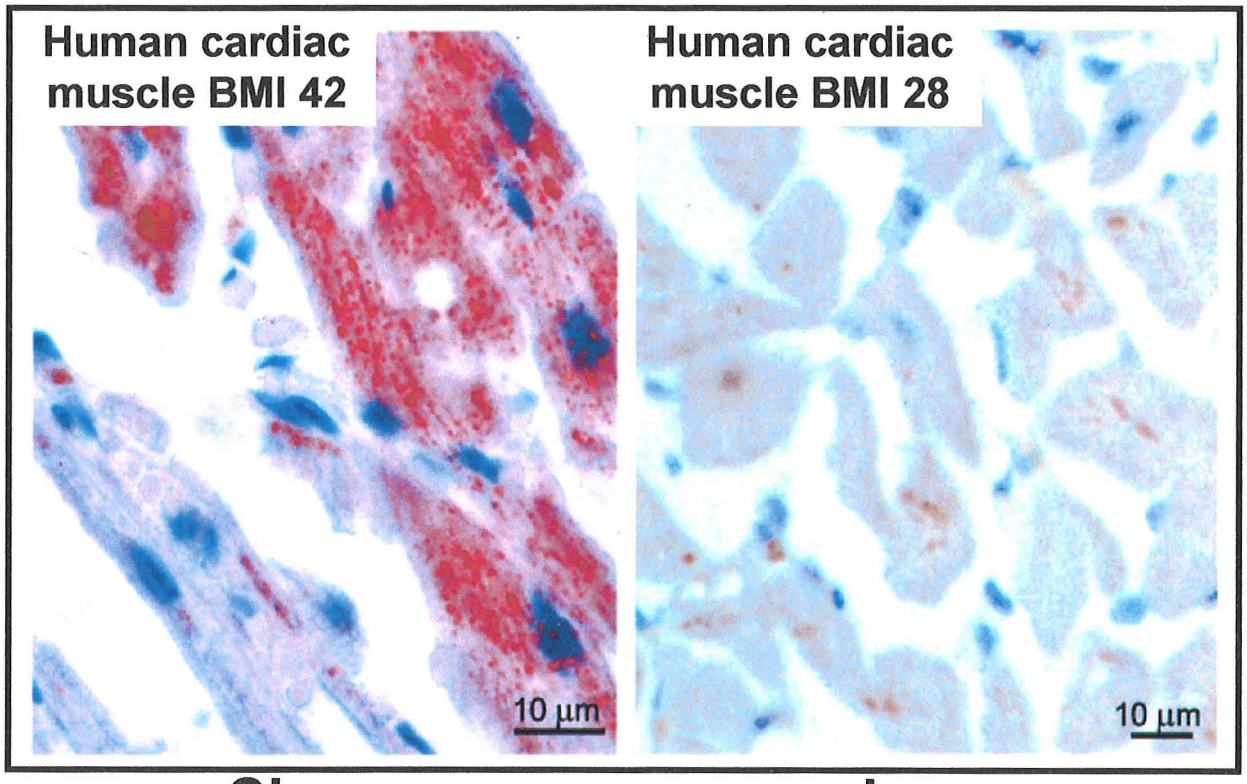
MEDICAL GRAND ROUNDS
June 21, 2001

FATTY HEART

(Lipotoxic Cardiomyopathy)



Obese **Lean**

(Courtesy of Lelio Orci from Unger & Orci, FASEB J 15:312-21, 2001)

Roger H. Unger, M.D.

This is to acknowledge that Roger H. Unger, M.D. has not disclosed financial interests or other relationships with commercial concerns related directly or indirectly to this program. Dr. Unger will not be discussing "off-label" uses in his presentation.

Roger H. Unger, M.D.
Professor of Internal Medicine

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. In recent years his studies have dealt with liporegulation and the lipotoxic diseases that result from disorders of fatty acid homeostasis.

CASE REPORT

J.E.Y. is a 51-year-old obese black male admitted in November, 1999, for dyspnea, paroxysmal nocturnal dyspnea, orthopnea, pedal and upper extremity edema and increased abdominal girth. He had a history of dilated cardiomyopathy first noted in 1991 and obesity for at least 20 years. Extensive work-ups failed to reveal the etiology of the heart disease. A catheterization in 1992 demonstrated normal coronary arteries. Repeated blood pressure determinations failed to reveal hypertension.

Physical exam revealed an obese black male in obvious respiratory distress. He had S-3 and S-4 gallops and bibasilar rales, jugular venous distention and pitting edema of feet and ankles.

Laboratory tests, including liver and thyroid function, were within normal limits. Blood glucose was 75 ml/dl, cholesterol 182 ml/dl and triglycerides 207 mg/dl. Echocardiography revealed 4-chamber dilatation of heart with severely depressed LVSF and RVSF. An endomyocardial biopsy was reported as negative but stains for lipid content were not made.

He was treated with i.v. lasix and had a 12-pound diuresis, reaching a dry weight of 208 lbs. He was discharged on digoxin, lasix, metropolol, lysinopril and lesargin. The final diagnosis was hypertensive cardiovascular disease (without hypertension) or postviral myocarditis (without evidence of antecedent viral infection).

Commentary: In this obese patient the commonly diagnosed causes of heart disease were excluded and an endocardial biopsy was read as "negative". Nevertheless, his disorder was ascribed to hypertension, despite the complete lack of supporting evidence. No attempt was made to determine whether or not he might have a fatty heart.

HISTORY OF FATTY HEART

Fatty heart was first described by William Harvey in the 17th century (Harvey, 1628). Originally known as "cor adiposum" ("fatty heart"), it was familiar to clinicians for over 300 years but now seems to have disappeared from the clinical diagnostic vocabulary, ironically, in the midst of an American pandemic of obesity, the disorder with which it is most often associated. The term "fatty heart" (Figure 1) was used to refer to two different and probably unrelated conditions, lipomatous and lipotoxic cardiomyopathy.

A. Lipomatous cardiomyopathy: In this very rare disorder, epicardial fat becomes massively enlarged, sometimes to the point of encasing and restricting the myocardium, and/or infiltrates from the epicardium into the myocardium like a lipoma (Saphir & Corrigan, 1933; Shirani & Roberts, 1993). This idiopathic

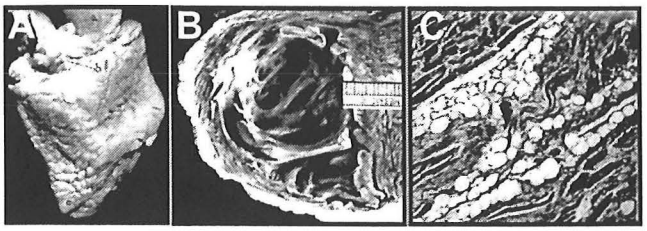


Fig. 1: Lipomatous cardiomyopathy. A) Appearance of the heart. B) Cross section of the heart. C) Fat metaplasia in an area of myocardial fibrosis. Note the hypertrophy of individual muscle fibers (Hematoxylin & eosin; X127)

disease appears to be a primary hyperplasia or lipomatosis of epicardial adipocytes and is ultimately fatal. The condition is often referred to as "lipomatous hypertrophy" of the atrial septum and there is a relationship between these deposits and atrial arrhythmia, which occurs in 40% of patients. When the atrial septal thickness exceeds 3 cm, the frequency of arrhythmia is 60%. In some patients cardiac fat is so great that their heart floats in water ("floating heart") (Roberts & Roberts, 1983). These hearts exhibit a high frequency of cardiac rupture during myocardial infarction.

B. Lipotoxic cardiomyopathy: This is the common form of fatty heart. It was originally referred to as "fatty degeneration" and regarded as a nonspecific consequence of injury. Any circumstance that interferes with or blocks myocardial fatty acid (FA) oxidation and/or increases intracellular lipids can cause accumulation of lipids within the cardiac muscle. The etiology could therefore range from local or generalized hypoxia to overnutrition.

The early descriptions of "fatty degeneration" of cardiomyocytes that occurred in the absence of known injury to the myocardium (4) are indistinguishable from the "lipotoxic cardiomyopathy" of obese rodents studied extensively in congenitally obese ZDF rats (Zhou *et al.*, 2000). Both varieties of fatty heart were described by Corvisart in 1812 (Corvisart, 1812) and by the great French physician Laennec in 1838 (Laennec, 1838). In 1928 fatty heart was linked to obesity for the first time, but the cardiac problems were attributed to weakness owing to lack of exercise in the obese. Amad et al. (Amad *et al.*, 1965) reported that patients weighing from 34-240% above the predicted ideal for 20-40 years all had increased heart weight and left ventricular thickness (Table 1). No studies of intramyocyte fat were reported; this is unfortunate because quite probably it might have identified lipotoxic cardiomyopathy years before the U.S. epidemic of obesity first became apparent by 1980.

In 1930 Zarday reported that 90% of obese individuals had circulatory disturbances, as determined by measurement of pulse rate, arterial and venous pressure and vital capacity. But in 1931 the dean of Ameri-

Table 1 (Amad *et al.*, 1965)

A. Clinical findings from 12 very obese subjects

Subject	Age	Sex	Height	Body weight kg	Ideal body weight kg	Predicted heart weight gm	Heart weight gm
1	42	M	167	225	67	291	1100
2	58	M	175	209	70	304	900
3	45	M	169	147	64	279	450
4	33	M	175	113	70	304	420
5	46	M	178	109	74	323	500
6	64	M	179	99	74	323	540
7	65	F	167	159	65	262	620
8	39	F	168	147	64	258	400
9	35	F	155	140	57	230	500
10	75	F	162	136	54	215	420
11	58	F	171	127	65	262	645
12	47	F	157	108	59	239	410

B. Microscopic findings in the heart of 12 very obese subjects

Subject	Epicardium	Myocardium	Coronary vessels
1	Normal amount of fat	Marked hypertrophy of all myocardial fibers with minimal focal perivascular fibrosis	Normal
2	Large amount of epicardial fat	Mild to moderate hypertrophy; small amount of fat infiltration between peripheral myocardial fibers	Normal
3	Normal amount of fat	Moderate hypertrophy of myocardial fibers; very small foci of fatty tissue about the major coronary branches; no myocardial fibrosis	Normal
4	Normal amount of fat	Diffuse hypertrophy; small amount of perivascular infiltrating fat	Minimal intimal proliferation
5	Normal amount of fat	Diffuse hypertrophy; no fatty infiltration or fibrosis	Medial hypertrophy
6	Slight increase in epicardial fat	Diffuse hypertrophy; no myocardial fibrosis; no fatty infiltration	Minimal focal intimal thickening by fibrosis
7	Normal amount of fat	Moderate hypertrophy of myocardial fibers; occasional foci of myocardial fibrosis; no fatty infiltration	Normal
8	Normal amount of fat	Diffuse hypertrophy; no fatty infiltration or fibrosis	Normal
9	Normal amount of fat	Diffuse hypertrophy; no fatty infiltration or fibrosis	
10	Normal amount of fat	Diffuse hypertrophy; slight patchy intersititial fibrosis; no fatty infiltration	Normal
11	Moderate increase in epicardial fat	Marked diffuse hypertrophy; occasional small foci of fibrosis; no fatty infiltration	Normal
12	Normal amount of fat	Diffuse hypertrophy; no fatty infiltration or fibrosis	Normal

can cardiologists, Paul Dudley White (White, 1931), considered the clinical significance of what he called "cor adiposum" to be obscure. He said, "The truth probably lies between the two extremes that the fatty heart is a common and dangerous condition and that it does not exist at all." This statement may have been a major factor in the ensuing decline of clinical awareness of the disorder.

During the past decade, with the prevalence of obesity at unprecedented heights, clinical cardiologists have once again begun to examine the relationship between obesity and cardiac function, aided by the availability of modern noninvasive technologies (Itoh *et al.*, 1996). Alpert *et al.* (Alpert *et al.*, 1989; Alpert & Hashimi, 1993; Alpert *et al.*, 1995 & 1995a) studied left ventricular mass, systolic function and diastolic relaxation in 50 morbidly obese subjects whose body weight was twice the ideal. They found left ventricular hypertrophy and impaired systolic function and diastolic filling. In individuals with increased left ventricular mass, exercise produced no increase in left ventricular ejection fraction, i.e., when the left ventricular mass reaches a certain level, reserve function for exercise is nonexistent. Most importantly, the longer duration of morbid obesity, the higher the left ventricular mass, the worse the left ventricular systolic function and the greater the impairment of left ventricular diastolic filling, evidence of the time-dependence of the disorder. Weight loss decreased the left ventricular mass and improved left ventricular function and diastolic filling. This pattern of FA-induced heart disease in obese human males, i.e., initial hypertrophy followed by late functional decompensation, is reminiscent of the initial fatty acid-induced β-cell hyperplasia and compensation and late β-cell depletion and decompensation observed in the FA-induced disease of the pancreatic islets of ZDF rats (Unger & Orci, 2001) (Figure 2).

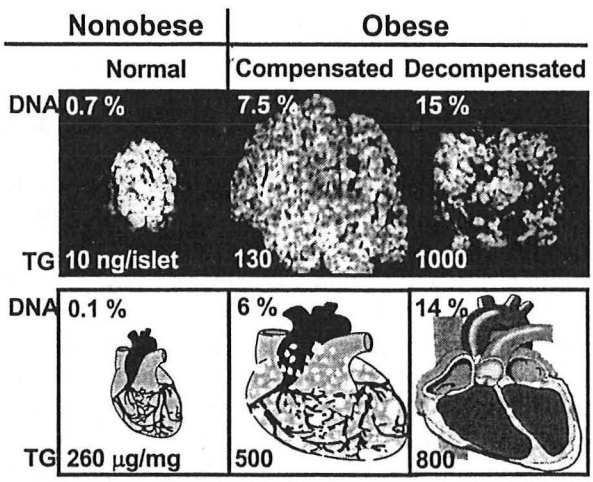


Fig. 2: The relationship of the chronology of lipid overaccumulation in the pancreatic islets stained for insulin (above) and the heart. The early hyperplasia of β-cells coincides with cardiac hypertrophy and late apoptosis with failure of both organs resulting in overt diabetes and lipotoxic cardiomyopathy.

FATTY HEART RAISES CLINICAL AND ETHICAL QUESTIONS

In the year 2001 not a single one of academic cardiologists polled had ever made a diagnosis of fatty heart, despite recent (between 1990 and 2000) studies indicating its widespread existence and evidence of its preventability.

Given its clear association with obesity, current clinical unawareness of such a serious and treatable disease during an obesity epidemic in the U.S. would appear to underscore a defect in health care education and delivery, particularly since studies in rat suggest that the syndrome is treatable with drugs currently in use for other purposes. Most cardiologists would argue that there is no clinical proof that this disorder really exists in man. The purpose of this Grand Rounds is to consider evidence that fatty heart is a common cause of cardiac morbidity and mortality in obese humans and that it can be prevented and arrested by available medications.

MECHANISMS OF STEATOSIS, LIPOTOXICITY AND LIPOAPOPTOSIS

Definitions: Steatosis = triacylglycerol (TG) deposition in nonadipose tissues; Lipotoxicity = functional changes secondary to steatosis; Lipoapoptosis = lipid-induced apoptosis.

A. Steatosis: An increase in fatty acid uptake and/or synthesis, on one hand, and/or a decrease in fatty acid oxidation on the other, will cause steatosis. There are multiple causes for lipid overaccumulation in tissues, but by far the most prevalent mechanism in the U.S. is a caloric mismatch caused by a hypercaloric diet coupled with a sedentary lifestyle. Steatosis can be subclassified etiologically as follows:

1. Enhanced lipogenesis plus decreased FA oxidation: The combination of enhanced lipogenesis and reduced β-oxidation will obviously cause far more steatosis than either abnormality alone. The combined abnormality can be caused by: 1) AMPK deficiency, and 2) lack of leptin action.

a) Deficient 5'AMP-activated protein kinase (AMPK) (15-21): The role of malonyl CoA was discovered in 1977 (McGarry *et al.*, 1977). Malonyl CoA, the first committed step in lipogenesis, is the product of the enzyme acetyl CoA carboxylase. In addition to entering the lipogenic pathway, malonyl CoA is a powerful inhibitor of carnitine palmitoyl transferase-1 (CPT-1), the rate-limiting enzyme for mitochondrial oxidation of long-chain fatty acids. Acetyl CoA carboxylase (ACC) is thus at the intersection of fatty acid oxidation and lipogenesis. Its product, malonyl CoA, prevents what would otherwise be a futile cycle by inhibiting oxidation while itself providing substrate for fatty acid synthesis (McGarry & Brown, 1997). When ACC is knocked out, the absence of malonyl CoA results in uncontrolled fatty acid oxidation and actually causes weight loss (Abu-Elheiga *et al.*, 2001). ACC is physiologically inactivated by phosphorylation by 5'AMP-activated protein kinase (AMPK) (Winder & Hardie, 1999). AMPK is thus the master switch in metabolism of fatty acids. Underexpression of AMPK would leave ACC in a fully active form, producing malonyl CoA for the synthesis of long-chain fatty acids, while inhibiting their oxidation by CPT-1. Although no human or animal disease has yet been attributed to underexpression of AMPK, it would be surprising if such did not exist, at least in secondary form in syndromes of leptin deficiency or resistance.

b) Lack of leptin action: In normal animals the rising leptin levels associated with obesity upregulate β-oxidation of fatty acids in nonadipose tissues through expression of PPARα, the transcription factor that increases the expression of the oxidative enzymes CPT-1 in mitochondria and acyl CoA oxidase (ACO) in peroxisomes. This is accompanied by an increase in uncoupling proteins (UCP-2 and -3), which allows the unwanted energy of compensatory oxidation to be dissipated as heat. At the same time there is overexpression of lipogenic transcription factors such as SREBP-1c, PPARγ and the lipogenic enzymes ACC, FAS and glycerol phosphate acyl transferase (GPAT). Even during overnutrition, nonadipose tissues remain relatively free of FA.

By contrast, tissues that have been genetically deprived of leptin action, whether because of a leptin deficiency, as in congenital generalized lipodystrophy or a loss-of-function mutation in the leptin gene (*ob/ob* mouse), or because of loss-of-function mutations in the

or a loss-of-function mutation in the leptin gene (*ob/ob* mouse), or because of loss-of-function mutations in the leptin receptor (OB-R), as in the *db/db* mouse and the *fa/fa* ZDF rat, exhibit generalized steatosis. All of the organs studied exhibit a breakdown in the liporegulatory system that prevents overaccumulation of lipids in nonadipocytes. The ability of their tissues to oxidize labeled palmitate is thus reduced, while the ability to esterify labeled palmitate and/or to synthesize fatty acids from labeled glucose is markedly enhanced. These are precisely the changes that one would predict if AMP kinase were not phosphorylating and thus inactivating ACC. Studies now under way should indicate the role of AMPK in the phenotype of leptinlessness. The expression profile of unleptinized nonadipose tissue is shown in Figure 3.

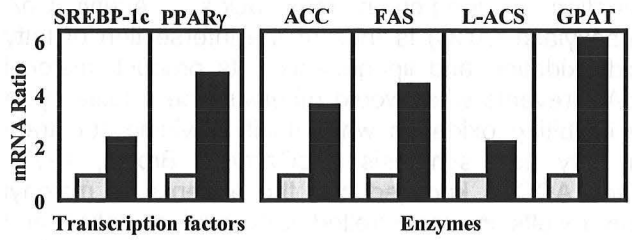


Fig. 3: Expression profile of the machinery of lipogenesis in a nonadipose tissue, the pancreatic islets. "Ratio" is mRNA relative to β-actin. Gray = lean wild-type (+/+) ZDF controls; black = obese (fa/fa) ZDF rats.

2. Increased FA influx alone with normal FA oxidation:

a) <u>Acyl CoA synthase overexpression</u>: Elegant transgenic studies of Schaffer's group (Chiu *et al.*, 2001) have demonstrated that myocardium-specific expression of a lipogenic gene, long-chain acyl CoA synthase, causes lipotoxic cardiomyopathy. There was a marked accumulation of cardiomyocyte TG associated with cardiac hypertrophy, left ventricular dysfunction and premature death. Terminal deoxynucleotidal transferase-mediated dUTP-ligand labeling staining and cytochrome C release strongly suggest that cardiac myocyte death occurred through lipoapoptosis, demonstrating that increased influx without impaired FA oxidation can cause lipotoxic damage.

b) <u>Lipoprotein lipase (LPL) overexpression</u>: Shulman's group overexpressed lipoprotein lipase in the heart and observed a similar accumulation of lipids via enhanced release from circulating triglycerides (Kim *et al.*, 2001). These studies also provide powerful support for the lipotoxic hypothesis and demonstrate that even without overnutrition or underoxidation increased influx of fatty acids can trigger lipoapoptosis.

3. Decreased FA oxidation without increased lipogenesis:

a) <u>PPAR knockout</u>: In normal animals with functioning leptin receptors, PPARα is expressed in nonadipose tissues, particularly the liver, at normal levels. When FA influx into these tissues is increased as a consequence of overnutrition, FA serve as ligands that activate and increase the expression of PPARα; this nuclear receptor upregulates enzymes of fatty acid oxidation such as mitochondrial carnitine palmitoyl transferase 1 (CPT-1) and peroxisomal acyl CoA oxidase (ACO). Additionally, PPARα can induce uncoupling proteins (UCP) -1, -2 and -3 (Kelly *et al.*, 1998), thus dissipating as heat the energy generated by oxidation of the unneeded surplus of fatty acids. Knockout of PPARα results in underexpression of at least 7 mitochondrial fatty-acid metabolizing enzymes (Watanabe *et al.*, 2000; Leone *et al.*, 1999). Cardiac abnormalities, including abnormal mitochondria, abnormal caveoli and fibrosis appear in the myocardium of PPARα null mice in an age-dependent manner, particularly when treated with etomoxir, a CPT-1 inhibitor (Djouadi *et al.*, 1999 & 1999a) (Figure 4). Clearly, then PPARα plays a regulatory role in the lipid homeostasis of cardiomyocytes and other nonadipose tissues (Keller *et al.*, 1993). When it is knocked out or underexpressed (as in unleptinized tissues), protection against steatosis is diminished.

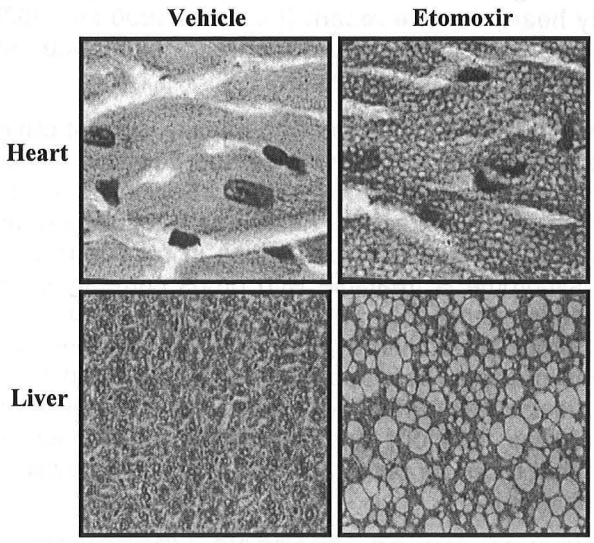


Fig. 4: Histologic sections of heart and liver prepared from male PPARα -/- mice treated with a single dose of the CPT-1 inhibitor, etomoxir (Djouadi et al., 1999).

b) <u>CPT-1 inhibition by etomoxir</u> (Paumen *et al.*, 1997): This blocks FA oxidation and increases apoptosis (Figure 4).

c) <u>Carnitine deficiency</u>: In hereditary systemic carnitine-deficient mice, cardiac hypertrophy develops early. The lipid content in the heart is 6-fold higher than controls (Kuwajima *et al.*, 1998).

long-chain acyl CoA dehydrogenase (LCAD) produce mice with cardiac lipidosis, cardiomyopathy and sudden death (Kurtz *et al.*, 1998).

e) Decreased peroxisome proliferator-activated receptor γ coactivator-1 (PGC-1): PGC-1 regulates mitochondrial biogenesis (Wu *et al.*, 1999; Lehman *et al.*, 2000) and is also a PPARα coactivator (Vega *et al.*, 2000). A deficit in any of the nonadipose tissues in which it is expressed would reduce FA oxidation.

f) Other causes of underoxidation: A deficit in any of the enzymes involved in β-oxidation of fatty acids can lead to accumulation of lipids in non-adipocytes, including cardiomyocytes, without an increase in FA influx.

B. Lipotoxicity

1. Lipotoxicity of β-cells: In the Zucker Diabetic Fatty (ZDF) (*fa/fa*) rat the onset of obesity occurs at approximately 4 weeks of age. As mentioned, the increase in adipocyte TG is accompanied by a relatively parallel accumulation of TG [in liver, skeletal muscle, heart muscle (Lee *et al.*, 2001), kidneys and pancreatic islets (Lee *et al.*, 1994)]. Of all of these tissues the β-cells are perhaps the most vulnerable to lipid-induced apoptosis and thus provide the best tissue in which to study lipotoxicity because the FA that enter in excess of their oxidative needs are essentially trapped and thus must inevitably drift into nonoxidative pathways of fatty acid metabolism. In tissues such as skeletal and cardiac muscle, FA oxidation can be augmented by an increase in physical activity, which may in part account for the salutary effects of exercise on health. Liver cells also can divest themselves of a substantial portion of their lipid overload through increased secretion of very low-density lipoprotein particles. Because such options are not available to β-cells, the intracellular FA excess must be metabolized via nonoxidative pathways, predisposing them to be early cellular victims of FA overload (Figure 5).

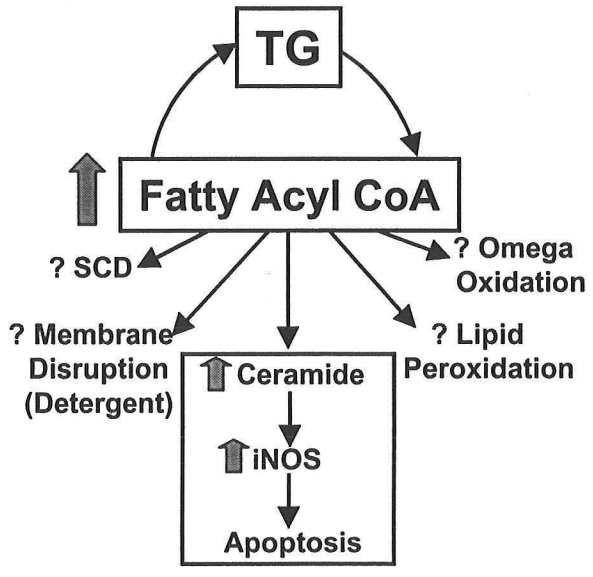


Fig. 5: Putative pathways of lipoapoptosis. Obesity-related damage to other tissues in other species may also involve the ceramide pathway or one or more of the other still untested routes marked with a "?". All require the presence of an excess of unoxidized fatty acyl-CoA.

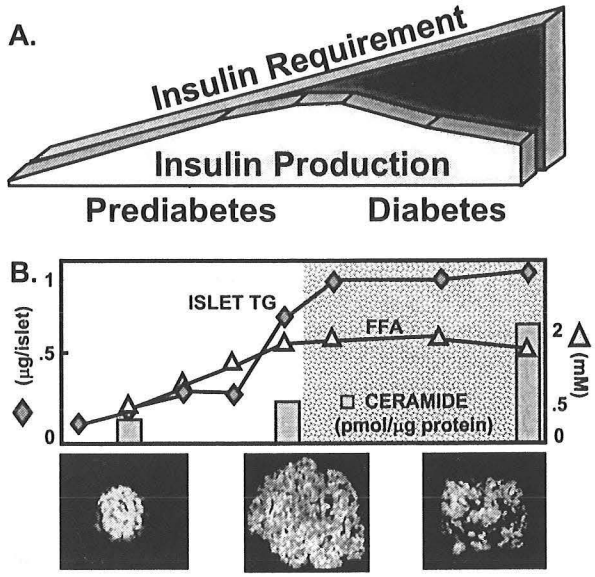


Fig. 6: A) Schematic representation of the relative changes in insulin requirements and insulin production as ZDF rats progress from preobesity to overt diabetes. B) Longitudinal course of plasma free FA levels (mM) and islet TG levels (μg/islet) and ceramide content in preobese, obese prediabetic and obese diabetic ZDF fa/fa rats. Lipid abnormalities appear ~2 weeks prior to the onset of diabetes (stippled zone), consistent with a lipotoxic mechanism.

a) The pattern of β-cell response to the lipid overload: The pattern is biphasic (Figure 6). Initially, as intraislet lipid content rises from normal to approximately 10x normal, there is increased proliferation of β-cells and a 4-fold increase in β-cell mass together with increased secretion of insulin (Unger & Orci, 2001). The hyperinsulinemia is in part secondary to the 4-fold increase in the number of β-cells, and in part the result of a decline in the Km for glucose usage by islets (Milburn *et al.*, 1995). This means that in these enlarged islets insulin release at basal and even sub-basal levels of glucose is far above normal. This combination of changes provides sufficient insulin production to maintain normoglycemia in the face of rising insulin resistance (Figure 6A). The changes that occur *in vivo*, the increase in β-cell replication and the enhanced insulin secretion, can be duplicated *in vitro* by culturing normal islets in the presence of 1 or 2 mm long-chain fatty acids, BrdU incorporation and insulin release increase strikingly (Figure 7A).

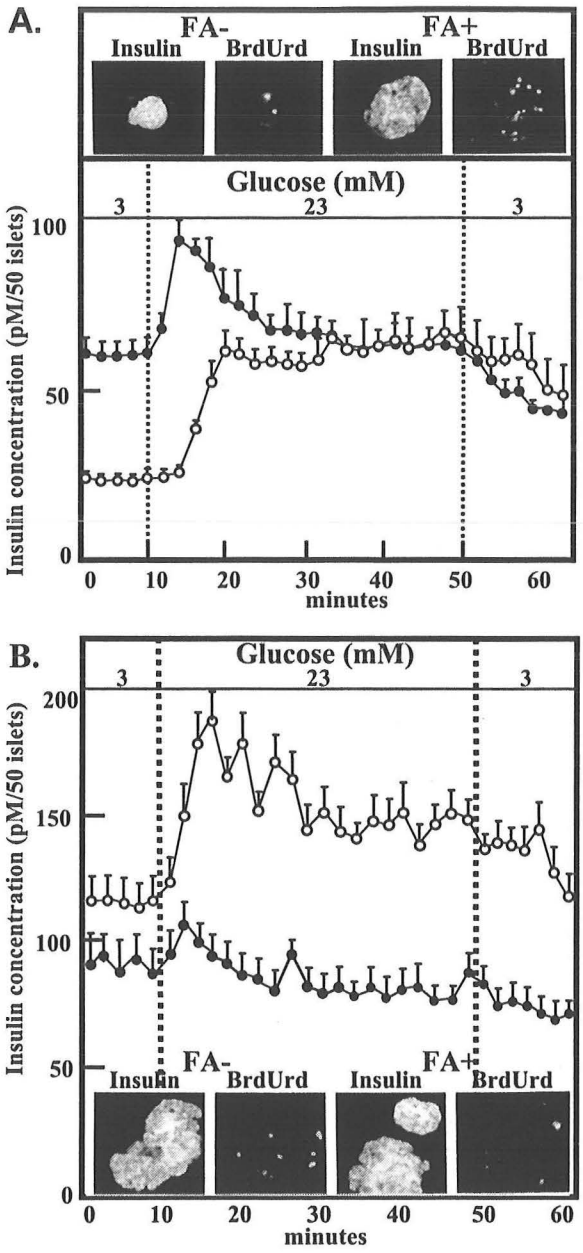


Fig. 7: A) Increase in BrdUrd incorporation (evidence of cell division) and in glucose-stimulated insulin secretion in normal islets from 6-wk-old male Wistar rats cultured in 0 (o— o) or 2 mM FA (•—•). B) Decrease in glucose-stimulated insulin secretion and BrdUrd incorporation in hyperplastic islets from 6-wk-old obese prediabetic male ZDF (fa/fa) rats cultured in 0 (o— o) or 2 mM FA (•—•). Note the differences in the basal levels of insulin secretion between the normal and hyperplastic islets.

In this compensated prediabetic stage of obesity in the ZDF *fa/fa* rats, the rate of replication has far exceeded apoptosis. But as the intraislet lipid content rises even further (Figure 6B), the β-cells that are the most lipid-laden develop severe mitochondrial alterations together with a ~10-fold increase in DNA laddering, signifying a marked increase in apoptosis. The rate of apoptosis now exceeds the rate of β-cell replication, causing a net loss of β-cells and a decline in insulin production to below the levels required to compensate for insulin resistance (Figure 6A) (Unger & Orci, 2001). At this point, overt diabetes appears. Ultimately, the mass of β-cells recedes to approximately the original level that it had been during the preobese stage of the disease, i.e., the 4-fold increase in β-cell mass observed during the compensated stage of β-cell hyperplasia has essentially disappeared (Figure 6B).

This loss of β-cell mass and secretory function is also attributed to the lipid excess, since they can be duplicated *in vitro* by culturing hyperplastic islets from compensated prediabetic rats in 1 or 2 mM FA; BrdU incorporation and insulin release are dramatically lowered (Figure 7B). In other words the response of compensated hyperplastic TG-laden islets to FA is the polar opposite of that of normal islets.

2. Lipotoxic heart disease: The same biphasic pattern occurs in the heart. However, cardiomyocytes are probably terminal cells, so that hyperplasia is not possible and apoptotic cells cannot be replaced. Consequently, depending on the magnitude and duration of the FA overload, a continuous drop-out of

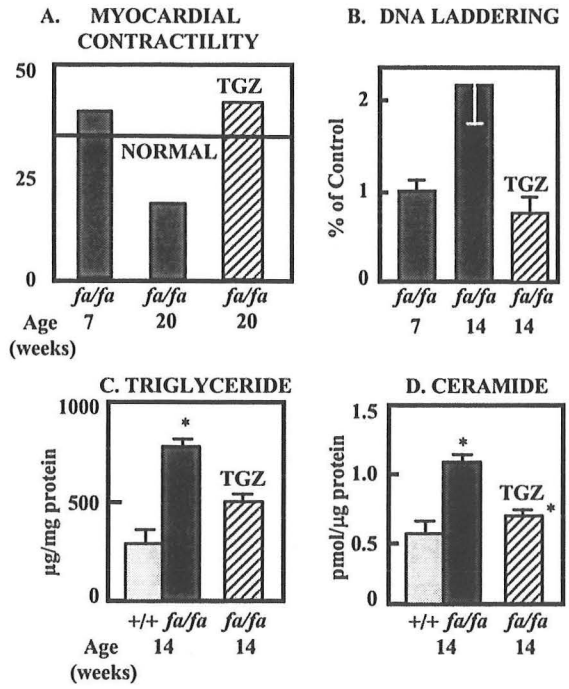


*Fig. 8: A) Cardiac lipotoxicity: Myocardial contractility, showing functional loss in 20-wk-old fa/fa ZDF rats prevented by treatment with the antisteatotic drug troglitazone (TGZ). B) DNA laddering, an index of apoptosis in hearts of obese fa/fa ZDF rats at age 14 weeks. The increase in laddering and the loss of contractility are blocked by treatment of the rats for 6-wk with the antisteatotic agent troglitazone (TGZ) beginning at 7-wks of age. Data are expressed as fold change from the 7-wk-old baseline in fa/fa, which is 10x that of lean +/+ rats at that age. C) Cardiac triacylglycerol (TG) and D) ceramide content of 14-wk-old ZDF (fa/fa) rats and wild-type controls (+/+). *p<0.01.*

cardiomyocytes at even a slow rate will ultimately leave the heart unable to meet the increased workload required to perfuse the enlarging adipose tissue mass at rest and to move it during physical activity. Thus, even a low 0.1% level of apoptosis of cardiac tissues can, in time, substantially deplete the functional capacity of the heart.

Indeed, as ZDF animals age from 7 weeks to 20 weeks, they develop echocardiographic evidence of impaired myocardial contractility (Figure 8A) (Zhou *et al.*, 2000). This functional loss has been ascribed to increased apoptosis of cardiomyocytes; DNA laddering, an index of apoptosis, rises to more than 7 times that of wild-type controls at 7 weeks of age, but contractility still appears to be normal, at least at rest (Figure 8B). However, at 20 weeks of age, when DNA laddering is approximately 30 times that of wild-type controls, contractility has declined to less than 50% of controls (Figure 8A) (Zhou *et al.*, 2000). The functional loss is almost certainly a consequence of progressive dropout of myocardial cells.

a) Measurements of myocardial lipids: TG content of the myocardium is significantly increased at 7 weeks of age and rises progressively thereafter (Figure 8C); morphometric quantification of lipid droplets by electron microscopy discloses an even more dramatic increase (Figure 9). A statistically significant increase of ceramide (Figure 8D) suggests that it is involved in the cardimyocyte apoptosis, as was the case in the mouse model of lipotoxic cardiomyopathy, in which cardiomyocyte-specific overexpression of acyl-CoA synthase caused an increase in cardiac TG and ceramide and resulted in lipid cardiomyopathy (Chiu *et al.*, 2001).

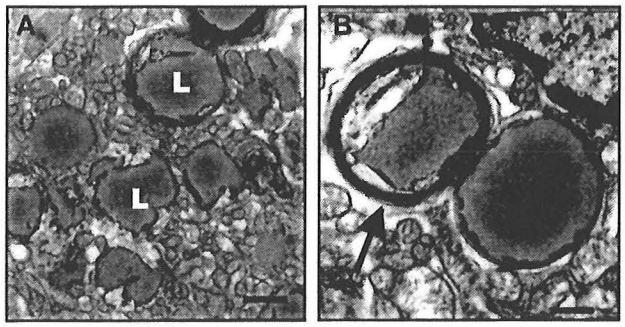


Fig. 9: Electron microscopy of cardiac tissue from 18-day-old MHC-ACS mice. 7500x magnification (A, bar = 1 μm) & 15,000x magnification (B, bar = 0.5 μm); lipid droplets (L). Some droplets at this stage are surrounded by multiple concentric layers of membrane (arrow). From Chiu et al., 2001.

Remarkably, treatment of obese ZDF rats with troglitazone, beginning at 7 weeks of age, reduces myocardial TG, measured both biochemically and by morphometry of lipid droplets, lowers ceramide content and prevents the loss of contractile function of the heart (Figure 8) (Zhou *et al.*, 2000).

Thus, in the ZDF rat, the metabolic abnormalities associated with their heart disease mimic to a remarkable degree those observed in their islets, and in both organs the metabolic changes and functional evidence of disease can be completely prevented by the antisteatotic agent, troglitazone. One would predict that AICAR, the activator of AMPK (Bergeron *et al.*, 1999; Russell *et al.*, 1999), or cerulenin, the inhibitor of FAS (Loftus *et al.*, 2000) would be even more effective.

C. Lipoapoptosis

1. General considerations:

a) Cell homeostasis: In multicellular organisms, the cell population must be regulated to assure optimal function of various organs. In many tissues this homeostasis of the cell population involves the removal of older, damaged, hypofunctional cells so that new healthy cells can replace them. Their removal involves programmed self-destruction, which in 1972 was named "apoptosis" by Currie and colleagues (Kerr *et al.*, 1972). This group noticed that such dying cells differed morphologically from cells undergoing pathological necrotic cell death.

b) Morphology of apoptosis: Apoptotic cell death differs from necrosis caused by noxious stimuli. Necrosis is characterized by cell membrane disruption, swelling, disintegration, cell content leakage and local inflammation. Apoptosis, by contrast, is characterized by "apoptotic bodies", fragments of dense DNA surrounded by apparently intact plasma membrane, and DNA condensation and fragmentation, the latter appearing as a "ladder" when separated on DNA-gel electrophoresis. There is no inflammatory response.

While apoptosis is a normally occurring means of cellular turnover, it may be accelerated by cellular stresses caused by thermal, hypoxic and cytotoxic factors such as cytokines, oxygen radicals and peroxynitrite ($ONOO^-$) and cause a net reduction in the functioning cell population of an organ.

c) Caspases: The morphologic changes of apoptosis are the result of the action of cysteine proteases known as "caspases" (Budihardjo *et al.*, 1999; Cikala *et al.*, 1999). They are specifically activated during apoptosis (Alnemri *et al.*, 1996). These enzymes have been highly conserved throughout evolution and are present in hydra, nemotodes, insects and man (ibid). Caspases have an active site cysteine and cleave substrates after aspartic acid residues (Thornberry & Lazebnik, 1998). They can be thought of as the "central executioners" of apoptosis, which can be prevented by blocking their activity.

d) DNA laddering: A major discovery was the demonstration of the mechanism of DNA fragmenta-

tion, which results in the DNA laddering used extensively as a marker for apoptotic cell death (Wyllie, 1980). This nuclease cuts genomic DNA between nucleosomes. The DNA ladder nuclease is known as caspase-activated DNase (CAD) and is present in living cells as an inactive complex with an inhibitory subunit called "ICAD" (Nagata, 2000). It is activated by caspase-3-mediated cleavage of the inhibitory subunit (Liu *et al.*, 1997; Enari *et al.*, 1998; Sakahira *et al.*, 1998).

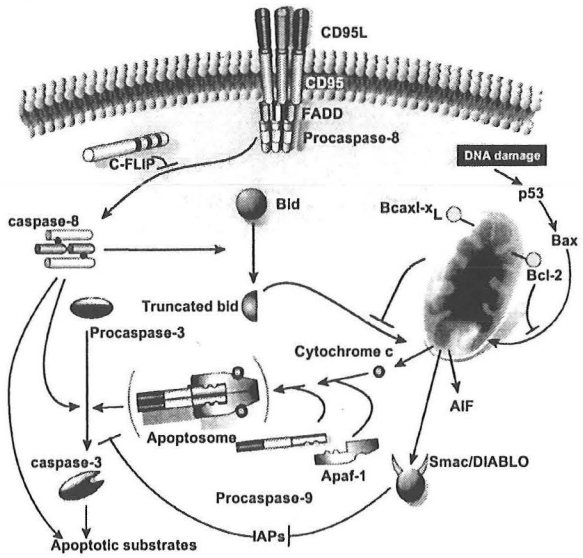


Fig. 10: Two of the apoptotic pathways. The caspase 8 (death receptor) pathway is triggered via cell surface receptors to CD95 ligand and TNFα. The other pathway is triggered by DNA damage such as can be caused by lipotoxicity. It shows Bcl-2 inhibitory Bax-induced mitochondrial extrusion of cytochrome c to associate with Apaf-1 and then precaspase 9 to form an apoptosome which, like the caspase 8 pathway, activates caspase 3.

e) <u>The caspase cascade</u>: CD95 ligand binds to CD95 and recruits via the Fas-associated death domain protein (FADD) multiple procaspase-8 molecules, thereby activating them (Figure 10). There are also other mechanisms for caspase activation. In the case of caspase-9, activation is a result of association with a dedicated protein cofactor, Apaf-1 (Li *et al.*, 1997; Zhou *et al.*, 1997). Cytochrome C is extruded from the inner mitochondrial membrane through a pore formed by proapoptotic Bcl_2 family members (Reed, 1997). Cyto C oligomerization of Apaf-1 recruits procaspase 9 into the apoptosome complex and activates caspase-9 (Figure 11). Caspase-8 and -10 contain a death effector known as DED; caspase-2 and -9 contain activation and recruitment domain (CARD).

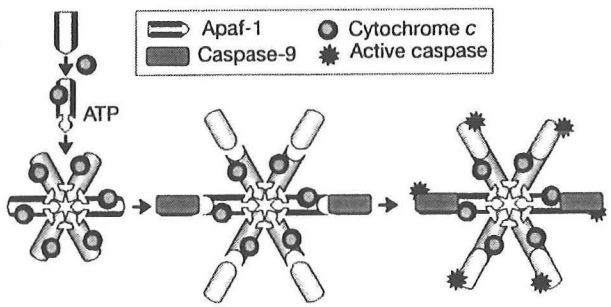


Fig. 11: A possible mechanism by which the cytochrome c extruded from mitochondria associates with Apaf-1 to activate caspase 9.

f) <u>Bcl_2 family</u>: A second set of apoptotic regulators are known as the Bcl_2 family. They have been divided into three groups. Group 1 has anti-apoptotic activity, whereas groups 2 and 3 promote cell

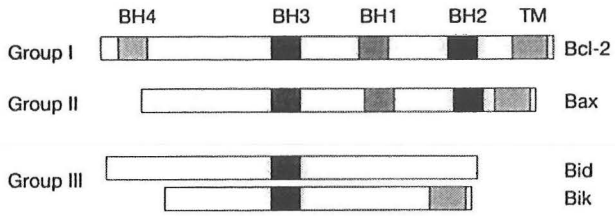


Fig. 12: Bcl-2 family members. Group 1 is antiapoptotic and Group II proapoptotic. Group III is diverse.

death (Figure 12). These family members can homodimerize or heterodimerize (Figure 13). If an excess of proapoptotic members, Bax, Bad, Bim and Bid, dimerize, apoptosis would be promoted (Figure 13A), while an excess of dimerized anti-apoptotic proteins,

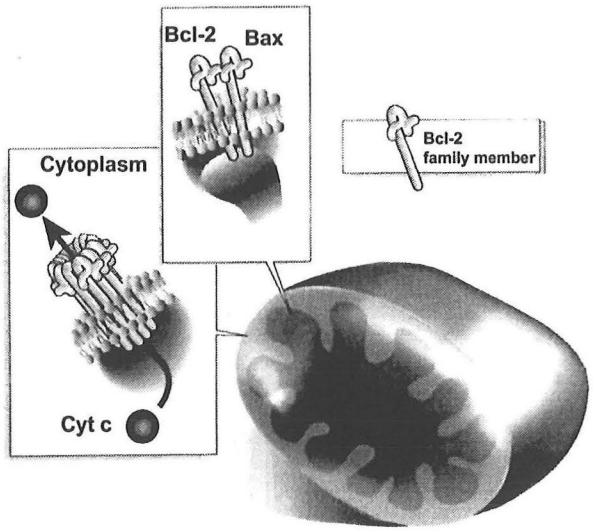


Fig. 13: A possible mechanism to explain the pro- and antiapoptotic effects of members of the Bcl-2 family. Oligomerization of a proapoptotic member, such as Bax, may form pores in the outer mitochondrial membrane through which cytochrome c is extruded from the intermembrane space into the cytoplasma (left). There is evidence that Akt can block apoptosis (Kennedy et al., 1997), perhaps by phosphorylation of certain of the proapoptotic family members and blocking oligomerization into pores. Bcl-2 association with Bax fails to produce a pore, thereby protecting against apoptosis. (See also Fig. 20)

NOTE: Figures 10-13 from Hengartner et al., 2000.

such as Bcl_2, would protect. Bcl_2 protects cells by direct binding to and sequestering of the Apaf-1 homologue – at least in *C. elegans* (Figure 13B). However, the precise mechanisms remain speculative.

g) Cytochrome-C: Cytochrome-C is an electron carrier involved in oxidative phosphorylation and located in the intramembrane space of the mitochondria. It is also required for activation of caspase-9 in the cytosol (Li *et al.*, 1997). The mechanism of how cytochrome-C crosses the outer membrane of the mitochondrium is unclear, but pro-apoptotic Bcl_2 family members enhance the process, while antiapoptotic Bcl_2 family members prevent it. It is not known if they promote apoptosis by forming channels or holes in the membrane, perhaps by forming a large pore channel following interaction with other proteins (Reed, 1997) (Figure 13A). One possibility is that apoptotic signals alter mitochondrial physiology in a manner that cause swelling and rupture of the outer membrane and release of intermembrane proteins such as cytochrome-C. Although the mechanism of its exit from the mitochondrium is not known, cytochrome-C exit is almost a universal feature of apoptotic cell death. It has been shown to occur in cardiac lipoapoptosis (Chiu *et al.*, 2001). In lipoapoptosis of β-cells severe mitochondrial alterations have been observed electron microscopically (Figure 14) (Higa *et al.*, 1999).

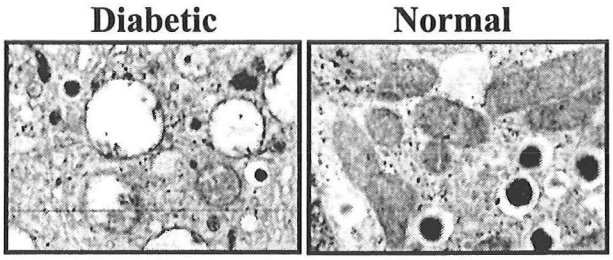


Fig. 14: Electron microscopic appearance of mitochondria in lipid-laden β-cells of obese fa/fa ZDF rats.

2. Lipoapoptic pathway: As mentioned above, whenever FA influx into a cell exceeds FA oxidation, the FA surplus must ultimately enter pathways of nonoxidative metabolism. Initially, TG appear to be the major initial lipid product, and they probably do no harm to the cell. They may even provide initially a protective buffer by diverting FA from entry into more deleterious pathways of lipid-inflicted damage. But ultimately hydrolysis of these TG stores will add to the already expanded FA pool, providing additional substrate for nonoxidative FA metabolism (Figure 5). Although unproven, increased DAG may, through its phosphorylation to phosphatidic acid, maintain protein kinase C activity, and thus play a role in cardiac (Dhalla *et al.*, 1997) and perhaps β-cell hypertrophy. In pancreatic islet cells (Shimabukura *et al.*, 1998;

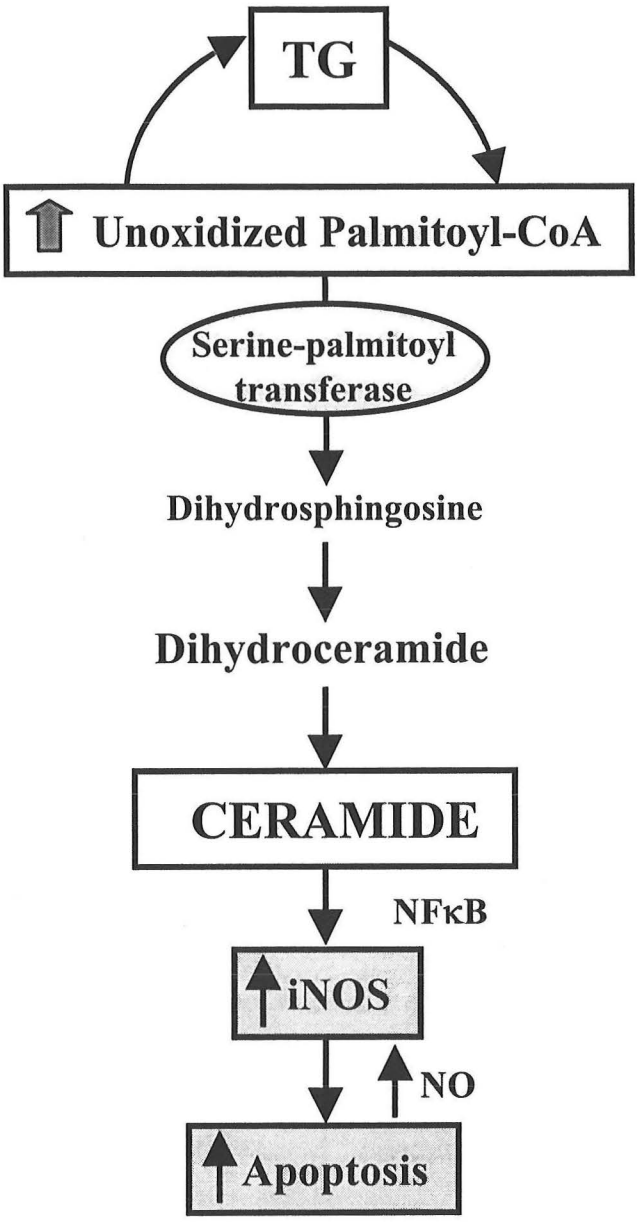


Fig. 15: Putative pathway of lipoapoptosis. Condensation of unoxidized palmitoyl-CoA and L-serine catalyzed by serine-palmitoyl transferase generates ceramide. Ceramide upregulates the expression of inducible nitric oxide synthase (iNOS) by activating NFκB. Alternatively, it may reduce Akt phosphorylation thereby reducing its antiapoptotic effects (see text).

Shimabukura *et al.*, 1998a), and probably in cardiac myocytes (Zhou *et al.*, 2000; Chiu *et al.*, 2001), the ceramide pathway (Figure 15) seems to be the most important of the destructive routes, although direct pathways independent of ceramide, such as lipid peroxidation, detergent effects, etc., have not been excluded. In leptin resistance, the enzyme serine palmitoyl transferase (SPT) (Weiss & Stoffel, 1997) is expressed at high levels (Figure 16) (Shimabukuro *et al.*, 1998 & 1998a), thereby increasing the condensation of palmitoyl CoA and serine to form dihydrosphingosine, the first step in *de novo* ceramide

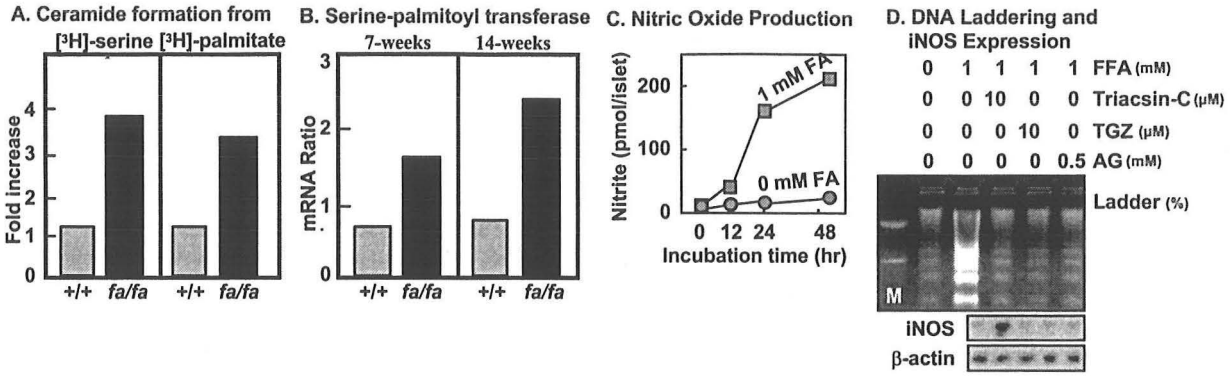


Fig. 16: Evidence for ceramide excess, iNOS induction excess and NO formation in the pathogenesis of fatty acid-induced apoptosis. A) Comparison of [^{3}H]-ceramide formation for [^{3}H]-serine or [^{3}H]-palmitate. B) mRNA of serine palmitoyl transferase, the enzyme that catalyzes condensation of L-serine and palmitoyl CoA in 7-wk-old and 14-wk-old normal (+/+) and obese (fa/fa) ZDF rats. Fa/fa rats become diabetic at age 14 wks as a consequence of apoptotic depletion of their β-cells. C) Comparison of the effects of a 1 mM 2:1 oleate:palmitate mixture on nitric oxide production (nitrite) in isolated islets of prediabetic fa/fa ZDF rats. D) DNA laddering and iNOS expression in islets from fa/fa ZDF rats cultured in medium without supplementation, in medium containing the 1 mM fatty acid mixture by itself, or with triacsin-C or with troglitazone, both of which reduce islet lipid content, or with aminoguanidine (AG), an iNOS inhibitor. DNA laddering is shown and quantified as percent of total DNA.

biosynthesis (Figure 15). The profound mitochondrial alterations (Figure 14) (Higa *et al.*, 1999) and apoptosis identified in the pancreatic β-cells of leptinless *fa/fa* ZDF rats are believed to be the result of excessive *de novo* ceramide formation, coupled with underexpression of the antiapoptotic factor, Bcl_2 (Shimabukuro *et al.*, 1998a). The inhibitor of ceramide synthesis, fumonicin-B_1, completely blocks FA-induced changes, suggesting that ceramide is a major mediator of lipotoxicity and apoptosis in islets of ZDF rats. This does not exclude the possibility that other pathways of lipotoxicity dominate in other tissues (Listenberger *et al.*, 2001).

a) How do increased FA and/or ceramide lead to lipoapoptosis? Several pathways may be involved.

1) The Akt pathway: Akt, also known as protein kinase-B (PKB), is a serine-threonine kinase involved in crucial phosphorylations that seem to account for insulin-stimulated translocation of Glut-4 to the plasma membranes (Cho *et al.*, 2001). Akt is required for insulin-mediated glucose uptake in certain cells, and also for insulin- and IGF-1-mediated survival signals (antiapoptosis) (Aikawa *et al.*, 2000). Akt, in turn, is phosphorylated and thus activated by phosphotidylinositol-3'-OH-kinase (PI3' kinase). Akt may promote cell survival by phosphorylating and thereby inhibiting components of the cell death machinery, probably members of the Bcl_2 family. For example, if Ser-112 and Ser-136 of the BAD moledule are unphosphorylated, it induces cell death by forming proapoptotic homodimers or heterodimers. But when BAD is phosphorylated at Ser-136, BAD-induced apoptosis is blocked.

The simplest (if incomplete) way to answer this question is to accept the evidence provided by Shulman's group that certain lipid moieties can block the action of PI3 kinase to phosphorylate Akt. High levels of both long-chain fatty acids and ceramide appear to interfere with Akt phosphorylation and thus give rise to both impaired insulin-stimulated glucose uptake in skeletal muscle and apoptosis (Navarro *et al.*, 2000). Thus this concept would explain both apoptosis and insulin resistance with a similar mechanism and raise the possibility that in skeletal muscle the resistance leads to muscle loss.

2) The NO pathway: Other mechanisms may also prevail (Basu *et al.*, 1998; Di Paola *et al.*, 2000). Ceramide can upregulate inducible nitric oxide synthase by activating NκB. This causes an increase in nitric oxide production and the resulting rise in tissue peroxynitrite provides a source of damaging reactive molecules. Although cytokines increase ceramide by activating the enzyme sphingomyelinase, a major pathway in cytokine-induced, ceramide-mediated apoptosis, in obesity-related apoptosis ceramide appears to be synthesized *de novo* from the condensation of L-serine and palmitoyl CoA, catalyzed by the enzyme serine palmitoyl transferase. An increase in this pathway will occur whenever tissues palmitoyl CoA levels are increased.

c) Lipoapoptosis of cardiomyocytes: Apoptosis involving caspase-3 and -8 have been induced by ceramide in cardiomyocytes (Downward, 1998). Incubation of cultured cardiomyocytes with palmitate decreases their ability to oxidize fatty acids by raising intracellular malonyl CoA levels and lowering the activity of 5'AMPK; associated with these effects are increases in intracellular triglyceride and ceramide, together with an increase in caspase-3-like activity and DNA laddering. In addition, fatty acids have been

shown to reduce Bcl_2 expression in certain nonadipose tissues (pancreatic islets) (Shimabukuro *et al.*, 1998a).

Thus, there is an extraordinarily complex and generally redundant apoptogenic system in place, as if to make certain that no impaired cell is left behind. In this manner each of the organs can be maintained in an optimal condition through weeding out of its least fit cells. However, if this process is accelerated or the replacement process decelerated, cellular depletion will leave the organ in a functionally compromised state.

Schaffer's group has demonstrated most specifically the apoptotic consequences of FA overload (Figure 17A) in their cardiomyocyte-specific ACS-overexpressing mice. The high TUNEL staining and evidence of cyto-c extrusion into the cytosol (Figure 17B) in the presence of increased TG and ceramide coupled with symptoms of congestive heart failure and pre-mature death (Figure 17C) provide the most powerful evidence for the existence of lipotoxic heart disease in rodents.

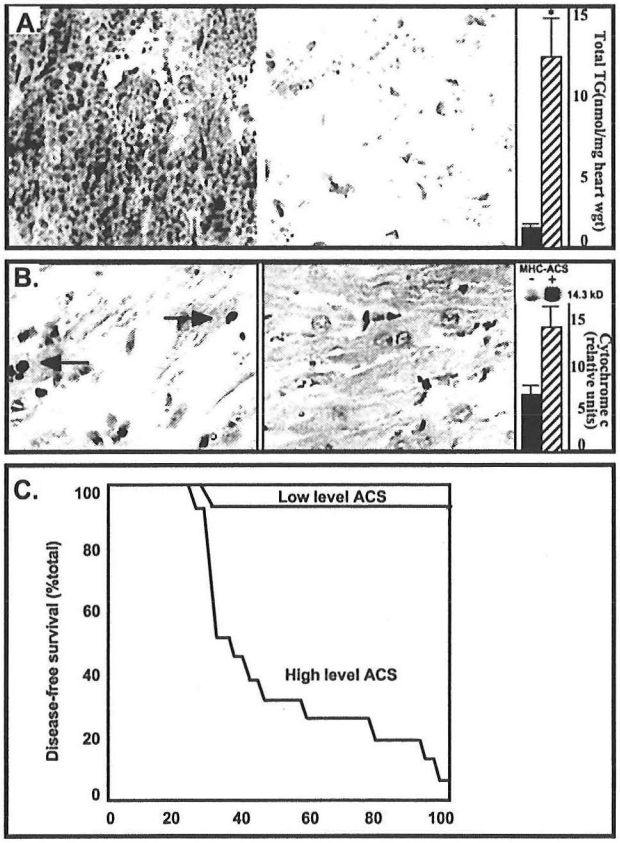


Fig. 17: A) Oil red O staining of myocardium from transgenic mice overexpressing acyl CoA synthase in myocardiocytes and showing dramatic increase in lipid compared to wild-type controls (right). B) Evidence of lipoapoptosis with positive TUNEL staining(arrows) in nuclei of cardiomyo-cytes and cytochrome c extrusion into the cytoplasm. C) the incidence of congestive heart failure or death in transgenic mice overexpressing acyl CoA synthase at low or high levels. From Chiu et al., 2001.

D. Does cardiac lipotoxicity really occur in man?

Findings obtained in monogenic disorders of intracellular lipid homeostasis in rodent models of obesity do not necessarily prove that the islet and cardiac abnormalities ("metabolic syndrome") associated with diet-induced, nongenetic human obesity necessarily have a similar pathogenesis. Certainly the heterogeneity of the age of onset and in the severity of the complications in nongenetic human obesity are in striking contrast to their homogeneity of the monogenic disorders in rodents. Nevertheless, for the following reasons the burden of proof lies on those who deny the existence of human lipotoxic heart disease: 1) there are multiple clinical similarities between the rodent syndrome of lipotoxicity and human metabolic syndrome; 2) over half of the American population is now classified as overweight and therefore at risk for cardiac lipotoxicity, a disorder not currently recognized by American clinicians; 3) the photomicrograph (cf cover page) shows that intramyocyte lipid overaccumulation can occur in human obesity (Unger & Orci, 2001); 4) Szczepaniak et al. (Szczepaniak *et al.*, 2001) report that myocardial fat can be estimated noninvasively using NMR (an example of an NMR study in a ZDF rat appears in Figure 18); and 5)

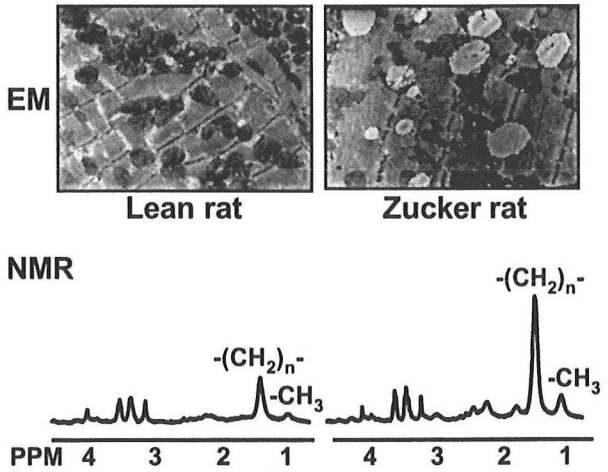


Fig. 18: Comparison of intramyocardial lipids by electron microscopy (EM) and by nuclear magnetic resonance (NMR) in a normal lean rat and an obese ZDF (fa/fa) rat. This demonstrates the feasibility of estimating intramyocardial fat noninvasively in man. Courtesy L. Orci & L. Sczcepaniak.

thiazolidinediones, which prevent both the Type 2 diabetes (Higa *et al.*, 1999) and lipid cardiomyopathy of obese ZDF rats (Zhou et al., 2000), ameliorate the Type 2 diabetes of humans (Cavaghan et al., 1997), suggesting that cardiac lipotoxicity of obese humans might also respond. It therefore seems imperative to develop effective strategies to cope with what could well be a prevalent heart disease, and to determine if it can be arrested by dietary restriction, a nontoxic thiazolidinedione, an AICAR-like agent, or FAS inhibitors.

E. Diet-induced lipotoxicity: Whatever happened to antisteatosis? Initially the vast majority of overnourished individuals have a perfectly normal liporegulatory system. Based on the evidence that leptin is an antisteatotic hormone (Lee *et al.*, 2001), nonadipose tissues such as the heart should be well protected by compensatory oxidation. As obesity increases, hyperleptinemia rises in proportion to the expanding fat mass to inhibit ectopic lipogenesis and enhance oxidation of any surplus of incoming fatty acids. Yet clinical experience indicates that obese individuals almost always develop insulin resistance, attributed to increased lipid deposition in skeletal muscle (McGarry, 1992), and often develop overt diabetes, which signifies a concomitant loss of β-cells, presumably through the same lipotoxic mechanisms identified in islets and heart of *fa/fa* ZDF rats (Lee *et al.*, 1994; Unger, 1995; Lee *et al.*, 1997; Zhou *et al.*, 2000). If so, this must mean that leptin-mediated antisteatotic protection ultimately wanes, either because of hypoleptinemia, leptin resistance or both.

1. Evidence for relative hypoleptinemia: The elevated leptin levels in young obese patients correlate with total body fat (Caro *et al.*, 1996; Moller *et al.*, 1998). However, as obese patients age, the correlation is lost as age-related hypoleptinemia relative to the body fat appears. This loss of correlation may be particularly marked in patients with abdominal obesity. Abdominal adipocytes produce less leptin and they contribute relatively little to the antisteatosis of obesity (Minocci *et al.*, 2000). If this is correct, it means that when excess calories are deposited primarily in the abdominal region of the body, leptin production may not be sufficient to elicit the desired antisteatotic effect. This may explain why patients with abdominal obesity are the most prone to develop the lipid-mediated complications grouped together under the term "metabolic syndrome".

2. Evidence for leptin resistance: There is also evidence for postreceptor leptin-resistance. Factors such as suppressor of cytokine signaling (SOCS) -1 and -3 (Bjorbaek *et al.*, 1998) in leptin's target tissues may attenuate its peripheral actions and thus contribute to age-related loss of antisteatosis (Wang *et al.*, 2001). Taken together, we suspect that diet-induced lipotoxicity and lipoapoptosis can occur in man and that they reflect combined overaccumulation and underoxidation of fatty acids resulting from hypoleptinemia and leptin resistance.

3. Preventing and treating lipotoxicity: Any maneuver that reduces ectopic deposition of lipid appears to prevent lipotoxicity. This includes caloric restriction (Ohneda *et al.*, 1995) and thiazolidinedione (TZD) treatment. In leptin-unresponsive *fa/fa* ZDF rats, overexpression of a fully functional leptin receptor in islets (Wang *et al.*, 1998) or liver (Lee *et al.*, 2001) will, in the presence of leptin lower the TG levels. In islets it decreases SPT expression and upregulates Bcl_2 (Shimabukuro *et al.*, 1998b), changes that reduce ceramide formation and prevent apoptosis, respectively. *In vivo* treatment of prediabetic *fa/fa* ZDF rats with the TZD troglitazone prevents the overaccumulation of lipids, including ceramide, and completely prevents the mitochondrial degeneration (Figure 14) and the apoptosis in the islets (Higa *et al.*, 1999); it also prevents all changes in the heart (Zhou *et al.*, 2000).

a. <u>*Old drugs*</u>: Treatment with troglitazone or with inhibitors of inducible nitric oxide synthase (iNOS) both *in vivo* and *in vitro* also prevents β-cell depletion by apoptosis in islets of ZDF rats (Figure 19A). *In vivo* treatment protects them from diabetes (Figure 19B) (Shima-bukuro *et al.*, 1997), suggesting that iNOS upregulation by FA and high levels of NO production may be a factor in the apoptosis.

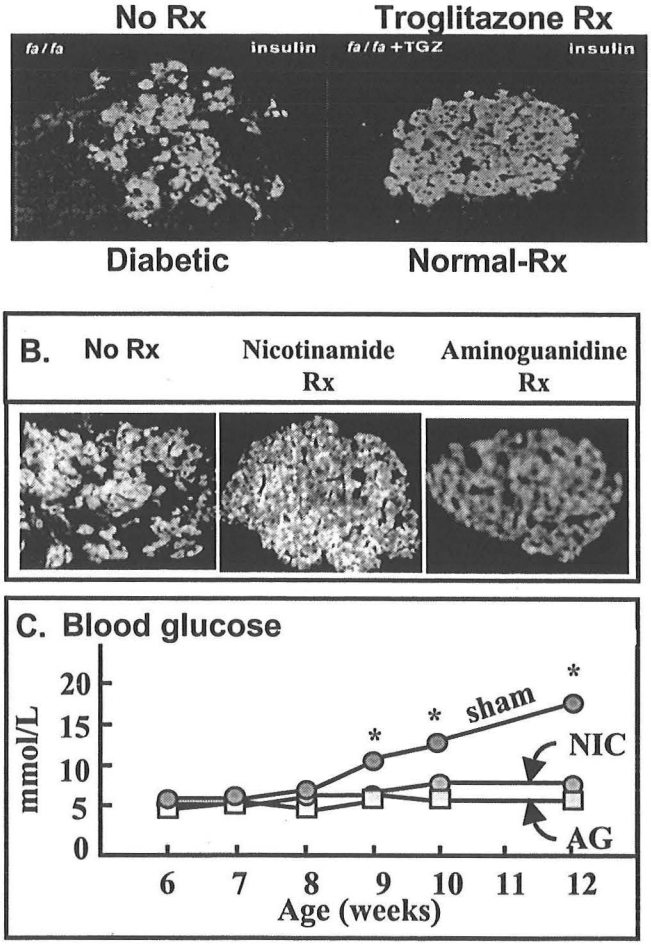


*Fig. 19: A) Effects of TGZ treatment on insulin-positive β-cells. B) Effects of iNOS inhibitory therapy for 6 wk. Immunofluorescent staining of pancreas of sham-, NIC- and AG-treated obese fa/fa ZDF rats. C) Effects of NIC and AG treatment on blood glucose (mean ± SEM) of obese prediabetic fa/fa ZDF rats compared to untreated +/+ controls. *p<0.05 vs. untreated lean fa/+ ZDF group.*

b. <u>*Potential drugs*</u>: But there are some even more promising drugs on the horizon. One is 5-

Table 2: Pharmacologic Rx of Steatosis/Lipotoxicity

Agent	Target	Rationale	Status	Downside
Troglitazone	PPARγ/PGC-1	Reduces steatosis, ceramide, lipoapoptosis	Available	Increases obesity
Aminoguanidine	iNOS	↓ NO, ↓ lipoapoptosis	?	?
Nicotinamide	iNOS	↓ NO, ↓ lipoapoptosis	?	?
AICAR	AMPK/ACC	↓ malonyl CoA; ↑ FA oxidation	?	?
Cerulenin	FAS	Inhibits both FA synthesis and appetite	?	?
C75	FAS	Inhibits both FA synthesis and appetite	?	?
Leptin	Leptin receptor (OB-Rb)	Should reduce steatosis but not appetite or obesity	Failed to reduce obesity	Painful on injection
Fibrates	PPARα	Should reduce steatosis in liver and other PPARα-expressing tissues	Available	Doesn't decrease obesity
HIV protease inhibitors	SREBP-1c site 1 or 2 proteases	AIDS patients develop lipodystrophy during protease inhibitor Rx	Hypothetical	?

aminoimidazole-4-carboximide-1-β-D-Ribo-furanoside (AICAR), an AMPK activator that increases FA oxidation by inhibiting ACC-catalyzed and malonyl CoA production, decreases lipogenesis and enhances insulin sensitivity, thus correcting the liporegulatory defect in nonadipose tissues via antisteatosis and, unlike the other pharmacologic interventions, might possibly have an antiobesity effect (Bergeron *et al.*, 2001). Similarly, inhibitors of FAS such as cerulenin and C75, cause dramatic weight loss and decreased feeding in mice (Loftus *et al.*, 2000). Finally, protease inhibitors used in AIDS may cause lipoatrophy via unknown mechanisms. Conceivably they might have an antiobesity action.

WHEN TO SUSPECT AND DIAGNOSE FATTY HEART (Table 2)

Fatty heart should be suspected in any obese individual. It can be assumed to be present in any lipodystrophic patient.

A. Specific diagnosis: This requires a myocardial biopsy with staining for Oil Red O.

B. Noninvasive diagnosis: The magnetic resonance spectroscopic technics pioneered by Szczpaniak and coworkers now provide a relatively specific noninvasive means of making the diagnosis.

C. Indirect evidence of fatty heart: Evidence from the Shulman and Birnbaum laboratories suggests that the insulin resistance (resistance to insulin-stimulated glucose transport into skeletal muscle) of obesity and lipoapoptosis share a common pathway. Long-chain fatty acids and/or ceramide block phosphorylation of Akt2, which is crucial for translocation of Glut-4 to the plasma membrane. Akt2 is also important in mediating the survival signals mediated by insulin and IGF-1, as depicted in Figure 20A. By blocking the association of IRS-2 and Akt2, elevated tissue lipids, FAcyl CoA and/or ceramide not only cause insulin resistance but they promote apoptosis by failing to inactivate proapoptotic factors such as Bad (Figure 20B). We therefore suspect that a common mechanism exists for insulin resistance and apoptosis and that insulin resistance may simply be an early harbinger of a slow

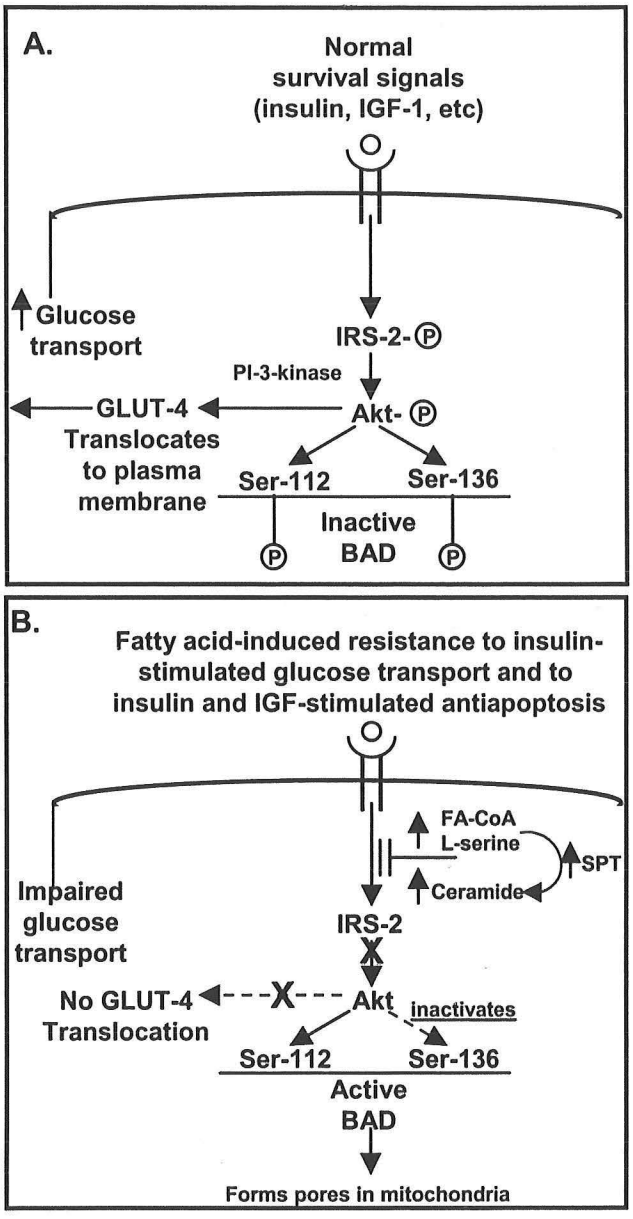


Fig. 20: A) A naïve depiction of a central role for Akt in mediating insulin and IGF-1-stimulated glucose transport and antiapoptosis. B) Possible mechanism by which intracellular lipid excess impairs insulin sensitivity early on and over years destroys these cells via lipoapoptosis (Kennedy et al., 1997). See also Fig. 13.

lipotoxic process taking place in skeletal and cardiac muscle cells that culminates ultimately in lipoapoptosis. We suspect that whatever is happening in skeletal muscle is also happening in cardiac muscle (Figure 21). Consequently, the presence of insulin resistance in an obese person is presumptive evidence of increased intramyocyte lipids both in skeletal and cardiac muscle. Fasting hyperinsulinemia in excess of 70 μU/ml in a normoglycemic person, we guess, would be correlated with intramand skeletal muscle and would also be correlated with the BMI. While this is, of course, hypothetical, the hypothesis is readily testable. If confirmed, this would provide a simple screening test for more specific diagnostic procedures for fatty heart, and this is supported by Figure 21, showing simultaneous overaccumulation of lipids in skeletal and cardiac muscle.

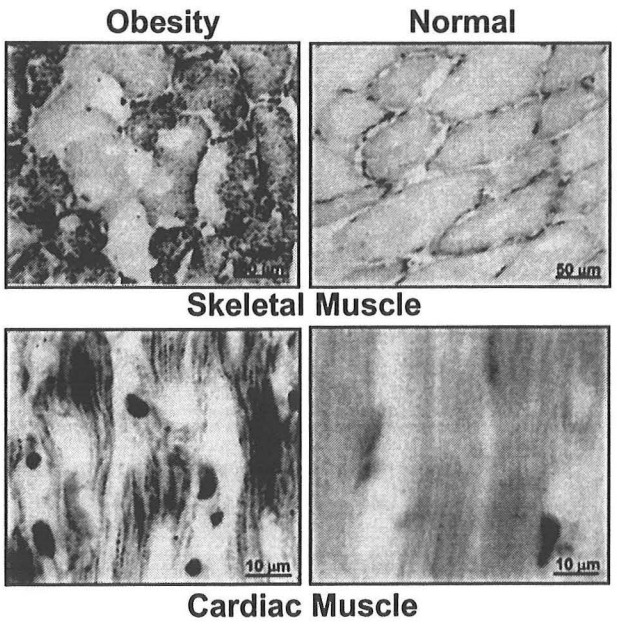


Fig. 21: Comparison of staining with oil red O in skeletal and cardiac muscle of obese fa/fa and normal +/+ ZDF rats. Overaccumulation of lipid in the two muscle organs occurs simultaneously.

ACKNOWLEDGMENTS

We wish to thank Kay McCorkle and Susan Kennedy for their remarkable creativity, respectively, in the illustrations and the organization of the text presentation.

Professor Lelio Orci, Chairman, Dept. of Morphology at the University of Geneva, contributed most of the photomicrographs. Lidia Sczcepaniak, Ph.D., provided the magnetic resonance spectroscopy data and Dr. Paul Grayburn the echocardiograms; Shirley Waggoner provided technical help; Drs. Yan-Ting Zhou, Young Lee, Michio Shimabukura, Tetsuya Kakuma and Asad Karim carried out much of the experimental work presented here.

This work was supported in part by the Department of Veterans Affairs Institutional Support, National Institutes of Health (NIH) (DK02700-41), and the NIH/ Juvenile Diabetes Foundation Diabetes Interdisciplinary Research Program.

Bibliography (by subject)

Lipotoxicity

Billheimerr DW, Buja LM, Parkey RW, Bonte FJ, Willerson JT. 1978. Fatty acid accumulation and abnormal lipid deposition in peripheral and border zones of experimental myocardial infarcts. *J Nucl Med* 19:276-83

Chien KR, Bellary A, Nicar M, Mukherjee A, Buja LM. 1983. Induction of a reversible cardiac lipidosis by a dietary long-chain fatty acid (erucic acid). Relationship to lipid accumulation in border zones of myocardial infarcts. *Am J Pathol* 112:68-77

Chiu HC, Kovacs A, Ford DA, Hsu FF, Garcia R, Herrero P, Saffitz JE, Schaffer JE. 2001. A novel mouse model of lipotoxic cardiomyopathy. *J Clin Invest* 107:813-22

Chu KC, Sohal RS, Sun SC, Burch GE, Colcolough HL. 1969. Lipid cardiomyopathy of the hypertrophied heart of gold thioglucose obese mice. *J Pathol* 97:99-103

Dhalla NS, Xu YJ, Shen SS, Tappia PS, Panagia V. 1997. Phosphatidic acid: A potential signal transducer for cardiac hypertrophy. *J Mol Cell Cardiol* 29:2865-71

Hickson-Bick DL, Buja ML, McMillin JB. 2000. Palmitate-mediated alterations in the fatty acid metabolism of rat neonatal cardiac myocytes. *J Mol Cell Cardiol* 32:511-9

Higa M, Zhou Y-T, Ravazzola M, Baetens D, Orci L, Unger RH. 1999. Troglitazone prevents mitochondrial alterations, β-cell destruction, and diabetes in obese prediabetic rats. *Proc Natl Acad Sci USA* 96:11513-8

Kim JK, Fillmore JJ, Chen Y, Yu C, Moore IK, Pypaert M, Lutz EP, Kako Y, Velez-Carrasco W, Goldberg IJ, Breslow JL, Shulman GI. 2001. Tissue specific overexpression of lipoprotein lipase causes tissue specific insulin resistance. *Proc Natl Acad Sci USA* (in press)

Kurtz DM, Rinaldo P, Rhead WJ, Tian L, Millington DS, Vockley J, Hamm DA, Brix AE, Lindsey JR, Pinkert CA, O'Brien WE, Wood PA. 1998. Targeted disruption of mouse long-chain acyl-CoA dehydrogenase gene reveals crucial roles for fatty acid oxidation. *Proc Natl Acad Sci USA* 95:15592-7

Kuwajima M, Lu K, Sei M, Ono A, Hayashi M, Ishiguro K, Ozaki K, Hotta K, Okita K, Murakami T, Miyagawa J, Narama I, Nikaido H, Hayakawa J, Nakajima H, Namba M, Hanafusa T, Matsuzawa Y, Shima K. 1998. Characteristics of cardiac

hypertrophy in the juvenile visceral steatosis mouse with systemic carnitine deficiency. *J Mol Cell Cardiol* 30:773-81

Lee Y, Hirose H, Ohneda M, Johnson JH, McGarry JD, Unger RH. 1994. β-cell lipotoxicity in the pathogenesis of non-insulin-dependent diabetes mellitus of obese rats: Impairment in adipocyte-β-cell relationships. *Proc Natl Acad Sci USA* 91:10878-82

Lee Y, Hirose H, Zhou Y-T, Esser V, McGarry JD, Unger RH. 1997. Increased lipogenic capacity of the islets of obese rats. A role in the pathogenesis of NIDDM. *Diabetes* 46:408-13

Milburn JL, Hirose H, Lee YH, Nagasawa Y, Ogawa A, Ohneda M, BeltrandelRio H, Newgard CB, Johnson JH, Unger RH. 1995. Pancreatic β-cells in obesity: Evidence for induction of functional, morphologic and metabolic abnormalities by increased long-chain fatty acids. *J Biol Chem* 270:1295-99

Ohneda M, Inman LR, Unger RH. 1995. Caloric restriction in obese prediabetic rats prevents β-cell depletion, loss of β-cell GLUT-2 and glucose incompetence. *Diabetologia* 38:173-9

Saphir O, Corrigan M. 1933. Fatty infiltration of the myocardium. *Arch Int Med* 52:410-28

Seip M, Trygstad O. 1996. Generalized lipodystrophy, congenital and acquired (lipoatrophy). *Acta paediatr Suppl* 413:2-28

Unger RH. 1995. Lipotoxicity in the pathogenesis of obesity-dependent NIDDM. Genetic and clinical implications. *Diabetes* 44:863-70

Unger RH, Orci L. 2001. Diseases of liporegulation: New perspective on obesity and related disorders. *FASEB J* 15:312-21

Wang M-Y, Koyama K, Shimabukuro M, Mangelsdorf D, Newgard CB, Unger RH. 1998. Overexpression of leptin receptors in pancreatic islets of Zucker diabetic fatty rats restores GLUT-2, glucokinase and glucose-stimulated insulin secretion. *Proc Natl Acad Sci USA* 95:11921-6

Zhou Y-T, Grayburn P, Karim A, Shimabukuro M, Higa M, Baetens D, Orci L, Unger RH. 2000. Lipotoxic heart disease in obese rats: Implications for human obesity.*Proc Natl Acad Sci USA* 97:1784-9

Akt (PKB)

Aikawa R, Nawano M, Gu Y, Katagiri H, Asano T, Zhu W, Nagai R, Komuro I. 2000. Insulin prevents cardiomyocytes from oxidative stress-induced apoptosis through activation of PI3 kinase/Akt. *Circulation* 102:2873-9

Downward J. 1998. Mechanisms and consequences of activation of protein kinase B/Akt. *Curr Opin Cell Biol* 10:262-7

Fujio Y, Nguyen T, Wencker D, Kitsis RN, Walsh K. 2000. Akt promotes survival of cardiomyocytes in vitro and protects against ischemia-reperfusion injury in mouse heart. *Circulation* 101:660-7

Galetic I, Andjelkovic M, Meier R, Brodbeck D, Park J, Hemmings BA. 1999. Mechanism of protein kinase B activation by insulin/insulin-like growth factor-1 revealed by specific inhibitors of phosphoinositide 3-kinase – Significance for diabetes and cancer. *Pharmacol Ther* 82:409-25

Hong F, Nguyen VA, Shen X, Kunos G, Gao B. 2000. Rapid activation of protein kinase B/Akt has a key role in antiapoptotic signaling during liver regeneration. *Biochem Biophys Res Commun* 279:974-9

Kenedy SG, Wagner AJ, Conzen SD, Jordan J, Bellacosa A, Tsichllis PN, Hay N. 1997. The PI 3-kinase/Akt signaling pathway delivers an antiapoptotic signal. *Genes Dev* 11:701-13

Kulik G, Klippel A, Weber MJ. 1997. Antiapoptotic signaling by the insulin-like growth factor I receptor phosphatidylinositol 3-kinase, and Akt. *Mol Cell Biol* 17:1595-606

Majewski M, Nieborowska-Skorska M, Salomoni P, Slupianek A, Reiss K, Trotta R, Calabretta B, Skorski T. 1999. Activation of mitochondrial Raf-1 is involved in the antiapoptotic effects of Akt. *Cancer Res* 59:2815-9

Matsuzaki H, Tamatani M, Mitsuda N, Namikawa K, Kiyama H, Miyake S, Tohyama M. 1999. Activation of Akt kinase inhibits apoptosis and changes in Bcl-2 and Bax expression induced by nitric oxide in primary hippocampal neurons. *J Neurochem* 73:2037-46

Navarro P, Valverde AM, Rohn JL, Benito M, Lorenzo M. 2000. Akt mediates insulin rescue from apoptosis in brown adipocytes: Effect of ceramide. *Growth Horm IGF Res* 10:256-66

Summers SA, Garza LA, Zhou H, Birnbaum MJ. 1998. Regulation of insulin-stimulated glucose transporter GLUT4 translocation and Akt kinase activity by ceramide. *Mol Cell Biol* 18:5457-64

Westfall SD, Hendry IR, Obholz KL, Rueda BR, Davis JS. 2000. Putative role of the phosphatidylinositol 3-kinase-Akt signaling pathway in the survival of granulose cells. *Endocrine* 12:315-21

Zimmerman S, Moelling K. 1999. Phosphorylation and regulation of Raf by Akt (protein kinase B). *Science* 286:1741-4

Apoptosis General Considerations

Adams JM, Cory S. 1998. The Bcl-2 protein family: Arbiters of cell survival. *Science* 281:1322-6

Alnemri ES, Livingston DJ, Nicholson DW, Salvesen G, Thornberry NA, Wong WW, Yuan J. 1996. Human ICE/CED-3 protease nomenclature. *Cell* 87:171

Antonsson B, Martinou JC. 2000. The Bcl-2 protein family. *Exp Cell Res* 258:50-7

Budihardjo I, Oliver H, Lutter M, Luo X, Wang X. 1999. Biochemical pathways of caspase activation during apoptosis. *Annu Rev Cell Dev Biol* 15:269-90

Chandra J, Zhivotovsky B, Zaitsev S, Juntti-Berggren L, Berggren PO Orrenius S. 2001. Role of apoptosis in pancreatic β-cell death in diabetes. *Diabetes* 50(Suppl 1):S44-7

Cikala M, Wilm B, Hobmayer E, Bottger A, David CN. 1999. Identification of caspases and apoptosis in the simple metazoan Hydra. *Curr Biol* 9:959-62

Datta SR, Dudek H, Tao X, Masters S, Fu H, Gotoh Y, Greenberg ME. 1997. Akt phosphorylation of BAD couples survival signals to the cell-intrinsic death machinery. *Cell* 91:231-41

Earnshaw WC, Martins LM, Kaufmann SH. 1999. Mammalian caspases: Structure, activation, substrates and functions during apoptosis. *Annu Rev Biochem* 68:383-424

Enari M, Sakahira H, Yokoyama H, Okawa K, Iwamatsu A, Nagata S. 1998. A caspase-activated Dnase that degrades DNA during apoptosis, and its inhibitor ICAD. *Nature* 391:43-50

Eskes R, Desagher S, Antonsson B, Martinou J-C. 2000. Bid induces the oligomerization and insertion of Bax into the outer mitochondrial membrane. *Mol Cell Biol* 20:929-35

Feuerstein GZ. 1999. Apoptosis in cardiac diseases – new opportunities for novel therapeutics for heart diseases. *Cardiovasc Drugs Ther* 13:289-94

Feuerstein GZ,Young PR. 2000. Apoptosis in cardiac diseases: Stress- and mitogen-activated signaling pathways. *Cardiovasc Res* 45:560-9

Garrido R, Mattson MP, Hennig B, Toborek M. 2001. Nicotine protects against arachidonic-acid-induced caspase activation cytochrome c release and apoptosis of cultured spinal cord neurons. *J Neurochem* 76:1395-403

Gottlieb RA, Engler FL. 1999. Apoptosis in myocardial ischemia-reperfusion. *Ann NY Acad Sci* 874:412-26

Green DR, Reed JC. 1998. Mitochondria and apoptosis. *Science* 281:1309-12

Heinzl S. 1996. Heart failure: Oxidative stress and apoptosis. *Med Monatsschr Pharm* 19:319

Hengartner MO. 2000. The biochemistry of apoptosis. *Nature* 407:770-6

Kerr JF, Wyllie AH, Currie AR. 1972. Apoptosis: A basic biological phenomenon with wide-ranging implications in tissue kinetics. *Br J Cancer* 26:239-57

Kluck RM, Bossy-Wetzel E, Green DR, Newmeyer DD. 1997. The release of cytochrome c from mitochondria: A primary site for Bcl-2 regulation of apoptosis. *Science* 275:1132-6

Li H, Zhu H, Xu C-J, Yuan J. 1998. Cleavage of BID by caspase 8 mediates the mitochondrial damage in the Fas pathway of apoptosis. *Cell* 94:491-501

Li P, Nijhawan D, Budihardjo I, Srinivasula SM, Ahmad M, Alnemri ES, Wang X. 1997. Cytochrome c and dATP-dependent formation of Apaf-1/caspase-9 complex initiates an apoptotic protease cascade. *Cell* 91:479-89

Liu X, Zou H, Slaughter C, Wang X. 1997. DFF, a heterodimeric protein that functions downstream of caspase-3 to trigger DNA fragmentation during apoptosis. *Cell* 89:175-84

Lutter M, Fang M, Luo X, Nishijima M, Xie X-S, Wang X. 2000. Cardiolipin provides specificity for targeting of tBid to mitochondria. *Nature Cell Biol* 2:754-6

Mandrup-Poulsen T. 2001. β-cell apoptosis: Stimuli and signaling. *Diabetes* 50(Suppl 1):S58-63

Nagata S. 2000. Apoptotic DNA fragmentation. *Exp Cell Res* 256:12-8

Reed JC. 1997. Double identity for proteins of the Bcl-2 family. *Nature* 387:773-6

Rodriguez J, Lazebnik Y. 1999. Caspase-9 and APAF-1 form an active holoenzyme. *Genes Dev* 13:3179-84

Rucker-Martin C, Henaff M, Hatem SN, Delpy E, Mercadier JJ. 1999. Early redistribution of plasma membrane phosphatidylserine during apoptosis of adult rat ventricular myocytes in vitro. *Basic Res Cardiol* 94:171-9

Sakahira H, Enari M, Nagata S. 1998. Cleavage of CAD inhibitor in CAD activation and DNA degradation during apoptosis. *Nature* 391:96-9

Thornberry NA, Lazebnik Y. 1998. Caspases: Enemies within. *Science* 261:1312-6

Thornberry NA, Rano TA, Peterson EP, Rasper DM, Timkey T, Garcia-Calvo M, Houtzager VM, Nordstrom PA, Roy S, Vaillancourt JP, Chapman KT, Nicholson DW. 1997. A combinatorial approach defines specificities of members of the caspase family and granzyme B. Functional relationships established for key mediators of apoptosis. *J Biol Chem* 272:17907-11

Tsujimoto Y. 1998. Role of Bcl-2 family proteins in apoptosis: apoptosomes or mitochondria? *Genes to Cells* 3:697-707

Wang J, Zhen L, Klug MG, Wood D, Wu X, Mizrahi J. 2000. Involvement of caspase 3- and 8-like proteases in ceramide-induced apoptosis of cardiomyocytes. *J Card Failure* 6:243-9

Williams RS. 1999. Apoptosis and heart failure. *N Engl J Med* 341:759-60

Wu W, Lee WL, Wu YY, Chen D, Lin TJ, Jang A, Sharma PM, Wang PH. 2000. Expression of constitutively active phosphatidylinositol 3-kinase inhibits activation of caspase 3 and apoptosis of cardiac muscle cells. *J Biol Chem* 275:40113-9

Wyllie AH. 1980. Glucocorticoid-induced thymocyte apoptosis is associated with endogenous endonuclease activation. *Nature* 284:555-6

Xanthoudakis S, Nicholson DW. 2000. Heat shock proteins as death determinants. *Nature Cell Biol* 2:E163-5

Yang J, Liu X, Bhalla K, Kim CN, Ibrado AM, Cai J, Peng T-I, Jones DP, Wang X. 1997. Prevention of apoptosis by Bcl-2: Release of cytochrome c from mitochondria blocked. *Science* 275:1129-32

Yenush L, Zanella C, Uchida T, Bernal D, White MF. 1998. The pleckstrin homology and phosphotyrosine binding domains of insulin receptor substrate 1 mediate inhibition of apoptosis by insulin. *Mol Cell Biol* 18:6784-94

Yin XM, Wang K, Gross A, Zhao Y, Zinkel S, Klocke B, Roth KA, Korsmeyer SJ. 1999. Bid-deficient mice are resistant to Fas-induced hepatocellular apoptosis. *Nature* 400:886-91

Zou H, Henzel WJ, Liu X, Lutschg A, Wang X. 1997. Apaf-1, a human protein homologous to C. elegans CED-4, participates in cytochrome c-dependent activation of caspase-3. *Cell* 90:405-13

PPARα

Barger PM, Brandt JM, Leone TC, Weinheimer CJ, Kelly DP. 2000. Deactivation of peroxisome proliferator-activated receptor α during cardiac hypertrophic growth. *J Clin Invest* 105:1723-30

Brandt JM, Djouadi F, Kelly DP. 1998. Fatty acids activate transcription of the muscle carnitine palmitoyltransferase I gene in cardiac myocytes via the peroxisome proliferator-activated receptor α. *J Biol Chem* 273:23786-92

Djouadi F, Brandt JM, Weinheimer CJ, Leone TC, Gonzalez FJ, Kelly DP. 1999. The role of the peroxisome proliferator-activated receptor α (PPARα) in the control of cardiac lipid metabolism. *Prostaglandins Leukot Essent Fatty Acids* 60:339-43

Djouadi F, Weinheimer CJ, Kelly DP. 1999a. The role of PPARα as a "lipostat" transcription factor. *Adv Exp Med Biol* 466:211-20

Keller H, Mahfoudi A, Dreyer C, Hihi AK, Medin J, Ozato K, Wahli W. 1993. Peroxisome proliferator-activated receptors and lipid metabolism. *Ann NY Acad Sci* 684:157-73

Kelly LJ, Vicario PP, Thompson GM, Candelore MR, Doebber TW, Ventre J, Wu MS, Meurer R, Forrest MJ, Conner MW, Cascieri MA, Moller DE. 1998. Peroxisome proliferator-activated receptors γ and α mediate in vivo regulation of uncoupling protein (UCP-1, UCP-2, UCP-3) gene expression. *Endocrinology* 139:4920-7

Lehman JJ, Barger PM, Kovacs A, Saffitz JE, Medeiros DM, Kelly DP. 2000. Peroxisome proliferator-activated receptor γ coactivator-1 promotes cardiac mitochondrial biogenesis. *J Clin Invest* 106:847-56

Leone TC, Weinheimer CJ, Kelly DP. 1999. A critical role for the peroxisome proliferator-activated receptor α (PPARα) in the cellular fasting response: The PPARα-null mouse as a model of fatty acid oxidation disorders. *Proc Natl Acad Sci USA* 96:7473-8

Vega RB, Huss JM, Kelly DP. 2000. The coactivator PGC-1 cooperates with peroxisome proliferator-activated receptor α in transcriptional control of nuclear genes encoding mitochondrial fatty acid oxidation enzymes. *Mol Cell Biol* 20:1868-76

Watanabe K, Fujii H, Takahashi T, Kodama M, Aizawa Y, Ohta Y, Ono T, Hasegawa G, Naito M, Nakajima T, Kamijo Y, Gonzalez FJ, Aoyama T. 2000. Constitutive regulation of cardiac fatty acid metabolism through peroxisome proliferator-activated receptor α associated with age-dependent cardiac toxicity. *J Biol Chem* 275:22293-9

Wu Z, Puigserver P, Andersson U, Zhang C, Adelmant G, Mootha V, Troy A, Cinti S, Lowell B, Scarpulla RC, Spiegelman BM. 1999. Mechanisms controlling mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1. *Cell* 98:115-24

Leptin Resistance

Bjorbaek C, Elmquist JK, Frantz JD, Shoelson SE, Flier JS. 1998. Identification of SOCS-3 as a potential mediator of central leptin resistance. *Mol Cell* 1:619-25

Caro JF, Sinha MK, Kolaczynski JW, Zhang PL, Considine RV. 1996. Leptin: The tale of an obesity gene. *Diabetes* 45:1455-62

Lee Y, Wang M-Y, Kakuma T, Wang Z-W, Babcock E, McCorkle K, Higa M, Zhou Y-T, Unger RH. 2001. Liporegulation in diet-induced obesity. The antisteatotic role of hyperleptinemia. *J Biol Chem* 276:5629-35

Minocci A, Savia G, Lucantoni R, Berselli ME, Tagliaferri M, Calo G, Petroni ML, de Medici C, Viberti GC, Liuzzi A. 2000. Leptin plasma concentrations are dependent on body fat distribution in obese patients. *Int J Obes Relat Metab Disord* 24:1139-44

Moller N, O'Brien P, Nair KS. 1998. Disruption of the relationship between fat content and leptin levels with aging in humans. *J Clin Endocrinol Metab* 83:931-4

Schwartz MW, Peskind E, Raskind M, Boyko EJ, Porte D Jr. 1996. Cerebrospinal fluid leptin levels: Relationship to plasma levels and to adiposity in humans. *Nat Med* 2:589-93

Wang Z-W, Pan W-T, Lee Y, Kakuma T, Zhou Y-T, Unger RH. 2001. The role of leptin resistance in the lipid abnormalities of aging. *FASEB J* 15:108-14

Lipoapoptosis Pathway

Andrieu-Abadie N, Jaffrezou JP, Hatem S, Laurent G, Levade T, Mercaider JJ. 1999. L-carnitine prevents doxorubicin-induced apoptosis of cardiac myocytes: Role of inhibition of ceramide generation. *FASEB J* 13:1501-10

Basu S, Bayoumy S, Zhang Y, Lozano J, Kolesnick R. 1998. BAD enables ceramide to signal apoptosis via Ras and Raf-1. *J Biol Chem* 273:30419-26

Blazquez C, Geelen MJ, Velasco G, Guzman M. 2001. The AMP-activated protein kinase prevents

ceramide synthesis of de novo and apoptosis in astrocytes. *FEBS Lett* 489:149-53

Carlsson C, Borg LA, Welsh N. 1999. Sodium palmitate induces partial mitochondrial uncoupling and reactive oxygen species in rat pancreatic islets *in vitro*. *Endocrinology* 140:3422-8

Cho H, Mu J, Kim JK, Thorvaldsen JL, Chu Q, Crenshaw EB III, Kaestner KH, Bartolomei MS, Shulman GI, Birnbaum MJ. 2001. Insulin resistance and a diabetes mellitus-like syndrome in mice lacking the protein kinase Akt2 (PKB). *Science* 292:1728-31

de Vries JE, Vork MM, Roemen TH, de Jong YF, Cleutjens JP, van der Vusse GJ, van Bilsen M. 1997. Saturated but not mono-unsaturated fatty acids induce apoptotic cell death in neonatal rat ventricular myocytes. *J Lipid Res* 38:1384-94

Degli-Esposti M, McLennan H. 1998. Mitochondria and cells produce reactive oxygen species in virtual anaerobiosis: Relevance to ceramide-induced apoptosis. *FEBS Lett* 430:338-42

Di Paola M, Cocco T, Lorusso M. 2000. Ceramide interaction with the respiratory chain of heart mitochondria. *Biochemistry* 39:6660-8

Gudz TI, Tserng K-Y, Hoppel CL. 1997. Direct inhibition of mitochondrial respiratory chain complex III by cell-permeable ceramide. *J Biol Chem* 272:24154-8

Huwiler A, Brunner J, Hummel R, Vervoordeldonk M, Stabel S, van den Bosch, Pfeilschifter J. 1996. Ceramide-binding and activation defines protein kinase c-Raf as a ceramide-activated protein kinase. *Proc Natl Acad Sci USA* 93:6959-63

Kerner J, Hoppel C. 2000. Fatty acid import into mitochondria. *Biochem Biophys Acta* 1486:1-17

Kong JY, Rabkin SW. 2000. Palmitate-induced apoptosis in cardiomyocytes is mediated through alterations in mitochondria: Prevention by cyclosporin A. *Biochim Biophys Acta* 1485:45-55

Listenberger LL, Ory DS, Schaffer JE. 2001. Palmitate-induced apoptosis can occur through a ceramide-independent pathway. *J Biol Chem* 276:14890-5

McGarry JD, Brown NF. 1997. The mitochondrial carnitine palmitoyltransferase system. From concept to molecular analysis. *Eur J Biochem* 244:1-14

Obeid LM, Linardic CM, Karolak LA, Hannun YA. 1993. Programmed cell death induced by ceramide. *Science* 259:1769-71

Paumen MB, Ishida Y, Muramatsu M, Yamamoto M, Honjo T. 1997. Inhibition of carnitine palmitoyltransferase I augments sphingolipid synthesis and palmitate-induced apoptosis. *J Biol Chem* 272:3324-9

Selzner M, Bielawska A, Morse MA, Rudiger HA, Sindram D, Hannun YA, Clavien PA. 2001. Induction of apoptotic cell death and prevention of tumor growth by ceramide analogues in metastatic human colon cancer. *Cancer Res* 61:1233-40

Shimabukuro M, Koyama K, Lee Y, Unger RH. 1997. Leptin- or troglitazone-induced lipopenia protects islets from interleukin 1b cytotoxicity. *J Clin Invest* 100:1750-4

Shimabukuro M, Zhou Y-T, Levi M, Unger RH. 1998a. Fatty acid-induced β-cell apoptosis: A link between obesity and diabetes. *Proc Natl Acad Sci USA* 95:2498-502

Shimabukuro M, Wang M-Y, Zhou Y-T, Newgard CB, Unger RH. 1998b. Protection against lipoapoptosis of β-cells through leptin-dependent maintenance of Bcl-2 expression. *Proc Natl Acad Sci USA* 95:9558-61

Sparagna GC, Hickson-Bick DL. 1999. Cardiac fatty acid metabolism and the induction of apoptosis. *Am J Med Sci* 318:15-21

Sparagna GC, Hickson-Bick DL, Buja LM, McMillin JB. 2000. A metabolic role for mitochondria in palmitate-induced cardiac myocyte apoptosis. *Am J Physiol Heart Circ Physiol* 279:H2124-32

Sparagna GC, Hickson-Bick DL, Buja LM, McMillin JB. 2001. Fatty acid-induced apoptosis in neonatal cardiomyocytes: Redox signaling. *Antioxid Redox Signal* 3:71-9

Weiss B, Stoffel W. 1997. Human and murine serine-palmitoyl-CoA transferase – Cloning, expression and characterization of the key enzyme in sphingolipid synthesis. *Eur J Biochem* 249:239-47

Zhou H, Summers SA, Birnbaum MJ, Pittman RN. 1998. Inhibition of Akt kinase by cell-permeable ceramide and its implications for ceramide-induced apoptosis. *J Biol Chem* 273:16568-75

Obesity and the Heart

Alpert MA, Singh A, Terry BE, Kelly DL, el-Deane MS, Mukerji V, Villarreal D, Artis AK. 1989. Effect of exercise and cavity size on right ventricular function in morbid obesity. *Am J Cardiol* 64:1361-5

Alpert MA, Hashimi MW. 1993. Obesity and the heart. *Am J Med Sci* 306:117-23

Alpert MA, Lambert CR, Panayiotou H, Terry BE, Cohen MV, Massey CV, Hashimi MW, Mukerji V. 1995. Relation of duration of morbid obesity to left ventricular mass, systolic function and diastolic filling, and effect of weight loss. *Am J Cardiol* 76:1194-7

Alpert MA, Lambert CR, Terry BE, Cohen MV, Mukerji V, Massey CV, Hashimi MW, Panayiotou H. 1995a. Interrelationship of left ventricular mass, systolic function and diastolic filling in normotensive morbidly obese patients. *Int J Obes Relat Metab Disord* 19:550-7

Amad KH, Brennan JC, Alexander JK. 1965. The cardiac pathology of chronic exogenous obesity. *Circulation* 32:740-5

Berkalp B, Cesur V, Corapcioglu D, Erol C, Baskal N. 1995. Obesity and left ventricular diastolic dysfunction. *Int J Cardiol* 52:23-6

Bharati S, Lev M. 1995. Cardiac conduction system involvement in sudden death of obese young people. *Am Heart J* 129:273-81

Carpenter HM. 1962. Myocardial fat infiltration. *Am Heart J* 63:491-6

Corvisart JN. 1812. An essay on the organic diseases and lesions of the heart and great vessels, Philadelphia, A Finley, p. 153

Coughlin SS, Rice JC. 1996. Obesity and idiopathic dilated cardiomyopathy. *Epidemiology* 7:629-32

Duflou J, Virmani R, Rabin I, Burke A, Farb A, Smialek J. 1995. Sudden death as a result of heart disease in morbid obesity. *Am Heart J* 130:306-13

Harvey W. 1628. Anatomical examination of the body of Thomas Parr. In *Works of William Harvey* (translated from the Latin by Robert Willis), London, Sydenham Society, p. 589, 1847

Herrera MF, Deitel M. 1991. Cardiac function in massively obese patients and the effect of weight loss. *Can J Surg* 34:431-4

Itoh H, Yamamoto M, Tanimoto N, Okabayashi T, Arii K, Nakauchi Y, Takamatsu K, Suehiro T, Hashimoto K. 1996. Simple obesity with cardiomyopathy of obesity. *Intern Med* 35:876-9

Laennec RTH. 1838. *A treatise on diseases of the chest*, New York, Wood SS, Wood W (ed), p 682

Nakajima T, Fujioka S, Tokunaga K, Matsuzawa Y, Tarui S. 1989. Correlation of intraabdominal fat accumulation and left ventricular performance in obesity. *Am J Cardiol* 64:369-73

Roberts WC, Roberts JD. 1983. The floating heart or the heart too fat to sink: Analysis of 55 necropsy patients. *Am J Cardiol* 52:1286-9

Shirani J, Roberts WC. 1993. Clinical, electrocardiographic and morphologic features of massive fatty deposits ("lipomatous hypertrophy") in the atrial septum. *J Am Coll Cardiol* 22:226-38

Smith HL, Willius FA. 1933. Adiposity of the heart. A clinical and pathologic study of one hundred and thirty-six obese patients. *Arch Int Med* 52:911-31

Soloff LA. 1970. Arrhythmias following infusions of fatty acids. *Am Heart J* 80:671-4

Thakur V, Richards R, Reisin E. 2001. Obesity, hypertension and the heart. *Am J Med Sci* 321:242-8

White PD. 1931. *Heart Disease*, New York, Macmillan Company, p. 438

AMPK

Abu-Elheiga L, Matzuk MM, Abo-Hashema KAH, Wakil SJ. 2001. Continuous fatty acid oxidation and reduced fat storage in mice lacking acetyl-CoA carboxylase 2. *Science* 291:2613-6

Bergeron R, Russell RR III, Young LH, Ren JM, Marcucci M, Lee A, Shulman GI. 1999. Effect of AMPK activation on muscle glucose metabolism in conscious rats. *Am J Physiol* 276:E938-44

Chen ZP, McConell GK, Michell BJ, Snow RJ, Canny BJ, Kemp BE. 2000. AMPK signaling in contracting human skeletal muscle: Acetyl-carboxylase and NO synthase phosphorylation. *Am J Physiol Endocrinol Metab* 279:E1202-6

Foretz M, Carling D, Guichard C, Ferre P, Foufelle F. 1998. AMP-activated protein kinase inhibits the glucose-activated expression of fatty acid synthase gene in rat hepatocytes. *J Biol Chem* 273:14767-71

Hardie DG, Carling D. 1997. The AMP-activated protein kinase – fuel gauge of the mammalian cell? *Eur J Biochem* 246:259-73

Henin N, Vincent MF, Gruber HE, Van den Berghe G. 1995. Inhibition of fatty acid and cholesterol synthesis by stimulation of AMP-activated protein kinase. *FASEB J* 9:541-6

Kim J, Yoon M-Y, Choi S-L, Kang I, Kim S-S, Kim Y-S, Choi Y-K, Ha J. 2001. Effects of stimulation of AMP-activated protein kinase on insulin-like growth factor-1 and epidermal growth factor-dependent extracellular signal-regulated kinase pathway. *J Biol Chem* 276:19102-10

Merrill GF, Kurth EJ, Hardie DG, Winder WW. 1997. AICA riboside increases AMP-activated protein kinase, fatty acid oxidation and glucose uptake in rat muscle. *Am J Physiol* 273:E1107-12

Musi N, Fujii N, Hirshman MF, Ekberg I, Fröberg S, Ljungqvist O, Thorell A, Goodyear LJ. 2001. AMP-activated protein kinase (AMPK) is activated in muscle of subjects with Type 2 diabetes during exercise. *Diabetes* 50:921-7

Russell RR III, Bergeron R, Shulman GI, Young LH. 1999. Translocation of myocardial GLUT-4 and increased glucose uptake through activation of AMPK by AICAR. *Am J Physiol* 277:H643-9

Velasco G, Geelen MJ, Guzman M. 1997. Control of fatty acid oxidation by 5'AMP-activated protein kinase involves a malonyl-CoA-dependent and a malonyl-CoA-independent mechanism. *Arch Biochem Biophys* 337:169-75

Winder WW, Hardie DG. 1999. AMP-activated protein kinase, a metabolic master switch: Possible roles in Type 2 diabetes. *Am J Physiol* 277:E1-E10

Fatty Acids

McGarry JD. 1992. What if Minkowski had been ageusic? An alternative angle on diabetes. *Science* 258:766-70

McGarry JD, Mannaerts GP, Foster DW. 1977. A possible role for malonyl CoA in regulation of hepatic fatty acid oxidation and ketogenesis. *J Clin Invest* 60:265-70

Ruderman N, Flier JS. 2001. Chewing the fat – ACC and energy balance. *Science* 291:2558-9

Treatment

Bergeron R, Previs SF, Cline GW, Perret P, Russell RR III, Young LH, Shulman GI. 2001. Effect of 5-aminoimidazole-4-carboxamide-1-β-D-ribofurano-

side infusion on in vivo glucose and lipid metabolism in lean and obese Zucker rats. *Diabetes* 50:1076-82

Cavaghan MK, Ehrmann DA, Byrne MM, Polonsky KS. 1997. Treatment with the oral antidiabetic agent troglitazone improves β-cell responses to glucose in subjects with impaired glucose tolerance. *J Clin Invest* 100:530-7

Loftus TM, Jaworsky DE, Frehywot GL, Townsend CA, Ronnett GV, Lane MD, Kuhajda FP. 2000. Reduced food intake and body weight in mice treated with fatty acid synthase inhibitors. *Science* 288:2379-81

Shimabukuro M, Zhou Y-T, Lee Y, Unger RH. 1998. Troglitazone lowers islet fat and restores β-cell function of Zucker diabetic fatty rats. *J Biol Chem* 273:3547-50

Noninvasive Diagnosis of Fatty Heart

Kriegshauser JS, Julsrud PR, Lund JT. 1990. MR imaging of fat in and around the heart. *Am J Roentgen* 155:271-4

Szczepaniak L, personal communication

William Harvey, William Harvey was born in England in 1578. After earning a degree at Cambridge University at the age of twenty, he journeyed to Italy to study medicine at the University of Padua. Padua was the center for western European medical instruction at that time. Harvey graduated with honors in 1602 and returned to England where he earned yet another medical degree from Cambridge University. He then settled down to begin practicing medicine.

Harvey was fascinated by the way blood flowed through the human body. Most people of the day believed that food was converted into blood by the liver, then was consumed as fuel by the body. Harvey knew this was untrue through his firsthand observations of human and animal dissections. In 1628 Harvey published *An Anatomical Study of the Motion of the Heart and of the Blood in Animals* which explained how blood was pumped from the heart throughout the body, then returned to the heart and recirculated. The views this book expressed were very controversial and lost Harvey many patients, but it became the basis for all modern research on the heart and blood vessels. A second ground-breaking book published by Harvey in 1651, *Essays on the Generation of Animals*, is considered the basis for modern embryology.

Despite the uproar over each of Harvey's unconventional anatomical theories, he was recognized as a medical leader in his day. He was doctor to King Charles I of England and was appointed doctor of physic at Oxford. At the time of his death in 1657, Harvey's medical and scientific genius were celebrated throughout the European medical community.

Jean-Nicholas Corvisart

1755-1821

Jean-Nicholas Corvisart, an important figure in early 19th century French medicine, popularized percussion as a diagnostic tool, was the author of a major textbook oncardiovascular diseases and was Napoleon's personal physician.

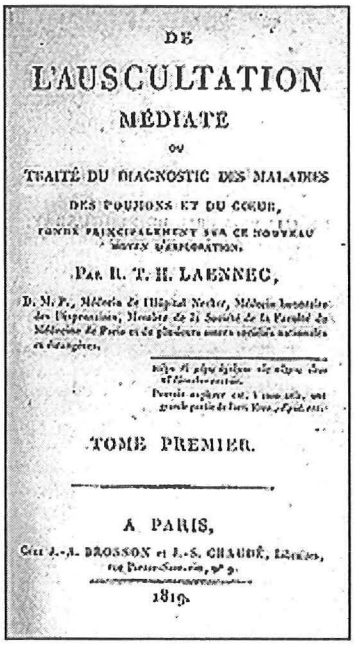
The Austrian physician Leopold von Auenbrugg (1722-1809) first described the diagnostic utility of chest percussion in 1761, in his book Inventum Novum. Ahead of his time, Auenbrugg's work was criticized and ignored and he was forced to retreat to private practice and other interests (including writing the libretto for a Salieri opera!). Corvisart became interested in percussion of the chest, translated Inventum Novum from Latin into French in 1808 (the year before von Auenbrugg died), and popularized the technique.

Corvisart became professor of medicine at the Collège de France in 1797. He acquired areputation as a skilled bedside diagnostician and an excellent teacher. His students included Laënnec, Dupuytren, Bichat and Bretonneau†. Corvisart's main interest was in cardiology. He described some of the clinical features of pericardial and valvular heart disease, including the palpatory "thrill" associated with mitral stenosis. In 1806 he published a major textbook entitled Essay on the Diseases and Organic Lesions of the Heart and Large Vessels.

Corvisart's most famous patient was the Emperor, Napoleon ("I do not believe in medicine,but I do believe in Corvisart"), whom he attended from 1804 until Napoleon's fall from power in 1815.

†René Laënnec (1781-1826) invented the stethoscope in 1816. Pierre Fidèle Bretonneau (1778-1862) gave diphtheria its name, and is credited with the first tracheotomy. Francois-Xavier Bichat (1771-1802) pathologist, described various "tissues". Guillaume Dupuytren (1777-1835) was a French surgeon.

R. T. H. Laennec
(1781-1826)


DE
L'AUSCULTATION
MÉDIATE
OU
TRAITÉ DU DIAGNOSTIC DES MALADIES
DES POUMONS ET DU CŒUR,
FONDÉ PRINCIPALEMENT SUR CE NOUVEAU
MOYEN D'EXPLORATION.
PAR R. T. H. LAENNEC,
TOME PREMIER.
A PARIS,
1819.

De l'auscultation mediate (On mediate auscultation)

René Théophile Hyacinthe Laennec studied medicine in Paris under Jean-Nicolas Corvisart, personal physician to Napoleon. He served as visiting physician to Necker Hospital, then succeeded his mentor as chair of medicine at the Collège de France in 1822. Just four years later, he died of tuberculosis.

Laennec is considered one of the greatest clinicians of his time. Like his teacher Corvisart, who popularized Leopold Auenbrugger's method of percussion to diagnose thoracic disorders, he specialized in the study of chest disease by auscultation. Laennec's contribution to the field was *De l'auscultation mediate* (1819), in which he introduced auscultation by means of an instrument he invented and named the stethoscope ("chest examiner"). His first stethoscope was simply a cylinder of paper. By 1819, it had evolved into a hollow wooden tube. In *De l'auscultation mediate*, Laennec described in unprecedented detail the audible symptoms of thoracic diseases. He also included diagrams of his new invention and made the stethoscope available for purchase from the publishers.

This is a first edition (in two volumes) published in Paris in 1819.