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# Banting Lecture 2001

## Dysregulation of Fatty Acid Metabolism in the Etiology of Type 2 Diabetes

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**R**eaders of this journal do not need to be reminded of the explosive increase in obesity/type 2 diabetes syndromes and their attendant staggering public health costs that are currently afflicting developed countries. Because of this alarming development and what it portends for the future if left unchecked, the American Diabetes Association, together with its counterparts around the world, as well as the World Health Organization, are faced with the daunting challenge of finding answers to two huge questions. First, what is the pathophysiological basis for these disorders? Second, why is their rate of appearance accelerating so rapidly at this particular juncture of human history, and what, if anything, can we do to intervene? The primary focus of this lecture will be on the first of these issues. I want to emphasize at the outset that I do not plan to dwell exclusively on our own studies in this area. Instead, I shall try to incorporate recent developments from a variety of other laboratories working in the field. Although these will of necessity have to be highly selective, I hope that collectively they will illustrate how we have come to a relatively new and exciting way of thinking about the etiology of type 2 diabetes.

### WHAT CAUSES TYPE 2 DIABETES?

It has been said in literary circles that James Joyce's *Ulysses* is probably the most frequently opened and least read book that has ever been published. In a similar vein, I sometimes think that the question of what causes type 2

diabetes might be one of the most frequently asked and least satisfactorily answered in the history of diabetes research. This is not meant to be an arrogant statement, nor indeed does it imply that I have a magical solution to the question at hand. Rather, it simply reflects the fact that at the dawn of this new millennium and in the 80th anniversary year of the discovery of insulin by Sir Frederick Banting and his colleagues, we are still grappling with the enormous complexity of a disease process in which almost every aspect of the body's metabolism goes awry.

Despite all that we don't know, there is general agreement that type 2 diabetes is tightly associated with obesity. It is also broadly accepted that, although undoubtedly polygenic and heterogeneous in its roots, the condition has two hallmark features: 1) insulin resistance, defined here as an impaired ability of the hormone to suppress hepatic glucose output and to promote peripheral glucose disposal and 2) compromised function of the pancreatic  $\beta$ -cell such that insulin secretion is insufficient to match the degree of insulin resistance. Less frequently emphasized, however, is that in the prediabetic phase of the condition, when insulin resistance is already in place, the  $\beta$ -cell actually hypersecretes insulin despite normal blood glucose levels. What has defied explanation in biochemical terms is precisely what causes this insulin resistance in the first place and how it relates in a temporal sense to the accompanying hyperinsulinemia. Also enigmatic has been why, in a presumably genetically programmed subset of obese individuals, the  $\beta$ -cell fails, leading to frank diabetes. Is it possible that one of the reasons for this impasse might relate to the fact that traditional views on the metabolic derangements of diabetes have been largely "gluco-centric" in nature and that a more "lipocentric" approach to the problem might be more rewarding (1)? In support of this line of thinking, it has long been known that, in addition to hyperglycemia, the type 2 diabetic individual almost invariably manifests a serious breakdown in lipid dynamics, often reflected by elevated levels of circulating free fatty acids (FFAs) and triglycerides (TG), together with excessive deposition of fat in various tissues including the muscle bed (2,3). Less clear has been whether this breakdown in lipid homeostasis is a result of the diabetic state or in fact is instrumental in its development. We and others have suspected for some time that the latter is probably the case. More specifically, we believe that a compelling argument can now be made for the notion that an abnormal accumulation of fat in muscle and other tissues plays an important role in the etiology of insulin

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Received for publication 28 September 2001 and accepted in revised form 8 October 2001.

Because of Dr. J. Denis McGarry's illness, this manuscript was finalized by Drs. Victoria Esser and Brian Weis under his direction.

ACC, acetyl-CoA carboxylase; AMPK, AMP-activated kinase; CPT I, carnitine palmitoyltransferase I; DM2, type 2 diabetes; EMCL, extramyocellular lipid; FAS, fatty acid synthase; FFA, free fatty acid; GSIS, glucose-stimulated insulin secretion;  $^1\text{H MRS}$ ,  $^1\text{H}$  magnetic resonance spectroscopy; HSL, hormone-sensitive lipase; IGT, impaired glucose tolerance; IMCL, intramyocellular lipid; IMGU, insulin-mediated glucose uptake; IRS-1, insulin receptor substrate-1; LCACoA, long chain acyl-CoA; MCD, malonyl-CoA decarboxylase; NA, nicotinic acid; NGT, normal glucose tolerance; PI, phosphatidylinositol; PKC, protein kinase C; PPAR, peroxisome proliferator-activated receptor; RQ, respiratory quotient; TG, triglycerides.

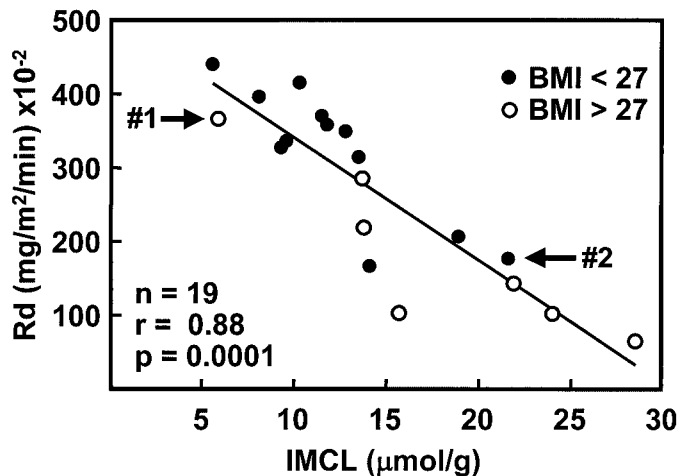


FIG. 1. Correlation between measurements of IMCL by  $^1\text{H}$  MRS and the rate of insulin-stimulated glucose disposal ( $R_d$ ) in NGT individuals who underwent a hyperinsulinemic-euglycemic clamp.

resistance and possibly also in the demise of the  $\beta$ -cell in type 2 diabetes. This thesis will be examined in more detail in the sections that follow.

**The fatty acid–muscle connection.** Work from a number of laboratories has shown that both in rodents and humans the TG content of muscle, as measured by Folch extraction of tissue samples followed by chemical analysis, bears a negative relationship to whole-body insulin sensitivity. A case in point is the study by Pan et al. (4) in Pima Indians in which each subject underwent a hyperinsulinemic-euglycemic clamp to determine whole-body insulin sensitivity and then provided a muscle biopsy for chemical measurement of total TG content. Although a rough negative relationship was found between these two parameters, there was considerable scatter among the data points. In this type of analysis, there is always uncertainty as to the anatomical location of the TG measured. In other words, how much of it is present within the myocyte, and how much arises from adipose tissue interspersed between the muscle fibers? We felt that variations in this extramyocellular lipid (EMCL) component might well interfere with the measurement of intramyocellular lipid (IMCL). We also became aware of the elegant studies of Schick et al. (5) suggesting that EMCL and IMCL can be distinguished noninvasively using the technique of  $^1\text{H}$  magnetic resonance spectroscopy ( $^1\text{H}$  MRS). This procedure detects resonances from protons associated with the methylene groups of fatty acids present in tissue TG. Importantly, these signals differ by  $\sim 0.2$  ppm depending upon whether they arise from TG within the muscle cell or from surrounding adipose tissue, allowing separate quantification of the two pools. Accordingly, 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  $^1\text{H}$  MRS of the soleus muscle to measure the quantity of IMCL. As seen from Fig. 1 (6), a remarkably tight negative relationship was found between the two parameters.

Indeed, IMCL was found to correlate more tightly with insulin resistance than any of the other commonly measured indexes, such as BMI, waist-to-hip ratio, or total

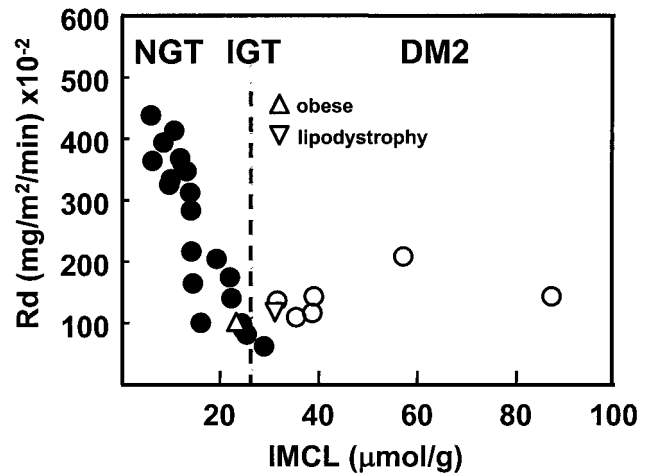


FIG. 2. Correlation between measurements of IMCL by  $^1\text{H}$  MRS and the rate of insulin-stimulated glucose disposal ( $R_d$ ) in NGT ( $\bullet$ ), IGT ( $\Delta$ ), and DM2 ( $\circ$ ) individuals who underwent a hyperinsulinemic-euglycemic clamp.

body fat. Similar findings have since been reported by other groups (7–10). Also evident from Fig. 1 is that one of the more obese individuals (#1, BMI  $32.8 \text{ kg/m}^2$ ) was one of the most insulin-sensitive but had one of the lowest IMCL values. Conversely, subject 2, with a BMI of only  $18.9 \text{ kg/m}^2$ , proved to be highly insulin-resistant but had a marked expansion of the IMCL pool. Thus, insulin sensitivity appears to correlate not so much with total body fat but with where the fat is located.

We have recently extended this type of analysis to patients with either impaired glucose tolerance (IGT) or frank type 2 diabetes (DM2). The results are shown in Fig. 2 (11), which includes for comparison the NGT subjects from Fig. 1.

Two points are noteworthy. First, the individuals with IGT or DM2 were no more insulin-resistant than the most insulin-resistant members of the NGT group; however, their IMCL content was sometimes massively increased, particularly when diabetes was also present. One implication might be that insulin sensitivity can fall to its nadir without the appearance of diabetes as long as  $\beta$ -cell compensation remains adequate, as suggested by DeFronzo et al. (12). Subsequently, when the  $\beta$ -cell fails, possibly because of lipotoxicity (see below), hyperglycemia ensues and the concomitant elevation of plasma FFA and VLDL levels causes even greater accumulation of fat in the muscle cell. Second, one of the two IGT subjects shown in Fig. 2 had congenital lipodystrophy and, by definition, essentially no subcutaneous fat. Here again, however, the low insulin sensitivity was accompanied by a marked increase in IMCL. We have found the same to be true in other patients with this condition (13).

It might be argued that the fat accumulation in muscle and its tight association with diminished insulin sensitivity shown in Figs. 1 and 2 are simply coincidental features of a group of individuals genetically programmed to develop both conditions in parallel but independently to varying degrees. To establish cause and effect, it is necessary to show that in healthy, insulin-sensitive subjects or animal models, manipulations that result in an increase or decrease of IMCL will cause reciprocal changes in insulin sensitivity. There is now good reason to believe that this is

the case. Brechtel et al. (14) subjected healthy male volunteers to a 5-h hyperinsulinemic-euglycemic clamp during which Intralipid and heparin were infused to raise the plasma FFA level. Using  $^1\text{H}$  MRS to measure IMCL in the tibialis anterior and soleus muscles, only modest changes were noted over the first 3 h of lipid infusion. However, by the 5-h time point, the IMCL content in both sites had increased by 61 and 22%, respectively. Interestingly, in an earlier study of similar design, Boden et al. (15) had demonstrated that artificial elevation of the plasma FFA concentration in healthy humans had little effect on insulin-mediated glucose disposal over the first 2–3 h but thereafter became markedly inhibitory. Similar results were reported by Roden et al. (16). In this case, the negative effect of lipid/heparin infusion on whole-body glucose removal began after a lag time of  $\sim 3.5$  h, and 2 h later both oxidative glucose disposal and muscle glycogen synthesis (as measured by  $^{13}\text{C}$ -MRS) were reduced by some 40–50%. Importantly, these changes were found to be associated with a fall rather than a rise in the muscle glucose-6-phosphate concentration. This suggested that under the conditions used, the FFA effect was exerted at the step of glucose transport and/or phosphorylation rather than through the classic Randle mechanism in which excessive fatty acid oxidation would be expected to cause an increase in the glucose-6-phosphate level secondary to inhibition of pyruvate oxidation (17). In a subsequent series of experiments, Shulman and colleagues (18) went on to pinpoint the inhibitory site of FFA to the glucose transport step. This was particularly significant in light of the fact that the Shulman group has also shown glucose transport to be the primary defect in insulin-mediated glucose metabolism in patients with type 2 diabetes (19). The same principle was established in obese nondiabetic and diabetic humans by Kelley et al. (20), although in this study an additional impairment of glucose phosphorylation appeared to exist in the diabetic subjects. That this derangement in muscle glucose transport/phosphorylation must be an early event in the etiology of type 2 diabetes is attested to by the fact that it is also present in normoglycemic first-degree relatives of such individuals (21), who also exhibit elevated IMCL levels (22).

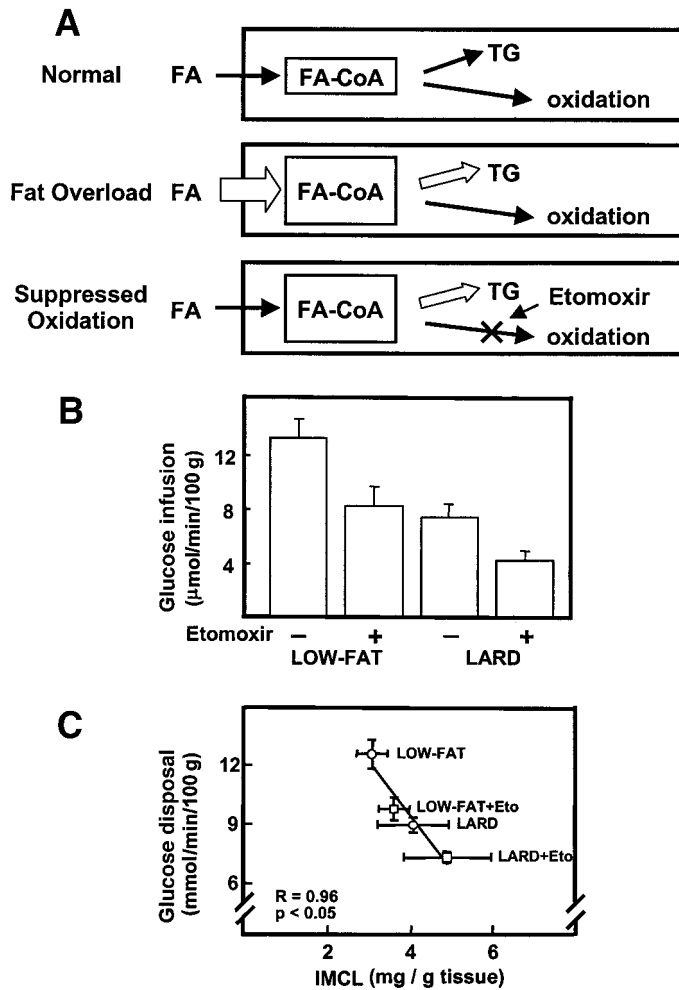
Taking the opposite approach, Man et al. (23) examined the effect of food restriction on a variety of parameters in the Otsuka Long-Evans Tokushima fatty rat, a model of type 2 diabetes. Of particular note was the marked reduction in total TG content of muscle, liver, and pancreas and the striking improvement in glucose tolerance of these animals. The same is true in the Zucker diabetic fatty (ZDF) rat (24).

Collectively, these findings indicate that 1) artificial elevation of the circulating FFA level can, over the course of a few hours, result in a very significant suppression of insulin-mediated glucose uptake (IMGU) into muscle in healthy people; 2) this effect correlates in a temporal sense with the accumulation of TG in skeletal muscle cells; 3) diet-induced lowering of tissue TG levels improves insulin sensitivity and essentially reverses the diabetes in animal models of type 2 diabetes; and 4) the increase in IMCL noted above in cross-sectional studies in humans (Figs. 1 and 2) is almost certainly instrumental in bringing about the associated decline in muscle insulin sensitivity.

**What is the TG marking?** Although the possibility cannot be excluded, it seems doubtful that TG itself interferes with insulin action in muscle. A more likely scenario is that the TG is acting as a surrogate marker for some other fatty acid-derived entity, such as long chain acyl-CoA species (LCACoA), and that the latter or an esterified product derived therefrom impacts negatively on IMGU. Persuasive evidence to this effect is now beginning to emerge. In a group of individuals with varying degrees of glucose tolerance, Ellis et al. (25) found a strong negative correlation between whole-body insulin sensitivity during a hyperinsulinemic-euglycemic clamp and the content of LCACoA measured in muscle biopsy samples. In a study of rats fed a high-fat diet, Ye et al. (26) showed that the resultant fall in IMGU into muscle was accompanied by a marked increase in plasma TG and muscle LCACoA levels. All of these abnormalities were ameliorated by the simultaneous administration of either a peroxisome proliferator-activated receptor (PPAR)- $\alpha$  or  $\gamma$  ligand and in direct proportion to the improvement in IMGU. We reasoned that if expansion of the LCACoA pool in muscle interferes with IMGU, it should be possible to bring about this situation in two ways: either by imposing a fat overload on the tissue, as in the studies of Ye et al. (26), or by inhibiting the process of mitochondrial fatty acid oxidation at the level of carnitine palmitoyltransferase I (CPT I), the enzyme responsible for converting LCACoA into long-chain acyl-carnitine (Fig. 3A). Accordingly, we fed normal rats a low-fat or a high-fat (lard) diet in the absence or presence of the CPT I inhibitor, etomoxir (27), for a period of 4 weeks. The animals were then subjected to a hyperinsulinemic-euglycemic clamp after an overnight fast using a  $3\text{-}[^3\text{H}]\text{glucose}$  infusion to measure the rates of glucose production ( $R_a$ ) and disposal ( $R_d$ ). As seen from Fig. 3B, feeding etomoxir, lard, or lard plus etomoxir produced increasing degrees of whole-body insulin resistance, as reflected in the stepwise fall in the glucose infusion rate required to maintain euglycemia during the clamp. This attenuation of insulin sensitivity was manifested by a reduction in both  $R_d$  and  $R_a$ . Moreover, the fall in  $R_d$  once again correlated with the rise in IMCL content of soleus muscle (presumably driven by expansion of the LCACoA pool) as measured using  $^1\text{H}$  MRS (Fig. 3C).

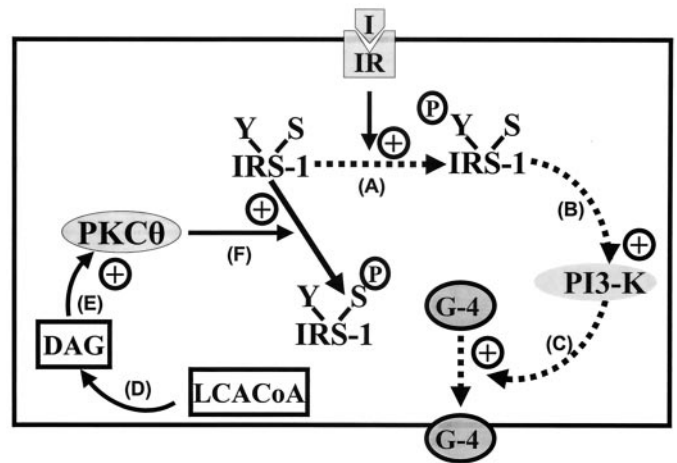
**How are the LCACoAs acting?** Assuming that an increase in muscle LCACoA levels interferes with IMGU into muscle, how might it do this? Evidence is mounting that the mechanism involves disruption of the insulin signaling cascade that normally leads to movement of GLUT4 transporters from an intracellular compartment to the muscle cell surface. This process is depicted schematically in Fig. 4 (dotted arrows). It involves a sequence of reactions in which binding of insulin to its receptor causes tyrosine phosphorylation of insulin receptor substrate-1 (IRS-1). This in turn leads to activation of phosphatidylinositol (PI) 3-kinase, a key player in GLUT4 translocation to the plasma membrane (28,29). It had been known from work in a number of laboratories that muscle from insulin-resistant obese humans (with or without diabetes) and Zucker rats exhibited reduced levels of insulin-stimulated IRS-1 tyrosine phosphorylation and PI 3-kinase activity (30,31). With this in mind, Griffin et al. (32) infused lipid and heparin into normal rats to raise plasma FFA levels for





**FIG. 3. A:** Schematic representation of intracellular disposition of fatty acids. Upper panel represents the disposition of fatty acids in the normal state. An overload of fatty acids results in enhanced TG production (middle panel). Fatty acids will also be converted into TG if their oxidative pathway is inhibited (lower panel). **B:** Influence of dietary fat content and etomoxir (Eto) on the rates of glucose infusion. **C:** Relationship between IMCL and glucose disposal in overnight-fasted rats before and during insulin infusion of  $3 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ . Animals were fed for 4 weeks either with low-fat diet (4% fat) or lard diet (41% fat) with or without 0.01% R-etomoxir, a CPT I inhibitor. Each value represents the mean  $\pm$  SE for six to seven determinations (reproduced from ref. 27).

5 h before conducting a hyperinsulinemic-euglycemic clamp. As expected, the lipid infusion caused a 40–50% reduction in muscle glucose oxidation and glycogen synthesis by impairing glucose transport. This was accompanied by a marked fall in insulin-mediated tyrosine phosphorylation of IRS-1, a 50% reduction in IRS-1-associated PI 3-kinase activity, and a fourfold increase in membrane-bound (active) protein kinase C (PKC)- $\theta$ . Furthermore, the same study design in healthy humans (18) also brought about a profound suppression of IRS-1-associated PI 3-kinase activity in skeletal muscle together with severe attenuation of insulin-mediated glucose transport. Those findings led the authors to propose an attractive hypothesis whereby an elevated concentration of LCACoA in the muscle cell generates a pool of diacylglycerol, which then serves to activate a serine kinase (possibly PKC $\theta$ ). The resultant phosphorylation of IRS-1 on a serine residue interferes with its ability to recruit and



**FIG. 4. IMGU in muscle.** In the absence of fatty acids, when insulin (I) binds to its receptor (IR), the sequence of events are as follows (dotted arrows). **A:** phosphorylation of the IRS-1 on a tyrosine residue, **B:** activation of phosphatidylinositol 3-kinase (PI3-K), and **C:** translocation of GLUT4 (G-4) to the membrane. In the presence of LCACoA, the insulin signaling cascade follows an abnormal pathway (solid arrows). **D:** generation of a diacylglycerol (DAG) pool; **E:** activation of PKC $\theta$  and phosphorylation of IRS-1 on a serine residue. This model is supported by work published by the laboratories of Shulman (18) and Kraegen (29).

activate PI 3-kinase. The net effect, therefore, is loss of the stimulus for GLUT4 translocation to the cell surface and thus blunting of IMGU (Fig. 4, solid arrows). Consistent with this notion, Bell et al. (33) found that the rapid reversal of muscle insulin resistance in high fat-fed rats after a single high-carbohydrate meal was associated with a fall in muscle LCACoA levels and loss of membrane-bound PKC $\theta$  activity.

While the above described studies make a strong case for LCACoA-mediated disruption of muscle glucose transport through the intermediacy of PKC $\theta$  activation, other mechanisms are not ruled out. For example, it is possible that if LCACoA levels become high enough they might also act to inhibit hexokinase, as suggested by Thompson and Cooney (34). In addition, Hawkins et al. (35) have emphasized the possible role of the glucosamine pathway in fat-induced muscle insulin resistance. Certainly, it is entirely possible that these mechanisms play a role in hyperglycemia-induced insulin resistance. However, its relative contribution to fat-induced impairment of insulin action on muscle under euglycemic conditions remains to be worked out.

**What sets the cellular LCACoA level?** It is evident, therefore, that an excessive lipid burden placed on the muscle cell in the form of nonesterified or VLDL-borne fatty acids can cause increased LCACoA levels and, secondarily, insulin resistance. However, this begs the question of what underlies the defect in insulin action in young first-degree relatives of type 2 diabetics in whom there might as yet be no significant disturbance in plasma lipid dynamics (as in the studies by Vaag et al. [36]). This calls attention to another key factor involved in control of the intracellular LCACoA concentration, namely, the malonyl-CoA/CPT I partnership. This central aspect of lipid metabolism first came to light in 1977 when we were trying to unravel the mysteries of hepatic ketone body production and its regulation. What emerged was a fascinating ar-

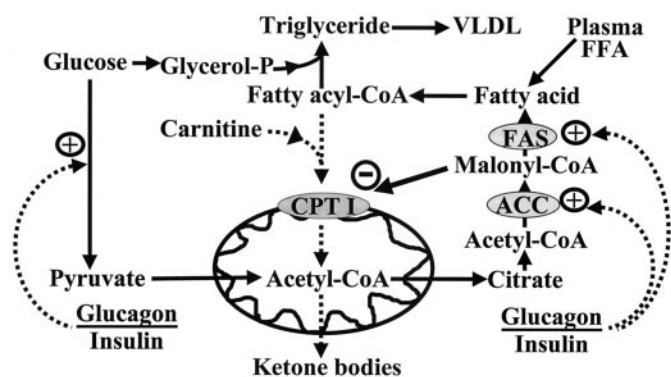


FIG. 5. Relationship between the pathways of fatty acid biosynthesis (solid arrows) and oxidation (dotted arrows) in the liver.

rangement whereby the capacity of the liver to oxidize fatty acids fluctuates dramatically with changes in nutritional state.

With carbohydrate feeding (high insulin/low glucagon), the liver actively converts glucose carbon to fatty acids and then to LCACoA (Fig. 5, solid arrows) (37). If the newly formed acyl-CoA were now to react with carnitine under the influence of CPT I present on the outer mitochondrial membrane, they would be converted back to acetyl-CoA through the process of  $\beta$ -oxidation, generating a futile cycle. What prevents this from happening is that during active lipogenesis, the pool of malonyl-CoA, produced from the acetyl-CoA carboxylase (ACC) reaction, increases and inhibits CPT I, thus ensuring that the LCACoA reacts with glycerol phosphate to form TG that are then exported from the liver in the form of VLDL. Conversely, during ketotic states (Fig. 5, dotted arrows), such as starvation or uncontrolled diabetes (low insulin/high glucagon), the pathway of fatty acid synthesis comes to a halt, the malonyl-CoA concentration falls, CPT I becomes deinhibited, and fatty acids reaching the liver readily enter the  $\beta$ -oxidation pathway to form ketone bodies.

Over the years, it has come to light that the malonyl-CoA/CPT I interaction is also at work in a variety of nonhepatic tissues, particularly heart and skeletal muscle (38–41). We have also come to realize that CPT I exists in at least two forms, the liver (L) and muscle (M) variants, with very different kinetic properties and sensitivities to malonyl-CoA (42). While liver and skeletal muscle express exclusively the L and M forms of the enzyme, respectively, heart contains both with the M isoform predominating (43). Interestingly, ACC has also been found to exist as two separate gene products, ACC1 and ACC2 with molecular sizes of 265 and 280 kDa, respectively (44). While they coexist in a variety of tissues, ACC1 dominates over ACC2 in lipogenic tissues such as liver and white fat while the opposite is true for nonlipogenic tissues such as heart and skeletal muscle (45). Moreover, ACC2 by virtue of its unique  $\text{NH}_2$ -terminal extension appears to associate with the outer mitochondrial membrane, possibly in close proximity to CPT I (46).

Unlike the situation in liver, where malonyl-CoA can be actively converted into fatty acids (Fig. 5, solid arrows), muscle contains very low levels of fatty acid synthase (FAS). Accordingly, in muscle cells, malonyl-CoA is

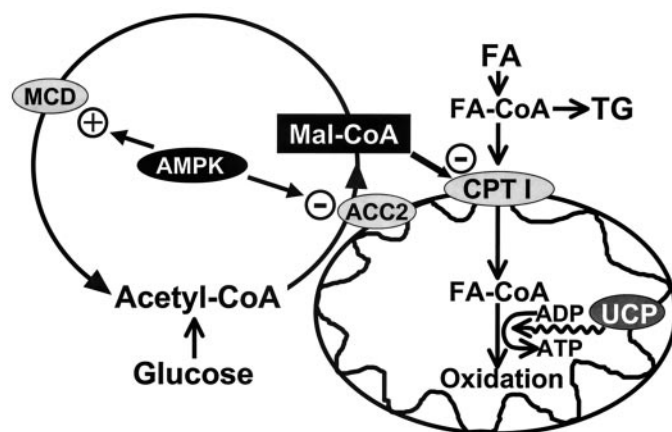


FIG. 6. Interaction of glucose and fatty acid metabolism in muscle. UCP, uncoupling protein; FA, fatty acids. Note that in this model, emphasis is placed on ACC2, the predominant form in muscle. However, it is recognized that ACC1 is also expressed in this tissue, and its contribution should be considered.

thought to act as a “fuel sensor” whose primary role is to regulate the rate of fatty acid oxidation (47). Furthermore, its concentration appears to be controlled by the relative activity of ACC2 and malonyl-CoA decarboxylase (MCD), both of which are regulated by AMP-activated kinase (AMPK), as depicted in Fig. 6. AMPK has also come to be recognized as part of the cell’s fuel sensing machinery (48). Under conditions of stress, such as hypoxia or exercise, the fall in the cellular ATP/AMP ratio results in phosphorylation and activation of AMPK by an upstream kinase (AMPKK). A well-established role of AMPK is the phosphorylation and inactivation of ACC (49). Importantly, studies by Ruderman et al. (50) suggest that in exercising muscle AMPK, in addition to lowering ACC activity, also causes phosphorylation and activation of MCD. The net result would be a rapid fall in the malonyl-CoA level and an enhanced capacity of the cell to oxidize fatty acids. Note that if ACC2 is physically juxtaposed to CPT I, the exercise-induced changes in malonyl-CoA concentration might be of much greater magnitude in the vicinity of CPT I than those measured in total tissue homogenates (51).

Could it be that AMPK acts as more than a stress signal to the muscle cell in regulating fatty acid metabolism? More specifically, is it possible that this enzyme is instrumental in the genesis of early insulin resistance in individuals prone to develop obesity and type 2 diabetes? One theoretical scenario might be that, for whatever reason, AMPK activity is lower than normal in such subjects. If so, this might be expected to cause an increase in the activity of ACC, leading to higher than normal levels of malonyl-CoA. The result would be a suppressed capacity of muscle (and possibly other tissues) to oxidize fatty acids, expansion of the LCACoA (and TG) pool, and diminished IMGU. It is not excluded that accompanying hyperinsulinemia could also favor dephosphorylation of AMPK and/or ACC and MCD by upregulation of the relevant phosphatases (52), giving further impetus to malonyl-CoA accumulation. To be sure, this is a speculative proposal. It would, however, be consistent with the findings by Ruderman and colleagues (50) that 1) muscle malonyl-CoA levels are increased in insulin-resistant animal models, 2) denervation of muscle in normal rats causes an increase in

malonyl-CoA content, and 3) the same effect can be achieved by exposure of normal muscle tissue to hyperinsulinemia and/or hyperglycemia. In this context, a recent and exciting development has been the creation of an ACC2 knockout mouse by Wakil et al. (53). This animal appears to eat the same or even more food than the wild type while maintaining a lower body weight because of a decreased fat mass. The implication is that the absence of ACC2 endows this animal with an enhanced capacity to burn fat. Although not directly measured, the impression is that the ACC2 null mouse also displayed enhanced insulin sensitivity (53). Of interest will be whether this trait can be conferred to the *ob/ob* or *db/db* mouse by introduction of the ACC2 null allele.

**Is inefficient fat oxidation a predictor of insulin resistance?** The concept that a lower than normal capacity to burn fat might be a predictor of insulin resistance receives support from both human and animal studies. For example, Astrup et al. (54) 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 (55). In a study with Pima Indians, Ravussin et al. (56) demonstrated that a reduced rate of energy expenditure is a risk factor for body weight gain. Kelley et al. (57) showed directly that in obese humans the muscle bed has a diminished capacity to oxidize fatty acids.

The above considerations lend weight to the notion that a subnormal ability of muscle to oxidize fatty acids is an important contributor to the genesis of insulin resistance. At first glance, this might seem at odds with the classic studies of Randle et al. (17) demonstrating that excessive oxidation of fatty acids interferes with insulin-mediated glucose uptake by muscle cells. The paradox might, however, be more apparent than real if we view the relationship between fatty acid metabolism and the etiology of type 2 diabetes as being biphasic. It is entirely possible that in the very early stages of the disease, i.e., before plasma FFA and VLDL levels begin to rise, the muscle cell displays a subtle defect in its ability to oxidize fatty acids, perhaps through dysregulation of the ACC/CPT I axis described earlier. The result would be a gradual accumulation of LCACoAs and the beginnings of insulin resistance (see above). Later in the course of the disease, particularly when insulin secretion begins to wane and plasma FFA and VLDL levels become chronically high (see below), muscle LCACoA levels likely rise to a point at which they offset the inhibition of CPT I by malonyl-CoA such that the rate of fatty acid oxidation accelerates. At this point, the Randle mechanism would undoubtedly come into play and exacerbate the degree of insulin resistance. A recent longitudinal study by Etgen and Oldham (58) using the ZDF rat is entirely in keeping with such a viewpoint. Before the appearance of diabetes (7 weeks of age), when insulin levels were very high, the fatty rats displayed a significantly higher RQ than their lean littermates. However, as insulin levels declined and hyperglycemia supervened, the RQ of the fatty rats gradually declined, and by

12 weeks of age (fulminant diabetes) it was significantly below the value of the lean animals.

**The fatty-acid- $\beta$ -cell connection.** There is little doubt that an important component of glucose-stimulated insulin secretion (GSIS) from the pancreatic  $\beta$ -cell involves the following sequence of steps: entry of glucose into the cell through a high-Km glucose transporter; phosphorylation of glucose by the high-Km member of the hexokinase family (glucokinase); metabolism of the glucose-6-phosphate so formed through glycolysis and the tricarboxylic acid cycle; elevation of the cytosolic ATP/ADP ratio; closure of the cell membrane  $K^+$ ATP channel; depolarization of the membrane; opening of the voltage-sensitive  $Ca^{2+}$  channel; and entry of  $Ca^{2+}$ , which triggers insulin release. It is also clear, however, that some additional component(s) of glucose metabolism is needed for the full effect of glucose to be manifested (59). Prentki and Corkey (60) raised the intriguing possibility that one such component involves an element of glucose-fatty acid cross-talk, in other words, that the CPT I/malonyl-CoA axis recognized in liver and later in muscle and heart may also be at work in the  $\beta$ -cell. They proposed that an elevation of the blood glucose level produces an increase in glucose-derived malonyl-CoA within the  $\beta$ -cell, just as in liver. This has the effect of suppressing CPT I activity and causing an expansion of the LCACoA pool behind the block. It was further suggested that the LCACoAs somehow synergize with glucose to enhance insulin secretion (59). As attractive as this hypothesis is, attempts to support it have met with mixed success (61–64), and for this reason my inclination is that final judgment on its validity should await more definitive experiments.

Regardless of whether the malonyl-CoA/CPT I interaction is central to GSIS, there can be no doubt that under certain circumstances fatty acids play an essential role in this process. A particularly striking example of this principle is seen in Fig. 7A. In these experiments, we examined the effect of a hyperglycemic clamp on plasma insulin and FFA levels in 18-h-fasted rats. Under control conditions, elevation of the blood glucose concentration produced the expected rise in insulin secretion and a concomitant fall in plasma FFA levels. Remarkably, however, when the FFA concentration was first brought to almost zero by prior infusion of the antilipolytic agent nicotinic acid (NA), the insulin response to glucose was virtually ablated (the same was found to be true for other insulin secretagogues such as arginine, leucine, and glyburide [65]). Conversely, when the NA infusion was accompanied by a lipid emulsion plus heparin to maintain high plasma FFA levels, GSIS was greatly enhanced (this effect could not be reproduced by replacement of ketone bodies, whose concentration paralleled that of the FFA in these experiments). Interestingly, when the same study was conducted in fed animals, the insulin response to glucose was the same regardless of whether the already low level of plasma FFA was reduced still further by NA infusion (Fig. 7B). But here again, artificial maintenance of a high FFA level profoundly stimulated GSIS. Qualitatively similar results were obtained when this protocol was applied to 48-h-fasted humans (66).

These studies established several important points. First, in the fasted rat, were it not for the elevated



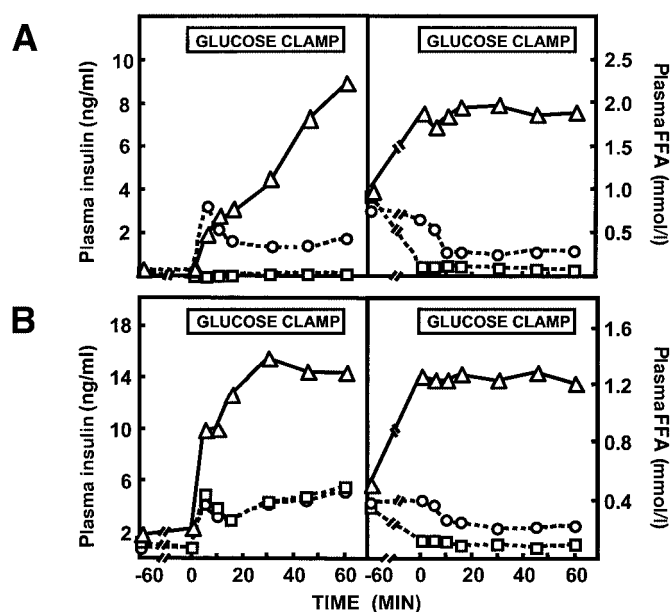


FIG. 7. Effect of NA on GSIS in 18- to 24-h-fasted (A) or fed (B) rats. At 0 min, the rats received an intravenous bolus of glucose to raise the plasma glucose concentration to ~14 mmol/l at +5 min. Thereafter, the plasma glucose concentration was clamped at ~11 mmol/l. In addition, they received vehicle (○), NA (□), or NA plus Intralipid (IL) and heparin (HEP) (△) from -60 min onward (reproduced from ref. 70).

concentration of circulating FFA, the pancreatic  $\beta$ -cell would be blind to glucose in terms of insulin secretion when the fast is terminated. Second, FFA promote a fall in their own plasma concentration during the fasted to fed transition by virtue of their ability to synergize with glucose to promote insulin secretion, which then exerts its antilipolytic effect on the fat cell. Third, the nonessentiality of external FFA for normal GSIS in the fed state raises the possibility that in this condition FFA are not needed for a robust insulin response to glucose. Alternatively, they might be internally available to the  $\beta$ -cell, perhaps by liberation from a storage pool of TG as a result of cAMP-mediated activation of hormone-sensitive lipase (HSL). In this regard, it is noteworthy that islets have been shown to contain HSL (67) and are known to display an increase in cAMP content in response to elevated glucose concentrations (68). If this were true, the absolute requirement for exogenous FFA for GSIS in the fasted state might be ascribed to depletion of the  $\beta$ -cell's TG reserve and/or to blunting of its cAMP response to glucose (68).

The profound insulinotropic effect of FFA seen in the experiments of Fig. 7 raised the question of whether all fatty acids are "created equal" in this regard. Initial indications that they are not came from a subsequent study in which endogenous circulating FFA in fasted rats were again eliminated by NA infusion and this time replaced by either soybean oil (the main constituent of Intralipid and composed largely of unsaturated TG) or the more saturated lard oil (69). The latter proved to be far more effective in promoting insulin secretion when the animals were challenged with glucose. The insulinotropic potency of individual fatty acids was then examined in the perfused pancreas from fasted rats (69). As seen from Fig. 8, GSIS was found to increase dramatically with chain length and the degree of saturation over the range tested. As dis-

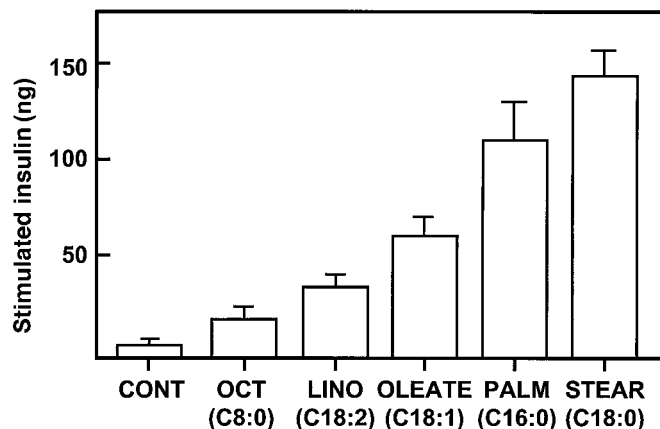


FIG. 8. Effect of different fatty acids on GSIS from the perfused pancreas of 18- to 24-h-fasted rats. Pancreata were perfused initially with 3 mmol/l glucose and then for 40 min with 12.5 mmol/l glucose in the absence or presence of the indicated fatty acid at a concentration of 0.5 mmol/l (with 1% albumin). CONT, control; OCT, octanoate; LINO, linoleate; PALM, palmitate; STEAR, stearate (reproduced from ref. 69).

cussed elsewhere (70), the mechanisms through which fatty acids in general and the more saturated species in particular synergize with glucose to promote insulin secretion remain to be elucidated.

#### Is there a darker side to the FFA- $\beta$ -cell interaction?

It is important to emphasize that the stimulatory effects of FFA on GSIS discussed above are physiological in nature, particularly during the fasted to fed transition. Circulating FFAs are also important in preventing starvation ketosis from developing into a pathological ketoacidosis. They do this by helping maintain a basal rate of insulin secretion, which serves to keep adipose tissue lipolysis in check. Without this feedback mechanism, as occurs in the uncontrolled type 1 diabetic, lipolysis becomes excessive, FFA levels rise dramatically, and hepatic ketone production is driven to a dangerously high rate. In a sense, this is also true in obese insulin-resistant states, where moderately elevated plasma FFA levels undoubtedly contribute to the hyperinsulinemia necessary to match the defect in insulin action. In this regard, it seems more than coincidental that the greater effectiveness of saturated versus unsaturated fatty acids to induce insulin resistance (71) is paralleled by the relative insulinotropic potency of these lipid substrates. Might this be part of nature's plan whereby the  $\beta$ -cell is kept informed as to how much insulin the muscle cell needs to dispose of incoming glucose at a normal rate?

The above point notwithstanding, there is evidence that chronic exposure of the  $\beta$ -cell to elevated FFA levels can be damaging to its function. Support for this notion has come mainly from studies with isolated islets exposed to high concentrations of FFA for periods of 24–48 h (72,73). Typically, this maneuver resulted in enhanced insulin secretion at low glucose concentrations, depressed proinsulin biosynthesis, depletion of insulin stores, and an impaired response of the  $\beta$ -cell to stimulatory concentrations of glucose, i.e., characteristics of type 2 diabetes. A seemingly similar phenotype was reported in intact rats during infusion of Intralipid to raise plasma FFA levels to 1–2 mmol/l for 48 h (74). This, however, has not been a consistent finding in rats (75) or humans (15), possibly due to differences in the experimental conditions employed in

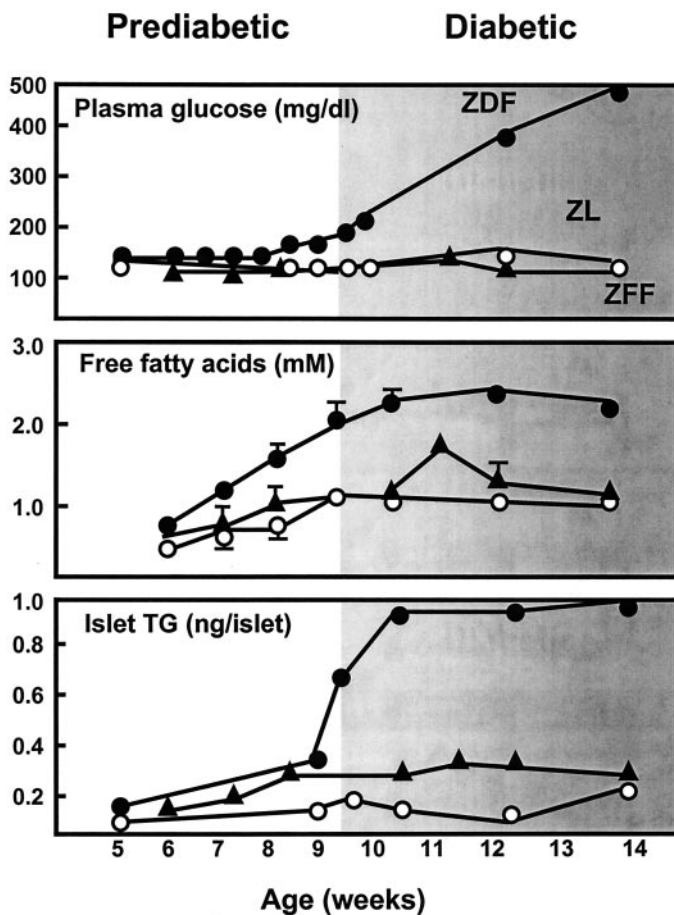


FIG. 9. Longitudinal studies of plasma glucose, plasma FFA levels, and TG content of islets in lean male ZDF rats (Fa/fa) (ZL) (○) and obese female ZDF rats (fa/fa) (ZFF) (▲), which do not develop diabetes, and in obese male ZDF rats (fa/fa) (ZDF) (●), which develop diabetes between the ages of 8 and 10 weeks.

the various laboratories. Perhaps the most persuasive case for islet lipotoxicity *in vivo* comes from studies with the ZDF rat, which, as noted earlier, shares many of the features of obesity-related type 2 diabetes in humans.

In a longitudinal study done in collaboration with Roger Unger's laboratory (76), we found, as had others (77), that when maintained on a 6% fat diet the male ZDF rat developed marked hyperglycemia after ~9 weeks of age (Fig. 9). This was not true in the fatty females or in lean littermates of the male animals. A distinguishing feature of the male ZDF rat was that he displayed a marked elevation of plasma FFA levels from 5 weeks of age onward. Even more striking, just before the appearance of diabetes, there was an abrupt and massive increase in islet TG content that coincided with severe disruption of islet morphology and  $\beta$ -cell function. Furthermore, diet restriction of these animals from 6 weeks of age greatly reduced their hyperlipidemia, hypertriglyceridemia, and islet fat accumulation. Under these circumstances, islet function was largely restored and hyperglycemia did not develop, *i.e.*, the entire phenotype of type 2 diabetes was prevented. The same relationship between increased TG content and deranged  $\beta$ -cell function was seen during long term exposure of rat islets to high fatty acid levels *in vitro* (78).

Subsequent studies from the Unger laboratory (79) have sought to elucidate the mechanisms underlying islet lipo-

toxicity. Possible candidates include fatty acid-mediated upregulation of inducible nitric oxide synthase and/or ceramide synthesis, either or both of which might lead to  $\beta$ -cell apoptosis, a documented event in the ZDF rat (79). Collectively, this body of work implicates fat accumulation in the islet as an important contributor to  $\beta$ -cell demise in the ZDF rat (and probably in other rodent models of type 2 diabetes). Whether this is also true in humans is not yet clear, in part because of the scarcity of suitable pancreas specimens for histochemical analysis from individuals with increasing degrees of  $\beta$ -cell malfunction.

In this context, it should be noted that the  $\beta$ -cell produces other polypeptides in addition to insulin. One of these is the 37-amino acid peptide amylin (80). Both rodents and humans co-secrete this hormone with insulin. However, unlike the rodent form, human amylin possesses a unique secondary structure that promotes its self-aggregation and precipitation as amyloid deposits in the islets of Langerhans. These deposits were first recognized in 1900 by Opie (81). It is now recognized that amyloid plaques in the islets are a common finding at autopsy in type 2 diabetes. The role that these deposits play in  $\beta$ -cell demise, versus the toxicity associated with FFA, remains to be elucidated. It should be noted that the  $\beta$ -cells in rodent models of obesity undergo apoptosis in the complete absence of islet amyloid deposits, suggesting that FFA toxicity alone may be sufficient to initiate the death of these islet cells.

**The fatty acid–liver connection.** As alluded to earlier, at some point in the genesis of type 2 diabetes, the fat cell appears to become refractory to the antilipolytic effect of insulin (mechanism unclear), such that plasma FFA levels begin to rise. Importantly, not only do their concentrations become elevated after an overnight fast, but they fail to suppress appropriately after meals, even though postprandial insulin levels may still be in the high range (82). Not surprisingly, the situation worsens as  $\beta$ -cell function deteriorates and insulin secretion is attenuated. In addition to exacerbating the insulin resistance in muscle, this hyperlipidemia can influence liver function in a variety of ways, particularly when the FFAs arise from expanded visceral fat stores from which they may reach the liver directly and at high concentration through the portal circulation (83). One effect appears to be an impairment in hepatic extraction of insulin, which will exaggerate the degree of hyperinsulinemia (83). In a setting of high insulin, which increases hepatic expression of sterol regulatory binding protein 1c and, consequently, of ACC and other lipogenic enzymes (84), tissue malonyl-CoA levels will rise. Under these conditions, fatty acid esterification will be favored over oxidation because of CPT I inhibition by malonyl-CoA, such that the fat content of the liver and VLDL secretion increases, contributing to the development of hypertriglyceridemia (70). If the intracellular LCACoA level rises sufficiently, it will tend to offset the inhibitory effect of malonyl-CoA on CPT I with the result that both fatty acid esterification and oxidation will be enhanced. It has long been known that increased fatty acid oxidation in liver promotes the pathway of gluconeogenesis, in part by maintaining pyruvate carboxylase in an active state (17). This, together with the fact that an increased fat burden on the liver is usually associated with upregulation of key



gluconeogenic enzymes (e.g., PEPCK and G6Pase), sets the scene for inappropriately high rates of hepatic glucose output (85).

The above considerations serve to emphasize an important point that is often overlooked when the term “insulin resistance” is applied to liver metabolism. Clearly, when circulating levels of insulin and FFA are both elevated, as occurs during the transition from simple obesity to obesity with IGT (12), the liver has become resistant to insulin in terms of the ability of the hormone to suppress glucose production. However, as demonstrated in animal models of this condition, such as the *ob/ob* mouse or the transgenic “fatless” mouse generated by Shimomura et al. (86), the liver is by no means insulin-resistant with respect to its lipogenic capacity. Indeed, under these conditions, the hyperinsulinemia turns the liver into a “fat-producing factory” with all of its negative downstream effects, including the genesis of hypertriglyceridemia.

#### SO WHERE DO ALL OF THE PROBLEMS BEGIN?

From the foregoing discussion, it is evident that, once established, the various derangements in lipid metabolism can have devastating consequences on glucose homeostasis. However, we also know that type 2 diabetes generally takes many years to fully manifest itself, and the burning question confronting us is, “When and at which body sites do the metabolic derangements first begin?” Because of the polygenic nature of the disorder, it is doubtful that one set of temporal events will apply in all cases.

As to the nature of this primary defect, the short answer is we don't know. However, is it possible that we are talking about the “thrifty gene” discussed by Neel (87) back in the 60s as a factor that would promote a little extra fat deposition and therefore during periods of food shortages confers a survival advantage on our ancestors? On the contrary, in the modern-day life with unlimited food availability and a sedentary lifestyle, this gene turns against us. On an even more speculative note, one wonders whether this primary defect might reside somewhere in the leptin signaling system (88,89). Note that in both animals and humans, inefficient leptin action leads to hyperphagia, decreased fat oxidation, increased tissue triglyceride levels, insulin resistance, and ultimately obesity/type 2 diabetes. Conversely, treatment of normal animals with leptin causes hypophagia, increased fat oxidation, complete depletion of tissue triglyceride, increased insulin sensitivity, and prevention of obesity/type 2 diabetes. Precisely how leptin accomplishes this remarkable feat is far from clear, but we suspect that part of the mechanism might be through the activation of the sympathetic nervous system (90,91). Consistent with this idea, it is interesting to recall that rodents lacking either leptin or the leptin receptor show low sympathetic activity and go on to develop obesity/type 2 diabetes. Conversely, treatment of normal animals with leptin produces increased sympathetic tone and dissipation of tissue triglyceride stores. If increased sympathetic activity could be shown 1) to alter the ACC/CPT I axis in muscle such that malonyl-CoA levels fall and 2) to maintain a tonic level of uncoupling activity of the respiratory chain, it would follow that a reduced level of sympathetic activity would result in decreased fatty acid oxidation, leading to insulin resis-

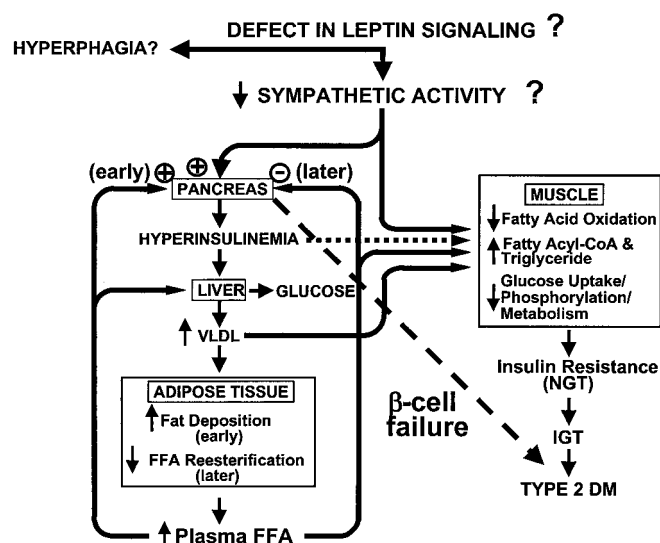


FIG. 10. Hypothetical steps in the etiology of type 2 diabetes. If there is a defect in the leptin signaling system that lowers the sympathetic activity, as seen in animal models, fatty acid oxidation capacity in the muscle will decrease (possibly by altering the ACC/CPT I axis). This will increase the IMCL concentration, which will lower glucose uptake, leading to insulin resistance. To compensate for the decreased insulin sensitivity in muscle, the pancreas increases insulin secretion to maintain NGT. Chronic hyperinsulinemia further diminishes fatty acid oxidation, raising the concentration of fatty acyl-CoA and TG, and interferes with the transport/phosphorylation pathways associated with normal glucose uptake. An elevated concentration of insulin will also increase the output of VLDL by the liver. Circulating VLDL will deposit in the muscle and adipose tissue, which in the early stages of the disease will increase fat deposition. If on top of this hyperphagia is added, the situation deteriorates. Eventually, the ability of the adipose tissue to reesterify FFA is overwhelmed, increasing their concentration in plasma. Excessive FFA will enhance glucose production by the liver, precipitating IGT. In an early stage, the increase of plasma FFA will exacerbate the already existing hyperinsulinemia. Later, they will, through their lipotoxic effect, lead to  $\beta$ -cell failure and overt type 2 diabetes.

tance by the mechanisms that we discussed earlier. A model for this hypothesis is depicted in Fig. 10.

#### WHY IS THE INCIDENCE OF TYPE 2 DIABETES INCREASING SO RAPIDLY, AND WHAT CAN BE DONE ABOUT IT?

As discussed, the combination of the modern diet and sedentary lifestyle has resulted in an increase in obesity/type 2 diabetes. It is evident that if people would eat fewer calories and increase their activity level, then this would reverse ectopic fat accumulation and lessen insulin resistance. However, compliance with such a strategy is clearly difficult. If society is not willing to make major lifestyle changes, are other interventions available? Some promising approaches have recently been introduced by the pharmaceutical industry. The use of PPAR- $\gamma$  agonists, in the form of the thiazolidinediones, has been shown to effectively decrease plasma concentrations of TG, VLDL, FFA, and glucose. The mechanism of their actions is not entirely understood, but it is clear that by activating the nuclear PPAR- $\gamma$  receptor, these agents promote preadipocyte differentiation into mature adipocytes with the capacity to store TG. By enabling the appropriate accumulation of triglyceride in adipose stores, these agents lessen the ectopic deposition of fat, the hallmark of insulin resistance (92).

An alternative way of reducing the fat burden would be to enhance its oxidation in tissues such as liver, pancreas, and skeletal muscle. Agonists to the PPAR- $\alpha$  receptor that is prevalent in these organs are currently in development. By upregulating the enzymes responsible for fatty acid oxidation, these agents would promote the utilization of fat for energy in these tissues. Clearly, the PPAR agonists, either by effecting the appropriate storage of fat in adipocytes or by promoting its oxidation in tissues such as liver or skeletal muscle, enhance insulin sensitivity (26). The use of these drugs are in the formative stages, and only time will prove whether or not they will be effective.

#### ACKNOWLEDGMENTS

It is only through the hard work and dedication of people too numerous to acknowledge individually that has permitted the telling of this story. I have named individuals whose work I consider integral to our current understanding of the field, but I recognize that such credit is highly selective.

With regard to my own career, I recognize first and foremost Dr. Daniel W. Foster, M.D., with whom I began my work in Dallas some 33 years ago. Since that time, there have been numerous assistant professors, postdoctoral fellows, graduate students, technicians, summer interns, and administrative staff members who have contributed to the work of my laboratory: Sean Adams, Laurence Baillet, Michael Boyd, Britt Britton, Nicholas Brown, Lucia Carulli, Songyuan Chen, Petra Contreras, Murphy Daniels, Peter Declercq, Olivier Descamps, Robert Dobbins, Victoria Esser, Larry Hirsch, Jennifer Hodges, Masamichi Kuwajima, Mark Liao, Guy Mannaerts, Jürgen Meier, Scott Mills, Rashmi Mullur, Christopher Newgard, Carlos Robles-Valdes, Anjan Sen, Michael Stark, Daniel Stein, Todd Swanson, Lidia Szczepaniak, Yoichi Takabayashi, Jorge Tamarit, Brian Weis, Keith Woeltje, Bruce White, and Kittie Wyne.

I also thank all of my collaborators over the years, both at U.T. Southwestern in Dallas and at many institutions around the world. Science, like few other human endeavors, thrives on the open sharing of ideas. My interaction with my many colleagues around the globe has been a constant source of inspiration and insight. Of course, none of my own work or the work cited in this lecture would be possible without the generous support of funding agencies such as the National Institutes of Health, the American Diabetes Association, the Juvenile Diabetes Research Foundation, the Center for Diabetes Research, the Chilton Foundation, the Clifton and Betsy Robinson Foundation, the Forrest C. Lattner Foundation, Merck, Novo Nordisk, Sandoz Pharmaceuticals, Sigma-Tau, and numerous private research foundations and corporations.

Last, but most important, I recognize my wife Angela and my three children, Margaret, John, and Denise, who have stood beside me for so many years. Their love and support have been the foundation upon which rests all that I have accomplished.

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