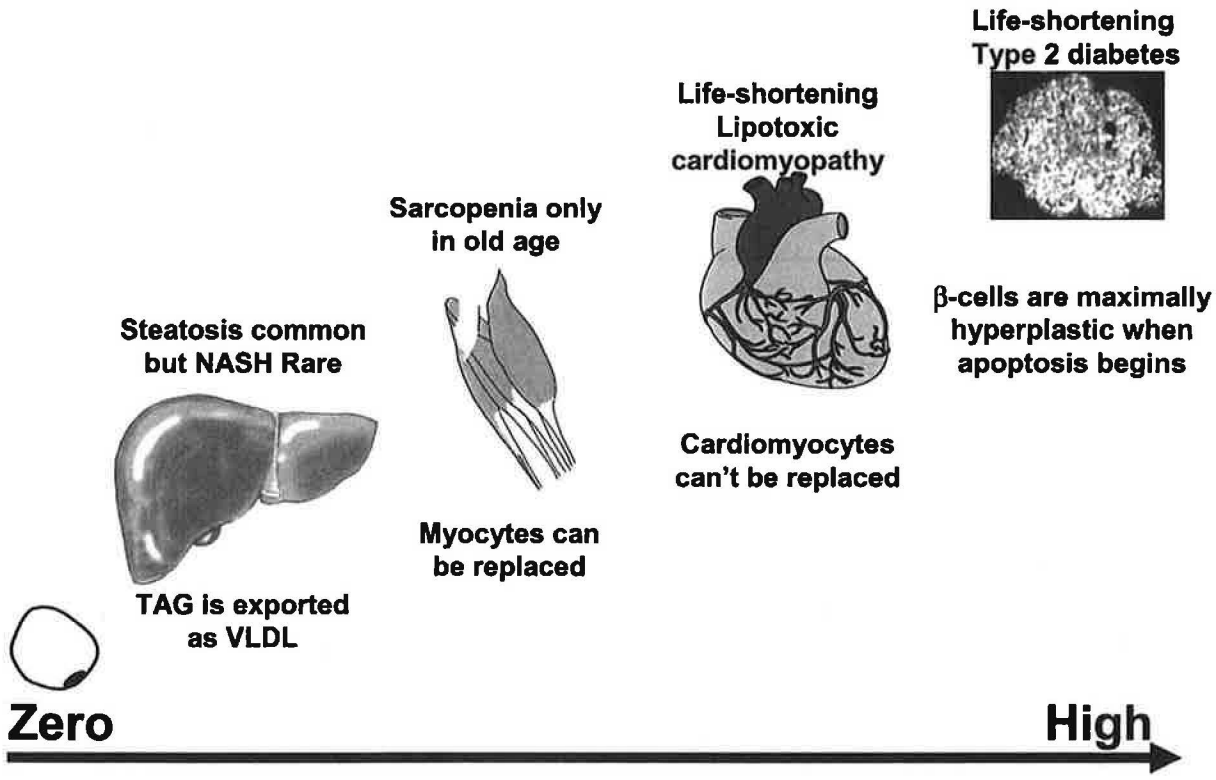


Figure 12. A comparison of the variable pathological consequences on lipid overload of various organs and the likely explanation for their wide difference in lipid tolerance.

The second and most potent argument against lipid overload as the cause of the metabolic syndrome of ZDF rats is the lack of direct *in vivo* evidence that lipids can kill normal cells in the absence of the myriad of associated diabetogenic risk factors, obesity, hyperlipidemia, insulin and leptin resistance, etc.

To determine if, in the absence of these risk factors, exposure to excess lipids can independently destroy perfectly normal cardiomyocytes and pancreatic β-cells *in vivo*, we tested this in the 2 most vulnerable of tissues in animals in which no diabetogenic risk factors were present.

Cardiomyocyte-Specific Lipotoxicity: Cardiomyocytes are terminal cells that cannot be replaced; when a cardiomyocyte is destroyed by lipid overload, cardiac function is permanently reduced. To prove that lipid excess could destroy cardiomyocytes, Jean Schaffer's group at Washington University, St. Louis, developed transgenic mice with cardiomyocyte-specific overexpression of acyl CoA synthetase (ACS) using the α-MHC promoter. These mice die prematurely of dilated cardiomyopathy due to FA-induced cardiomyocyte apoptosis resulting from the increased fatty acid transport (Chiu *et al*, 2001). This lethal syndrome could be completely prevented by reducing the high lipid content with either leptin treatment or α-lipoic acid, a leptinomimetic agent (Lee *et al*, 2004; Lee *et al*, 2006) (Figure 13). This indicates that, an excess of lipids is associated with destruction of normal cardiomyocytes in the absence of any diabetogenic, metabolic syndrome risk factors and that destruction can be completely prevented by lowering the cardiomyocyte fat.

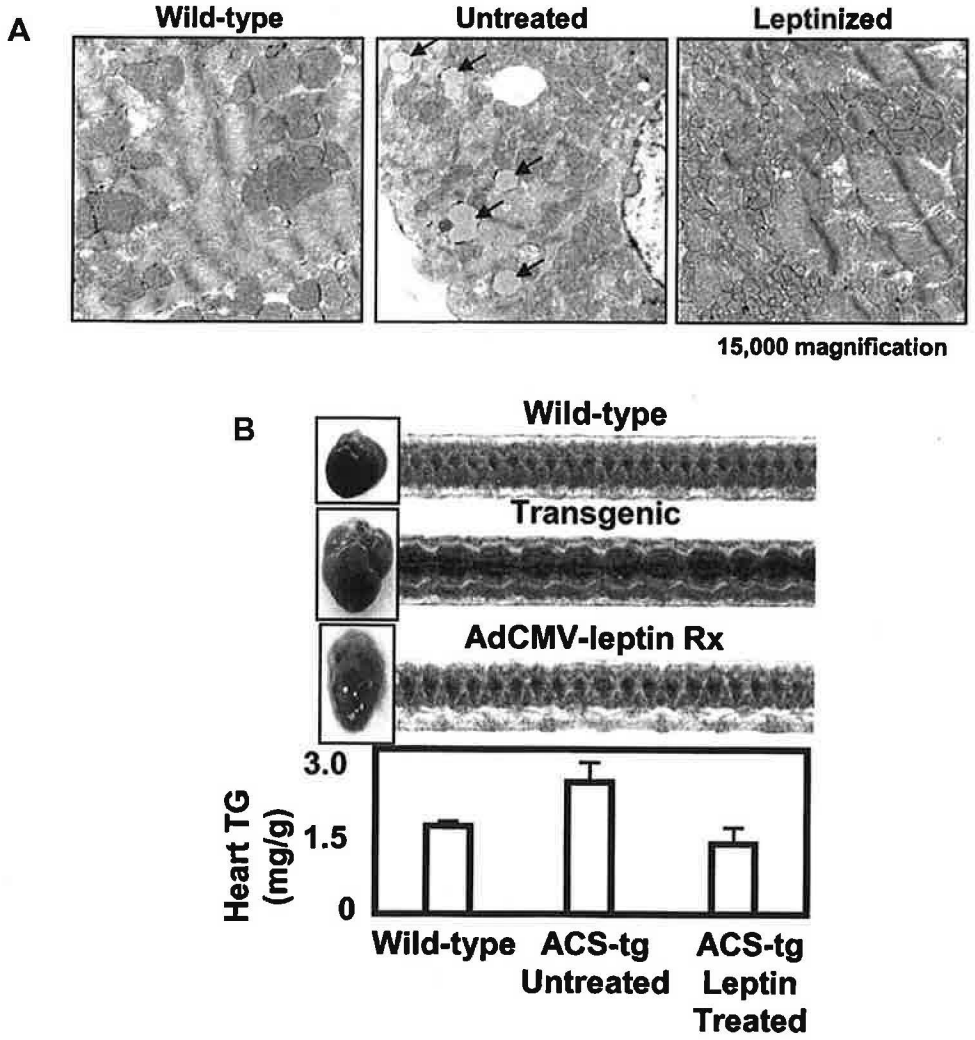


Figure 13. A. Electron microscope appearance of myocardial cells of three groups (wild-type, ACS-transgenic untreated and ACS-transgenic treated with AdCMV-leptin). Lipid vacuoles in cardiomocytes are limited to untreated mice and are marked by arrows. B. Gross appearance, echocardiographic tracing and TAG content of normal wild-type hearts and hearts of acyl CoA synthetase-transgenic (ACS-tg) mice untreated or made hyperleptinemic by adenoviral transfer of the leptin cDNA.

Beta Cell-Specific Lipotoxicity: *In vitro* exposure to FA mixtures impairs β-cell function of isolated rat islets (Zhou and Grill, 1994) and induces cytostatic and proapoptotic changes in isolated human islets (Lupi *et al*, 2002), but this has never been shown *in vivo*. To obtain specific *in vivo* evidence that a lipid surplus surrounding normal islets can destroy β-cells in the absence of any diabetogenic risk factors, and to identify the proximal cause of type 2 diabetes (T2D) and metabolic syndrome, normal pancreatic islets were transplanted into the liver of syngeneic recipient rats. The recipients were normal except for streptozotocin diabetes induced one week before transplantation. The rationale behind this model is that insulin hypersecretion by the transplanted islets would be driven by the high intraportal levels of nutrients and incretins, rather than by the usual stimuli, overnutrition, systemic hyperlipidemia, insulin and leptin resistance or obesity (Figure 14). The hepatocytes surrounding the transplants would respond to the high local insulin with upregulation of the lipogenic transcription factor, SREBP1c (Horton *et al*, 2002), and its lipogenic target enzymes, causing increased periinsular lipogenesis. The overabundance of the TAG in the VLDL surrounding the islets would be hydrolyzed by the islet lipoprotein lipase (Cruz *et al*, 2001) and the increased FA would flip-flop into the islets by diffusion. The effect of locally high lipid levels on β-cells would thus be tested *in vivo*.

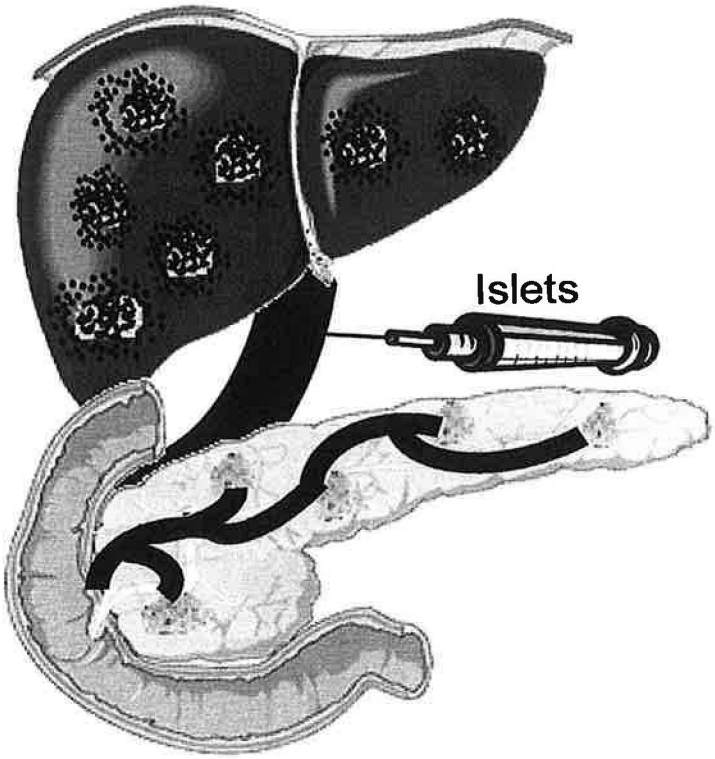


Figure 14. Rationale behind transplantation of normal islets into normal recipients to test the ability of peri-insular steatosis to destroy normal β-cells in the absence of any other of the known diabetogenic risk factors.

The foregoing predictions were borne out. As shown in Figure 15A, islets were surrounded by oil red O positive hepatocytes and total hepatic lipid levels were increased. This peri-insular steatosis was mediated by insulin-driven upregulation of SREBP-1, the lipogenic transcription factor (Horton *et al*, 2002) (Figure 15B), peri-insular evidence of which is demonstrated immunocytochemically in Figure 15C. β-cells were destroyed and replaced with fibrous tissue. However, when lipid levels were reduced by induction of hyperleptinemia or by diet restriction, both liver TAG content and β-cell apoptosis, demonstrated by TUNEL staining, were markedly reduced (Figure 15D), and β-cell loss and the hyperglycemia were prevented or greatly attenuated (Figure 16).

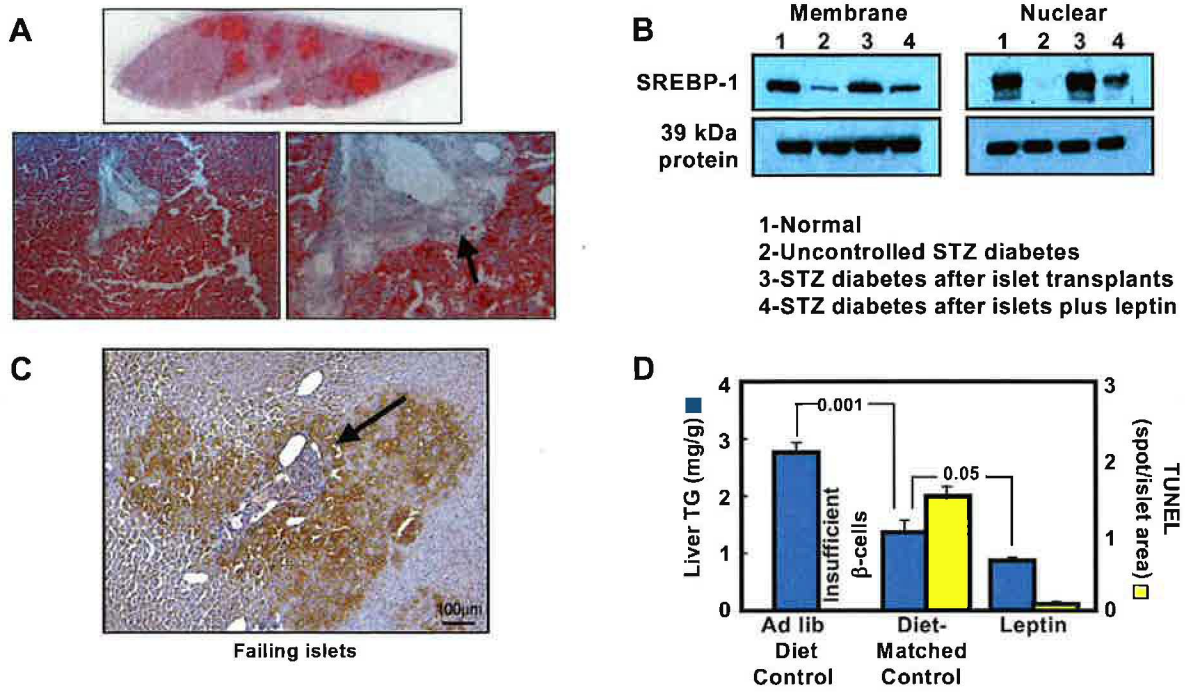


Figure 15 A. Oil red O staining of liver of islet transplant recipients showing at low and high power the lipid accumulation surrounding islets. Arrow points to the remnant of an islet presumably destroyed by lipotoxicity, B. Membrane and nuclear SREBP-1 protein, the insulin-induced lipogenic transcription factor considered responsible for the steatosis in A in uncontrolled diabetes (Lane 2), after islet transplants restore insulin action (Lane 3). Lane 4 shows that induction of hyperleptinemia lowers nuclear SREBP-1. C. Local SREBP-1 immunostaining of liver of a failing transplant recipient showing residual protein surrounding a still viable islet. D. TAG content (■) and TUNEL (□) stain for apoptosis of islet transplant recipients on *ad lib* diet restricted diet or after induction of hyperleptinemia. Surviving β-cells were too few to count TUNEL grains in the *ad lib* fed group.

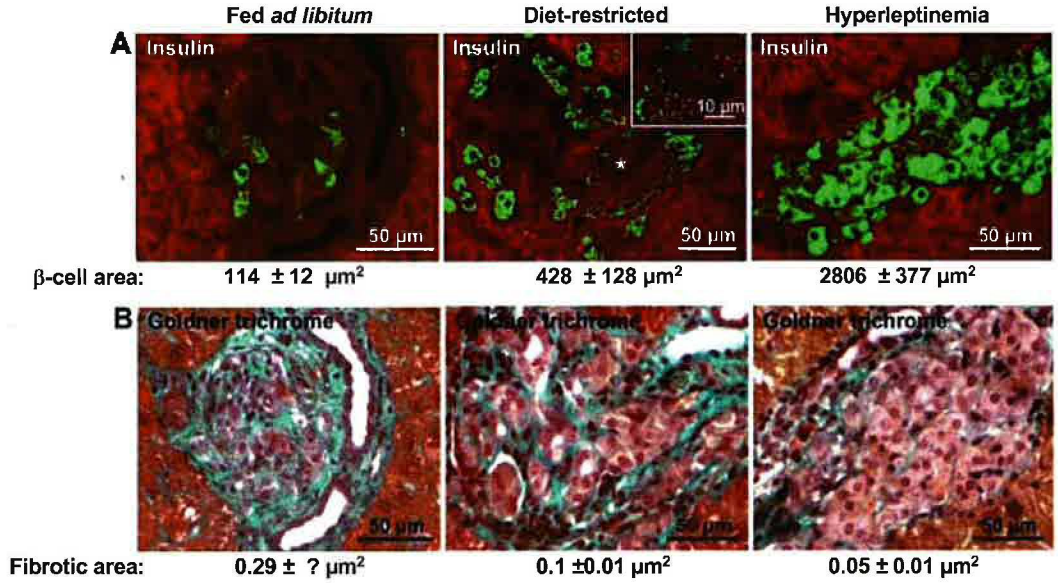


Figure 16 Lipopenic measures reduce destruction of β-cells in intrahepatic islet transplants. A. Insulin immunostaining and morphometry, showing prevention of β-cell loss by the lipopenic interventions (diet restrictions, hyperleptinemia) (cf. Figure 15D). B. Goldner trichrome staining for collagen showing prevention of poststeatotic fibrosis.

This proves 1) that β-cells can be destroyed by lipid overload in the absence of other potentially diabetogenic variables; 2) that the destruction caused by lipid overload results from overnutrition-driven, hyperinsulinemic upregulation of SREBP-1c-mediated lipogenesis, which causes the lipotoxicity (Figure 17); 3) that lipopenia prevents the destruction.

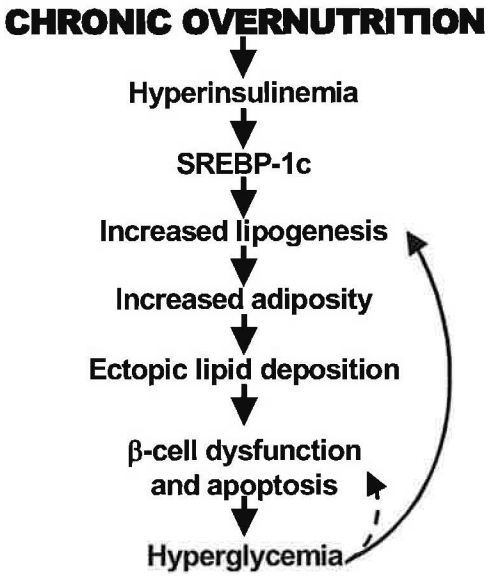


Figure 17. Putative pathogenesis of lipotoxic T2D. We postulate that T2D can be caused by overstimulation of insulin secretion by chronic overnutrition. The combined caloric excess and hyperinsulinemia induce excessive lipogenesis in liver and adipocytes that provide a source of ectopic lipids to islets and other organs. Fatty acids derivatives from imported fatty acids or from locally synthesized fatty acids can impair and destroy β-cells, thereby causing overt T2D. The coexistence of hyperglycemia, as in the suboptimally transplanted streptozotocin-diabetic islet recipients of this study, can accelerate the lipotoxicity by providing glucose, a substrate for lipogenesis, and/or by causing direct damage (broken arrow).

PRIMARY CONCLUSIONS

- **Lipid overload can destroy normal cardiomyocytes and β-cells.**
- **The β-cell destruction of type 2 diabetes can be prevented by lipid-lowering.**
- **Hyperinsulinemia, commonly driven by overnutrition, causes the lipid overload.**

SECONDARY CONCLUSIONS

- **High dose insulin treatment of obese, insulin-resistant, diabetic patients may further increase lipid overload.**
- **Caloric restriction and lipopenic agents prevents lipid overload and may improve outcome of islet transplantation.**

MECHANISMS OF LIPOAPOPTOSIS

Pathway of Lipid-Induced Apoptosis: The mechanism of the lipid-induced β-cell loss has been partially worked out in islets of leptin-unresponsive ZDF rats. Excess palmitoyl CoA condenses with L-serine to enter the ceramide pathway (Figure 18), catalyzed by the enzyme serine palmitoyl transferase (SPT).

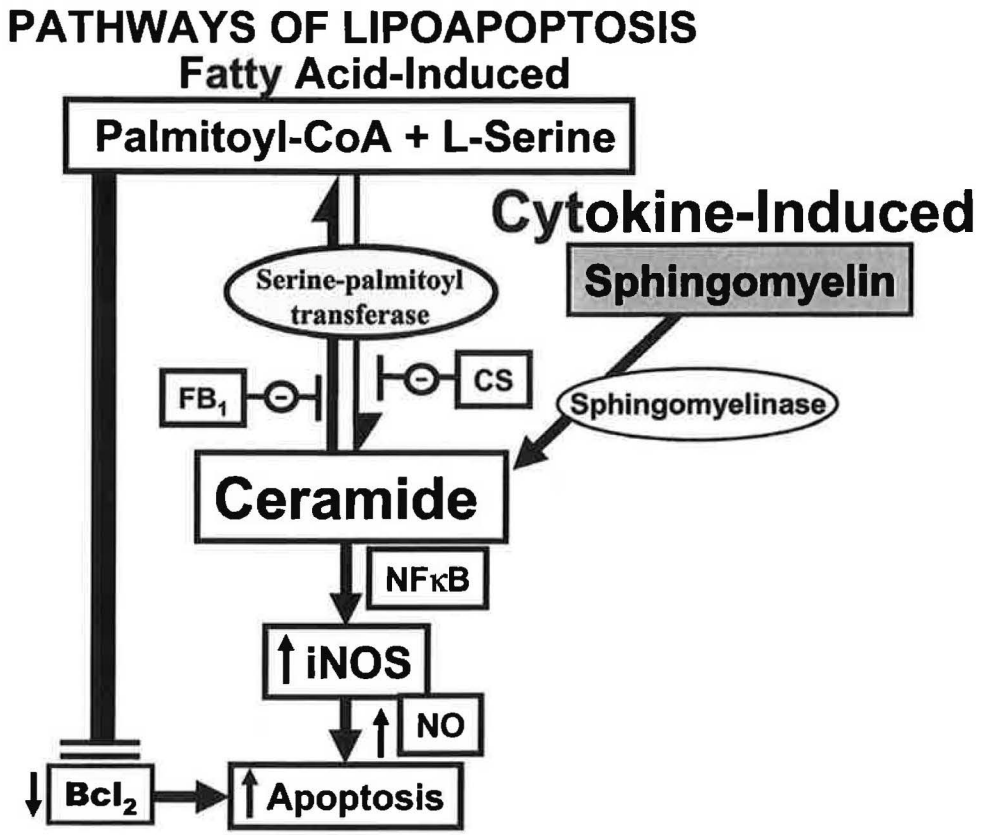


Figure 18. Unoxidized, unesterified palmitoyl CoA is condensed with L-serine, catalyzed by serine palmitoyl transferase, the rate limiting enzyme in the *de novo* ceramide pathway to apoptosis. L-cycloserine (CS) and fumonisin-B1 (FB1) both block ceramide synthesis. Palmitoyl CoA suppresses the anti-apoptotic protein (Bcl2), thereby facilitating lipoapoptosis, Cytokine-induced apoptosis, in which sphingomyelin provides a source of ceramide, may also be facilitated by lipid overload.

In islets of unleptinized islets SPT mRNA is upregulated and incorporation of ^{3}H-palmitate (Figure 19A) or ^{3}H-serine is increased. As shown in Figure 19B, ceramide induces inducible nitric oxide synthase (iNOS) and causes lipoapoptosis. In addition to the ceramide pathway, excess lipids may enter pathways to form reactive oxygen species (ROS). Any measure that reduces palmitoyl CoA will diminish or prevent apoptosis (Figure 19B,C). These include diet restriction (Ohneda *et al*, 1995), and AMP kinase-activating agents such as the thiazolidinediones (Higa *et al*, 1999) (Figure 19C), α-lipoic acid (Lee *et al*, 2006), leptin (Wang *et al*, 1998a) and AICAR (Yu *et al*, 2004). In addition, apoptosis can be prevented by blocking the ceramide pathway with cycloserine or fumonisin-B1 (Shimabukuro *et al*, 1998) and by the iNOS inhibitors aminoguanidine (Figure 19C) and nicotinamide (Shimabukuro *et al*, 1997b). Finally, palmitoyl CoA is a powerful suppressor of the antiapoptotic factor, Bcl2 (Shimabukuro *et al*, 1998) (Figure 20), explaining perhaps in part how lipid-depleting maneuvers such as hyperleptinemia attenuate the β-cell-killing activity of cytokines such as IL1β (Shimabukuro *et al*, 1997a).

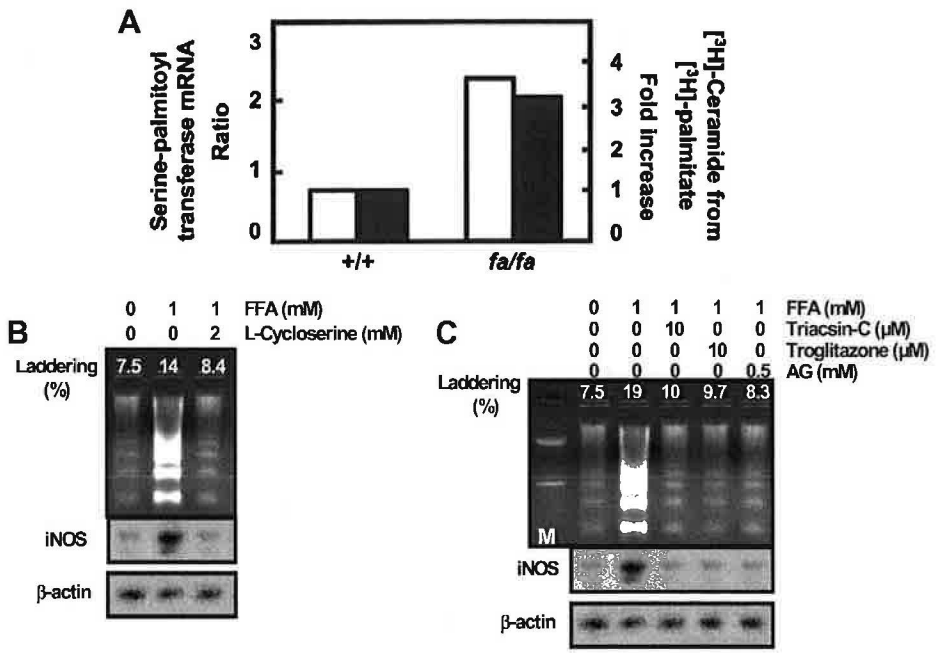


Figure 19. How surplus lipids destroy β-cells. A. mRNA of serine palmitoyl transferase (□) and rate of incorporation of ^{3}H-palmitate into ^{3}H ceramide (■) in normal (+/+) and in congenitally unleptinized *fa/fa* rats C. Laddering of DNA of *fa/fa* islets, an index of apoptosis, showing the preapoptotic effect of 1mM FFA and its inhibition by 2mM L-cycloserine, another blocker of ceramide synthesis. D. Triacsin-C, an acyl CoA synthetase inhibitor, troglitazone, which lowers ectopic lipids by activating AMPK and aminoguanidine (AG), an iNOS inhibitor, also reduce the DNA laddering.

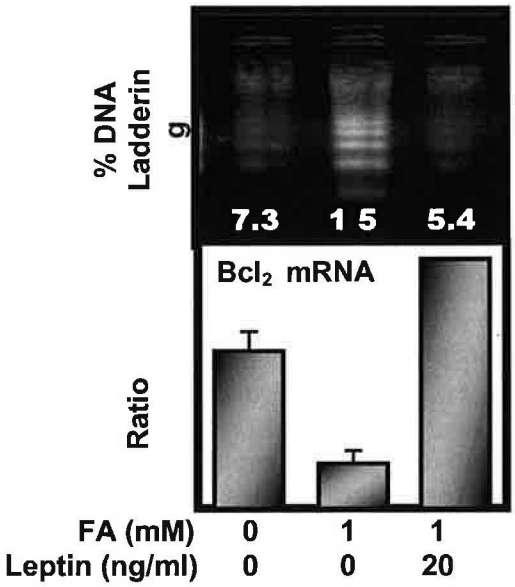
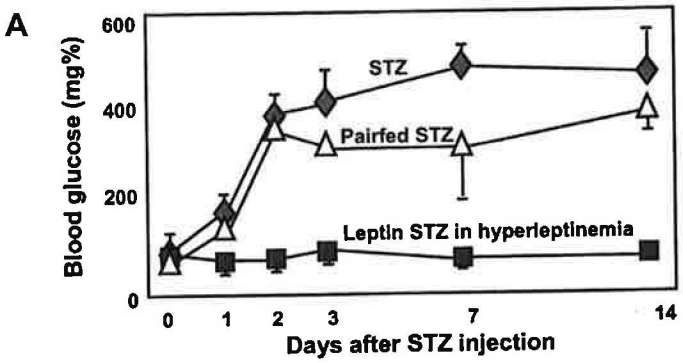


Figure 20. Effect of leptin on fatty acid-induced Bcl_2 mRNA and DNA laddering, an index of apoptosis.

LIPIDS, LEPTIN AND LONGEVITY: DESENSITIZING CELLS TO DESTRUCTION

The sirtuin gene family (Guarente, 2006) and the Klotho gene (Kurosu *et al*, 2006) both appear to prolong life. They both reduce intracellular lipids by completely different pathways, their only known common effect being the reduction in intracellular lipids. Diet restriction also prolongs life of many different organisms. Can lipopenia prolong the life of individual cells, organs and organisms? Could hyperleptinemia-induced lipopenia prevent disease and extend life? Certainly this seemed to be the case in the leptinized ACS-transgenic mice spared from premature death from lipotoxic dilated cardiomyopathy (Figure 13).

Protection of β-cells from Chemical Destruction: Since β-cells are so vulnerable to destruction by chemicals and immune factors, we have tested *in vivo* the effect of hyperleptinemia-induced lipopenia on the response of β-cells to various noxious substances. Two chemicals, streptozotocin and alloxan, are highly toxic to pancreatic β-cells. When they are administered at a high dose to normal animals, severe diabetes rapidly develops and all animals die from the metabolic consequences of severe insulin deficiency. However, if hyperleptinemia is induced 1 week before the streptozotocin treatment by the administration of adenovirus containing the leptin cDNA (AdCMV-leptin) (Chen *et al*, 1996), β-cell loss and lethal diabetes caused by administration of streptozotocin or alloxan are completely prevented. The blood glucose levels remain normal, as do the β-cells (Figure 21).



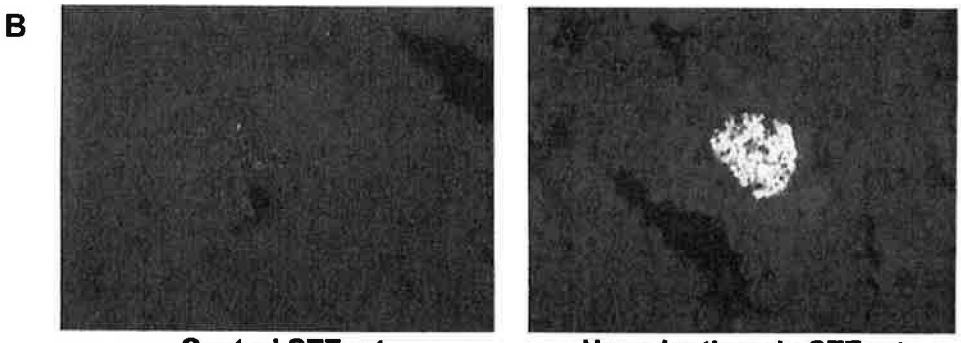


Figure 21. The effect of hyperleptinemia on β-cell sensitivity to streptozotocin (STZ)-induced destruction. A. Glucose levels after STZ in unleptinized, and leptinized rats. B. Insulin immunostaining of pancreas of unleptinized rat following STZ (100mg/kg) and a similar treated leptinized rat.

Protection of β-cells from Immunologic Destruction: An inverse relationship between the lipid content of β-cells and their susceptibility to IL1β-induced killing is suggested by the *in vitro* findings in Figure 22A (Shimabukuro *et al*, 1997a). Since IL-1β and other cytokines have been implicated in the

autoimmune β-cell destruction believed to cause type 1 diabetes (T1D) in humans (Nerup *et al*, 1994), it seemed possible that lipopenia might protect against this disease. An animal model of T1D has been developed in BB rats by Drs. Aldo Rossini and Dale Greiner at the University of Massachusetts by injecting polyinosinic:polycytidylic acid, a toll-like receptor agonist and KRV, a parvo virus (Mordes *et al*, 2004). The ability of hyperleptinemia-induced lipopenia to prevent this form of β-cell demise was tested in this BB rat variant, in which 100% of treated controls develop the disease within a period of 12 days. Whereas all untreated controls developed diabetes, thus far only one of six leptinized rats has developed diabetes within the period of observation (Figure 22B). These results raise the possibility that human T1D might be a preventable disease.

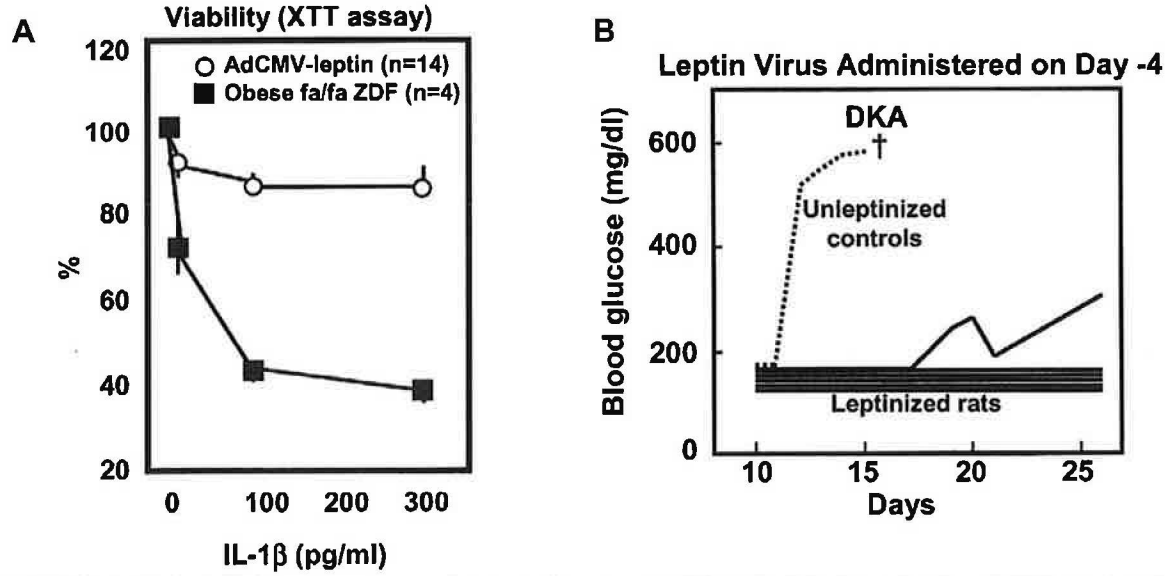


Figure 22. A. Effect of TG content on β-cell viability in islets isolated from AdCMV-leptin-induced wild-type (+/+) ZDF rat, pair-fed controls, free-feeding AdCMV-βgal controls and obese *fa/fa* ZDF rats. Islets were isolated 7 days after virus infusion and cultured for 24 hours with recombinant human IL-1b at the indicated concentrations. Data are expressed as the mean ± SEM. Viability is expressed as the percentage of the values observed in the absence of IL-1b. Numbers of experiments are in parentheses. B. Blood glucose levels of BBDR rats injected with poly I:C and infected with KRV. BBDR rats were treated with the adenovirus vector expressing leptin prior to initiation of the diabetes induction protocol consisting of poly I:C plus KRV treatment as described. One of the 6 leptinized BBDR rats developed diabetes on day 20 following KRV infection. All 6 of the unleptinized BBDR rats developed diabetes within 11-13 days after KRV infection ($p<0.001$ vs. adenovirus vector containing leptin treated group.

ARE HUMAN METABOLIC SYNDROME AND RODENT LIPOTOXICITY THE SAME?

The Similarities: There are many indications that human metabolic syndrome is a lipotoxic disease, the human counterpart of rodent lipotoxicity (Unger, 2003). 1) The organs impaired in rodent lipotoxicity are the same as in human metabolic syndrome. 2) In leptin-deficiency the abnormalities of metabolic syndrome respond to the administration of recombinant leptin in both rodents (Pelleymounter *et al*, 1995) and in humans with congenital generalized lipodystrophy (Oral *et al*, 2002), or congenital leptin deficiency (Farooqi *et al*, 1999). 3) In both species insulin resistance can be attributed to the ectopic deposition of lipids in target tissues of insulin, such as liver and skeletal muscle (Boden and Shulman, 2002), as suggested by McGarry in 1992. 4) In a histologic comparison, the heart of an obese human stained positively for lipids while a lean control was negative as in lipotoxic rats (Figure 23A). The actual TAG values measured biochemically in rats and by MRS in humans are placed in the photomicrographs.

Ectopic TAG Deposition in Human Organs: A major advance in our ability to study the mechanism of human metabolic syndrome has been pioneered by Lidia Szczepaniak in the Division of Hypertension in collaboration with Drs. Jon McGavock, Ildiko Lingvay, and Orson Moe. Using magnetic resonance spectroscopy, she is now able to survey ectopic TAG deposition in 5 organs of interest,

skeletal muscle, cardiac muscle, liver, pancreas and kidney. Figure 23B demonstrates that, as in leptin-resistant rodents, the TAG content of human heart, skeletal muscle, pancreas, liver and kidney is increased in humans with obesity, particularly if glucose tolerance is impaired or diabetes is present.

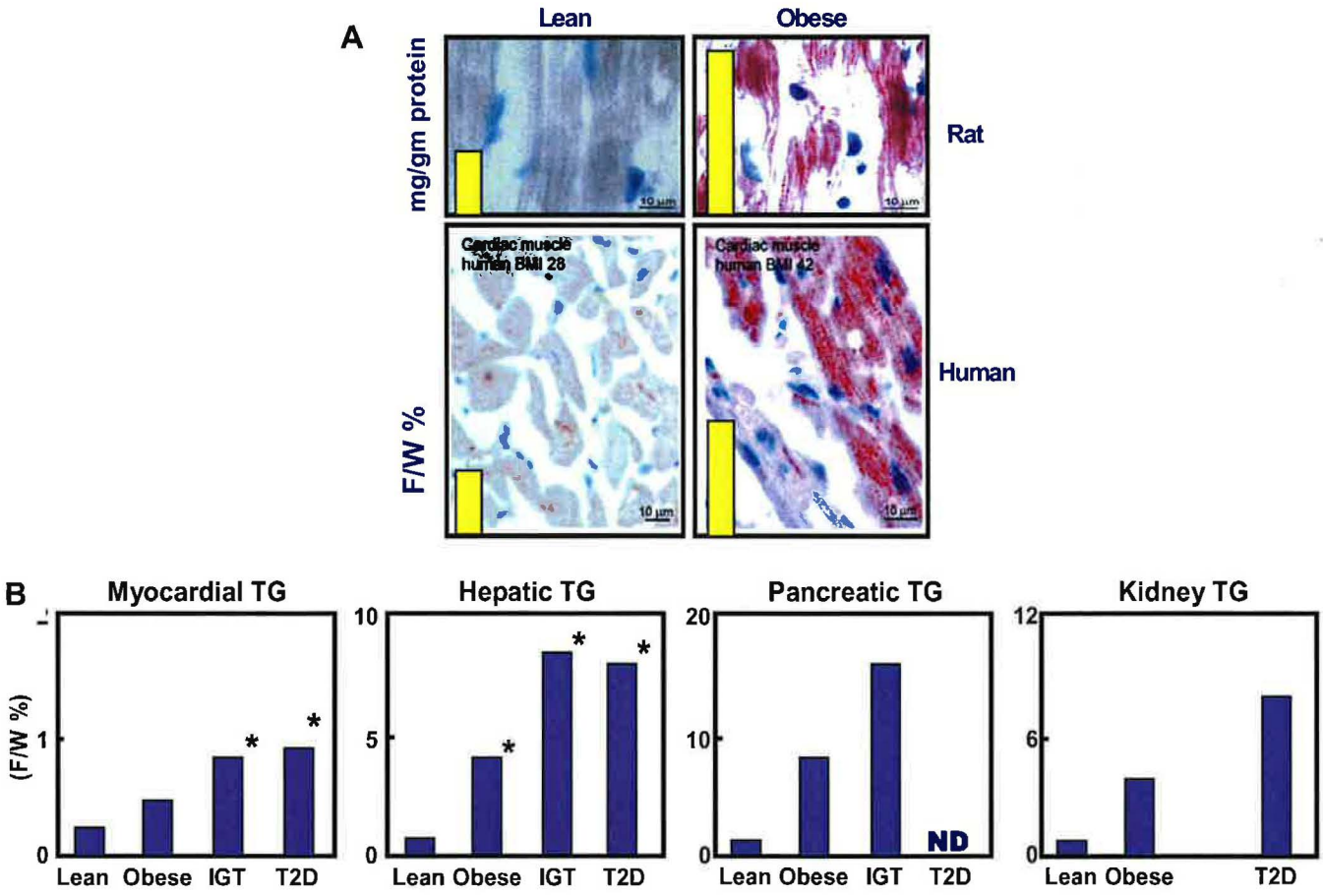


Figure 23. A. Comparison of oil red O staining for lipids in myocardium of a lean and obese rat and human. Bars represent differences in TAG. B. Ectopic lipid deposition measured by magnetic resonance spectroscopy in lean and obese humans with and without impaired glucose tolerance (IGT) and type 2 diabetes (T2D).

CLASSIFICATION OF FAT CELL INSUFFICIENCY IN HUMANS

Table I lists all clinical causes of human metabolic syndrome and indicates the presence of "adipocyte insufficiency" as it was defined. The severity of the accompanying metabolic syndrome is indicated. When obesity is absent or restricted, the onset of metabolic syndrome occurs sooner and is more severe. The three illustrative case reports in the appendix also support the concept that the metabolic syndrome is more severe in the absence of obesity.

TABLE 1. CLASSIFICATION OF ADIPOCYTE DISORDERS

PRIMARY

CONDITION	ADIPOCYTES	LEPTIN	LEPTIN RESISTANCE	ECTOPIC LIPIDS	METABOLIC SYNDROME	R_x
Congenital Generalized Lipodystrophy	Absent	Absent	0	++	Severe Early onset	Leptin
Adipocyte maturation arrest (Asian Indian Syndrome-Abate)	Normal	Normal	?	++?	Present	Diet AMPK activators
Leptin Receptor Deficiency (*Lepr* mutation)	Obese	High	++++	++++	Present	Diet TZD ? Exendin 4
Leptin Deficiency (*ob* mutation)	Obese	Absent	0	+++	Present	Leptin

SECONDARY

CONDITION	ADIPOCYTES	LEPTIN	LEPTIN RESISTANCE	ECTOPIC LIPIDS	METABOLIC SYNDROME	R_x
Supersizing	Nonobese	Relatively Low	?	+++	Severe	Stop Supersizing
DIO-early DIO-late	Obese	High Not as high	+ ++++	+/- ↑	Absent Present	Diet Restriction Diet Restriction and AMPK activators
Old Age	Variable	Relatively low	++++	↑	Mild	Diet Restriction
Hyperalimental 3° burns with hyperalimentation	Absent	Absent	?	↑	Severe	N/A
HAART lipodystrophy	Absent*	Low	?	?	Present	-
PCOS	Variable	?	?	?	Present	-
Cushing's	Localized	?	?	?	Severe	-

Obesity-Related Metabolic Syndrome: Diet-induced obesity (DIO) is by far the most common condition associated with metabolic syndrome, even though the metabolic syndrome may not appear for years after the onset of the obesity. During the initial healthy phase of DIO the adipocytes are able to accommodate the prevailing caloric surplus and minimize ectopic lipid deposition (Figure 24A). The onset of metabolic syndrome signifies that the caloric surplus is no longer being fully accommodated (fat cell insufficiency) and spillover of FA is occurring (Figure 24B).

Nonobese Metabolic Syndrome: A far more severe, earlier onset metabolic syndrome is caused by a subnormal adipocyte storage capacity, of which fatlessness, (e.g.,congenital generalized lipodystrophy), is the most extreme example (Figure 24C). There may also be a milder clinical variant, in which adipocytes are present and seemingly normal but are incapable of expanding in volume or number to accommodate a lipid surplus. This may be the "metabolically-obese," normal-weight syndrome of Ruderman *et al* (1981). We refer to this hypothetical disorder as "adipocyte maturation arrest". It may explain why metabolic syndrome and T2D can occur in nonobese people who can "eat anything they want without getting fat" (Figure 24D). Abate and Chandalia (Abate *et al*, 2005) have identified a K121Q polymorphism in the ENPP1/PC-1 gene in nonobese South Asians with this phenotype, which could be an example of this putative abnormality. Finally, it is possible for a normal individual to "supersize" food intake so that the influx of surplus calories is so rapid that the adipocytes cannot expand to accommodate it. The force-feeding of geese to produce paté de foie gras is an example of this cause of metabolic syndrome (Figure 24E).

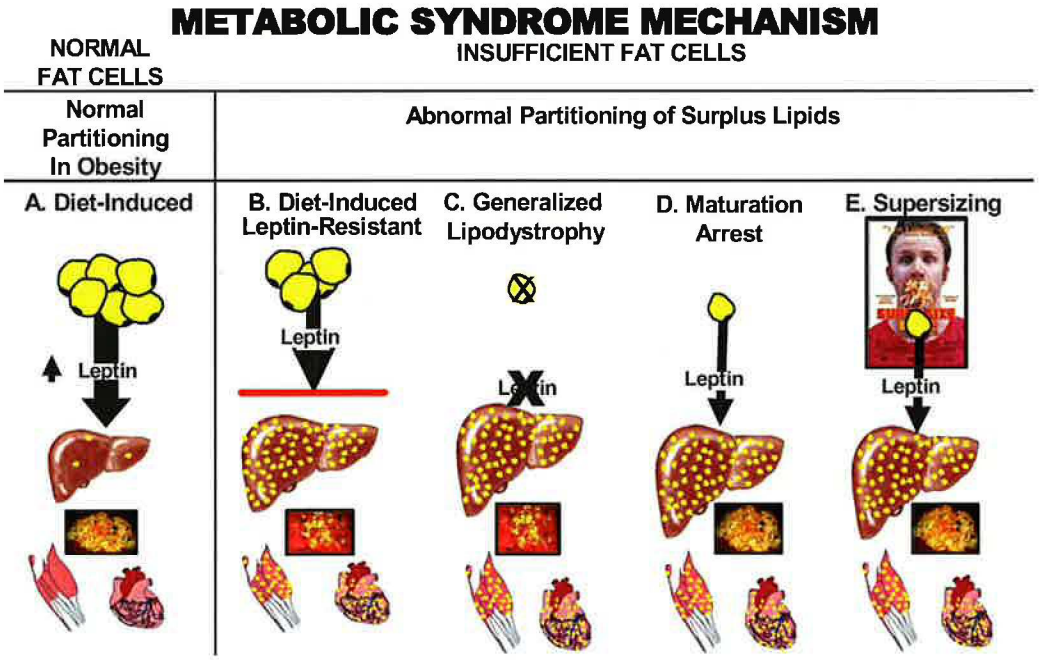


Figure 24. A. Normal partitioning of surplus calories in a sufficiently expanded adipocyte mass (far left). Abnormal partitioning resulting from relative fat cell insufficiency of diet-induced obesity (B), of generalized lipodystrophy (C), of maturation arrest (D) and of supersizing. Red bar signifies resistance to leptin action.

PREVENTION OF METABOLIC SYNDROME

Based on the evidence of ectopic lipid deposition gathered by the Szczepaniak group, it now seems appropriate to employ currently available interventions (Table 2) to reduce ectopic lipid deposition in individuals at risk for metabolic syndrome, rather than to wait for the syndrome to evolve.

TABLE 2. PREVENTING ECTOPIC LIPID DEPOSITION IN DIO

INTERVENTION	TARGET SITES	ACTIONS	FOOD INTAKE	↑ FA OXIDATION	INSULIN SECRETION	BODY WEIGHT
↓ Diet	General	↑ P-AMPK ↓ Hyperinsulinism ↓ Lipogenesis	↓	–	↓	↓
↑ Exercise	General	↑ P-AMPK ↓ Hyperinsulinism ↑ FA oxidation	–	↑	↓	↓
Bariatric Surgery	General	↑P-AMPK ↓ Hyperinsulinism ↓ Lipogenesis	↓	–	↓	↓
Rimonabant	Hypothalamus CB1 receptors Periphery	Blocks: anandamide and 2-AG	↓	↑	↓	↓
Leptin (not effective in DIO)	Hypothalamus Periphery	↓ P-AMPK ↑ P-AMPK	↓	↑	↓	↓
Adiponectin (low in DIO)	Periphery	↑ P-AMPK	?	↑	↓	↓
Thiazolidinediones	PPARγ Adiponectin	↑ P-AMPK	?	↑	↓	–
α-Lipoic acid	Hypothalamus Periphery	↓ P-AMPK ↑ P-AMPK	↓	↑	↓	↓
Metformin	Liver	↑ P-AMPK	↓	↑	↓	↓
Byetta (Exendin 4)	Hypothalamus Periphery	↑ P-AMPK	↓	?	↓	↓

Prevention of diabetes, heart disease and fatty liver alone would constitute a therapeutic triumph, even if the patient remains obese, since this goal can be achieved by diet restriction even without a major reduction in body weight. Figure 25 indicates that the increase in myocardial fat is accompanied by a significant decrease in diastolic filling in asymptomatic volunteers. Thus, clinically silent evidence of impaired heart function occurs long before the metabolic syndrome is manifest, and serious lipotoxic heart dysfunction can develop (Sharma *et al*, 2004). Figure 26 shows that the myocardial TAG content can be reduced in humans by thiazolidinediones therapy, as had been shown earlier in rats (Zhou *et al*, 2000).

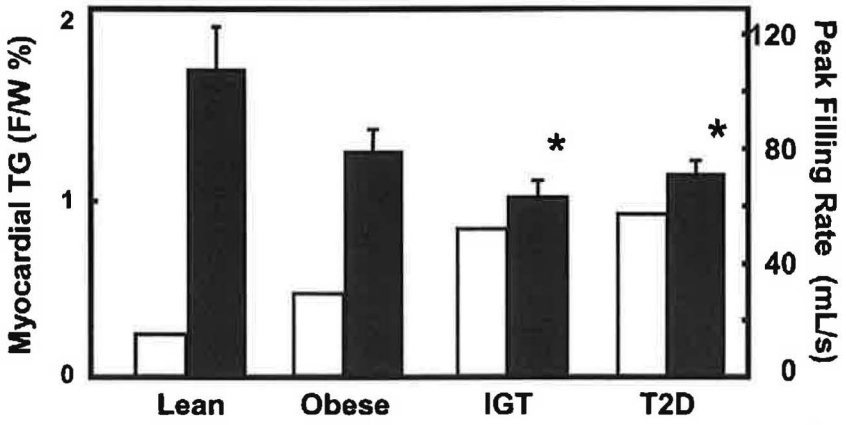


Figure 25. Cardiac steatosis (□) is associated with a measurable decline in diastolic filling (■), even in apparently healthy volunteers. McGavock et al, submitted to Circulation.

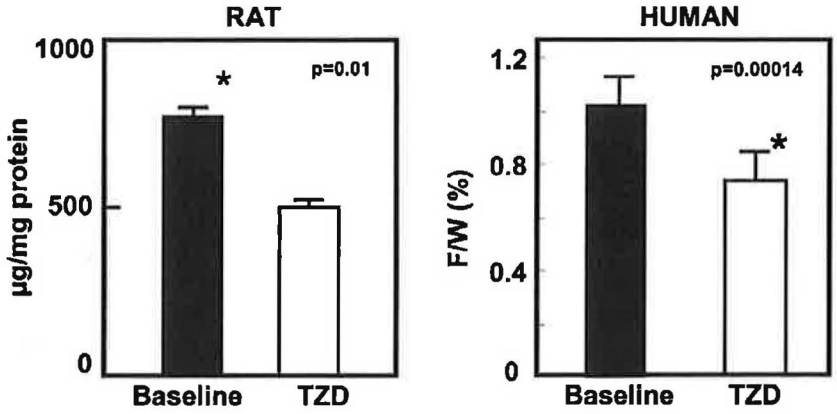


Figure 26 A. Effect of thiazolidinedione treatment of obese rats and obese humans on their elevated myocardial TAG.

Since MRS screening of populations for organ steatosis may not be practical in the foreseeable future, in nondiabetic overweight individuals correlations between ectopic organ TAG and plasma FFA (Figure 27) and other easily assayed plasma constituents may identify pre-metabolic syndrome. Intervention at this point might duplicate the benefit that cholesterol-lowering interventions have had on pre-coronary artery disease subjects.

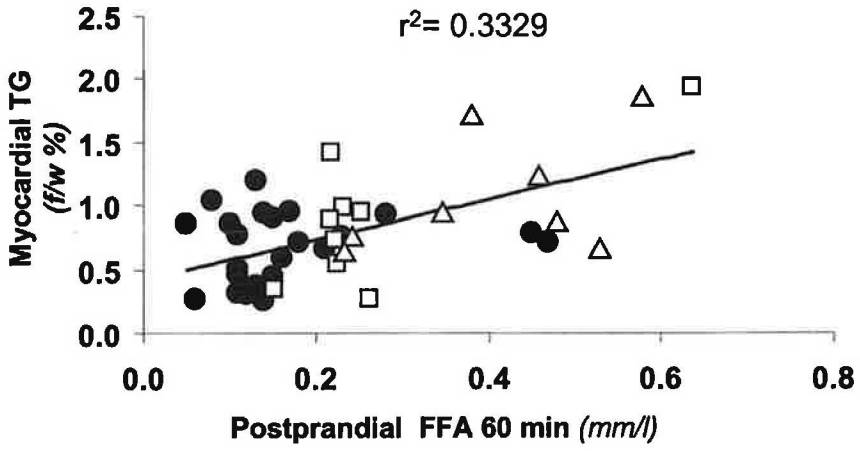


Figure 27. A myocardial TAG content measured by MRS as a function of 60 minute postprandial FFA levels in patients with normal oral glucose tolerance (●), impaired glucose tolerance (□) and diabetes (△). At present one might consider 0.2 mM as the cutoff level above which preventative measures should be instituted to prevent more severe cardiac steatosis.

Rational preventive strategy can be deduced from the evidence embodied in Figure 17, and the interventions listed in Table 2. Elimination of overnutrition-driven hyperinsulinemia, the primary perturbation that initiates the changes that lead to lipotoxicity, is the first essential goal. Serious food restriction, supplemented, if necessary, by anorexic drugs, should be a mandatory first priority to prevent progression of the disorder. The second goal is upregulation of fatty acid oxidation by exercise, supplemented, if necessary, by AMPK-activating drugs, to reduce ectopic lipid deposition in vital organs.

BIBLIOGRAPHY

1. Abate, N., Chandalia, M., Satija, P., Adams-Huet, B., Grundy, S.M., Sandeep, S., Radha, V., Deepa, R., and Mohan, V. (2005). ENPP1/PC-1 K121Q polymorphism and genetic susceptibility to type 2 diabetes. Diabetes *54*, 1207-1213.
2. Ahima, R.S., Qi, Y., Singhal, N.S., Jackson, M.B., and Scherer, P.E. (2006). Brain adipocytokine action and metabolic regulation. Diabetes *55 Suppl 2*, S145-154.
3. Berg, A.H., Combs, T.P., and Scherer, P.E. (2002). ACRP30/adiponectin: an adipokine regulating glucose and lipid metabolism. Trends in Endocrinology and Metabolism: TEM *13*, 84-89.
4. Boden, G., and Shulman, G.I. (2002). Free fatty acids in obesity and type 2 diabetes: defining their role in the development of insulin resistance and beta-cell dysfunction. European Journal of Clinical Investigation *32 Suppl 3*, 14-23.
5. Borradaile, N.M., and Schaffer, J.E. (2005). Lipotoxicity in the heart. Current Hypertension Reports *7*, 412-417.
6. Bray, G.A., and Greenway, F.L. (1999). Current and potential drugs for treatment of obesity. Endocrine Reviews *20*, 805-875.
7. Chen, G., Koyama, K., Yuan, X., Lee, Y., Zhou, Y.T., O'Doherty, R., Newgard, C.B., and Unger, R.H. (1996). Disappearance of body fat in normal rats induced by adenovirus-mediated leptin gene therapy. Proceedings of the National Academy of Sciences of the United States of America *93*, 14795-14799.
8. Chiu, H.C., Kovacs, A., Ford, D.A., Hsu, F.F., Garcia, R., Herrero, P., Saffitz, J.E., and Schaffer, J.E. (2001). A novel mouse model of lipotoxic cardiomyopathy. The Journal of Clinical Investigation *107*, 813-822.
9. Cruz, W.S., Kwon, G., Marshall, C.A., McDaniel, M.L., and Semenkovich, C.F. (2001). Glucose and insulin stimulate heparin-releasable lipoprotein lipase activity in mouse islets and INS-1 cells. A potential link between insulin resistance and beta-cell dysfunction. The Journal of Biological Chemistry *276*, 12162-12168.
10. Di Marzo, V., Goparaju, S.K., Wang, L., Liu, J., Batkai, S., Jarai, Z., Fezza, F., Miura, G.I., Palmiter, R.D., Sugiura, T., *et al.* (2001). Leptin-regulated endocannabinoids are involved in maintaining food intake. Nature *410*, 822-825.
11. dos Reis, R.M., Foss, M.C., de Moura, M.D., Ferriani, R.A., and Silva de Sa, M.F. (1995). Insulin secretion in obese and non-obese women with polycystic ovary syndrome and its relationship with hyperandrogenism. Gynecol Endocrinol *9*, 45-50.
12. Duffy, D., and Rader, D. (2007). Endocannabinoid Antagonism: Blocking the Excess in the Treatment of High-Risk Abdominal Obesity. Trends Cardiovasc Med *17*, 35-43.
13. Eyre, N.S., Cleland, L.G., Tandon, N.N., and Mayrhofer, G. (2006). Importance of the C-terminus of FAT/CD36 for plasma membrane localization and function in long-chain fatty acid uptake. J Lipid Res.
14. Farooqi, I.S., Jebb, S.A., Langmack, G., Lawrence, E., Cheetham, C.H., Prentice, A.M., Hughes, I.A., McCamish, M.A., and O'Rahilly, S. (1999). Effects of recombinant leptin therapy in a child with congenital leptin deficiency. The New England Journal of Medicine *341*, 879-884.
15.Ford, E.S., Giles, W.H., and Dietz, W.H. (2002). Prevalence of the metabolic syndrome among US adults: findings from the third National Health and Nutrition Examination Survey. Jama *287*, 356-359.
16. Ford, E.S., Abbasi, F., and Reaven, G.M. (2005). Prevalence of insulin resistance and the metabolic syndrome with alternative definitions of impaired fasting glucose. Atherosclerosis *181*, 143-148.
17. Guarente, L. (2006). Sirtuins as potential targets for metabolic syndrome. Nature *444*, 868-874.
18. Hajri, T., and Abumrad, N.A. (2002). Fatty acid transport across membranes: relevance to nutrition and metabolic pathology. Annual Review of Nutrition *22*, 383-415.
19. Halaas, J.L., Gajiwala, K.S., Maffei, M., Cohen, S.L., Chait, B.T., Rabinowitz, D., Lallone, R.L., Burley, S.K., and Friedman, J.M. (1995). Weight-reducing effects of the plasma protein encoded by the obese gene. Science *269*, 543-546.
20. Hamilton, J.A. (2003). Fast flip-flop of cholesterol and fatty acids in membranes: implications for membrane transport proteins. Current Opinion in Lipidology *14*, 263-271.
21. Hardie, D.G., Hawley, S.A., and Scott, J.W. (2006). AMP-activated protein kinase--development of the energy sensor concept. The Journal of Physiology *574*, 7-15.

22. Higa, M., Zhou, Y.T., Ravazzola, M., Baetens, D., Orci, L., and Unger, R.H. (1999). Troglitazone prevents mitochondrial alterations, beta cell destruction, and diabetes in obese prediabetic rats. Proceedings of the National Academy of Sciences of the United States of America *96*, 11513-11518.
23. Horton, J.D., Goldstein, J.L., and Brown, M.S. (2002). SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. The Journal of Clinical Investigation *109*, 1125-1131.
24. Hu, Z., Cha, S.H., Chohnan, S., and Lane, M.D. (2003). Hypothalamic malonyl-CoA as a mediator of feeding behavior. Proceedings of the National Academy of Sciences of the United States of America *100*, 12624-12629.
25. Hukshorn, C.J., van Dielen, F.M., Buurman, W.A., Westerterp-Plantenga, M.S., Campfield, L.A., and Saris, W.H. (2002). The effect of pegylated recombinant human leptin (PEG-OB) on weight loss and inflammatory status in obese subjects. Int J Obes Relat Metab Disord *26*, 504-509.
26. Jbilo, O., Ravinet-Trillou, C., Arnone, M., Buisson, I., Bribes, E., Peleraux, A., Penarier, G., Soubrie, P., Le Fur, G., Galiegue, S., *et al.* (2005). The CB1 receptor antagonist rimonabant reverses the diet-induced obesity phenotype through the regulation of lipolysis and energy balance. Faseb J *19*, 1567-1569.
27. Kakuma, T., Lee, Y., Higa, M., Wang, Z., Pan, W., Shimomura, I., and Unger, R.H. (2000). Leptin, troglitazone, and the expression of sterol regulatory element binding proteins in liver and pancreatic islets. Proceedings of the National Academy of Sciences of the United States of America *97*, 8536-8541.
28. Kahn, S.E., Hull, R.L., and Utzschneider, K.M. (2006). Mechanisms linking obesity to insulin resistance and type 2 diabetes. Nature *444*, 840-846.
29. Kurosu, H., Yamamoto, M., Clark, J.D., Pastor, J.V., Nandi, A., Gurnani, P., McGuinness, O.P., Chikuda, H., Yamaguchi, M., Kawaguchi, H., *et al.* (2005). Suppression of aging in mice by the hormone Klotho. Science *309*, 1829-1833.
30. LeBrasseur, N.K., Kelly, M., Tsao, T.S., Farmer, S.R., Saha, A.K., Ruderman, N.B., and Tomas, E. (2006). Thiazolidinediones can rapidly activate AMP-activated protein kinase in mammalian tissues. American Journal of Physiology *291*, E175-181.
31. Lee, Y., Naseem, R.H., Duplomb, L., Park, B.H., Garry, D.J., Richardson, J.A., Schaffer, J.E., and Unger, R.H. (2004). Hyperleptinemia prevents lipotoxic cardiomyopathy in acyl CoA synthase transgenic mice. Proceedings of the National Academy of Sciences of the United States of America *101*, 13624-13629.
32. Lee, Y., Naseem, R.H., Park, B.H., Garry, D.J., Richardson, J.A., Schaffer, J.E., and Unger, R.H. (2006). Alpha-lipoic acid prevents lipotoxic cardiomyopathy in acyl CoA-synthase transgenic mice. Biochemical and Biophysical Research Communications *344*, 446-452.
33. Liang,J., Cook,W.S., Fu, M., Ciociola, E., Livingston, E.H., Chandalia, M., Abate, N. (to be published). Effects of ENPP1 over-expression on adipogenesis.
34. Lupi, R., Dotta, F., Marselli, L., Del Guerra, S., Masini, M., Santangelo, C., Patane, G., Boggi, U., Piro, S., Anello, M., *et al.* (2002). Prolonged exposure to free fatty acids has cytostatic and pro-apoptotic effects on human pancreatic islets: evidence that beta-cell death is caspase mediated, partially dependent on ceramide pathway, and Bcl-2 regulated. Diabetes *51*, 1437-1442.
35. Matsuzawa, Y., Funahashi, T., Kihara, S., and Shimomura, I. (2004). Adiponectin and metabolic syndrome. Arteriosclerosis, thrombosis, and vascular biology *24*, 29-33.
36. McGarry, J.D., Mannaerts, G.P., and Foster, D.W. (1977). A possible role for malonyl-CoA in the regulation of hepatic fatty acid oxidation and ketogenesis. The Journal of Clinical Investigation *60*, 265-270.
37. Mordes, J.P., Bortell, R., Blankenhorn, E.P., Rossini, A.A., and Greiner, D.L. (2004). Rat models of type 1 diabetes: genetics, environment, and autoimmunity. ILAR Journal / National Research Council, Institute of Laboratory Animal Resources *45*, 278-291.
38. Nawrocki, A.R., Rajala, M.W., Tomas, E., Pajvani, U.B., Saha, A.K., Trumbauer, M.E., Pang, Z., Chen, A.S., Ruderman, N.B., Chen, H., *et al.* (2006). Mice lacking adiponectin show decreased hepatic insulin sensitivity and reduced responsiveness to peroxisome proliferator-activated receptor gamma agonists. The Journal of Biological Chemistry *281*, 2654-2660.
39. Neel, J.V. (1962). Diabetes mellitus: a "thrifty" genotype rendered detrimental by "progress"? American Journal of Human Genetics *14*, 353-362.

40. Nerup, J., Mandrup-Poulsen, T., Helqvist, S., Andersen, H.U., Pociot, F., Reimers, J.I., Cuartero, B.G., Karlsen, A.E., Bjerre, U., and Lorenzen, T. (1994). On the pathogenesis of IDDM. Diabetologia *37 Suppl 2*, S82-89.
41. Ohneda, M., Inman, L.R., and Unger, R.H. (1995). Caloric restriction in obese pre-diabetic rats prevents beta-cell depletion, loss of beta-cell GLUT 2 and glucose incompetence. Diabetologia *38*, 173-179.
42. Oral, E.A., Simha, V., Ruiz, E., Andewelt, A., Premkumar, A., Snell, P., Wagner, A.J., DePaoli, A.M., Reitman, M.L., Taylor, S.I., *et al.* (2002). Leptin-replacement therapy for lipodystrophy. The New England Journal of Medicine *346*, 570-578.
43. Orci, L., Cook, W.S., Ravazzola, M., Wang, M.Y., Park, B.H., Montesano, R., and Unger, R.H. (2004). Rapid transformation of white adipocytes into fat-oxidizing machines. Proceedings of the National Academy of Sciences of the United States of America *101*, 2058-2063.
44. Park, B.H., Wang, M.Y., Lee, Y., Yu, X., Ravazzola, M., Orci, L., and Unger, R.H. (2006). Combined leptin actions on adipose tissue and hypothalamus are required to deplete adipocyte fat in lean rats: implications for obesity treatment. The Journal of Biological Chemistry *281*, 40283-40291.
45. Pelleymounter, M.A., Cullen, M.J., Baker, M.B., Hecht, R., Winters, D., Boone, T., and Collins, F. (1995). Effects of the obese gene product on body weight regulation in ob/ob mice. Science *269*, 540-543.
46. Petersen, K.F., and Shulman, G.I. (2002). Pathogenesis of skeletal muscle insulin resistance in type 2 diabetes mellitus. The American Journal of Cardiology *90*, 11G-18G.
47. Pohle, T., Brandlein, S., Ruoff, N., Muller-Hermelink, H.K., and Vollmers, H.P. (2004). Lipoptosis: tumor-specific cell death by antibody-induced intracellular lipid accumulation. Cancer Research *64*, 3900-3906.
48. Rasmussen, M.S., Lihn, A.S., Pedersen, S.B., Bruun, J.M., Rasmussen, M., and Richelsen, B. (2006). Adiponectin receptors in human adipose tissue: effects of obesity, weight loss, and fat depots. Obesity (Silver Spring, Md *14*, 28-35.
49. Reaven, G.M. (2005). The insulin resistance syndrome: definition and dietary approaches to treatment. Annual Review of Nutrition *25*, 391-406.
50. Ruderman, N.B., Schneider, S.H., and Berchtold, P. (1981). The "metabolically-obese," normal-weight individual. The American Journal of Clinical Nutrition *34*, 1617-1621.
51. Samuel, V.T., Liu, Z.X., Qu, X., Elder, B.D., Bilz, S., Befroy, D., Romanelli, A.J., and Shulman, G.I. (2004). Mechanism of hepatic insulin resistance in non-alcoholic fatty liver disease. The Journal of Biological Chemistry *279*, 32345-32353.
52. Scherer, P.E., Williams, S., Fogliano, M., Baldini, G., and Lodish, H.F. (1995). A novel serum protein similar to C1q, produced exclusively in adipocytes. The Journal of Biological Chemistry *270*, 26746-26749.
53. Shaibi, G.Q., Cruz, M.L., Weigensberg, M.J., Toledo-Corral, C.M., Lane, C.J., Kelly, L.A., Davis, J.N., Koebnick, C., Ventura, E.E., Roberts, C.K., *et al.* (2007). Adiponectin Independently Predicts Metabolic Syndrome in Overweight Latino Youth. J Clin Endocrinol Metab.
54. Sharma, S., Adrogue, J.V., Golfman, L., Uray, I., Lemm, J., Youker, K., Noon, G.P., Frazier, O.H., and Taegtmeyer, H. (2004). Intramyocardial lipid accumulation in the failing human heart resembles the lipotoxic rat heart. Faseb J *18*, 1692-1700.
55. Shimabukuro, M., Higa, M., Zhou, Y.T., Wang, M.Y., Newgard, C.B., and Unger, R.H. (1998a). Lipoapoptosis in beta-cells of obese prediabetic fa/fa rats. Role of serine palmitoyltransferase overexpression. The Journal of Biological Chemistry *273*, 32487-32490.
56. Shimabukuro, M., Koyama, K., Lee, Y., and Unger, R.H. (1997a). Leptin- or troglitazone-induced lipopenia protects islets from interleukin 1beta cytotoxicity. The Journal of Clinical Investigation *100*, 1750-1754.
57. Shimabukuro, M., Ohneda, M., Lee, Y., and Unger, R.H. (1997b). Role of nitric oxide in obesity-induced beta cell disease. The Journal of Clinical Investigation *100*, 290-295.
58, Shimabukuro, M., Koyama, K., Chen, G., Wang, M.Y., Trieu, F., Lee, Y., Newgard, C.B., and Unger, R.H. (1997c). Direct antidiabetic effect of leptin through triglyceride depletion of tissues. Proceedings of the National Academy of Sciences of the United States of America *94*, 4637-4641.

59. Shimabukuro, M., Wang, M.Y., Zhou, Y.T., Newgard, C.B., and Unger, R.H. (1998b). Protection against lipoapoptosis of beta cells through leptin-dependent maintenance of Bcl-2 expression. Proceedings of the National Academy of Sciences of the United States of America *95*, 9558-9561.
60. Shimomura, I., Hammer, R.E., Ikemoto, S., Brown, M.S., and Goldstein, J.L. (1999). Leptin reverses insulin resistance and diabetes mellitus in mice with congenital lipodystrophy. Nature *401*, 73-76.
61. Unger, R.H. (2003). Minireview: weapons of lean body mass destruction: the role of ectopic lipids in the metabolic syndrome. Endocrinology *144*, 5159-5165.
62. Unger, R.H. (2003). Lipid overload and overflow: metabolic trauma and the metabolic syndrome. Trends in endocrinology and metabolism: TEM *14*, 398-403.
63. Unger, R.H. (2004). The hyperleptinemia of obesity-regulator of caloric surpluses. Cell *117*, 145-146.
64. Unger, R.H. (2005). Longevity, lipotoxicity and leptin: the adipocyte defense against feasting and famine. Biochimie *87*, 57-64.
65. Unger, R.H. (2006). Klotho-induced insulin resistance: a blessing in disguise? Nature Medicine *12*, 56-57.
66. Wang, M.Y., Koyama, K., Shimabukuro, M., Mangelsdorf, D., Newgard, C.B., and Unger, R.H. (1998). Overexpression of leptin receptors in pancreatic islets of Zucker diabetic fatty rats restores GLUT-2, glucokinase, and glucose-stimulated insulin secretion. Proceedings of the National Academy of Sciences of the United States of America *95*, 11921-11926.
67. Wang, M.Y., Koyama, K., Shimabukuro, M., Newgard, C.B., and Unger, R.H. (1998). OB-Rb gene transfer to leptin-resistant islets reverses diabetogenic phenotype. Proceedings of the National Academy of Sciences of the United States of America *95*, 714-718.
68. Williams, D.L., Baskin, D.G., and Schwartz, M.W. (2006). Leptin regulation of the anorexic response to glucagon-like peptide-1 receptor stimulation. Diabetes *55*, 3387-3393.
69. Yanovski, S.Z., and Yanovski, J.A. (2002). Obesity. The New England Journal of Medicine *346*, 591-602.
70. Yu, X., McCorkle, S., Wang, M., Lee, Y., Li, J., Saha, A.K., Unger, R.H., and Ruderman, N.B. (2004). Leptinomimetic effects of the AMP kinase activator AICAR in leptin-resistant rats: prevention of diabetes and ectopic lipid deposition. Diabetologia *47*, 2012-2021.
71. Zhang, Y., Proenca, R., Maffei, M., Barone, M., Leopold, L., and Friedman, J.M. (1994). Positional cloning of the mouse obese gene and its human homologue. Nature *372*, 425-432.
72. Zhou, Y.P., and Grill, V.E. (1994). Long-term exposure of rat pancreatic islets to fatty acids inhibits glucose-induced insulin secretion and biosynthesis through a glucose fatty acid cycle. The Journal of Clinical Iinvestigation *93*, 870-876.