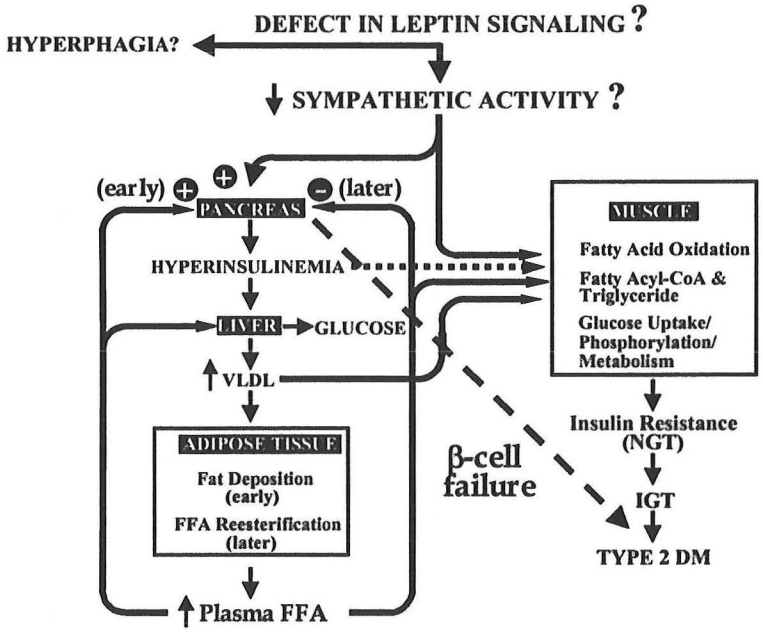
# THE ETIOLOGY OF TYPE 2 DIABETES MELLITUS: FOLLOW YOUR NOSE



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I am currently engaged in a number of clinical and basic research projects investigating the etiology of type 2 diabetes mellitus. Previous work has demonstrated the essential role that plasma fatty acids play in sustaining normal glucose-stimulated insulin secretion in fasted rats and human subjects. I have also worked on a collaborative effort utilizing novel $^{1}H$ NMR spectroscopic techniques to illustrate the strong correlation between intramyocellular lipid content and skeletal muscle insulin resistance. Current projects are evaluating the effects of high-fat feeding, pharmacologic inhibition of lipid oxidation, and leptin administration on insulin secretion and insulin sensitivity in rats and determining how these changes might be linked to alterations in muscle and islet triglyceride content. Because deficiencies of leptin and/or leptin signaling can precipitate the development of obesity/diabetes mellitus, it is conceivable that the primary function of leptin is to control lipid oxidation and lipolysis in a manner that prevents tissue lipid accumulation, thus maintaining normal glucose metabolism. We are exploring the biochemical pathways through which leptin regulates lipid metabolism.

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## Introduction

I never really planned to give a lecture at UT-Southwestern Medical Center discussing the possible role that disordered lipid metabolism plays in the etiology of type 2 diabetes mellitus. Although all of the research projects that I have completed in the past five years have been devoted to this theme, I realize that the concept is always indelibly linked to Denis McGarry and Roger Unger. The unfortunate events of the past several months have greatly altered even the best-laid plans. Earlier this year, Denis McGarry was selected as the 2001 recipient of The Banting Medal for Scientific Achievement, the highest honor accorded by the American Diabetes Association. He was diagnosed with a glioblastoma in May and was unable to deliver the Banting Lecture at the Annual Scientific Sessions of the ADA but a written version will appear in the January issue of *Diabetes*. I want to dedicate today's Grand Rounds to Denis McGarry because I feel it is important for this audience to recognize how his ideas have influenced recent developments in diabetes research.

Type 2 diabetes mellitus (type 2 DM) has truly reached epidemic proportions in the United States. A recent article in the *Journal of the Medical Association* states that the prevalence of the condition in the U.S. reached 7.3% in the year 2000, up from 5.1% in 1991 (1). According to current epidemiologic trends, the prevalence likely will rise even further as the population becomes progressively older and obesity more pervasive (1). The socioeconomic costs of diabetes are staggering as well. The disease costs the U.S. economy $105 billion annually while one out of every 10 U.S. healthcare dollars is spent for diabetes and one of four Medicare dollars pays for care in individuals suffering from diabetes (2). Furthermore, mortality from diabetes has increased and it is shocking to see just how poorly we are doing in combating the disease. Figure 1 illustrates that while we have achieved significant gains in reducing the deaths from other major conditions such as cardiovascular disease and strokes, age-adjusted death rates from diabetes have increased by 30% since 1980 (2). We may be in the midst of a revolution in medical care, but all of our research efforts have not turned the tide for people with diabetes.

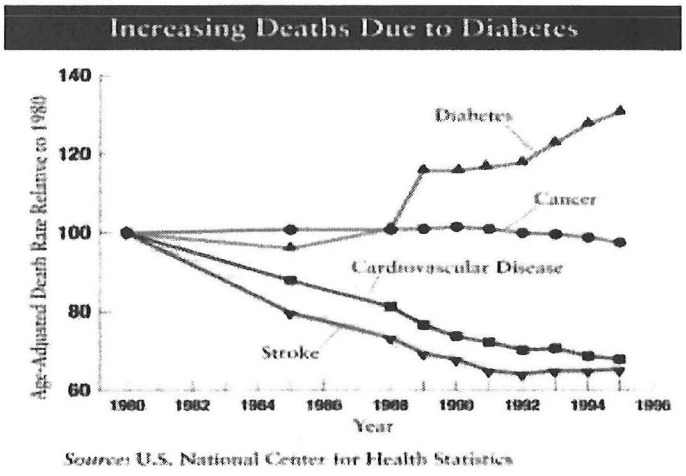


**Figure 1.** Comparison of trends in mortality for diabetes and some other major diseases.

Ten years ago (almost to the day), Denis McGarry recognized these trends and voiced a simple but yet provocative question. "Is it possible that our historical perception of the primary metabolic derangement in diabetes has carried the wrong emphasis?" In other words, "were we led astray by Oskar Minkowski's taste buds?" To understand the question, we must first reflect on two of the pivotal discoveries in diabetes research and understand how they have influenced future generations of scientists. On a momentous day in 1889, Minkowski noticed that urine collected from his pancreatectomized dogs attracted an inordinate number of flies. Legend has it that he then tasted the urine and was struck by its sweetness. From this simple, but astute, observation he established for the first time that the pancreas produced some substance that controlled the blood sugar level, and whose loss resulted in diabetes mellitus (3). A second milestone was reached about 30 years later when Banting and Best identified the active pancreatic polypeptide, insulin. Thus, in 1921 the concept of an insulin-glucose axis as the central component of fuel homeostasis was established and the term diabetes mellitus, or "sugar diabetes" as my aunt calls it, was etched in our consciousness. We can speculate what would have happened if Dr. Minkowski had lacked a sense of taste but possessed a very

good nose. Instead of tasting sugar, he would have smelled acetone and surmised that the removal of the pancreas significantly disrupted fatty acid metabolism. Subsequently, The work of Banting and Best would have illustrated that the preeminent role of insulin was the control of fat metabolism instead of glucose metabolism. Therefore, in Grand Rounds presented here at UT-Southwestern as well as a subsequent editorial published in *Science* (4) Denis challenged the diabetes world to set aside a century of "glucocentric" bias and consider "What if Minkowski had been ageusic?" Today, I would like to review what progress has been made in the intervening years regarding the merits of a "lipocentric" view type 2 diabetes with an emphasis on work that has been completed in Denis McGarry's laboratory.

**Insulin Resistance and Hyperinsulinemia in Obesity/Type 2 Diabetes Mellitus Syndromes**

In order to explore the defects underlying obesity/type 2 DM syndromes, we must first recognize the key clinical and physiologic features of the disorder. The most prominent feature is insulin resistance defined as impairment of the hormone's ability to suppress hepatic glucose production and stimulate glucose uptake in skeletal muscle. Normal glucose tolerance is maintained because concomitant basal and postprandial hyperinsulinemia match impaired insulin action. An additional often overlooked component of the syndrome is resistance to the antilipolytic actions of insulin in adipose tissue resulting in elevated plasma concentrations of nonesterified (free) fatty acids (FFA) and triglycerides (TG) that are packaged in the form of very low-density lipoproteins. These features are illustrated in Figure 2 showing cross-sectional data collected from normal, obese and diabetic individuals who stayed for 24 hours in the General Clinical Research here at UT-Southwestern. Each subject consumed a standardized diet and blood samples were drawn throughout the day for the measurement of plasma glucose, insulin, FFA and triglyceride concentrations. The diabetic individuals were easily distinguished from the other two groups because of their elevated plasma glucose levels. Plasma insulin concentrations in diabetic patients were higher than those of nonobese controls but insulin secretion was still not sufficient to normalize plasma glucose levels. Obese individuals were not frankly hyperglycemic but they displayed insulin resistance because very high plasma insulin concentrations accompanied the normal glucose concentrations. Triglyceride concentrations were elevated in both obesity and type 2 DM in the fasted state but FFA levels were indistinguishable between all three groups. As the subjects ate breakfast, lunch and dinner throughout the day, plasma lipid concentrations in obese and diabetic individuals diverged from normal so that they were elevated for much of the day.

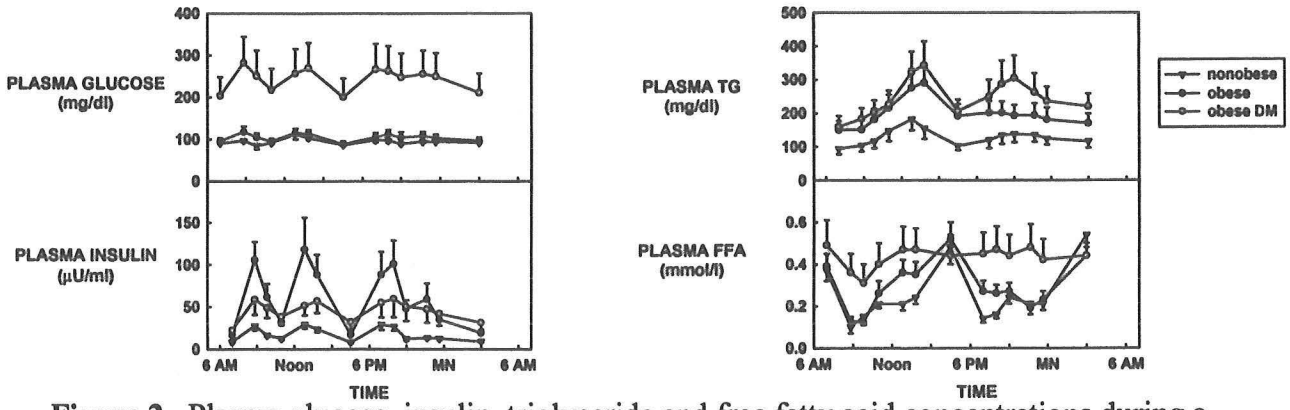


**Figure 2.** Plasma glucose, insulin, triglyceride and free fatty acid concentrations during a 24 hour day in nonobese, obese and diabetic individuals. All subjects consumed a standardized, eucaloric diet.

Since each of the physiologic derangements has been documented even before serious metabolic decompensation and clinical diabetes appear, separating the initial defect from subsequent compensatory changes has presented a difficult challenge. Type 2 DM is most easily diagnosed as a disorder of glucose/carbohydrate metabolism, such that the prevailing perception of its pathophysiology has been glucocentric. The most widely accepted scenario is that some as yet unidentified genetic defect(s) initially causes impaired insulin-mediated glucose disposal (IMGD) in skeletal muscle. Normal glucose tolerance is maintained by "compensatory" β-cell oversecretion of insulin until the resistance eventually reaches the point at which the β-cell response is inadequate to maintain normal glucose concentration, and uncontrolled hyperglycemia appears (5). However, one central tenet of this formulation, namely, that insulin resistance begets hyperinsulinemia, has never been rigorously proven. To the contrary, mice with a skeletal muscle-specific knockout of the insulin receptor display the predicted insulin resistance but they have normal fasting glucose and insulin levels as well as normoglycemia and insulinemia after glucose ingestion (6). In clinical studies, prepubertal obese children who have defective glucose disposal during hyperinsulinemic-euglycemic clamps simultaneously exhibit basal hyperinsulinemia and accentuated β-cell responses to hyperglycemia (7). Vaag *et al* (8) compared a cohort of 25-year-old first-degree relatives of patients with type 2 DM with age and weight matched controls lacking any family history of diabetes. Both groups showed similar basal fasting glucose, hemoglobin $A_{1C}$, FFA, and TG levels, but individuals with a positive family history of diabetes exhibited basal hyperinsulinemia and β-cell hyperresponsiveness to an oral glucose load (Figure 3). The same group of patients also had diminished IMGD. These data clearly establish an important although seldom-emphasized point, namely, that young, obese subjects and individuals with a family history of diabetes secrete more insulin than normal controls, even when blood glucose levels lie within the "normal" range.

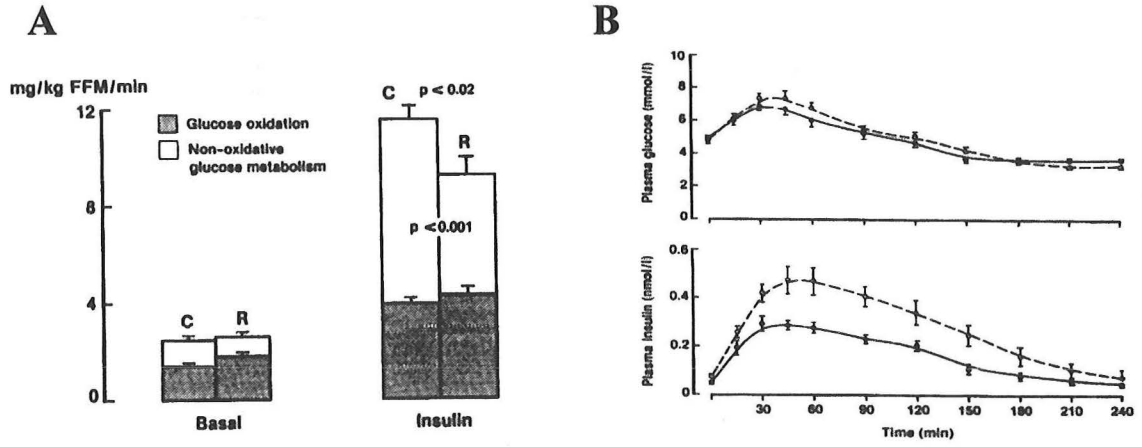


**Figure 3.** **(A)** Rates of total body glucose disposal, glucose oxidation and nonoxidative glucose metabolism in the basal state and during insulin infusion in 20 first-degree relatives of patients with type 2 DM (R) and 20 control subjects without a family history of diabetes (C). **(B)** Plasma glucose and insulin concentrations in the same patients during oral glucose tolerance tests.

Additional cross-sectional studies of genetically high-risk youths document that hyperinsulinemia predicts the subsequent development of type 2 DM in diverse populations (9). While the cited studies do not necessarily establish hyperinsulinemia as the initial defect in the development of glucose intolerance and type 2 DM, they do suggest that it is an early event arising concurrently with, or even antedating, the changes in IMGD in skeletal muscle. Therefore, if hyperinsulinemia and insulin resistance develop concurrently, it is possible that they both arise as the end result of a single,

common metabolic derangement that leads to the elevated plasma concentrations of TG and FFA that are characteristic of obesity/type 2 DM syndromes. Herein, I will outline how abnormal cellular fluxes of fatty acids lead to a pathologic accumulation of lipid substrates in plasma and metabolically active tissues that could precipitate hyperinsulinemia and insulin resistance (4). The ensuing discussion will focus on events occurring in skeletal muscle and pancreatic β-cells and the following questions will be addressed:

**Skeletal Muscle**

1. Is insulin resistance associated with increased intramyocellular lipid stores?
2. How does the accumulation of lipid substrates in skeletal muscle impair insulin action?
3. Why do the lipid substrates accumulate in skeletal muscle in the first place?

**Pancreatic β-cell**

1. Is glucose-stimulated insulin secretion affected by acute perturbations in plasma FFA concentrations?
2. How do fatty acids influence β-cell function?
3. Is hyperinsulinemia the result of prolonged exposure to elevated plasma FFA concentrations?
4. Can abnormal lipid metabolism have a link to β-cell failure?

## LIPIDS AND SKELETAL MUSCLE

### Is insulin resistance associated with increased intramyocellular lipid stores?

Evidence is mounting that elevated intramuscular lipid stores are associated with insulin resistance. For example, the malonyl-CoA content, and by inference the cytosolic concentration of fatty acyl-CoA, is increased in skeletal muscle of insulin resistant rats (10). Muscle TG levels in biopsy samples obtained from patients with type 2 DM are elevated (11). A particularly illustrative clinical study was completed by Pan *et al* (12) in which non-diabetic Pima Indian volunteers each underwent a hyperinsulinemic-euglycemic clamp to determine whole body insulin sensitivity and then provided a muscle biopsy for chemical analysis. Neutral lipids measured from Folch extracts of skeletal muscle biopsy samples correlated with insulin resistance better than did measures of adiposity such as body mass index (BMI) or body fat % (Figure 4). However, the scale for muscle triglyceride in the figure was logarithmic, indicating that the values exhibited considerable scatter. Because chemical measurement of total TG in muscle samples could not discriminate between fat present within myocytes and that associated with surrounding adipose tissue, such variability could easily be predicted for a series of samples obtained via a procedure in which a percutaneous needle was blindly placed through skin and layers of subcutaneous fat into the muscle bed.

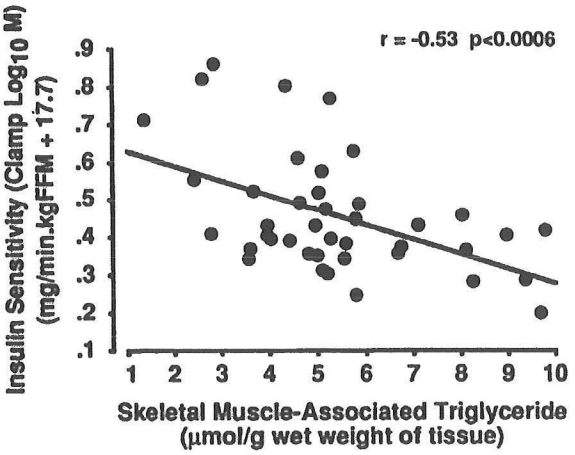


**Figure 4.** Relationship between the logarithm of insulin sensitivity and skeletal muscle-associated triglyceride concentration. FFM, fat-free mass by hydrodensitometry.

Elegant studies performed by Schick and colleagues (13) pointed to a better method in which image-guided proton magnetic resonance spectroscopy (HMRS) can be used to measure noninvasively the lipid within myocytes (14). The spectroscopy technique distinguishes intramyocellular lipid (IMCL) from extramyocellular lipid (EMCL) due to bulk magnetic susceptibility differences introduced by the physical manner in which lipids are stored in myocytes and adipocytes. Therefore, it provides a quantitative measure of true cell-associated lipids that can only be qualitatively assessed in muscle biopsy samples by oil-red O staining or electron microscopy. The quantitative accuracy of method has been validated in numerous animal models by comparing lipid content measured using HMRS to results obtained from biochemical analyses (14). Spectroscopy reduces the variability of intramyocellular lipid measurements as well, since the CV≈10% *vs.* 20-50% (14) in standard biopsy determinations.

In order to show that insulin resistance was associated with increased intramyocellular lipid stores, we recruited a group of healthy volunteers with normal glucose tolerance (NGT) who underwent a hyperinsulinemic-euglycemic clamp for assessment of whole body insulin sensitivity followed by a proton spectroscopy study to measure IMCL. In this cross-sectional study insulin resistance correlated more tightly with IMCL (R=-0.88, Figure 5) than with any other factor, including BMI, body fat %, waist-hip ratio, and age (15). Other groups have recently reported comparable findings using HMRS, electron microscopy and computed tomography (16-20). We have extended the analysis to include patients with either impaired glucose tolerance (IGT) or frank type 2 DM. The results are also shown in Figure 5 in comparison to the values obtained for individuals with normal glucose tolerance. The individuals with IGT and type 2 DM were no more insulin resistant than many of the obese patients with normal glucose tolerance but their IMCL content was often markedly increased particularly when diabetes was present. It seemed that insulin sensitivity could fall to its nadir without the appearance of diabetes as long as β-cell compensation remained adequate, but when insulin secretion failed, hyperglycemia ensued and the concomitant elevation of plasma FFA and TG levels exacerbated the accumulation of fat in muscle without causing additional decrements in insulin action.

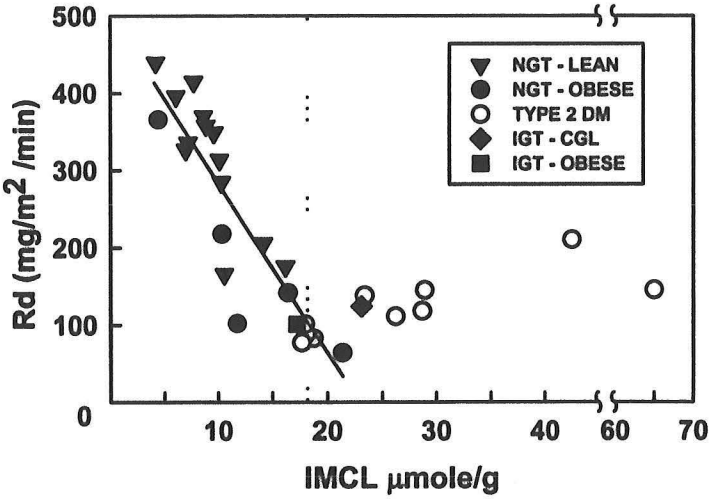


**Figure 5.** Relationship between insulin sensitivity (Rd) determined by hyperinsulinemic-euglycemic clamp studies and the intramyocellular lipid (IMCL) concentration measured using proton spectroscopy. Study subjects had normal glucose tolerance (NGT), impaired glucose tolerance (IGT) or type 2 DM.

The results described above demonstrate an association between IMCL and insulin resistance but association is not the same as causation. One could argue that these two features are simply two coincidental findings in a group of individuals genetically programmed to develop both conditions in a parallel but independent fashion. Cause and effect can only be established by performing experiments in which manipulations that augment or deplete IMCL are temporally and quantitatively linked to reciprocal alterations of insulin sensitivity.

Recent studies by Boden *et al* (21) have directly tested whether changes in insulin resistance produced by acute changes in plasma FFA levels involved corresponding changes in IMCL. Hyperinsulinemic-euglycemic clamps were conducted in normal, healthy volunteers in the presence or absence of an additional infusion of Intralipid + heparin and serial measures of intramyocellular lipid content were obtained by HMRS at the beginning and end of the study. Plasma insulin and glucose concentrations were similar in the two groups but the plasma FFA levels shown in Figure 6 diverged considerably. Insulin infusion alone suppressed lipolysis and plasma FFA levels decreased from 548±65 μmol/l at baseline to 42±10 μmol/l after four hours. On the other hand, FFA values in the insulin+lipid group rose from 516±75 to 1,206±142 μmol/l. Insulin sensitivity was assessed by tracking the rate at which exogenous glucose had to be infused to maintain euglycemia. The glucose infusion climbed to 49±5 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ in the insulin only group and to 28±2 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ in the insulin+lipid group. The difference between the two groups only became apparent after two hours and reached statistical significance at the 210-minute time point. IMCL levels fell 5±3% (p=NS) in the more insulin sensitive insulin only group but myocellular lipid deposition in the insulin+lipid exhibiting impaired insulin action built up by 9±5% ($p<0.04$). Individually, IMCL rose in 6 of 7 patients with a range of 5-38%. The demonstration that acute changes in IMCL were accompanied by the development of insulin resistance, taken together with previous reports of a close correlation between the two parameters, supports the notion that intramyocellular accumulation of lipid substrates is a step in the development of lipid-induced insulin resistance.

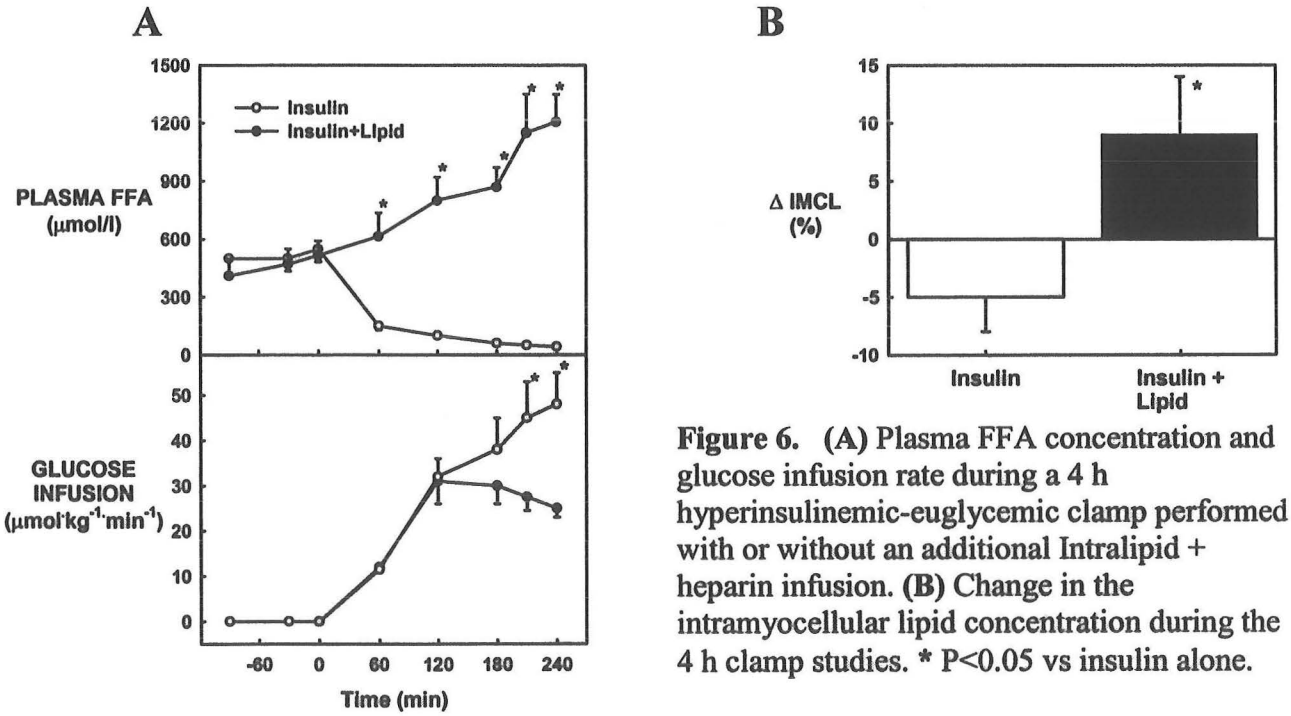


**Figure 6.** **(A)** Plasma FFA concentration and glucose infusion rate during a 4 h hyperinsulinemic-euglycemic clamp performed with or without an additional Intralipid + heparin infusion. **(B)** Change in the intramyocellular lipid concentration during the 4 h clamp studies. * $P<0.05$ vs insulin alone.

**How does the accumulation of lipid substrates in skeletal muscle impair insulin action?**

Almost forty years ago Philip Randle presented the hypothesis that glucose and lipid oxidation were reciprocally regulated (22). From acute studies in cultured myocytes, he suggested a classic scenario whereby accelerated fatty acid oxidation increases intramitochondrial acetyl-CoA/CoA and $NADH/NAD^+$ ratios resulting in allosteric inhibition of pyruvate dehydrogenase (PDH). Intramitochondrial citrate levels also increase under conditions of increased fatty acid oxidation and

decreased flux through isocitrate dehydrogenase. Citrate inhibits phosphofructokinase and blocks the conversion of glucose-6-phosphate (G-6-P) to fructose 1,6-bisphosphate. Increased G-6-P allosterically inhibits hexokinase and would result in the build-up of intracellular free glucose and substrate inhibition of glucose transport into the muscle cell. Although this pathway is indubitably operative to some extent in the acute sense, it is likely that other mechanisms come into play during a long-term compensatory response to protracted intracellular fatty acid excess.

One oft-quoted study has recently examined the effect of lipid infusion on cellular levels of glucose and G-6-P using an Intralipid/heparin protocol similar to that detailed above. Roden *et al* (23) found a negative effect of lipid infusion on whole body glucose disposal that was again evident after 3½ hours, a full two hours after they detected significant changes in glucose oxidation and glycogen synthesis as measured by $^{13}C$ NMR analysis. Importantly these changes were associated with a fall rather than a rise in the intracellular G-6-P signal and suggested that the fatty acid effect in this setting was exerted at the step of glucose transport/phosphorylation rather than through the classic Randle mechanism. A number of investigators have tried to build upon these findings and determine the nature of the changes in intracellular free glucose and G-6-P levels in obesity/type 2 DM syndromes but controversy exists because different results are reported from groups utilizing widely varied techniques (24, 25).

Laying this controversy aside, what are the likely mechanisms through which prolonged elevations of cellular lipids could lead to insulin resistance? The quantitative NMR spectroscopy techniques really measure the size of the cellular triglyceride pool that represents a storage form of fatty acids. The muscle TG pool is a metabolically active pool of fat consisting of small oil droplets located in close proximity to mitochondria, thereby providing fuel for oxidation. Triglycerides are formed by esterification from long chain fatty acyl-CoA (LCFA-CoA) species and glycerol-3-phosphate while lipolysis releases fatty acids that are then linked to CoA and carnitine for transport into the mitochondria for oxidation. Therefore it is likely that triglycerides simply serve as a surrogate marker for the activated LCFA-CoA moieties that alter insulin signaling within the cell. Persuasive evidence to this effect is now emerging. Thus in a group of individuals with varying degrees of glucose tolerance, Ellis *et al* (26) found a strong negative correlation between whole body insulin sensitivity and the content of LCFA-CoA measured in muscle biopsies. In a study of rats fed a high fat diet, Ye *et al* (27) showed that the resultant fall in insulin-mediated glucose uptake into muscle was accompanied by a marked increase in plasma TG and muscle LCFA-CoA levels. All of these abnormalities were ameliorated by the administration of either a PPAR-$\alpha$ or -$\gamma$ ligand in direct proportion to the improvement in insulin action.

A number of hypotheses have been put forth to explain the mechanism through which muscle LCFA-CoA levels interfere with insulin signaling to inhibit the movement of insulin-sensitive glucose transporters (GLUT-4) from their intracellular compartment to the cell surface. The most completely developed hypothesis incorporates important changes in the phosphorylation of insulin receptor substrate-1 (IRS-1, 28). Under normal circumstances, the activation of GLUT-4 by insulin involves a sequence of reactions in which binding of insulin to its receptor initiates tyrosine phosphorylation of IRS-1. This in turn activates phosphatidylinositol 3-kinase (PI3-kinase), triggering a cascade of events culminating in GLUT-4 translocation to the plasma membrane (29-31). Work from a number of laboratories has shown that skeletal muscle of insulin resistant obese humans (with or without diabetes) and Zucker rats exhibits reduced tyrosine phosphorylated IRS-1 and PI3-kinase activity following insulin stimulation (32, 33). In an interventional study, lipid and heparin were infused into normal rats to raise plasma FFA levels for 5h before a hyperinsulinemic-euglycemic clamp was conducted. The experimental maneuver reduced glucose oxidation and glycogen synthesis by one-half (28). Impaired insulin-mediated glucose disposal was accompanied by a fall in tyrosine phosphorylation of IRS-1, a 50% reduction in IRS-1-associated PI3-kinase activity and a 4-fold

increase in membrane-bound (active) protein kinase Cθ (PKCθ). Furthermore, applying a similar study design in healthy humans also demonstrated a profound suppression of IRS-1 associated PI3-kinase activity in skeletal muscle together with severe attenuation of insulin-mediated glucose disposal (34). Thus it is believed that an elevation of cellular LCFA-CoA generates a pool of diacylglycerol that activates a serine kinase (PKCθ or one of the atypical PKC isozymes). Serine phosphorylation of IRS-1 interferes with tyrosine phosphorylation by the insulin receptor and limits the ability of IRS-1 to recruit and activate PI3-kinase. The signal for GLUT-4 translocation is blunted and glucose transport is diminished. Consistent with this notion, Bell *et al* (35) found that the rapid reversal of muscle insulin resistance in high-fat fed rats after a single carbohydrate-enriched meal was associated with a fall in both muscle LCFA-CoA levels and the loss of membrane-bound PKCθ activity.

Time and space do not permit an exposition detailing the other possible mechanisms through which lipids may alter insulin-signaling pathways responsible for stimulating glucose transport in muscle. The interested reader is referred to excellent reviews presented by the following authors: 1) Randle Hypothesis (22); 2) PKC isoforms (30); 3) ceramides (36); 4) glucosamine (37, 38); 5) peroxisomal proliferator activator receptors (39).

**Why do the lipid substrates accumulate in skeletal muscle in the first place?**

Up to this point, the discussion has centered on establishing a causal link between the intracellular accumulation of lipid derived moieties and impaired insulin action in skeletal muscle. I would now like to turn to what I believe is the more perplexing issue. Instead of working forward in time to explain what the lipid substrates are doing, let us look back in time to understand how lipids accumulate in skeletal muscle in the first place. The two broad processes that could lead to excessive lipid accumulation in skeletal muscle include substrate oversupply, and impaired utilization. We have extensively covered one model of substrate oversupply in which Intralipid and heparin are infused over a period of hours resulting in impaired insulin action and increased IMCL. The infusion studies of Boden *et al* (21) presented in Figure 6 showed that over a short time period intramyocellular lipid levels were correlated to plasma FFA concentrations. Since the infusion technique has some limitations as a model of insulin resistant states such as obesity and type 2 DM, it is important to see if such a relationship also holds true over a more protracted time period. Referring back to Figure 2, we can see the time course of plasma FFA and TG concentrations throughout the day for patients with normal glucose tolerance, obesity or type 2 DM. Because the majority of the subjects also volunteered for the studies revealing the relationship between IMCL and insulin resistance that were depicted in Figure 5, we can show that the average daily plasma FFA concentration correlated with the IMCL content. The data shown in Figure 7 provide an important missing piece of the puzzle, namely that obese, insulin resistant individuals have elevated plasma FFA levels that are associated with increased lipid deposition in skeletal muscle.

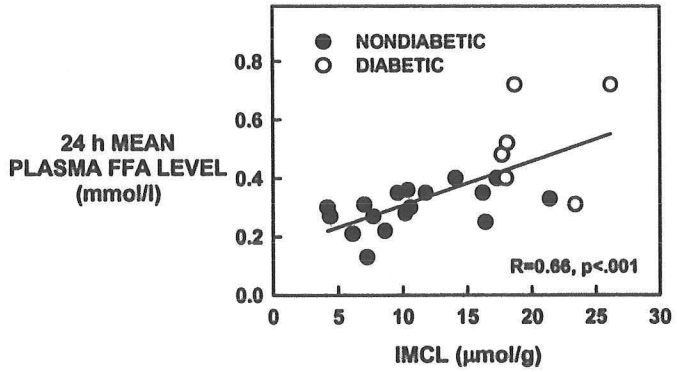


**Figure 7.** Relationship between plasma FFA levels averaged over 24 h and the intramyocellular lipid content in both diabetic and nondiabetic subjects.

It is quite straightforward to predict that overindulgence and high fat diets as part of the Western lifestyle leads to an overabundance of lipid substrates that eventually begets lipid accumulation in metabolically active tissues such as liver, skeletal muscle and the β-cell. Before placing all of the blame for the problem of diabetes squarely on overeating, we must also look closely at fatty acid utilization. As an alternative to an oversupply of lipids, an initial impairment of fatty acid oxidation in skeletal muscle could also result in the accumulation of IMCL associated with increased insulin resistance and elevated plasma FFA levels. Therefore, we measured whole body oxygen consumption and respiratory quotient by indirect calorimetry in the group of patients included in Figure 7. Figure 8 (open circles) shows that elevated muscle lipid content is very weakly associated with elevated net fatty acid oxidation. This finding would seem to argue against impaired fat utilization as an initial defect promoting the accumulation of IMCL, but it is important to remember that net fatty acid oxidation is determined by both the capacity to oxidize fatty acids in tissues and the availability of fatty acid substrates. If the data are reexamined as umol of fat oxidized per umol triglyceride measured in the skeletal muscle, we can see (closed circles) that the capacity for fatty acid oxidation is relatively impaired. This interpretation is consistent with the work of Kelley *et al* (40) showing that in obese humans the muscle bed has a diminished capacity to oxidize fatty acids. Therefore it is quite possible that impaired glucose tolerance and diabetes follow an extended pre-obese, pre-insulin resistant time period characterized by a decreased capacity to burn fat. Evidence from clinical studies in post-obese patients supports this concept. Astrup *et al* (41) showed that obese women who achieved a normal body weight by caloric restriction did not increase lipid oxidation appropriately in response to a fat meal when compared with women who had never been obese. In a similar study after dietary induced weight loss in moderately obese women, an elevated respiratory quotient (RQ) correlated with subsequent weight gain when the dietary restriction was removed (42). In a study with Pima Indians, a population group with an inordinately high prevalence of type 2 diabetes, Ravussin *et al* (43) demonstrated that a reduced rate of energy expenditure is a risk factor for future body weight gain.

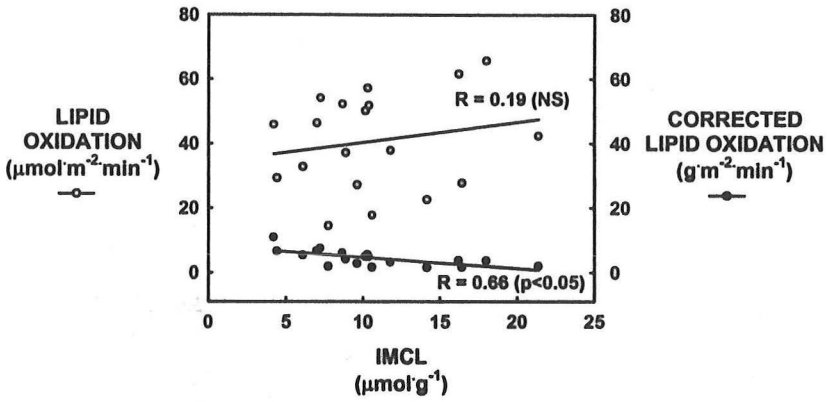


**Figure 8.** Relationship between whole body lipid oxidation determined by indirect calorimetry and intramyocellular lipid content. Open circles represent raw data while closed circles show lipid oxidation corrected for substrate availability in the soleus muscle.

One of the limitations of clinical investigation is the Herculean effort required to perform a longitudinal study examining the progressive changes occurring in intermediary metabolism as an individual progresses from the pre-obese state to obesity followed by insulin resistance, impaired glucose tolerance and type 2 DM. It is much easier to collect such longitudinal information in rodent models of obesity/type 2 DM. A recent study performed by Etgen and Oldham (44) with Zucker diabetic fatty (ZDF) rats is entirely consistent with the concept that impaired glucose tolerance and diabetes follow an extended period of impaired lipid oxidation. Prior to the appearance of diabetes (7

weeks of age), when insulin levels were very high, the fatty rats displayed a significantly higher RQ (signifying relatively increased glucose versus fat oxidation) than their lean littermates. However, as insulin levels declined and hyperglycemia supervened, the RQ of the fatty rats gradually fell so that by 12 weeks of age it was significantly below that of lean animals. Unfortunately, the authors of this study did not measure muscle lipid content, but others have found elevated muscle LCFA-CoA levels in the ZDF rats (Gary Cline, personal communication).

Pharmacologic interventions provide an alternative model for studying the longitudinal effects of impaired lipid oxidation in a group of animals lacking any genetic predisposition to obesity and type 2 DM. We have reported the results of studies in which etomoxir was administered to inhibit mitochondrial fatty acid oxidation at the level of CPT I, the enzyme responsible for converting LCFA-CoA into long chain acylcarnitine for transport into the mitochondria (45). To explore the effects of diet and CPT I inhibition, normal rats received either low- or high-fat (lard) diets in the absence or presence of etomoxir. After four weeks, animals were subjected to a hyperinsulinemic-euglycemic clamp after an overnight fast using a 3-[$^3$H]glucose infusion to measure the rates of glucose production and disposal. As can be seen in Figure 9, both etomoxir and increased dietary fat intake elicited whole body insulin resistance as reflected in the fall in the glucose infusion rate required during the clamp. Combining etomoxir and a high fat diet resulted in an even greater attenuation of insulin sensitivity that affected both glucose disposal and production. Separate groups of rats were studied with a combination of whole body and high-resolution proton spectroscopy techniques to measure total body fat mass and intramyocellular lipid accumulation in samples harvested from the soleus muscle. Expanded total fat stores and increased skeletal muscle lipid stores were detected in animals receiving either etomoxir or the lard diet and the greatest effect was seen with the two interventions were combined. As a culminating step, Figure 10 illustrates the strong negative correlation that existed between insulin sensitivity and IMCL. In summary, these results establish a "proof of concept" that an incipient defect in fatty acid oxidation, particularly when combined with excess dietary fat intake, can initiate the cascade toward obesity, tissue lipid deposition and insulin resistance.

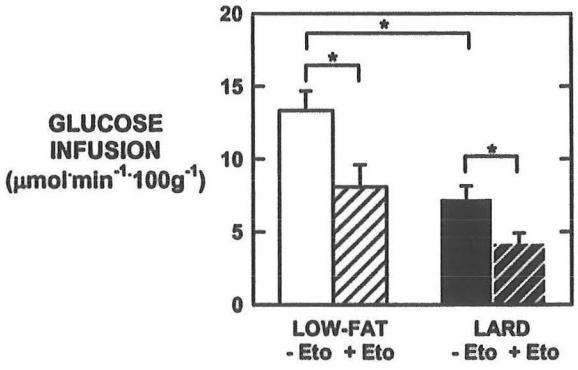


**Figure 9.** Influence of dietary fat content and etomoxir (Eto) on the glucose infusion rate in overnight-fasted rats during insulin infusion at 3 mU/kg/min. Each value represents the mean ± SE of 6-7 determinations. *, $p<0.05$ between linked groups.

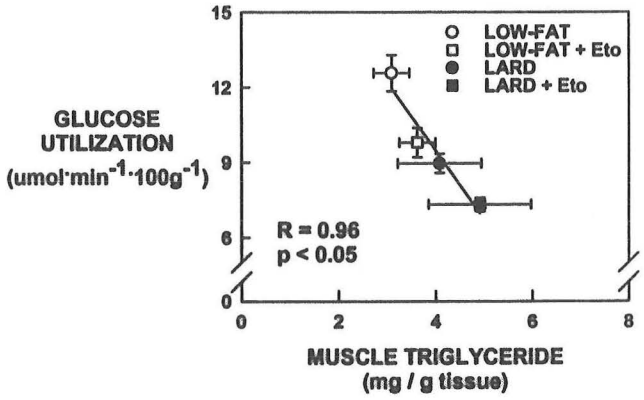


**Figure 10.** Correlation between glucose utilization and soleus muscle lipid content in rats receiving low-fat and lard diets either alone or supplemented with the CPT I inhibitor, etomoxir (Eto). Each value represents the mean ± SE of 4-7 determinations.

## LIPIDS AND THE β-CELL

We cannot forget that insulin resistance alone does not equate to type 2 DM. There are a lot of obese, insulin resistant people in this world that do not have, nor ever will have, type 2 DM. The progression from normal glucose tolerance to impaired glucose tolerance to clinical type 2 DM requires specific alterations of β-cell function. The remainder of the Grand Rounds will review recent findings regarding the role of lipids in the regulation of insulin secretion. The contribution of fatty acids to impaired insulin action in the liver and skeletal muscle has been confirmed by numerous investigators (23, 28, 46), but the role of these lipid substrates as modulators of β-cell function is less clearly defined. That fatty acids can considerably enhance glucose-stimulated insulin secretion (GSIS) in intact animals and humans was recognized in early studies from a number of laboratories (47-50), but since many interventions that modulate FFA concentrations also alter glucose uptake, it was often felt that changes in insulin sensitivity could explain most of the fluctuations in plasma insulin concentrations. Subsequent studies specifically designed to monitor insulin secretion patterns following manipulation of the plasma FFA concentration have, however, generated renewed interest in the importance of these substrates in governing β-cell function. A number of complexities must be considered when discussing modulation of GSIS by fatty acids. 1) Acute perturbations in plasma FFA concentrations appear to play an important physiologic role in sustaining basal insulin secretion in the fasted state and assuring efficient nutrient-stimulated insulin secretion when the fast is terminated. 2) Prolonged elevations of plasma FFA and islet lipid concentrations may also be an instrumental part of the adaptive hyperinsulinemic response in obese, insulin resistant individuals that protects against glucose intolerance. 3) Because the term fatty acids actually refers to a group of chemical compounds that possess structural differences based upon their chain length and number of C=C bonds, the fatty acid composition of lipids may be as important as their concentration. 4) Persistent lipacidemia could conceivably have pathologic consequences leading to β-cell failure in humans and animal models that are genetically predisposed to type 2 DM. These complexities will be reviewed in the following discussion.

### Is glucose-stimulated insulin secretion affected by acute perturbations in plasma FFA concentrations?

Although I was not aware of this fact until recently, the story relating acute elevations in plasma free fatty acids to stimulation of insulin secretion pretty much began with Leonard Madison and Willis Seyffert right here at UT-Southwestern (48). They performed experiments in mongrel dogs after the placement of an end-to-side portacaval shunt that diverted the portal blood flow into the peripheral venous system and isolated the liver from the rest of the splanchnic circulation. The primary purpose of their protocol was to examine changes in hepatic glucose production and peripheral glucose utilization following the infusion of lipid + heparin that elevated plasma FFA concentrations to levels normally seen during the fasted state. The acute elevation of plasma free fatty acids produced a 37% decrease in hepatic glucose output and a 30% inhibition of peripheral glucose utilization. The altered glucose kinetics were a consequence of both an increase in insulin secretion from 45±5 to 64±9 mU/l and a fall in glucagon secretion. The interpretation of these results was given as follows:

> These data suggest that the feedback of free fatty acids on secretion of insulin and glucagon plays an important physiologic role during starvation in the safe transition from carbohydrate to fat metabolism without the danger of progressive ketoacidosis. It is likely that during starvation plasma free fatty acids contribute

to the control and maintenance of a low but vital concentration of plasma insulin (48).

Additional studies in human subjects were completed about that same period of time and the human data also pointed to an effect of plasma free fatty acids in driving what was termed "minor" variations in insulin secretion. Because the insulin secretion always seemed to fluctuate in accord with alterations of glucose utilization, the overall interpretation gleaned from the early investigations was that fatty acid infusions primarily worsened insulin sensitivity and the pancreas merely responded by releasing additional insulin.

As far as I can tell, no follow-up studies ever tested the assertion that elevated fatty acid concentrations were important for the maintenance of plasma insulin concentrations during food deprivation in humans. When I began working with Denis McGarry, a study was planned that would address this issue. Healthy, lean, human volunteers who had fasted for a period of 48 h received a constant infusion of saline from -180 to +60 min (Figure 11). At the 0 min time point the plasma glucose concentration was acutely raised by intravenous bolus of glucose and was then allowed to decline. The study was repeated on a second occasion with the inclusion of an infusion of NA from -150 min onwards, and on a third with the further addition of Intralipid plus heparin (51). In all protocols the basal glucose concentration was clamped at its initial level. As seen from the figure, the effect of prior depletion of circulating FFA by NA infusion had quite striking effects on pancreatic β-cell function. Basal insulin concentrations fell significantly from 19.2 to 10.8 pmol/l (as noted previously in studies with rats and humans (52)) and the incremental areas under the C-peptide and insulin curves in response to the glucose challenge were also reduced by some 50%. All parameters were normalized, however, when the initial plasma FFA level was maintained by coinfusion of the lipid emulsion plus heparin. Clearly, in the 48 h-fasted human, the elevated plasma FFA concentration was essential not only to allow a normal insulin response to an incoming glucose load, but also to maintain the low basal level of circulating insulin. Similar results were obtained when the fasting period was shortened to 24 h (51). However, after an overnight fast, lowering of the plasma NEFA level with NA had no impact on GSIS, although basal insulin concentrations again fell by ~50% (51).

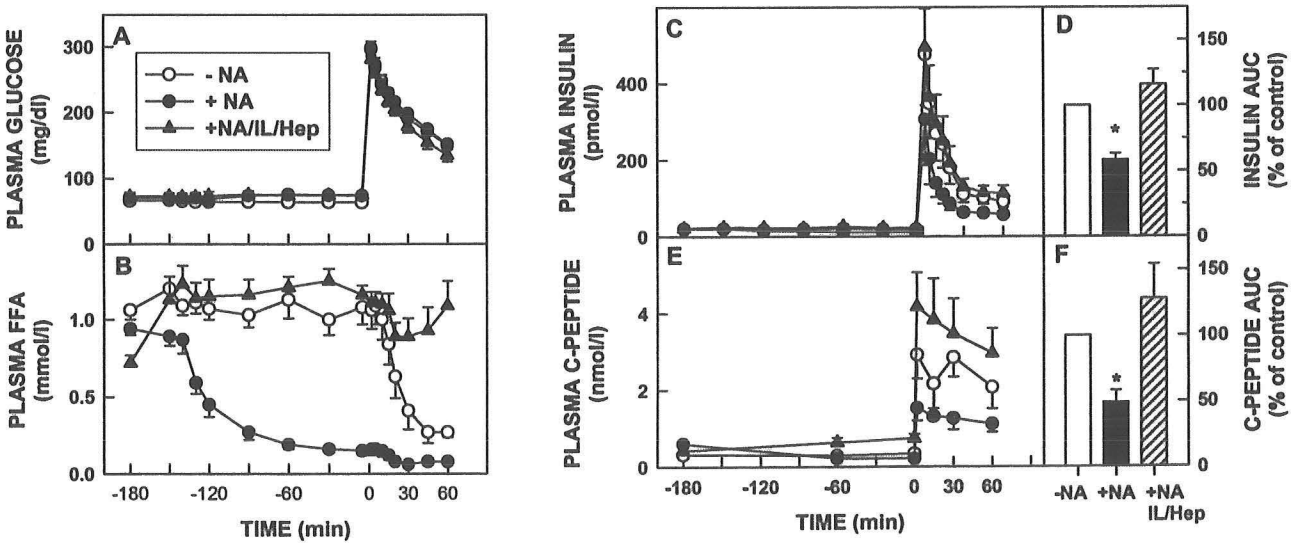


**Figure 11.** Effect of nicotinic acid in the absence and presence of lipid infusion on GSIS in 48 h-fasted nonobese humans. When used, Intralipid (IL) and heparin (Hep) were given from -180 min onwards; nicotinic acid infusion (NA) began at -150 min. AUC, stimulated area under the curve. *, $p<0.05$ compared with -NA control.

It thus appeared that in healthy, non-obese humans who have fasted for only ~14h, basal but not stimulated rates of insulin secretion rely upon circulating FFA. However, when the fasting period was increased to 24-48 h, ~50% of the beta cell's response to both basal and stimulatory glucose became fatty acid dependent. Interestingly, when overnight fasted obese volunteers were tested, a different picture was obtained. In this case the NA infusion resulted in a somewhat slower fall in plasma FFA levels, but this was nevertheless associated with a reduction, albeit modest (~25-30%), in both basal and glucose-stimulated insulin output (51). The attenuated responses were of a similar magnitude to the control values in the lean subjects.

**How do fatty acids influence β-cell function?**

It is generally agreed that in order to stimulate insulin secretion, glucose must first enter the β-cell via the GLUT-2 glucose transporter and then be metabolized to a point beyond pyruvate in a process initiated by the high $K_m$ enzyme, glucokinase. This in turn is thought to cause an increase in the intracellular ATP:ADP ratio, closure of the cell surface $K^+_{ATP}$ channels, cell depolarization and opening of the voltage-sensitive $Ca^{2+}$ channels, leading to a rise in intracellular $Ca^{2+}$ $[Ca^{2+}]_i$ and activation of exocytosis. Additional mechanisms contribute, however, to the regulation of insulin secretion in the in vivo setting. One of these, referred to as the $K^+_{ATP}$ channel-independent pathway, augments the response to a raised $[Ca^{2+}]_i$ generated through the more classical pathway. A second, referred to as the $K^+_{ATP}$ channel-independent, $Ca^{2+}$ -independent pathway of glucose signaling, appears to involve a GTP-dependent step that is activated through the combined effects of protein kinases A and C (52).

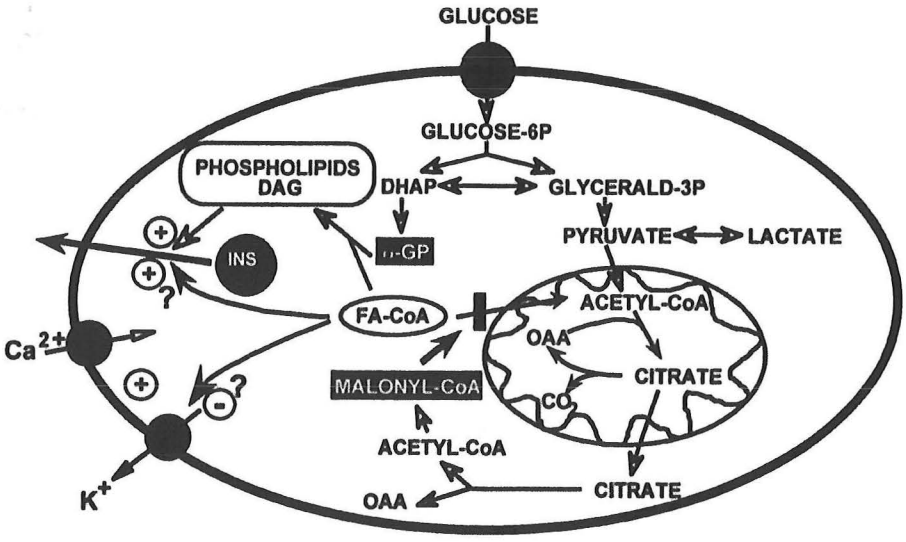


**Figure 12.** Model showing possible mechanisms through which fatty acids can alter glucose-stimulated insulin secretion. DHAP, dihydroxyacetone phosphate; DAG, diacylglycerol; OAA, oxaloacetate; α-GP, α-glycerol phosphate.

Although details of these partially overlapping signaling systems remain incomplete, yet another element must now be brought into the discussion. The powerful influence of glucose metabolism on the intracellular disposition of fatty acids and the potential role of this interaction in stimulus-secretion must be accommodated in the model. Studies in isolated rodent islets and insulin-secreting cell lines led to the suggestion that stimulus-secretion coupling within the β-cell might use an

element of glucose-fatty acid cross-talk analogous to that controlling fatty acid oxidation and ketone body production in liver (53-55). The model that has emerged is shown in Figure 12 and can be summarized as follows. An increase in glucose concentration is sensed by glucokinase, allowing metabolism of the hexose through glycolysis to pyruvate. In the beta cell, just as in liver and other tissues, the $C_3$ unit can be converted into citrate in mitochondria. Some fraction of the citrate is oxidized in the tricarboxylic acid cycle, generating $CO_2$ and ATP, while the remainder may leave the mitochondria and be converted in the cytosolic compartment into malonyl-CoA via the sequential action of ATP-citrate lyase and ACC. The increase in malonyl-CoA concentration is expected to suppress CPT I activity, and therefore fatty acid oxidation, resulting in an increase in the cytosolic concentration of long chain fatty acyl-CoA which then acts as a signaling molecule for insulin secretion, working in concert with the rise in $[Ca^{2+}]_i$ caused by alterations in the $K^+_{ATP}$ and $Ca^{2+}$ channel activities described earlier. The precise site/s of action of LCFA-CoAs in this model remains unclear, although several theoretical possibilities exist. One would be that they react with glycerol-3-phosphate, also derived from glucose metabolism, to form (a) phospholipids that might be required for reworking of the cell membrane during the exocytotic event or (b) a discrete pool of diacylglycerol that serves to activate PKC, an enzyme previously implicated as a player in the insulin secretory process (56). Alternatively, the LCFA-CoA might have a more direct effect, such as facilitation of insulin vesicle trafficking (57), alteration of ion channel activity or enhancement of the docking and/or fusion of the insulin vesicle with the cell membrane.

Before progressing any farther, I must interject a cautionary disclaimer. The LCFA-CoA model presented above represents a model of what might be happening in the β-cell but it may not depict the actual scenario. A number of studies in isolated islets and insulin secreting cell lines support the schema but other data present a much different picture. Space does not permit a point-counterpoint comparison of different models and the reader is referred to additional references, many of them emanating from Chris Newgard's laboratory (58-61).

**Is hyperinsulinemia the result of prolonged exposure to elevated plasma FFA concentrations?**

In short, present data indicate that prolonged exposure to elevated plasma FFA concentrations suppresses the acute insulin response to glucose. The most prominent study investigated short- and long-term effects of an infusion of lipid + heparin on insulin secretion in healthy subjects. Individuals received Intralipid plus heparin for 24 h (62). All subjects underwent an intravenous glucose tolerance test after an overnight fast (baseline), at 6 h and 24 h of lipid infusion. The lipid infusion caused a 3-fold rise in plasma FFA concentrations with no difference between the 6 and 24 h time points. Compared to the baseline acute insulin response (AIR) of 63±8 mU/l, short-term lipid infusion was associated with a significant increment in the AIR to 86±12mU/l as hown in Figure 13. In contrast, long-term lipid delivery inhibited AIR (31±5 mU/l) compared to the baseline and 6 h results. This study demonstrated that short- and long-term exposures of β-cells to high plasma FFA concentrations have opposite effects on GSIS.

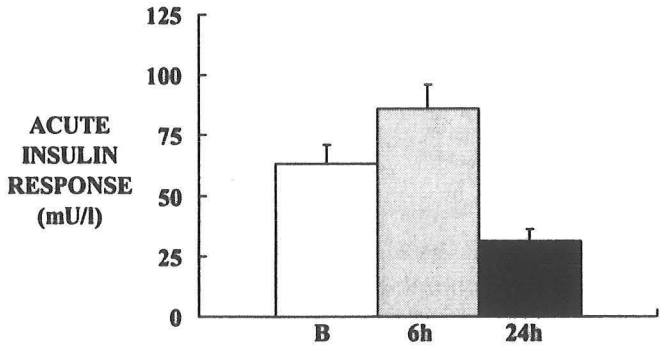


**Figure 13.** Acute insulin response to an intravenous glucose bolus at baseline (B) and after 6h or 24h of Intralipid infusion. All results are mean ± SD of 12 determinations. *, $p<0.01$, **, $p<0.001$ vs. baseline.

A similar protocol performed by a second group confirmed the results of Paolisso *et al* (62). Glucose-stimulated insulin secretion was examined in healthy young men after acute (90 min) or chronic (48 h) elevation of plasma FFA levels with a lipid + heparin infusion (63). GSIS was studied in response to a graded glucose infusion that permitted comparison of the insulin secretory rate over a range of plasma glucose concentrations (Figure 14). The glucose infusion rate during a separate hyperglycemic clamp provided an additional index of insulin sensitivity. In the acute studies, insulin sensitivity was lower during lipid infusion but it was precisely matched by significantly higher GSIS. The net result was that glucose disposal measured as the disposition index (DI = insulin sensitivity x insulin secretion) was unchanged. In the chronic studies, there was no difference in absolute GSIS between control and lipid infusion, but there was a reduction in insulin sensitivity with the increment in FFA levels. Because the expected compensatory increase in insulin secretion was lacking, the DI was significantly diminished. Even though chronic lipid + heparin infusion did not result in an absolute decrement in the insulin secretory rate, as was observed in the prior study, it could be said that insulin secretion was relatively diminished.

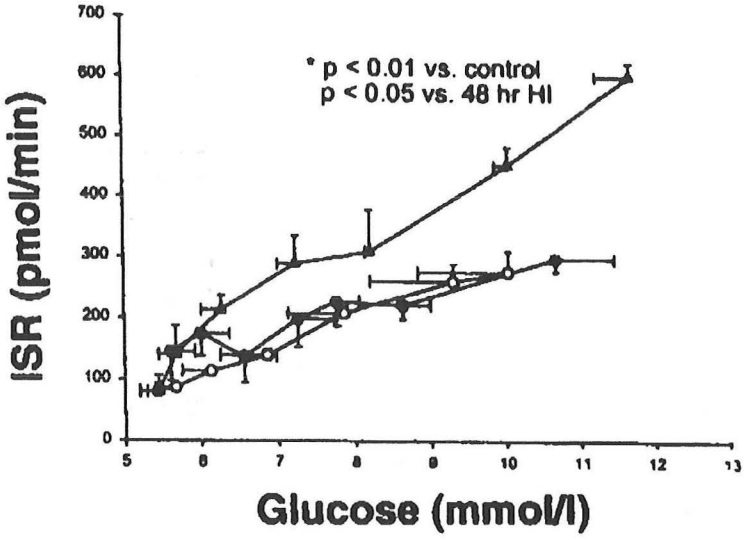


**Figure 14.** Insulin secretory rate versus plasma glucose concentration during graded glucose infusion studies. Secretion was measured after 90 min (▲) and 48 h (●) of Intralipid+heparin infusion for comparison to control (○) studies.

We have extensively covered one model of lipid oversupply in which Intralipid and heparin were infused over a period of hours or days before insulin sensitivity and insulin secretion were evaluated. However, the lipid infusion technique has many limitations as a model of insulin resistant states such as obesity and type 2 DM. First, infusion protocols often result in large deviations of plasma FFA and TG concentrations that far exceed the smaller deviations observed in the 24 h time course experiments discussed earlier. Secondly, infusion studies last for only a few hours, whereas the progression to clinical type 2 DM moves forward over years and decades. Finally, the lipid preparations approved for administration contain exclusively soybean oil that is highly enriched with unsaturated fatty acids (25% oleic acid and 50% linoleic acid). It is documented that the saturated fatty acids that are prevalent in animal species have more pronounced effects on insulin resistance and hyperinsulinemia (64). A number of epidemiologic studies actually indicate that the intake of saturated fat is more closely linked to insulin resistance and diabetes than is total fat intake. In the Normative Aging Study, a highly saturated fat diet was independent predictor of both fasting and postprandial plasma insulin concentrations (65). The study estimated that increasing dietary saturated fat from 8% to 14% of total energy would lead to an 18% increase in fasting insulin and a 25% increase in postprandial insulin levels (65). In a separate clinical study, saturated fat intake was associated with an increase of plasma insulin values 2 h following an oral glucose load (66).

Despite the interesting epidemiologic evidence, relatively few interventional clinical trials have been completed due to the challenges inherent in regulating the quantity and quality of fat consumed by study subjects for weeks at a time. The few trials that have been completed have primarily monitored changes in plasma lipoproteins or insulin resistance. We are aware of only one study in humans that has examined the effect of prolonged (3 weeks) ingestion of saturated fat on insulin secretion and glucose metabolism during repeated intravenous glucose tolerance tests (IVGTT). Young, healthy women (n= 6-8) receiving a diet with increased palmitic acid or stearic acid had a 22% (p=0.208) or 17% (p=0.069) increase in insulin secretion, respectively (67). The study did not achieve a statistically significant effect, but changes elicited by the experimental diets may be more evident in larger studies with older, insulin-resistant subjects that have higher rates of insulin secretion. We are currently recruiting for a clinical trial of this type.

Preliminary results are available from a dietary intervention trial in rats that compared glucose-stimulated insulin secretion and insulin resistance following prolonged exposure to either saturated or unsaturated fat. Rats received a low-fat diet (LOW-FAT) or one enriched with saturated fat (LARD) or unsaturated fat (SOY) for 4 weeks. The impact of dietary fat intake on glucose-stimulated insulin secretion was assessed using hyperglycemic clamp studies. Figure 15 displays plasma glucose, FFA and insulin concentrations measured during the experiments. Plasma glucose values were similar for each of the three diet treatments during both the basal and the hyperglycemic clamp periods. Postprandial FFA concentrations in the SOY and LARD diet groups were elevated two-fold in comparison to the LOW-FAT animals and remained higher than those in the LOW-FAT rats during the hyperglycemic period despite some suppression by insulin. As plasma glucose levels were elevated by intravenous administration of glucose, insulin output was augmented in the LARD group relative to that of the LOW-FAT and SOY animals. Compared with the LOW-FAT group, the rats receiving LARD had an increased area under the insulin curve, while those on SOY actually tended to have suppressed insulin levels. Whole body insulin sensitivity for each of the three diet groups was assessed by recording the glucose infusion rates during hyperglycemic-euglycemic clamps. Both of the high-fat diet groups elicited insulin resistance, a finding that stands in stark contrast to the impact on insulin secretion. Clearly, these results establish that prolonged exposure to diverse types of fat differentially influences GSIS in rats independent of effects on insulin sensitivity.

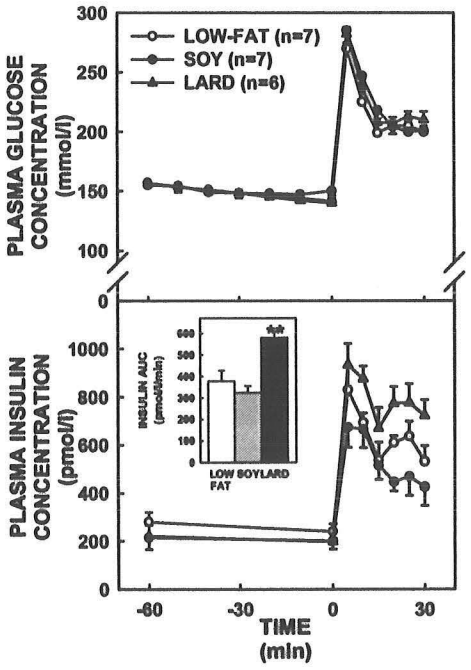


**Figure 15.** Plasma glucose and insulin concentrations during hyperglycemic clamp studies in fed rats. The animals received low-fat (n=7), soybean oil (n=7) or lard (n=6) diets for 4 weeks before studies were performed. **, $p<0.05$ versus control and soy studies.

Because dietary supplementation with highly unsaturated soybean oil tended to suppress GSIS while lard oil elicited enhanced insulin output, it is possible that the β-cell response during 24 or 48 h Intralipid infusions may have been compromised due to the unsaturated nature of the lipid infusion. A collaboration was initiated with Adria Giacca and Gary Lewis of the University of Toronto, who had performed the 48 h lipid + heparin infusion in studies previously presented in Figure 14 (63). As shown in

Figure 16, rats receiving a 48 h infusion of a soybean oil-based lipid emulsion had mildly suppressed plasma insulin and C-peptide concentrations during two-step hyperglycemic clamp studies (68). On the other hand, infusing animal fat in the form of lard oil significantly boosted plasma insulin and C-peptide responses as was seen during diet experiments. It is evident from these results that a great deal more work must be done before we can fully understand the relationship between fatty acids and insulin secretion.

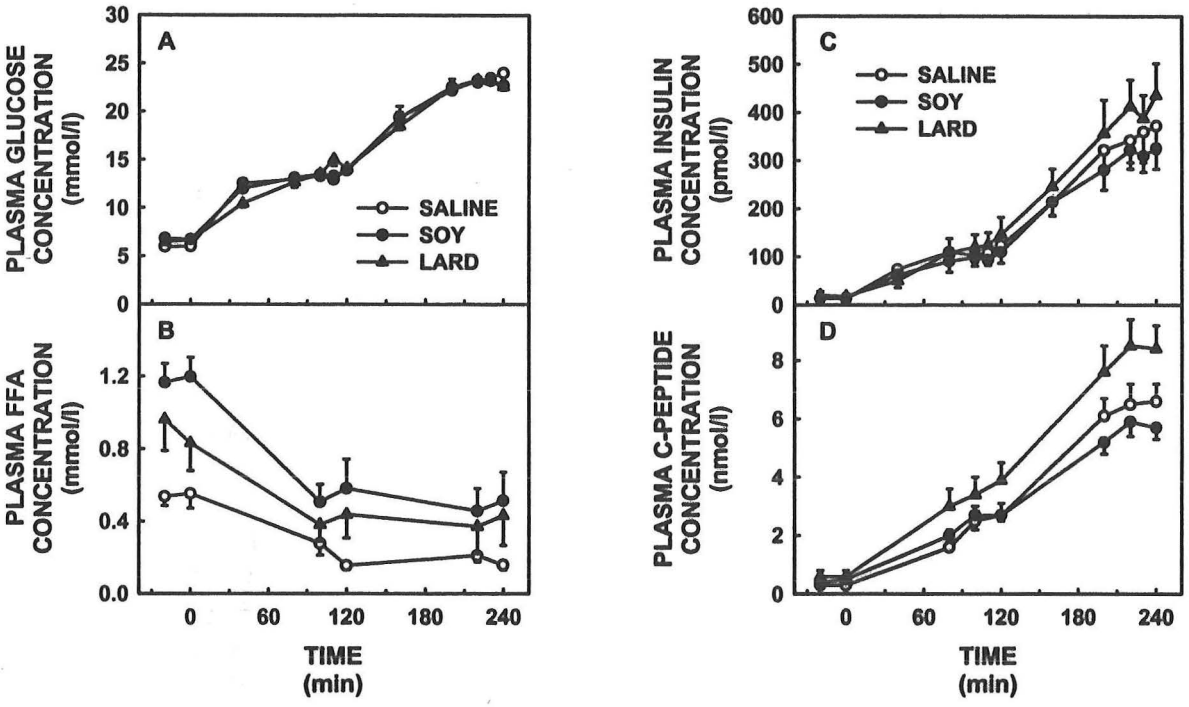


**Figure 16.** Plasma glucose, FFA, insulin and C-peptide concentrations during two-step hyperglycemic clamp studies in overnight-fasted rats. The animals had received infusions of saline (n=9), soybean oil (9) or lard oil (n=9) for 48 h before the studies were performed. Values represent mean ± SE.

**Can abnormal lipid metabolism have a link to β-cell failure?**

The final topic to be included in this discussion is the possible contribution of disordered lipid metabolism to the ultimate demise of β-cell function that signals the advent of clinical type 2 DM. In the final analysis, insulin secretory failure ultimately determines why some obese, insulin resistant individuals develop hyperglycemia and others do not. The abnormal progression of GSIS over time has been well-documented in human subjects by Ralph DeFronzo and his colleagues (5). Initially, basal and postprandial hyperinsulinemia match insulin resistance according to what has been termed "Starling's Law of the pancreas." Eventually as hyperglycemia increases, impaired insulin responses are noted including the loss of first-phase insulin release following a glucose bolus, delayed and blunted secretion following ingestion of a mixed meal, loss of rapid phase oscillations and increased release of proinsulin. The biochemical and molecular basis for β-cell failure remains unknown and is difficult to examine in human subjects because it is not possible to separate prospectively those individuals in whom glycemic control will deteriorate. If such individuals could be identified, it would be impractical to monitor their clinical course over decades and impossible to obtain islet tissue for morphologic, biochemical and genetic analysis.

Out of necessity, investigators have turned to animal models of type 2 DM. Based upon the previous discussion, we must consider the feasibility of diet-induced diabetic models. Although the high-fat diets mentioned above successfully triggered insulin resistance and hyperinsulinemia, no evidence of lipotoxicity was noted in normal Sprague-Dawley rats. Our experience is not unique because it matches that of the Garvan Institute in Sydney, Australia. They have maintained normal rats on high-fat diets for a year or more, but they have never succeeded in producing a diabetic rat. This comes as no surprise for clinicians. Nearly 40% of adults in Dallas County would meet the criteria for diet-induced obesity but only a relatively small proportion of them develops type 2 DM. Evidently, a second factor must come into play. Is it possible that this second defect renders the β-cell particularly susceptible to "lipotoxicity" in the setting of dietary impropriety and/or diminished lipid utilization? Data gathered from the ZDF rat suggest that this is the case.

The ZDF rat provides a model for common human type 2 diabetes (69, 70). It was originally derived from the Zucker fatty rat, which carries a spontaneous mutation in the leptin receptor (fa gene) (71) that causes hyperphagia and obesity. The non-inbred model of obesity predictably exhibits hyperinsulinemia and insulin resistance, but overt type 2 DM is not part of the phenotype. In this regard, these animals are similar to most obese humans. The ZDF subline was developed from the Zucker fatty line by selectively inbreeding those animals with the highest blood sugars (70). Animals of the ZDF subline are diabetes-prone and nearly all ZDF *fa/fa* males develop diabetes by 12 weeks of age. In contrast, obese female rats in the ZDF subline are insulin resistant as well but they do not become diabetic under normal circumstances. The vulnerability for progression to type 2 DM is genetically transmitted and can be lost within a rat colony if breeding is not managed carefully. The identity of the gene mutation remains unknown but it has several important characteristics. First, it seems to be imprinted early on in the development of the pancreas because fetal rat islets obtained from animals of the ZDF subline demonstrate less glucose sensitivity and diminished insulin/proinsulin synthesis compared to Zucker fatty rats (70). Secondly, full expression of the diabetic phenotype is environmentally determined because the homozygous male rats lacking normal leptin receptors (ZDF *fa/fa*) develop diabetes while lean heterozygous (ZDF *fa/+*) or wild-type (ZDF +/+) rats remain unaffected. Thirdly, expression of the diabetic phenotype is sensitive to dietary fat intake as shown in Figure 17 because diabetes in males appears at progressively younger ages when they consume diets containing increased amounts of fat up to 50% of total calories. The "diabetes-resistant" females of the line also develop diabetes on very high fat intakes of 50% or more. Finally, it is known that the transition to the diabetic state is preceded in time by a marked increase in islet triglycerides that could very well signal the "last rites" of the failing β-cell (72).

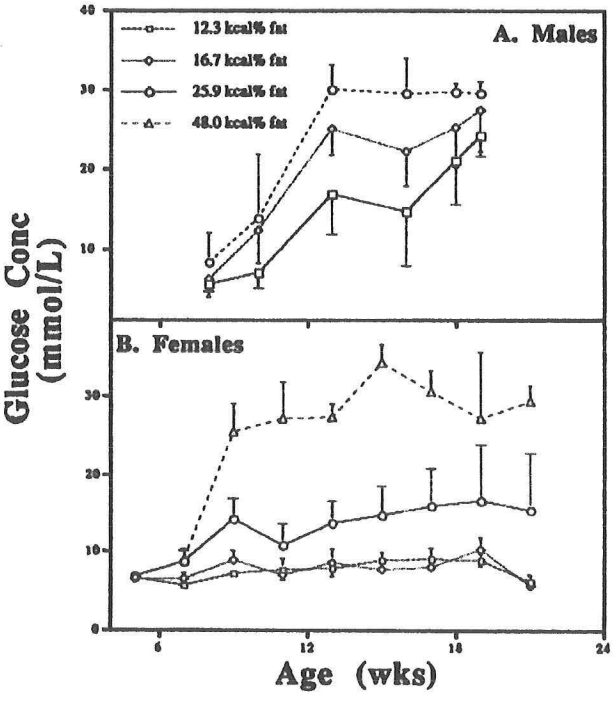


**Figure 17.** Plots of mean glucose concentrations as a function of time for obese male and female ZDF rats receiving diets ranging from 12.3 to 48.0 kcal% fat. Values represent mean ± SD for 5-10 rats.

Much more remains to be learned from this animal model that so distinctly resembles the human phenotype of type 2 DM. However many lines of evidence point

to disordered lipid metabolism as having a vital part in the etiology of initial hyperinsulinemia and insulin resistance and ultimate β-cell failure, at least in ZDF rats.

**Conclusion**

In conclusion, I would like to present an overall schema outlining hypothetical steps in the etiology of type 2 DM. Leptin resistance is the classic neurohormonal alteration that influences lipid metabolism but additional neurotransmitters are being discovered that modulate feeding behavior and metabolism. Although the interaction between substances such as leptin, neuropeptide Y, insulin, CCK, MCHC, orexins, etc., involves a large number of competing and redundant pathways, relatively few pathways are used to transmit information from the brain to peripheral tissues. As an example, leptin resistance in the hypothalamus due to a genetic defect or the result of a high-fat diet results in hyperphagia, decreased sympathetic tone, and increased adrenal cortisol secretion in response to a release of hypothalamic control of corticotropin releasing hormone. These events might limit sympathetic activity, as seen in animal models, and alter the ACC/CPT I axis in a manner that decreases the fatty acid oxidation capacity in skeletal muscle. This will increase the intramyocellular lipid concentration and reduce glucose uptake leading to insulin resistance. At the same time, the pancreas increases insulin secretion to maintain normal glucose tolerance (NGT). Chronic hyperinsulinemia further diminishes fatty acid oxidation and augments intracellular fatty acyl-CoA and TG concentrations, but it also interferes with the normal transport/phosphorylation pathways that are activated by insulin. In the liver, elevated insulin concentrations increase VLDL synthesis. Circulating VLDL provides substrate for fat deposition in adipose tissue resulting in obesity as an early event, particularly when hyperphagia is present. Eventually the capacity of the adipose tissue to esterify fatty acids is overwhelmed and the plasma FFA concentration increases. Excessive plasma FFA levels will enhance glucose production by the liver and impair glucose uptake in skeletal muscle, but they will also stimulate the β-cell and exacerbate hyperinsulinemia. In early stages, the insulin secretion compensates for insulin resistance, although impaired glucose tolerance (IGT) may develop. As a later event in genetically susceptible individuals, excessive exposure to lipid substrates could trigger β-cell failure and overt type 2 diabetes mellitus.

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