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Disordered Fat Storage and Mobilization in the Pathogenesis of Insulin Resistance and Type 2 Diabetes

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The primary genetic, environmental, and metabolic factors responsible for causing insulin resistance and pancreatic β-cell failure and the precise sequence of events leading to the development of type 2 diabetes are not yet fully understood. Abnormalities of triglyceride storage and lipolysis in insulin-sensitive tissues are an early manifestation of conditions characterized by insulin resistance and are detectable before the development of postprandial or fasting hyperglycemia. Increased free fatty acid (FFA) flux from adipose tissue to nonadipose tissue, resulting from abnormalities of fat metabolism, participates in and amplifies many of the fundamental metabolic derangements that are characteristic of the insulin resistance syndrome and type 2 diabetes. It is also likely to play an important role in the progression from normal glucose tolerance to fasting hyperglycemia and conversion to frank type 2 diabetes in insulin resistant individuals. Adverse metabolic consequences of increased FFA flux, to be discussed in this review, are extremely wide ranging and include, but are not limited to: 1) dyslipidemia and hepatic steatosis, 2) impaired glucose metabolism and insulin sensitivity in muscle and liver, 3) diminished insulin clearance, aggravating peripheral tissue hyperinsulinemia, and 4) impaired pancreatic β-cell function. The precise biochemical mechanisms whereby fatty acids and cytosolic triglycerides exert their effects remain poorly understood. Recent studies, however, suggest that the sequence of events may be the following: in states of positive net energy balance, triglyceride accumulation in "fat-buffering" adipose tissue is limited by the development of adipose tissue insulin resistance. This results in diversion of energy substrates to nonadipose tissue, which in turn leads to a complex array of metabolic abnormalities characteristic of insulin-resistant states and type 2 diabetes. Recent evidence suggests that some of the biochemical mechanisms whereby glucose and fat exert adverse effects in insulin-sensitive and insulin-producing tissues are shared, thus implicating a diabetogenic role for energy excess as a whole. Although there is now evidence that weight loss through reduction of caloric intake and increase in physical activity can prevent the development of diabetes, it remains an open question as to whether specific modulation of fat metabolism will result in improvement in some or all of the above metabolic derangements or will prevent progression from insulin resistance syndrome to type 2 diabetes. (*Endocrine Reviews* 23: 201–229, 2002)

Abbreviations: ACC, Acetyl-CoA carboxylase, apo B, apolipoprotein B; ASP, acylation-stimulating protein; CoA, coenzyme A; CPT-1 carnitine palmitoyl-transferase-1; DAG diacylglycerol; DGAT, acyl coenzyme A:diacylglycerol acyltransferase; DI, disposition index; DNL, *de novo* lipogenesis; FABP, fatty acid binding protein; FAT, fatty acid translocase (transporter); FATP, fatty acid transport protein; GLUT, glucose transporter; GSIS, glucose-stimulated insulin secretion; HSL hormone-sensitive lipase; IKK-β, IκB kinase β; IMTG, intramyocellular triglyceride; iNOS, inducible nitric oxide; IRS, insulin-resistance syndrome; LCFA-CoA, long-chain fatty acyl CoA; LPL lipoprotein lipase; MTP, microsomal transfer protein; NAC, *N*-acetyl-L-cysteine; NADH dihydronicotinamide adenine dinucleotide; PFK-1 phosphofructokinase-1; ROS, reactive oxygen species; S_i insulin sensitivity index; SREBP-1, sterol-regulatory element-binding protein-1; UCP2, uncoupling protein 2; VLDL, very low density lipoprotein; ZDF, Zucker diabetic fatty.

I. Introduction

UPTAKE, SYNTHESIS, AND storage of adipose tissue triglycerides and mobilization of this energy source as FFA are processes that are highly regulated by genetic, nutritional, hormonal, and paracrine factors. In this review we will present evidence incriminating a net positive energy balance and disordered fat storage and mobilization as central factors in the pathogenesis of many of the metabolic features of the insulin resistance syndrome (IRS) and type 2 diabetes. We will begin by reviewing evidence that fat storage and mobilization from fat storage sites is abnormal at a very early stage in IRS. We will also critically examine the evidence for a role of abnormal fatty acid metabolism in skeletal muscle and intestinal absorption of fatty acids in IRS. We will then discuss the consequences of these abnormalities for hepatic lipoprotein production, insulin action in muscle and liver, insulin clearance, and pancreatic β-cell function. It is beyond the scope of this review to discuss the many other putative effects of fatty acids, such as those that have been described on endothelium, myocardium, carcinogenesis, and atherosclerosis, to mention a few. This theory in no way precludes an important role for peptides and hormones secreted by the adipose tissue that have been shown to link adiposity to insulin resistance and type 2 diabetes (1, 2).

II. Abnormalities of FFA Metabolism in Obesity, Insulin Resistance, and Type 2 Diabetes

Fasting plasma FFAs have generally been found to be elevated when examined in large, well-characterized populations of individuals with obesity, IRS (see Ref. 3 for definition), and type 2 diabetes (4, 5). Postprandial FFA levels may also be higher in obese, insulin-resistant individuals (6) and in subjects with type 2 diabetes (7, 8). Prospective epidemiological studies have suggested that elevated plasma FFA is an independent predictor of progression to type 2 diabetes in Caucasians and Pima Indians (9–11). Although some studies did not find elevation of fasting plasma FFA in first-degree relatives of patients with type 2 diabetes (12, 13), other studies have shown that elevated fasting plasma FFA correlated with low insulin-mediated glucose disposal in these individuals (14, 15).

Plasma FFA concentration reflects a balance between release (from the intravascular lipolysis of triglyceride-rich lipoproteins and lipolysis of adipose tissue triglyceride stores) and uptake (predominantly re-esterified in adipose tissue and liver and oxidized in muscle, heart, liver, and other tissues). In the postabsorptive state, the systemic FFA concentration is determined largely by the rate of FFA entry into the circulation, but postprandially, the rate of uptake, particularly by adipose tissue, is also a critical determinant of plasma FFA concentration.

A. Hormone-sensitive lipase (HSL) and insulin suppression of lipolysis

Because insulin has a potent suppressive effect on HSL, the enzyme which is the principal regulator of FFA release from adipose tissue, there has been an intense focus on determining whether resistance of HSL to insulin in IRS and type 2 diabetes is the predominant abnormality accounting for increased flux of FFAs from adipose tissue in these conditions.

A number of *in vitro* studies have failed to demonstrate increased HSL and basal lipolytic rate in adipose tissue from obese individuals (16) or resistance to insulin's suppressive effect on HSL (Refs. 16 and 17 and reviewed in Ref. 18). There has been some confusion in the literature because of differences in the denominator used to ascertain the true lipolysis rate (*i.e.*, in relation to fat cell number, per unit lipid weight or cell surface area; reviewed in Ref. 18). Some studies have actually shown that the sensitivity or maximum insulin-induced inhibition of adipose tissue lipolysis was greater in obese subjects than in normal weight controls (17, 19).

In vivo, the rate of FFA turnover per unit of lean body weight does appear to be elevated in obese individuals (20–28). A number of studies have shown a diminished suppressive effect of insulin on FFA rate of appearance in obese and nonobese insulin-resistant humans (20, 21, 29) and in those with type 2 diabetes (25, 30). Resistance to insulin's suppressive effect on HSL also appears to be present postprandially in IRS and type 2 diabetes (6). Confusion regarding whether HSL activity in individual adipocytes is actually resistant to the suppressive effect of insulin arises because, when normalized per total body fat, lipolysis appears in fact to be normal or reduced in obese individuals (20, 28, 31, 32). In other words, the diminished whole-body insulin-suppressive effect on FFA rate of appearance seen in obese individuals may be largely due to a mass effect of the overall expansion of body fat depots.

A reduction in insulin-mediated suppression of fasting lipolysis *vs.* control subjects of the same age and weight has been found in most (13, 33, 34), but not in all (34), studies performed in glucose-tolerant first-degree relatives of patients with type 2 diabetes. This suggests that abnormal insulin-mediated suppression of plasma FFA appearance rate is a very early defect in those genetically predisposed to develop type 2 diabetes.

B. Adipose tissue uptake and intracellular esterification of fatty acids (see Fig. 1)

Although insulin plays an important role in the suppression of HSL, an additional major mechanism of insulin action is in stimulating postprandial glucose uptake and FFA esterification (2, 6, 20, 35–42). Riemens *et al.* (36) recently challenged the notion that the elevated FFA transport rate during fasting in patients with type 2 diabetes is due to impaired insulin-mediated suppression of HSL activity and suggested that the main abnormality is rather an elevated rate of escape of FFA from esterification in adipose tissue. We have shown previously that postprandial fatty acids become markedly elevated in type 1 diabetic subjects after ingestion of a high-fat meal when insulin is underreplaced in the periprandial period, emphasizing the important role that insulin plays in postprandial fatty acid disposal (43). Although the adipose tissue in lean individuals can switch from a negative to a positive FFA balance during the transition from fasting to the postprandial state, the adipose tissue FFA balance remains negative postprandially in insulin-resistant obese individuals, despite the presence of hyperinsulinemia (41). Lean, glucose-tolerant first-degree relatives of patients with type 2 diabetes have an increase in postprandial glucose and triglyceride excursion and less suppression of plasma FFA after a mixed meal compared with matched control subjects without a family history of diabetes (12). The precise mechanisms causing this postprandial elevation of plasma FFA are not known.

FFA esterification in fat cells is dependent on the supply of glycerol-3-phosphate derived from insulin-mediated glucose uptake and glycolysis in the adipocyte (Fig. 1), and insulin-mediated glucose uptake is diminished in insulin resistance (2). Less is known about direct insulin-stimulatory effects on esterification enzymes, and it is not entirely clear whether insulin directly stimulates the enzyme that catalyzes the final step in triglyceride synthesis, acyl coenzyme A:diacylglycerol acyltransferase [DGAT (44, 45)]. Although insulin directly stimulates enzymes responsible for *de novo* fatty acid synthesis, d*e novo* fatty acid synthesis in the adipocyte is quantitatively insignificant in normal physiological conditions.

The activity of lipoprotein lipase (LPL) is an important first step in plasma triglyceride clearance and FFA delivery to the adipocyte, particularly in the postprandial state (46). Insulin and glucose have been shown to stimulate adipose tissue LPL activity and to reduce LPL activity in muscle, implying a preferential postprandial partitioning of lipoprotein-derived fatty acids toward adipose tissue and away from muscle (47). In obesity and type 2 diabetes, insulin activation of LPL in adipose tissue is delayed, and LPL activity in skeletal muscle is increased instead of decreased by hyperinsulinemia (48, 49). The importance of LPL in tissue FFA uptake has recently been demonstrated by experiments in

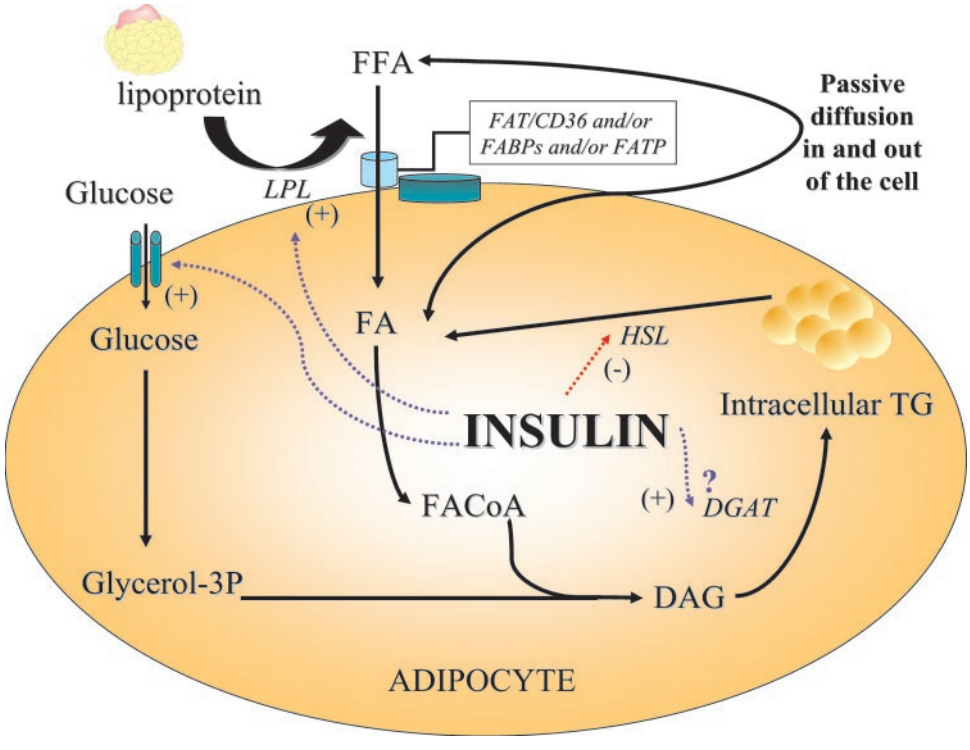

FIG. 1. Adipocyte: role of insulin in the stimulation of adipose tissue fatty acid uptake, esterification, and storage. *Solid lines* indicate flux of metabolic substrates, and *dashed lines* indicate stimulatory or inhibitory effects of insulin. + Indicates a stimulatory effect of insulin, and - indicates an inhibitory effect of insulin. Insulin promotes FFA uptake into the adipocyte by stimulating the LPL-mediated release of FFA from lipoprotein triglyceride. Fatty acids enter the adipocyte both by diffusion down a concentration gradient as well as by facilitated transport. Insulin regulation of fatty acid transporters such as FAT/CD36, FABPs, and/or FATP is not known. Insulin stimulates glucose transport into the adipocyte, thereby increasing the availability of glycerol-3 phosphate (Glycerol-3P) for triglyceride (TG) synthesis. Insulin may have a direct stimulatory effect on lipogenic enzymes such as DGAT. By inhibiting HSL, it reduces the intracellular lipolysis of cytosolic triglycerides, thereby promoting adipocyte triglyceride storage.

which either muscle-specific or liver-specific overexpression in mice induces marked tissue lipid accumulation (50). Although LPL may be viewed as a first step leading to the uptake of FFA by adipose tissue, it is clear that the deposition of FFA is also regulated downstream of LPL (46).

We are only beginning to elucidate the mechanisms of fatty acid transporter regulation in IRS and type 2 diabetes, and there is still considerable controversy as to whether the cellular uptake of fatty acids occurs predominantly by facilitated transmembrane transport or by passive diffusion (for reviews, see Refs. 51–53). The "scavenger" receptor CD36 has recently been identified as a fatty acid receptor/transporter (54), with particular abundance in adipose tissue, heart, and skeletal muscle but with low expression in kidney and liver (53). A deficiency in CD36, a protein analogous to CD36/fatty acid transporter (FAT) in humans, has been reported to underlie the metabolic abnormalities of the insulin-resistant spontaneously hypertensive rat (55, 56) and has been associated with functionally significant impairment of intracellular FFA transport (57, 58). Furthermore, transgenic expression of CD36 in the spontaneously hypertensive rat ameliorates insulin resistance and lowers serum fatty acids (59), perhaps by improving FFA uptake in adipose tissue. Mice with CD36 overexpression targeted to muscle tissue (MCK/CD36) have less body fat and lower serum FFAs and very low density lipoprotein (VLDL) triglycerides but elevated plasma glucose and insulin, suggesting that they are insulin resistant (60). One may speculate that the increased FFA uptake and oxidation in muscle tissues of these animals impairs muscle glucose utilization, thereby inducing insulin resistance in a fashion analogous to that seen in mice with muscle-specific LPL overexpression (50). In contrast, the uptake of fatty acids by heart, skeletal muscle, and adipose tissues from CD36-null mice is markedly reduced (by 50–80%), whereas that of glucose is increased severalfold (61). CD36 deficiency is present in 2–3% of the Japanese population, and recent evidence suggests that it may be associated with insulin resistance, dyslipidemia (62), and absence of myocardial uptake of FFA tracers *in vivo* (63). An association between IRS or diabetes and mutations in CD36 has not yet, however, been reported in other human populations. At the present time, the link between CD36 deficiency and the development of insulin resistance in humans cannot be incorporated into a consistent model due to our lack of knowledge regarding the functional consequence of CD36 deficiency on FFA metabolism in the various tissues *in vivo*. Animal models of obesity, insulin resistance, and type 2 diabetes are generally characterized by an increase, not a decrease, in adipose tissue fatty acid binding and transport proteins (64). Furthermore, marked compensation of other functionally redundant proteins can occur, which could limit the physiological impact of any deletion or defect of fatty acid binding proteins in adipose tissue (65). To date, there has been no demonstrated defect in adipose tissue fatty acid uptake caused by a defect in any of the FFA transport or binding proteins in humans (66).

The production of acylation stimulating protein (ASP), a proteolytic cleavage product of the third component of complement, is stimulated by hydrolyzed chylomicrons and is an important regulator of adipocyte fatty acid esterification by increasing the activity of diacylglycerol acyltransferase through a PKC-dependent pathway (35). There is controversy in the literature regarding the physiological importance of ASP, because some (67) but not others (68) have described abnormalities of postprandial lipoprotein metabolism in ASP-null mice. Although a blunted response to ASP in IRS and type 2 diabetes cannot as yet be ruled out, evidence in support of such a defect is currently lacking. ASP levels are increased in obesity (69), and adipocytes from obese humans remain responsive to ASP (70).

C. Total fat mass and regional fat depots

Aside from putative intrinsic abnormalities in adipocytes, these two other factors have important bearing on adipose tissue fat storage and release in IRS and type 2 diabetes. Firstly, because the pool of FFAs in adipocytes is released into the circulation in relation to its size, the greater overall fat mass of adipose tissue in obese individuals will result in an elevation of fatty acid flux to nonadipose tissues, even in the absence of a qualitative abnormality in adipose tissue metabolism (31). Secondly, there is an undisputed relationship between "central" fat distribution (*i.e.*, fat in the visceral and sc abdominal region) and features of the IRS (71), although the causal nature of this relationship (72, 73) and the relative importance of visceral *vs.* sc abdominal fat remains a matter of debate (72, 74, 75). Visceral fat cells are more sensitive than sc fat cells to the lipolytic effect of catecholamines and less sensitive to the antilipolytic and fatty acid re-esterification effect of insulin (reviewed in Ref. 2), a phenomenon which could further enhance FFA flux in those who are predisposed to store fat in the visceral area. Furthermore, the venous effluent of visceral fat depots leads directly into the portal vein, resulting in greater FFA flux to the liver in viscerally obese individuals than in those with predominantly sc obesity. Although visceral fat depots have been estimated to represent only approximately 20% of total body fat mass in men and 6% in women (76, 77), approximately 80% of hepatic blood supply is derived from the portal vein (78). Furthermore, total splanchnic blood supply increases postprandially (79) as might the proportion of lipolysis from splanchnic *vs.* sc fat (because of increased insulin and sympathetic activation after meals). Thus, the contribution of visceral fat to hepatic FFA uptake and systemic FFA appearance could be more substantial in the postprandial than in the fasting state.

D. Fat diversion from adipose to nonadipose tissue

The net result of increased FFA lipolysis and diminished FFA fractional esterification in IRS and type 2 diabetes is diversion of FFAs toward nonadipose tissues such as liver (Fig. 2), muscle, heart, and pancreatic β-cells. Extreme examples, at opposite ends of the spectrum, of adipose tissue capacity to take up and store incoming fatty acids are illustrated by the clinical conditions of congenital lipoatrophy and massive obesity. In humans (80) and animal models of lipoatrophy (81–83), in which there is absence of adipose tissue, nonadipose tissues accumulate cytosolic triglycerides to a massive extent and manifest many of the consequences

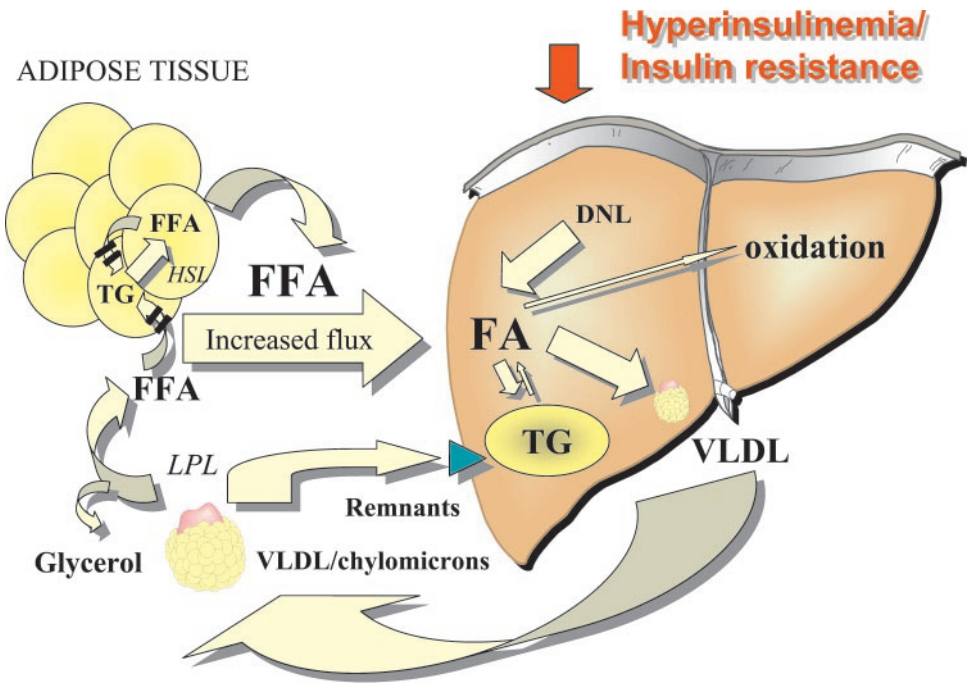

FIG. 2. Role of fatty acids in overproduction of hepatic VLDL and fatty liver infiltration. In insulin resistance and type 2 diabetes, there is defective esterification and re-esterification of fatty acids in adipose tissue, as well as possibly reduced insulin-mediated suppression of HSL, the rate-limiting enzyme for adipose tissue triglyceride mobilization. Fatty acid flux from adipose tissue is elevated in these conditions, and FFAs released by lipolysis of plasma triglyceride-rich lipoproteins (VLDL and chylomicrons) are diverted from adipose tissue to other organs, where they can exert their deleterious effects. Increased FFA flux to the liver in IRS and type 2 diabetes increases the hepatocyte fatty acid pool size. In the presence of hepatic hyperinsulinemia/insulin resistance, hepatic DNL is increased and esterification of incoming fatty acids is relatively favored over oxidation. Esterified fatty acids are either stored as cytosolic triglycerides (TG) or directed toward VLDL synthesis. The majority of fatty acids released from the cytosolic triglyceride stores are re-esterified and recycled to the cytosol or secreted in VLDL. A high VLDL production rate raises the plasma VLDL concentration, as well as the concentration of intestinally derived chylomicrons because of competition for removal between chylomicrons and VLDL. High plasma concentrations of triglyceride-rich lipoproteins (VLDL and chylomicrons) lead to an increase in the release of FFAs and generation of remnants as a result of lipolysis by LPL. FFAs and remnants of triglyceride-rich lipoproteins contribute to increase the hepatocyte fatty acid pool, thereby setting up a vicious cycle and further driving VLDL production.

of extreme insulin resistance. Furthermore, all aspects of the fatless mouse phenotype are alleviated in a dose-response fashion with surgical implantation of adipose tissue (81). Based on his studies with animal models of lipodystrophy, Shulman (84) has recently proposed that insulin resistance develops because of an imbalance of fat distribution between tissues. Consistent with this hypothesis is the observation that some massively obese individuals have surprisingly few manifestations of the IRS (85, 86). Normoglycemic and normolipidemic obese individuals display improved postprandial fat storage compared with lean subjects (87). Individuals with morbid obesity (body mass index > 40 kg/m^2) have been recently shown to display a greater meal-derived storage capacity than weight- and age-matched subjects after successful gastric bypass surgery, despite a slightly lower postprandial plasma insulin response (88). Presumably, the more efficient adipose tissue fat-storing capacity in these individuals could confer relative protection against lipotoxicity in nonadipose tissues.

There appears to be a reciprocal channeling of fuels between muscle and fat when one or the other tissue becomes preferentially insulin resistant. For example, there is preferential channeling of energy fuels toward fat rather than muscles during fat infusion in Zucker rats, related to down-regulation of muscle and simultaneous up-regulation of adipose tissue transporters and genes involved in glucose and fatty acid uptake and disposal (89). Similarly, mice with targeted disruption of glucose transporter (GLUT)4 in muscle and consequent muscle insulin resistance have a redistribution of substrate from muscle to adipose tissue (90). The converse also appears to be true, as down-regulation of GLUT4 and glucose transport selectively in adipose tissue has recently been shown to cause insulin resistance in muscle (91), perhaps by diverting FFAs and other fuels from adipose to nonadipose tissues, although the mechanism is not currently known. Ob/Ob mice lacking aP2, an adipose tissue fatty acid binding protein, have reduced adipose tissue lipolysis and increased adipose tissue mass, together with paradoxical reduction in plasma lipids and improvement in insulin sensitivity and insulin secretion (92), suggesting that enlargement of adipose tissue mass may protect against insulin resistance and diabetes in some circumstances. This concept of adipose tissue acting as a sink to protect other tissues from the toxic effects of excessive exposure to energy substrates is further supported by the finding that overexpression of GLUT4 in adipose tissue in mice is associated with an increase in adipose tissue mass and improved whole-body insulin sensitivity (93, 94). It is likely that the majority of individuals who fall along the spectrum between lipodystrophy and massive obesity have a genetically deter-

mined set point at which adaptive adipose tissue insulin resistance limits further adipose tissue fat accumulation, with consequent spillover of fat to nonadipose tissues (Fig. 3).

E. Abnormal fatty acid metabolism in skeletal muscle

Intramyocellular triglyceride (IMTG) accumulation has been associated with muscle insulin resistance in humans (95–98). IMTG is also elevated in lean, glucose-tolerant offspring of two parents with type 2 diabetes mellitus compared with individuals without a family history of diabetes and is associated with lower glucose disposal (14). Somewhat paradoxically, however, triglycerides have also been shown to accumulate in the muscle tissue of highly physically trained athletes (99). As pointed out in a recent review on this topic by Kelley and Goodpaster (100), muscle triglyceride may not have adverse metabolic consequences in muscle that has the capacity for efficient lipid utilization.

The mechanism accounting for the relationship between muscle triglyceride accumulation and insulin resistance is not known. It remains an open question as to whether muscle triglyceride accumulation is merely a marker or plays a causative role in the insulin resistance. A key issue is whether triglycerides accumulate in muscle tissue of insulin-resistant individuals as a result of a primary defect in fatty acid oxidation, increased total FFA flux to muscle, or due to an imbalance between FFA uptake, esterification, triglyceride lipolysis, and fatty acid oxidation. Muscle from obese, insulin-resistant individuals and type 2 diabetic patients has been shown to have reduced capacity for uptake and oxidation of fatty acids derived from the plasma FFA pool during fasting and exercise (101–105). These changes could perhaps be attributed to defects of fatty acid oxidation at the carnitine palmitoyl-transferase-1 (CPT-1) and post-CPT-1 levels (106). Furthermore, weight reduction using low-calorie diets in patients with type 2 diabetes has been shown to reduce plasma FFA flux during fasting but not exercise, without significant change in plasma-derived FFA oxidation or muscle mitochondrial oxidative enzymes (102, 107). Prolonged pharmacological inhibition of muscle CPT-1 in rats has also been associated with IMTG accumulation and development of insulin resistance (108). These findings have been interpreted to suggest that impaired muscle fatty acid oxidation is the primary defect causing the IMTG accumulation and muscle insulin resistance in patients with obesity, IRS, and type 2 diabetes (100). Impaired muscle FFA oxidation in these conditions could also be the result of excessive chronic exposure to FFA, because the elevation of malonyl-coenzyme A (CoA) due to energy excess has been associated with reduced muscle fat oxidation through inhibition of CPT-1 (109, 110). It should be pointed out that the reduction of plasma-derived FFA oxidation seen in patients with obesity and diabetes has been shown by some to occur in association with unaltered or even elevated total fat oxidation and with elevated muscle triglyceride lipolysis (104, 105). Thus, while the capacity for fat oxidation appears to be reduced, total fat

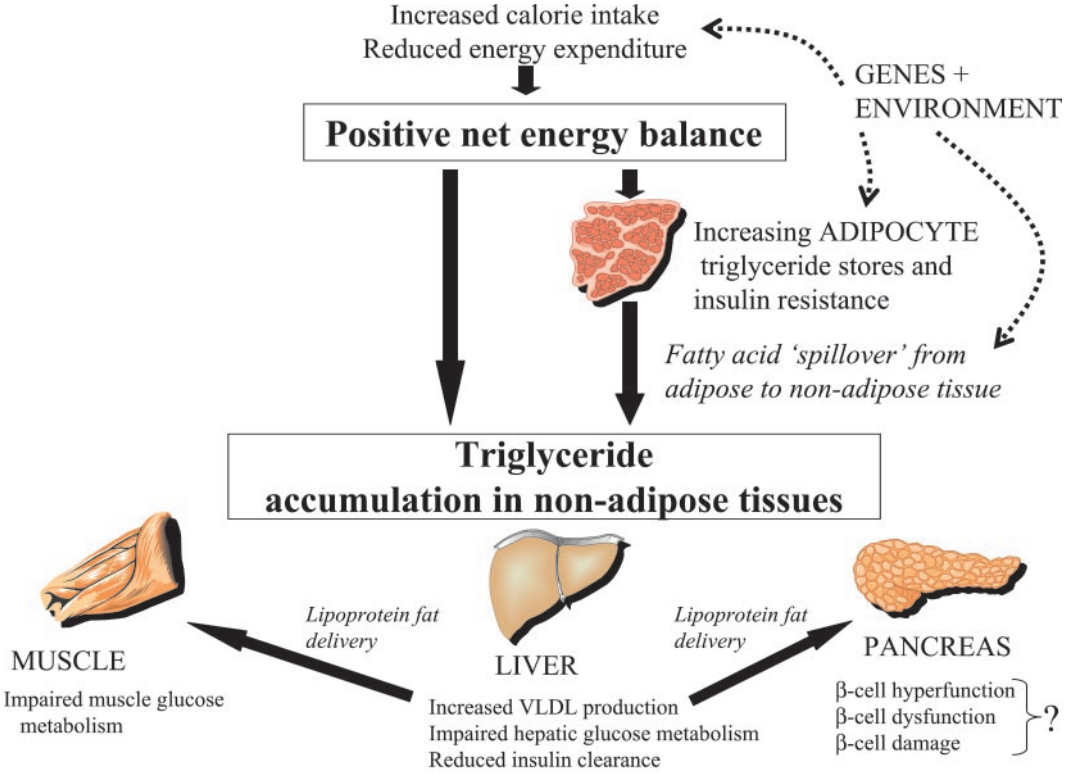

FIG. 3. Positive net energy balance exceeds the buffering capacity of adipose tissue, leading to glucolipotoxicity. Positive net energy balance, resulting from increased calorie intake and reduced energy expenditure, leads to an accumulation of triglyceride in many tissues, particularly in adipose tissue. The accumulation of triglyceride in adipose tissue leads to increased lipolysis by a mass effect. This, associated with the development of adipocyte insulin resistance, results in net spillover of fatty acids to nonadipose tissue, which further increases extraadipocytic triglyceride storage, leading to many of the typical features that characterize the insulin-resistant state and type 2 diabetes.

oxidation may be increased because of the mass action effect of increased FFA delivery from plasma and from increased intracellular triglyceride stores. Furthermore, net fat oxidation is not reduced in obese individuals in response to elevation of plasma FFAs using iv infusion of heparin and lipid emulsion (111). In addition, weight loss secondary to fat malabsorption after bariatric surgery in morbidly obese individuals corrects both the low respiratory quotient and insulin resistance seen in these individuals, suggesting that improvement in insulin resistance with correction of obesity is associated with reduction of lipid oxidation relative to carbohydrate oxidation (112). Because plasma FFA delivery itself, as well as glucose delivery and plasma insulin levels, may determine the rate of muscle FFA oxidation (113–116), these factors should also be carefully controlled for in *in vivo* experiments before drawing any conclusion regarding the presence of a primordial defect in muscle FFA oxidation in patients at risk for or with established type 2 diabetes.

Skeletal muscle has a high fractional extraction of FFAs in the postabsorptive state, and lipid oxidation accounts for the majority of its energy production (100). Some studies in humans have suggested that muscle fatty acid binding and transport proteins may be altered in obesity and type 2 diabetes. Skeletal muscle cytoplasmic fatty acid binding protein (FABP) content has been shown to be reduced together with reduced *in vivo* muscle plasma FFA uptake and oxidation in obese type 2 diabetic patients (105), but not in glucose-tolerant obese subjects (102). As mentioned above in the discussion of CD36, muscle-specific overexpression of the CD36/FAT is associated with insulin resistance (60). The skeletal muscle expression of another FAT protein, FATP-1, was found to be reduced in obese women with or without type 2 diabetes, but not in men (51, 66, 117), and their potential role in intramyocellular triglyceride accumulation and insulin resistance remains unclear. Despite the fact that the efficiency of skeletal muscle FFA uptake and utilization in the postabsorptive state has been shown to be impaired in obese patients with type 2 diabetes (118, 119) and in nondiabetic individuals with visceral obesity (120), we need to be cautious in interpreting this observation to mean that total 24-h fatty acid flux to muscle is reduced in insulin resistance and type 2 diabetes. Experimental evidence suggests that excessive FFA delivery to muscle from the circulation can be a source of muscle triglyceride accumulation (121–124). An extramuscular defect of fatty acid metabolism could contribute to the intramyocellular triglyceride accumulation and the skeletal muscle lipotoxic effects seen in obesity and type 2 diabetes.

F. Potential abnormalities in intestinal fatty acid uptake

It has been proposed that gain-of-function mutations of FABP-2, a FABP highly expressed in the small intestine, could result in postprandial lipid abnormalities, insulin resistance, and diabetes (125). A common polymorphism of the intestinal FABP2 gene (A54T) that results in higher affinity of FABP2 for long-chain fatty acids *in vitro* has been associated with an increased prevalence of insulin resistance or diabetes in some populations (126–129) but not in others (130–134). *In vivo*, this polymorphism has been inconsistently associated with increased total body fat oxidation and a small elevation of plasma FFA levels in different populations (126, 135, 136). The association with higher postprandial triglyceride and lipoprotein excursion has also been found in some (136, 137) but not all studies (134, 135). Although increased intestinal absorption of FFA has been postulated to be the cause of these abnormalities, this has not yet been convincingly demonstrated in humans (138). It is therefore likely that A54T polymorphism of the FABP2 gene could play some role in abnormal FFA metabolism and be linked with the development of insulin resistance and type 2 diabetes by an unknown mechanism in some populations, such as the Pima Indians, but not in others.

G. Protective role of leptin and adiponectin against lipotoxicity

Unger and colleagues (139–142) have proposed that the physiological role of the hyperleptinemia that accompanies caloric excess is to protect nonadipocytes from steatosis and lipotoxicity by preventing up-regulation of lipogenesis and by increasing fatty acid oxidation. These researchers argue convincingly against the conventional view that the physiological role of leptin is to prevent obesity during overnutrition. Leptin has been shown to be antilipogenic in some tissues (143) and up-regulates fatty acid oxidation (144). Leptin-deficiency states, including lipodystrophic syndromes, are associated with massive nonadipose tissue fat accumulation due to increased lipogenesis and reduced fatty acid oxidation (145), with adverse consequences of nonadipose tissue lipid overaccumulation. Adenoviral-mediated leptin overexpression in normal rats is antilipogenic and up-regulates β-oxidation (144). Transgenic overexpression of leptin rescues the insulin resistance and diabetes in a mouse model of lipoatrophic diabetes (146).

In humans, hyperleptinemia characterizes obesity, insulin-resistant states, and type 2 diabetes, suggesting that leptin resistance, not leptin deficiency, may be involved in the pathophysiology (147). The reduction of plasma leptin concentration after bariatric surgery in morbidly obese individuals occurs independently of the reduction of fat mass but correlates with the reduction of plasma insulin levels, suggesting that resistance to leptin and insulin are closely linked in humans (148). Although leptin resistance could play a role in extra-adipose tissue fat deposition and lipotoxicity, it could also be a consequence of elevated fatty acid availability to tissues (149, 150). Elevated plasma FFA could lead to relative suppression of leptin release by the adipose tissue, contributing to impaired leptin signaling in insulin-resistant states (151). Therefore, hyperleptinemia/leptin resistance may also be a consequence of abnormal FFA partitioning. Nevertheless, the important role of leptin in regulating rates of lipogenesis and fatty acid oxidation illustrates that factors in addition to fat spillover from adipose to nonadipose tissues may regulate the magnitude of triglyceride accumulation in nonadipose tissues in states of caloric overload.

Recently the adipocyte-derived hormone adiponectin has been shown to reverse insulin resistance associated with both lipoatrophy and obesity (152). Decreased expression of adiponectin was shown to correlate with insulin resistance in

mouse models of insulin resistance. Insulin resistance in lipoatrophic mice was completely reversed by the combination of physiological doses of adiponectin and leptin, but only partially by either adiponectin or leptin alone. Adiponectin reduced the triglyceride content of muscle and liver in obese mice by increasing the expression of fatty acid oxidation and energy dissipation in muscle.

H. Summary of the abnormalities of FFA metabolism in obesity, IRS, and type 2 diabetes

Adipose tissue storage, release of fatty acids, and its control by insulin are grossly abnormal in IRS well before the development of type 2 diabetes. In the postabsorptive period, basal lipolysis is elevated and suppression by insulin diminished. In the postprandial period, there is likely to be a net diversion of fat away from adipose tissue depots and toward nonadipose tissues. FFA efflux from an enlarged and lipolytically active visceral fat depot plays a major role in the elevation of fatty acids, which are then free to exert their biological effects in nonadipose tissues.

A high capacity for efficient triglyceride accumulation in adipose as well as nonadipose tissue may have presented a survival advantage in the past, during times of starvation, thus accounting for selection of a "thrifty genotype" as originally proposed by Neel (153) in 1962. This phenotype is hypothesized to be characterized by low oxidative or fat oxidative capacity and a tendency toward a positive energy balance (154). With current high-calorie, high-fat diets and sedentary lifestyle, such a thrifty genotype would accumulate excess tissue triglyceride stores, despite resistance to glucose disposal (155). As indicated in Fig. 3, in the presence of a positive net energy balance there is ongoing accumulation of triglyceride in both adipose and nonadipose tissues. In addition, adipose cells could adaptively limit further fat accumulation by becoming insulin resistant, thereby diverting fat to nonadipose tissues. Perhaps a corollary of the thrifty genotype theory is that those whose adipocytes are able to most effectively protect themselves against ongoing caloric overload, *i.e.*, by developing resistance to insulin's anabolic effects, are also those most likely to develop extra-adipocyte fat overload, with consequent metabolic manifestations of insulin resistance. Perhaps the accumulation of adipose tissue represented an evolutionary disadvantage to those engaged in hunter-gatherer lifestyles. Cytosolic triglyceride accumulation in nonadipose tissues such as muscle and liver is linked to the development of insulin resistance as these tissues also attempt to protect themselves from energy overload. Insulin resistance imposes a chronic stress on pancreatic β-cells, which may fail to hypersecrete insulin, as the same mechanisms that lead to insulin resistance may ultimately result in β-cell dysfunction and damage (see Fig. 3 and *Section VII*).

III. Dyslipidemia and Fatty Liver Infiltration in IRS and Type 2 Diabetes

The hypertriglyceridemia of IRS and type 2 diabetes is primarily due to VLDL overproduction, with reduced VLDL clearance playing a role in some instances, particularly when there is marked insulin deficiency or poor glycemic control in type 2 diabetes (156). Some of the other prominent features of the dyslipidemia of IRS and type 2 diabetes, such as low high density lipoprotein cholesterol and small, dense LDL particles, may be secondary to VLDL overproduction, as we have previously reviewed (157, 158). Hepatic VLDL production is primarily substrate driven, with the most important regulatory substrates being FFAs (159). FFAs are taken up by the liver in proportion to their delivery rate (160, 161). Hepatic fractional extraction of FFA is high (20–30%; Refs. 161–163) and does not appear to be a primary site of hormonal regulation, although it was reported to be reduced after a glucose load (163–165) and to be increased under conditions of increased hepatic FFA oxidation such as exercise (161) and prolonged starvation (166). Hepatic fractional extraction of FFA, however, is not affected in type 1 diabetic patients (161) or in depancreatized dogs (167). In the liver, depending on the nutritional and hormonal state of the organism, fatty acids are either predominantly oxidized or are esterified to form triglycerides, which are then either stored in the cytosol or secreted in VLDL.

The production rate of apolipoprotein B (apo B) is an important regulatory step in VLDL production, but the apo B transcription and translation rate does not regulate the pathway under most physiological conditions (168–170). Regulation of apo B occurs primarily at the posttranslational level, either during its translocation into the endoplasmic reticulum lumen or its rate of degradation. Protection against proteolysis is critically dependent on neutral lipid availability and is facilitated by a number of chaperone proteins and microsomal transfer protein [MTP (168)]. MTP catalyzes the transfer of lipids to the apo B molecule and is an important factor involved in the assembly of apo B-containing lipoproteins (171, 172). Primary rat hepatocytes, incubated *in vitro* with high concentrations of insulin for 3 d, no longer respond to insulin suppression of VLDL apo B secretion and secrete higher basal levels of VLDL apo B (173).

FFAs have been shown to directly stimulate hepatocyte VLDL triglyceride synthesis and secretion in HepG2 cells (174–180) and cultured hepatocytes (181, 182). Although it is generally believed that the rate of apo B secretion is determined by the extent of its intracellular degradation, several studies have shown that protection from degradation is insufficient to drive apo B secretion in the absence of available core lipoprotein lipids. Addition of oleate can rescue the protected apo B polypeptides and induce their lipidation and extracellular secretion in some but not all model systems. It has also been previously suggested that oleate treatment of HepG2 cells facilitates translocation of newly synthesized apo B across the endoplasmic reticulum membrane, which in turn reduces early degradation (183). However, whether or not this protection of early degradation stimulates apo B extracellular secretion appears to differ among cell types. In HepG2 cells (176, 179, 184), a rat hepatoma cell line (180), and freshly isolated rabbit hepatocytes (185), exogenous oleate significantly stimulates apo B secretion. In contrast, this is not the case in McArdle H7777 cells (186) and primary rat (187, 188), hamster (189), or human (190) hepatocytes, although oleate may increase the stability of apo B. Overall, the effect of oleate on the stability and secretion of apo B appears to be

dependent on the cell type (primary *vs.* transformed cell line), turnover of the triglyceride/fatty acid in the cells, size of the cellular triglyceride pool, and duration of incubation with oleate.

Because FFAs entering the hepatocyte are predominantly re-esterified and enter a cytoplasmic pool before secretion in VLDL, the size of the cytoplasmic triglyceride pool, rather than the availability of extracellular oleic acid, correlates with VLDL secretion (181). Although it is well recognized that plasma FFAs stimulate VLDL production (191) and are an important source of VLDL triglyceride fatty acids (192–195), an important contribution to the hepatocyte fatty acid pool also comes from three sources other than plasma FFAs: 1) *de novo* lipogenesis (DNL), 2) cytoplasmic triglyceride stores, and 3) intracellular lipolysis of lipoproteins taken up directly by the liver (Fig. 2). Hepatic re-esterification of plasma FFAs contributes the majority of fatty acids to VLDL triglycerides (195), with sources such as DNL, hepatic triglyceride stores, and lipoprotein remnants contributing somewhat less (194, 195). The contribution to VLDL triglycerides from plasma FFAs is lower in hypertriglyceridemic than in normotriglyceridemic individuals (195).

A. The contribution of de novo lipogenesis to elevated VLDL production

Chronic hyperinsulinemia and carbohydrate ingestion stimulate the production of newly synthesized fatty acids [DNL (196–199)], by stimulating the activity of lipogenic enzymes in the liver (200) and by increasing the transcription of the genes for fatty acid synthase and acetyl-coenzyme A carboxylase [ACC (170, 201)]. Recent studies suggest that the mechanism by which insulin and perhaps glucose stimulate transcription of these lipogenic enzymes is by increasing transcription of sterol-regulatory element-binding protein-1c (SREBP-1c), a member of a family of regulated transcription factors (202). Despite down-regulation of the IRS-2-mediated insulin signaling pathway in insulin-resistant states, there appears to be up-regulation of SREBP-1c and chronic stimulation of DNL (and reduced fatty acid oxidation) in the liver (203, 204), which can in turn enhance intracellular availability of triglyceride, promoting fatty liver and driving VLDL assembly and secretion.

Under nonstimulated conditions, the contribution of DNL to VLDL triglyceride fatty acid is exceedingly small, estimated to be less than 5% in the postabsorptive state (194, 195, 205). Even with carbohydrate feeding, which usually stimulates DNL, newly synthesized fatty acids account for the minority of VLDL-triglyceride fatty acids (195, 206–209). Nevertheless, even though DNL may not be a quantitatively significant contributor to VLDL triglyceride production, it appears to be an important marker of the relative rate of fatty acid re-esterification *vs.* oxidation (206), and there is a well-established correlation between the rates of DNL and the secretion of VLDL (210). Elevation of malonyl-CoA, which is the product of acetyl-CoA carboxylase, the rate-limiting enzyme in hepatic DNL, inhibits CPT-1 activity, thus resulting in diversion of fatty acids from an oxidative to a re-esterification pathway (211, 212). Conditions associated with high rates of DNL, such as high carbohydrate ingestion, hyperglycemia, and hyperinsulinemia, are invariably associated with a shift in cellular metabolism from lipid oxidation to triglyceride esterification, increasing the availability of liver triglyceride for VLDL synthesis and secretion. In accordance with the notion that the total capacity to secrete VLDL correlates with the rate of DNL (213, 214), hyperglycemia in the presence of constant FFA availability increases VLDL production in humans (215).

B. Contribution of hepatic cytosolic triglyceride stores to VLDL overproduction and fatty liver infiltration (nonalcoholic steatohepatitis)

There is debate about the quantitative contribution of cytosolic triglyceride stores to VLDL triglyceride production, but it does appear that the majority of FFAs esterified upon entering the hepatocyte enter this storage pool, at least temporarily, before their incorporation into VLDL (216–220). This intracellular triglyceride storage depot likely serves as a buffer, providing temporary disposal of potentially toxic FFA when their delivery to the liver exceeds its oxidative and VLDL secretory capacity. It also provides a means of regulating VLDL production in the face of widely fluctuating plasma FFA concentrations. Stored triglyceride turns over fairly rapidly, but only a minor proportion of the released fatty acids are used for VLDL assembly, the remainder being recycled back into the storage pool (216). Fatty acids released from lipolysis of stored triglycerides appear to be preferentially channeled into re-esterification rather than oxidative pathways (216). The cytosolic triglyceride droplets are not incorporated into VLDL *en bloc* across the endoplasmic reticulum membrane, but first have to be hydrolyzed (216). Hydrolysis of cytosolic triglycerides appears to be partial, to the level of diacylglycerol, followed by remodeling of some of its acyl chains, before re-esterification to form secretory triglyceride (221). Hydrolysis may also proceed to monoglyceride and FFA. The lipase involved in this process and the details of its regulation are not yet known (216). The partitioning of re-esterified triglycerides between secretory and storage (cytosolic) pathways can be acutely regulated and is a potentially important site for the regulation of VLDL secretion (221, 222). Secretion of the esterified fatty acids as VLDL triglycerides is limited by the availability of a number of factors other than the hepatocyte triglyceride pool size *per se*, including cholesteryl esters, apo B synthesis and translocation across the endoplasmic reticulum membrane, phospholipids, rate-limiting enzymes such as MTP, *etc.*

Fatty liver frequently coexists with obesity, type 2 diabetes, and metabolic features of IRS and responds to their amelioration (223–230). The capacity of the liver to esterify and store incoming fatty acids as cytosolic triglycerides appears to be quite considerable under metabolic conditions when triglyceride synthesis exceeds the combination of hepatic fatty acid oxidation and VLDL-triglyceride secretion (216).

C. Hepatic lipoprotein remnant uptake also contributes to VLDL production

Postlipolysis remnants of triglyceride-rich lipoproteins, taken up by receptor-mediated mechanisms, are hydrolyzed

in hepatic lysosomes, thereby contributing to the intracellular fatty acid and cholesteryl ester pool and stimulating VLDL secretion in a fashion similar to that of FFAs (231, 232). The quantitative contribution of fatty acids derived from remnant uptake is not known but could be quite substantial, particularly in the postprandial state. A self-perpetuating cycle is thus set up, in which elevated FFAs drive VLDL secretion, and FFAs derived from the elevated circulating pool of triglyceride-rich lipoproteins positively feed back on VLDL production by further increasing the FFA pool in the hepatocyte (Fig. 2).

D. The role of resistance to insulin action in the hepatocyte and chronic hyperinsulinemia per se in facilitating VLDL synthesis and secretion

The preceding discussion has emphasized the importance of fatty acid availability in the hepatocyte in driving VLDL production. Nevertheless, increased FFA availability *per se* is not sufficient to explain the high VLDL production rates seen in IRS and type 2 diabetes. The role of resistance to insulin action in the hepatocyte and chronic hyperinsulinemia *per se* in facilitating VLDL synthesis and secretion has been the focus of intense investigation for many years (159, 233, 234). There is still not widespread agreement regarding the acute effects of insulin on VLDL production, but the majority of studies have demonstrated that insulin acutely inhibits VLDL production, shown in both *in vitro* (170, 235, 236) and *in vivo* experiments in fasting humans (191, 237–241). The nutritional state of the organism, fed or fasted, has recently been shown to modify the acute effect of insulin on VLDL production (242), perhaps due to a switch in fatty acid partitioning in the hepatocyte (222, 243, 244). The acute inhibitory effect of insulin on VLDL production in fasting individuals appears to be independent of the profound FFA suppression induced by hyperinsulinemia *in vivo* (191), although this has been disputed by others (245). Chronically insulin-resistant hyperinsulinemic, obese humans and those with type 2 diabetes are resistant to the acute inhibitory effect of insulin on VLDL production (237, 241), in keeping with similar findings in hepatocytes derived from fructose-fed and insulin-resistant rats (197, 198, 246). The relationship between peripheral tissue insulin resistance with regard to insulin's antilipolytic effect and VLDL secretion rate may not be direct and could depend on other concomitant factors found in insulin-resistant individuals. For example, we recently found that VLDL secretion rate in healthy individuals is not predicted by the sensitivity of insulin-mediated suppression of plasma FFA concentration in the postabsorptive state but appears to be much more dependent on body mass index (247). Furthermore, VLDL oversecretion is not present in recipients of combined kidney-pancreas transplantation with systemic venous anastomosis of their pancreatic graft who display marked peripheral tissue insulin resistance without gross hepatic insulin resistance/hepatic overinsulinization, compared with graft recipients with portal venous anastomosis or healthy subjects (248). VLDL oversecretion associated with the insulin resistance syndrome in humans may therefore depend of the combination of peripheral tissue insulin resistance, hepatic insulin resistance/overinsulinization, and/or the presence of visceral obesity.

We have recently shown in the fructose-fed hamster, an animal model of insulin resistance whose lipoprotein physiology has a number of similarities to that of humans, and *in vitro* in cultured hamster hepatocytes, that hepatic VLDL-apo B overproduction is associated with whole-body insulin resistance and attenuated hepatic insulin signaling (249–251). Fructose feeding was associated with hyperinsulinemia, enhanced MTP expression in the liver, increased intracellular apo B stability, and facilitated assembly of apo B-containing lipoproteins leading to VLDL oversecretion (249). Induction of insulin resistance was accompanied by a considerable rise in hepatic VLDL-apo B and VLDL-triglyceride production. Although there was an increase in total apo B secretion, the apo B fraction secreted as VLDL was more prominently enhanced in fructose-fed hamsters, suggesting an increase in both the number of VLDL particles and the proportion secreted as VLDL. Enhanced apo B secretion appeared to be caused by increased intracellular stability of apo B, elevated levels of MTP, and enhanced assembly of VLDL particles, with no apparent changes in apo B translocational status in the endoplasmic reticulum. Control studies showed that insulin resistance induced these changes rather than being direct effects of fructose itself. More recently, we obtained molecular evidence (251) for impairment of hepatic insulin signaling and insulin resistance, including reduced tyrosine phosphorylation of the insulin receptor, IRS-1 and IRS-2, and suppressed activity of PI3K associated with IRS proteins. Importantly, changes in the insulin signaling pathway coincided with drastic suppression of ER-60, a cysteine protease previously shown to be associated with apo B in HepG2 cells that has been postulated to play an important role in the degradation of apo B in the endoplasmic reticulum lumen (252). These changes were also accompanied by an increase in the secretion of apo B.

The rate of assembly and secretion of apo B-containing lipoproteins is critically linked with the expression level of MTP as demonstrated in an elegant series of studies in knockout mouse models (253) as well as a model of adenoviral-mediated overexpression (254). Chronic modulation of hepatic apo B secretion in insulin-resistant states may be mediated through changes in expression of MTP. The promoter region of the MTP gene has an insulin response element, which is negatively regulated by the hormone (255). Thus, it is reasonable to suggest that in insulin-resistant states, there may be a chronic up-regulation of MTP expression and protein levels due to resistance to suppressive effects of insulin on MTP gene expression, leading to hepatic VLDL overproduction.

Based on these recent data, we hypothesize that attenuated insulin signal transduction in hepatocytes causes suppression of ER-60 protease expression, which may contribute to the observed increase in apo B stability. Furthermore, impairment of hepatic insulin signal transduction may negate a negative regulatory effect of insulin on MTP expression, leading to the overexpression of this key protein, which may further facilitate VLDL assembly and secretion. Enhanced hepatic FFA flux to the liver, as observed in insulin-resistant states, can provide ample lipid substrate for the high rate of

VLDL assembly and secretion. An important additional factor may be up-regulation of SREBP-1c and chronic stimulation of DNL (and reduced fatty acid oxidation) in the liver (204), which can in turn enhance intracellular availability of triglyceride and drive VLDL assembly and secretion. The important factor responsible for up-regulation of SREBP-1c seems to be hyperinsulinemia *per se* rather than insulin resistance (204). Thus, an interaction between hepatic insulin resistance, hyperinsulemia, increased flux of FFA, and enhanced DNL may be essential to induce the VLDL overproduction state in IRS.

E. Summary of the mechanisms of VLDL overproduction in relation to fat maldistribution between adipose and nonadipose tissue

In summary, VLDL overproduction occurs as a result of a composite set of factors, which includes increased flux of fatty acids from extrahepatic tissues to the liver and directly from lipoprotein remnant uptake, increased hepatic *de novo* fatty acid synthesis, preferential esterification *vs.* oxidation of fatty acids, reduced posttranslational degradation of apo B, and overexpression of MTP. These conditions, together with resistance to the normal acute suppressive effect of insulin on VLDL secretion, act in concert to channel fatty acids into secretory and storage rather than degradative pathways. The increased flux of FFAs to the liver arise from adipose tissue resistance to insulin action, as described above, but we do not know whether the hepatic effects of IRS and type 2 diabetes arise as a result of insulin resistance or hyperinsulinemia *per se*. It is possible that the changes in liver metabolism outlined above may be due to both increased and reduced insulin action, with some biochemical pathways in the liver remaining responsive to insulin (*i.e.*, DNL and triglyceride esterification), whereas others are down-regulated (*i.e.*, insulin-suppressive pathways of apo B biogenesis) in IRS and type 2 diabetes.

IV. Effects of FFA on Muscle Glucose Metabolism

It is well established that FFAs impair glucose metabolism in insulin-sensitive tissues, such as muscle and liver (reviewed in Ref. 84). Recent studies in muscle have shown that there are multiple mechanisms responsible for this impairment (Table 1), but they may be initiated by a single event, *i.e.*, increased energy availability, and may serve one predominant function, *i.e.*, that of preventing further accumulation of intracellular energy substrates. There is general agreement, as discussed below, that an elevation of FFAs impairs cellular glucose uptake. Recently, however, controversy has arisen regarding the inhibitory effect of FFAs on glucose oxidation that has been postulated to account for the FFA-mediated inhibition of glucose uptake (*i.e.*, the "Randle hypothesis"). Although this issue remains unresolved, we will attempt to provide a balanced analysis of existing data.

A. Effects of the Randle cycle on muscle glucose metabolism

One of the mechanisms whereby FFAs have been postulated to impair glucose metabolism, by substrate competition, was first described by Randle *et al.* (256) based on studies in the isolated perfused rat heart and diaphragm. According to the concept of substrate competition, glucose uptake is limited in tissues that can utilize both FFAs and glucose when the tissue energy needs are satisfied by increased FFA availability. FFA oxidation results in production of acetyl-CoA and reduced coenzymes [dihydronicotinamide adenine dinucleotide (NADH) and dihydroflavine adenine dinucleotide]. Acetyl-CoA can decrease glucose oxidation by inhibiting pyruvate dehydrogenase. NADH can inhibit the Krebs' cycle, and according to the classical Randle hypothesis, the resulting accumulation of citrate would inhibit phosphofructokinase-1 (PFK-1), thereby increasing glucose-6-phosphate. In muscle, glucose-6-phosphate inhibits hexokinase, and the consequent rise in intracellular glucose would ultimately decrease glucose uptake.

In skeletal muscle, which accounts for the bulk of whole-body insulin-mediated glucose uptake, *in vitro* studies only partially confirmed Randle's hypothesis, as FFAs reduced glucose oxidation but did not consistently reduce glucose uptake (257–259). *In vivo,* FFA elevation obtained by Intralipid plus heparin infusion (Intralipid is a triglyceride emulsion and heparin activates LPL, thereby hydrolyzing the Intralipid triglycerides to FFAs and glycerol) consistently reduced glucose oxidation and decreased glucose uptake in the majority of hyperinsulinemic clamp studies in rats (260–264), dogs (265–267), and humans (118, 268–277). Under the latter conditions, most of the glucose uptake occurs in skeletal muscle. In addition, leg (118) and forearm balance studies (271, 272) and studies using positron emission tomogra-

TABLE 1. Mechanism of FFA effect

	Impaired glucose metabolism in muscle	Impaired glucose metabolism in liver	Reduced insulin clearance	β-Cell hyperfunction	β-Cell dysfunction	β-Cell apoptosis
Randle's cycle	+	+	+?	–	+?	–
PKC activation	+	+?	+?	+	+?	?
Glucosamine pathway	+	+?	?	+?	+?	?
Oxidative stress	+?	+?	?	–	+?	+?
iNOS induction	+?	?	?	–	+	+
Ceramide synthesis	+?	?	?	–	+?	+

+, Established mechanism.
+?, Suggested mechanism.
?, No information.
–, No reported effect.

phy (277) have localized the reduction in whole-body glucose uptake to muscle. In these *in vivo* studies, the effect of FFA on glucose uptake was studied for a longer time than in *in vitro* preparations. The results showed that the expected reduction in glucose uptake is delayed (261, 268, 269, 274) and is more often associated with decreased (264, 269, 274), rather than increased (262), muscle content of glucose-6-phosphate. An early increase in muscle glucose-6-phosphate concentration followed by a decrease when glucose transport is inhibited, as found in rats (264), could still be consistent with Randle's hypothesis. Nevertheless, studies in humans based on nuclear magnetic resonance measurements of glucose-6-phosphate (274, 275) could not detect such an early increase. In addition, FFAs may induce a delayed impairment in glycogen synthesis, which exceeds that expected from the reduction in glucose uptake (261, 269). It is therefore evident that in muscle, FFAs have effects on glucose metabolism other than or beyond those postulated by the classical Randle cycle (*i.e.*, inhibition of PFK-1 by citrate).

B. Effects of long-chain fatty acyl-CoA (LCFA-CoA) and PKC activation on muscle glucose metabolism

Some studies have linked the impairing effects of FFAs on muscle glucose metabolism with increased LCFA-CoA (see for Ref. 278 review). LCFA-CoAs accumulate in the cytosol when increased FFA inflow is associated with malonyl-CoA inhibition of CPT-1 (the enzyme that transports fatty acid into the mitochondria for oxidation). In states of energy, excess glucose, insulin, and citrate (note the link with the Randle's cycle) activate ACC, the enzyme that synthesizes malonyl-CoA, and the rise in malonyl-CoA is paralleled by a rise in cytosolic LCFA-CoA. LCFA-CoAs have allosteric effects on purified enzyme preparations such as glycogen synthase (279); however, the physiological importance of these effects *in vivo* in muscle is uncertain. The impairment in glucose metabolism induced by the Randle cycle and/or allosteric effects of LCFA-CoA could become more evident when glucose metabolism is stimulated by insulin, as suggested by the findings that: 1) Intralipid infusion had little or no effect on peripheral glucose uptake in obese insulin resistant subjects (280) and in patients with type 2 diabetes (281), and 2) FFA impaired insulin-mediated glucose utilization to a greater extent than non-insulin-mediated glucose utilization *in vitro* (258) and *in vivo* (270, 271, 282). Recent studies by Shulman and colleagues (262, 275), however, have also demonstrated that insulin signaling is directly and specifically impaired by FFAs. This specific impairment may occur through LCFA-CoA and/or FFA esterification pathways, as muscle triglyceride content, which is a source of cytosolic LCFA-CoA [via the action of HSL (283) and is a marker of overactive esterification pathways], consistently correlates with insulin resistance (14, 97).

By their esterification to diacylglycerol (DAG), and perhaps directly, LCFA-CoAs stimulate PKC activity (284). PKC has inhibitory effects on insulin action, due to serine-threonine phosphorylation of the insulin receptor and other intermediates in the insulin signaling cascade (285). PKC can also directly inhibit glycogen synthase (286). In rat skeletal muscle, Intralipid plus heparin infusion increased membrane-bound (active) PKCθ (263). In addition, transgenic mice with inactivation of PKCθ have recently been shown to be protected from lipid-induced defects in insulin action and signaling in skeletal muscle (287), suggesting a direct role of PKCθ in the development of fat-induced insulin resistance in skeletal muscle. Other studies have found that high-fat feeding increased DAG and the percentage of membrane-associated PKCϵ and θ (288). Overexpression of PKCϵ, in particular, in skeletal muscle is believed to be causally related to the development of nutritionally induced insulin resistance and diabetes in the sand rat [*Psammomys obesus* (289)], perhaps in part by increasing degradation of the insulin receptor. Recent studies in muscle cell lines have suggested that ceramides, which can be derived from palmitoyl-CoAs via *de novo* synthesis, can also inhibit insulin signaling (Ref. 290, and for review see Ref. 139).

LCFA-CoA may also affect GLUT4 translocation by acylating proteins involved in membrane fusion processes (291), although further studies are required to investigate this mechanism in muscle.

C. Effects of FFAs on the hexosamine pathway and oxidative stress in muscle

A pathway that has been linked with the FFA-induced impairment of muscle glucose metabolism by some authors (264) but not by others (292) is the hexosamine pathway. This is also an energy-sensing pathway, which is stimulated by increased glucose uptake under conditions of hyperglycemia and hyperinsulinemia. FFAs could stimulate this pathway via increased fructose-6-phosphate due to FFA-induced inhibition of PFK-1. PFK-1 could be inhibited by citrate or by depletion of xylulose-5-phosphate (see Ref. 293 for the latter mechanism). The mechanism of hexosamine-induced insulin resistance is unknown, although *O*-glycosylation of insulin signaling molecules or transcription factors may be implicated (294).

Another pathway that may be involved in the FFA-induced impairment in glucose metabolism is oxidative stress. FFAs can directly increase reactive oxygen species (ROS) via peroxidation reactions (295) and via mitochondrial production (296). FFAs can also indirectly increase ROS via hexosamine biosynthetic products (297). Again, the oxidative stress pathway is shared by hyperglycemia/hyperinsulinemia (295). Recent data obtained in collaboration with Dr. I. G. Fantus (University of Toronto) and co-workers suggests that iv infusion of *N*-acetyl-L-cysteine (NAC), an antioxidant, abolishes hyperglycemia and glucosamine-induced insulin resistance (298) and prevents, in part, FFA-induced insulin resistance in rats (299). Infusion of reduced glutathione, an antioxidant, partially prevented FFA-induced insulin resistance in humans (273). The biochemical mechanisms of oxidative stress-induced insulin resistance are unknown; however, it is well known that ROS can affect both signal transduction and gene expression, perhaps via redox modification of critical molecules. It is known that both oxidative stress (300) and the glucosamine pathway (301) can induce PKC activation. Perhaps linked to FFA-induced oxidative stress and PKC activation is the activation of IκB kinase β (IKK-β), a serine-threonine kinase that phosphorylates the

insulin receptor and IRSs, thus inhibiting their tyrosine kinase phosphorylation. The latter pathway has recently been implicated in FFA-induced inhibition of insulin signaling and action, because high-dose salicylate, an inhibitor of IKK-β (302), prevented FFA-induced insulin resistance in skeletal muscle *in vivo*, and IKK-β-knockout mice did not exhibit altered skeletal muscle insulin signaling and action after lipid infusion (303, 304). Also perhaps linked to oxidative stress and to synthesis of ceramides is the induction of inducible nitric oxide (iNOS) in muscle, which has recently been implicated in insulin resistance in the high-fat-fed rat (305).

D. Effects of FFAs on the gene expression of enzymes involved in muscle glucose metabolism

By activating all the signaling pathways described above, FFA can indirectly influence gene expression (306). FFAs and their eicosanoid derivatives can also directly affect gene expression by binding to PPARs (307). These nuclear receptors induce genes of peroxisomal and mitochondrial fatty acid oxidation (307), thus potentially up-regulating the Randle cycle. Paradoxically, however, PPAR activation increases muscle insulin sensitivity, presumably because of induction of uncoupling proteins, which dissipate intracellular energy and reduce intracellular triglycerides. This may be viewed as a protective mechanism whereby fat accumulation tends to be self-limited. Fat accumulation also depends on the type of fatty acid. n-3 fatty acids, which preferentially activate PPARs, are associated with less muscle fat accumulation and increased insulin sensitivity compared with saturated fatty acids (306). Fatty acid activation of PPARγ in the adipocyte, perhaps by increasing adipocyte insulin sensitivity and by stimulating adipogenesis, may also indirectly improve muscle insulin sensitivity *in vivo* by modulating a fat-derived signaling molecule or FFA flux from adipose to muscle tissue (308).

V. Effects of FFA on Hepatic Glucose Metabolism

A. Controversial role of FFA in the regulation of hepatic glucose production in vivo

Most of the *in vivo* literature regarding the effect of FFA on hepatic glucose metabolism refers to the acute effect of Intralipid and heparin on glucose production. Intralipid plus heparin increases FFA as well as glycerol, which is a gluconeogenic precursor, and in almost all of the studies, a glycerol control was not performed. However, glycerol infusion had negligible effects on glucose production in both dogs (309) and humans (269), whereas we have shown that direct infusion of FFA (oleate) can increase glucose production during low-dose insulin clamps in dogs (310). In a number of studies, which were mostly conducted at basal insulin levels, Intralipid plus heparin increased gluconeogenesis but not glucose production, consistent with a compensatory reduction in glycogenolysis (311–315). This decrease in glycogenolysis may have been due, in part, to small changes in portal insulin concentrations induced by FFA stimulation of insulin secretion or to FFA-induced changes in plasma glucose (313). However, a compensatory reduction in glycogenolysis was also found in studies in which basal insulin and glucose levels were clamped (312). This is consistent with an intrahepatic autoregulatory mechanism, which has mainly been attributed to glucose-6-phosphate stimulation of glycogen synthase and inhibition of phosphorylase (316).

Hepatic autoregulation may break down, as evidenced by the increase in basal glucose production induced by Intralipid plus heparin in other studies (270, 282). The breakdown of autoregulation is facilitated under hyperinsulinemic clamp conditions (265, 269, 276, 280, 317), presumably because, at hyperinsulinemia, glycogenolysis is already maximally suppressed. It is also possible that, as is the case in muscle, FFAs eventually impair insulin signaling, leading to an increase of both glycogenolysis and gluconeogenesis and perhaps also to a decrease of hepatic glucose uptake (318). The latter is currently controversial (319, 320).

The effect of FFA on hepatic glucose production during hyperinsulinemic clamps, however, is more controversial than the effect of FFA on peripheral glucose uptake, as in some studies (260, 262, 270, 281). Intralipid plus heparin failed to increase glucose production. The negative results could be explained, in part, by the high rate of insulin infused, which completely suppressed glucose production, independent of FFA (262, 270, 281). In addition, in most studies, plasma glucose-specific activity was not maintained constantly during the clamp, which leads to an underestimation of glucose production, particularly in the early non-steady-state periods of the clamp (321). Due to this methodological problem, the time course of the effect of FFA on glucose production could not be accurately estimated in most studies (280, 317), although there is some suggestion that it might be more rapid than the time course of the effect of FFA on peripheral glucose uptake (269, 280, 317).

B. Effects of FFA oxidation on hepatic glucose metabolism

Some of the mechanisms that have been implicated in the FFA-induced impairment of hepatic glucose metabolism are shown in Table 1. The classical Randle hypothesis has been expanded to include FFA-induced stimulation of gluconeogenesis. As is the case with FFA-induced inhibition of glycolysis, this pathway is also related to FFA oxidation. Acetyl-CoA derived from FFA oxidation stimulates pyruvate carboxylase, and the increased NADH is necessary to produce glyceraldehyde-3-phosphate from 1,3 bisphosphoglycerate. Citrate-induced inhibition of PFK-1 (which reduces glycolysis) has been demonstrated in the perfused rat liver (322) and in isolated hepatocytes exposed to FFA (323). In the liver, in contrast to muscle, the increased content of glucose-6-phosphate (324) from reduction of glycolysis and stimulation of gluconeogenesis should not affect glucose uptake because liver glucokinase, unlike muscle hexokinase, is not inhibited by glucose-6-phosphate. However, translocation of glucokinase [*i.e.*, dissociation of glucokinase from its binding protein, which correlates with glucokinase activity (325)] is inhibited by fructose-6-phosphate, NADH (which increases after FFA oxidation), and directly by LCFA-CoA (325, 326). As is the case with muscle, FFA oxidation might be inadequate to fully account for the FFA-induced changes in glu-

cose metabolism in liver. In fact, Randle's expanded hypothesis might not entirely explain why FFAs mainly affect the insulin-mediated suppression of glucose production rather than basal glucose production, consistent with an impairment in hepatic insulin signaling. In addition, in the high-fat-fed rat, the resistance of glucose production to insulin was not ameliorated by inhibitors of FFA oxidation (327).

C. Other effects of FFA on hepatic glucose metabolism

LCFA-CoAs accumulate in liver, when increased FFA exposure is combined with inhibition of fatty acid oxidation due to elevated malonyl-CoA (328). Numerous allosteric effects of LCFA-CoA on purified hepatic enzyme preparations, including an inhibition of glucokinase (329), inhibition or stimulation of glucose-6-phosphatase (330), inhibition of glycogen synthase (279), and stimulation of glycogen phosphorylase (331), have been described; however, the physiological importance of these effects *in vivo* is uncertain. LCFA-CoAs stimulate PKC in hepatocytes (332). Phosphorylation by PKC can directly influence enzyme activity [for example, PKC reduces hepatic glycogen synthase activity (286)] and impair hepatic insulin signaling. Accordingly, hepatic triglyceride content, which is proportional to cytosolic LCFA-CoA, seems to correlate with hepatic insulin resistance (333). Our recent data show that in liver, FFAs increase glucose production in the basal state and induce hepatic insulin resistance. The increase in basal glucose production is not progressive over time and is associated with increased hepatic citrate content (334). Hepatic insulin resistance is progressive over time and is associated with a progressive increase in PKCδ membrane translocation (335).

Little is known about the role of the hexosamine pathway and of oxidative stress in the FFA-induced insulin resistance in the liver. However, transgenic mice with selective overexpression of glutamine-fructose amidotransferase in the liver (the rate-limiting enzyme for increasing flow through the hexosamine pathway) show hepatic insulin resistance (336). Furthermore, studies in collaboration with Dr. I. G. Fantus (299) suggest that the antioxidant NAC partially prevents FFA-induced hepatic insulin resistance in rats.

In the liver as well as in muscle, FFAs induce enzymes of FFA oxidation, including CPT-1 (337), an effect mediated by PPARs (338). In addition, in the liver, polyunsaturated fatty acids repress ACC gene expression by inhibiting SREBP-1 (338). This could also contribute to the establishment of a chronic Randle cycle by decreasing malonyl-CoA, which inhibits CPT-1. PPAR response elements have been shown on genes of enzymes that are not involved in the Randle cycle, such as phosphoenolpyruvate carboxykinase (339) and glucokinase (327). In addition, glucose-6-phosphatase mRNA and protein are induced by Intralipid infusion *in vivo*, an effect that may be PPAR dependent (340). Paradoxically, however, the predominant effect of PPAR activation is to increase rather than decrease hepatic insulin sensitivity, presumably by limiting fat accumulation. In the liver, PPAR-independent effects account for the repression of glycolytic and lipogenic enzymes by n-3 and n-6 fatty acids (the mechanism is through inhibition of SREBP-1) and by fatty acyl-CoA [the mechanism is through inhibition of hepatocyte nuclear factors (338)]. These effects may also improve hepatic glucose metabolism by limiting fat overload.

D. Effects of FFA on insulin resistance: concluding remarks

In summary, increased provision of FFAs in a setting of increased energy availability leads to insulin resistance, thus preventing further intracellular accumulation of energy substrates. It is unclear whether this response is entirely maladaptive or also provides some advantage in terms of avoidance of massive obesity and perhaps avoidance of cell toxicity from tissue fat overload, at least in cardiac muscle (341) and liver (230). The trade-off is a tendency to increased circulating energy substrates (fat and glucose) and compensatory hyperinsulinemia. Hepatic insulin resistance and hyperinsulinemia in a setting of elevated FFA influx to the liver lead to increased production of VLDL particles (reviewed in Ref. 159), which are also a source of FFA for peripheral cells and contribute to the fat overload (Fig. 2).

VI. Effect of FFA on Hepatic Insulin Extraction

In insulin-resistant states, peripheral hyperinsulinemia is caused both by insulin hypersecretion and by reduced hepatic extraction of insulin (342). Because approximately 50% of the insulin secreted by the pancreas is removed on first pass by the liver before reaching the peripheral circulation, a reduction in hepatic insulin extraction would lead to a substantial increase in peripheral insulin levels.

One of the factors that may account for the impaired hepatic insulin extraction in obesity is elevated circulating FFA levels. In the *in situ*-perfused rat liver, physiological FFA concentrations caused a decline in hepatic insulin extraction (343). We have found that an elevation of circulating FFA from an Intralipid plus heparin infusion decreases hepatic insulin extraction *in vivo* in dogs (265). In further studies, the impairment in hepatic insulin extraction appeared to be greater when equimolar oleate infusion was given portally *vs.* peripherally to selectively elevate the hepatic FFA levels and thus mimic visceral obesity (310). In agreement with our findings, Hennes *et al.* (344) showed in humans that Intralipid plus heparin decreased whole-body insulin clearance (which includes both hepatic and peripheral insulin extraction) during hyperglycemic clamps. We have obtained similar findings in humans (345) but only after prolonged Intralipid plus heparin infusion. On the contrary, others failed to show changes in hepatic insulin extraction after 48 h of Intralipid plus heparin infusion performed during a 48-h hyperglycemic clamp (346). The reason for the discrepancy between these studies is unclear but may be related to the experimental protocol, as 48 h of hyperinsulinemia/hyperglycemia in the latter study might have been sufficient to decrease hepatic insulin extraction independent of FFA.

The majority of studies in humans did not show differences in peripheral insulin levels when hyperinsulinemic euglycemic clamps were carried out with or without Intralipid plus heparin infusion (see, for example, Refs. 269, 280, and 317). Because insulin was infused peripherally in these studies, however, the impact of the liver on the resultant peripheral insulin levels was less than with physiological

portal insulin delivery. Furthermore, in most of these studies the duration of the Intralipid plus heparin infusion was not long. In rats, the reduction in insulin clearance that we observed during a hyperinsulinemic clamp was greater after 7 h than 2 h of the Intralipid plus heparin infusion (334).

Hepatic insulin extraction depends on insulin binding to its receptor. In isolated rat hepatocytes, low physiological concentrations of FFA reduced insulin binding and degradation in proportion to a decreased receptor number (347, 348). Fatty acid oxidation may partly mediate the effect of FFA on insulin binding (349) by increasing the rate of insulin receptor internalization and/or decreasing the rate of receptor recycling (Table 1). In addition, FFAs may activate PKC (Ref. 332; in this study α- and β-isoenzymes were analyzed by hydroxyapetite chromatography and the β-isoenzyme was found to be preferentially translocated), which can increase insulin receptor internalization (350). In our preliminary studies on isolated rat hepatocytes, PKC inhibition abolished the FFA-induced reduction in insulin binding (351). We are currently determining the isoform of PKC involved. *In vivo* in rats, the progressive reduction of insulin clearance induced by Intralipid and heparin was associated with a progressive increase in PKCδ translocation (334, 335). Interestingly, PKC activation has also been implicated in FFA-induced insulin resistance, which would explain the association between impaired insulin extraction and sensitivity (265). The FFA-mediated reduction in hepatic insulin extraction may be viewed as an adaptive mechanism to generate peripheral hyperinsulinemia, and thus, to partially overcome the peripheral insulin resistance induced by FFAs. This adaptive mechanism could relieve, in part, the stress on pancreatic β-cells imposed by insulin resistance (352).

VII. Effects of FFA and Islet Triglyceride Stores on Pancreatic β-Cells

A. Acute effects of FFAs on insulin secretion

Fatty acids are actively taken up and metabolized by β-cells and can regulate β-cell enzymes, ion channels, and genes (353, 354). It has long been recognized that FFAs acutely (<6 h) increase glucose-stimulated insulin secretion [GSIS (345, 355–357)], and it has recently been demonstrated that this increase is proportional to the FFA chain length and degree of saturation (358). In the latter study (358), it was demonstrated that more saturated animal fat was far more potent in acutely facilitating insulin secretion *in vivo* and that the insulinotropic effects of individual fatty acids in a perfused rat pancreas model increased and decreased dramatically with chain length and degree of unsaturation, respectively. Acute lowering of plasma FFAs with nicotinic acid results in a reduction in basal plasma insulin in both nonobese and obese healthy fasted individuals (359, 360) and in patients with type 2 diabetes (360). The prevailing FFA concentration also appears to play an important role in maintaining β-cell responsiveness to glucose during fasting (359).

The precise mechanisms responsible for the acute effects of FFAs on insulin secretion are still debated. Intracellular FFAs are rapidly converted to fatty acyl-CoA, which can be oxidized to produce ATP. However, contrary to glucose, ATP generation followed by closure of the K-ATP channels is not the main mechanism of the acute stimulatory effect of FFAs on insulin secretion. Instead, the key factor appears to be accumulation of cytosolic LCFA-CoA when FFA oxidation is inhibited by glucose-derived malonyl-CoA (353, 354, 361). This cytosolic accumulation of LCFA-CoA could then stimulate the K-ATP-independent insulin secretory pathway through activation of PKC (362, 363) and/or more directly by increasing the secretory granule fusion process (364). Of note, the acute effect of FFAs on insulin secretion does not appear to be specific for a glucose stimulus, which suggests that final common events in stimulus secretion coupling may be involved (365). Some of these mechanisms (PKC activation) may be operational in both β-cells and peripheral tissues and thus link insulin resistance and hyperinsulinemia at the cellular level. Another of these mechanisms may be increased hexosamine flux. Recent findings in transgenic mice with selective overexpression of glutamine-fructose amidotransferase in the β-cell suggest that increased hexosamine flux may lead to insulin hypersecretion with secondary insulin resistance (366).

B. Chronic effects of FFAs on insulin secretion

In contrast to the stimulatory effect of acute exposure to FFAs, Sako and Grill (367) have shown that prolonged Intralipid infusion (>24 h) results in reduced GSIS in the perfused rat pancreas at high glucose concentration, suggesting that chronic FFA elevation inhibits GSIS. Several *in vitro* studies in β-cell lines and in rodent and human islets have subsequently confirmed that insulin secretion at high glucose concentrations is impaired in a time-dependent fashion by exposure to FFAs (368–375). Islets from prediabetic Zucker diabetic fatty (ZDF) rats and from fructose-fed insulin-resistant rats appear to be more susceptible to this FFA-mediated desensitization of GSIS (370, 374). Under the same conditions, however, basal insulin secretion at low glucose concentrations was elevated in normal rodent islets and islet cell lines in most studies (367, 368, 370–374, 376), but not in human islets (377). Furthermore, insulin secretion at low glucose concentration is either unchanged or decreased by FFAs in islets from ZDF prediabetic rats or prediabetic Otsuka Long-Evans Tokushima fatty rats (370, 373).

The precise mechanism responsible for this "β-cell lipotoxicity", a term coined by Unger (378) in 1995, has been debated. This term has been applied to describe FFA-induced functional impairments in GSIS as well as reduction in β-cell mass. The functional effect of chronically elevated FFAs on insulin secretion, in contrast to the acute enhancing effect, appears to be specific for glucose *in vitro* (368) and *in vivo* (379), and at least part of the effect requires FFA oxidation (367, 368, 377). An early hypothesis was that chronic exposure of β-cells to FFAs may result in diminished glucose oxidation [via a "Randle-like" effect (367, 368, 377)]. Prolonged exposure to FFAs may also lead to decreased GLUT2 and glucokinase expression, thereby decreasing the glucose-sensing capacity of the β-cell (373, 380). Other studies, however, have shown that prolonged exposure to FFAs does not decrease and may even increase glucose utilization and ATP generation, and reduces glucose oxidation only slightly in

β-cells (370, 372, 381), casting doubts on FFA-mediated impairment of glucose metabolism as an important mechanism for the β-cell lipotoxic effect.

Several other potential mechanisms have been proposed to explain the functional β-cell defect induced by FFAs, such as direct activation of the ATP-sensitive K^+ channels (382), down-regulation of PKC (372), or inhibition of specific PKC isoforms (363), induction of uncoupling protein 2 [UCP2 (383)], induction of oxidative stress (296, 375) and perhaps iNOS (384), and increased synthesis of ceramides (385). In addition, FFAs decrease insulin biosynthesis (368, 376, 377, 380, 386), alter proinsulin processing (387), and decrease insulin gene transcription (324, 327) by unclear mechanisms. Furthermore, reduced β-cell mass may be caused by FFA-induced stimulation of apoptosis, which has been repeatedly demonstrated in *in vitro* studies and has been linked to FFA-mediated induction of iNOS, increase in ceramide synthesis, and perhaps oxidative stress (296, 388–391). Some of the biochemical mechanisms of β-cell lipotoxicity have also been implicated in the FFA-induced impairment in insulin action (Table 1) and may be common to glucotoxicity as well. Most of these lipotoxic mechanisms appear to be linked to fatty acid esterification rather than oxidation. For example, palmitate-induced reduction of rat islet insulin mRNA levels was shown to depend on induction of fatty acid esterification pathway at high glucose levels (392, 393). Interestingly, prolonged *in vitro* exposure of β-cells to FFAs, triglycerides, or glucose leads to induction of lipogenic genes and/or to increased fatty acid esterification and intracellular fat deposition (373, 381, 393–395), which have been associated with the development of β-cell dysfunction in animal models of type 2 diabetes (370). These intracellular triglycerides can be hydrolyzed by HSL, which is expressed and active in β-cells (396) and, therefore, may constitute an *in situ* reservoir of long-chain fatty acids.

Furthermore, depletion of intracellular triglycerides in ZDF rat islets by activating intracellular FFA oxidation using leptin receptor overexpression with leptin treatment or troglitazone treatment restores the FFA-induced defects in cellular ultrastructure, mitochondrial integrity, glucose sensing, insulin biosynthesis, and GSIS (397, 398). UCP-2 has been implicated in the functional secretory defect chronically induced by FFAs and can decrease insulin secretion by decreasing ATP production from glucose (399). Paradoxically, however, adenovirus-mediated transfer of UCP-2 in pancreatic β-cells from Zucker diabetic rats has been shown to increase fatty acid oxidation and improve insulin secretion (400).

C. Effect of chronically elevated FFAs on insulin secretion in vivo

The question of whether chronically elevated plasma FFAs actually impair GSIS *in vivo*, particularly in humans, remains controversial, with some groups showing an impairing effect of FFAs, whereas others claim that chronically elevated FFAs actually facilitate insulin secretion. As we will discuss, it is possible to explain the apparently discrepant findings from the various studies that have been reported in humans.

In vivo insulin secretion needs to be interpreted in relation to concurrent changes in insulin sensitivity and perhaps also insulin clearance. There is a well-described hyperbolic relationship between insulin secretion and insulin sensitivity (S_I), implying that the product of insulin secretion and S_I is a constant [called the disposition index (DI); Ref. 401]. In individuals with normal β-cell function, a decline in S_I should be followed by a compensatory increase in insulin secretion, thus maintaining the body's ability to dispose of glucose (401). This relationship suggests that insulin sensitivity and insulin secretion are linked through a negative feedback loop and/or that common biochemical mechanisms link insulin resistance and β-cell hypersecretion. In situations, such as in type 2 diabetes, in which β-cell function is defective and cannot fully compensate for the decline in S_I, there is a decline in DI but not necessarily in absolute insulin secretion. Because elevation of plasma FFAs results in a reduction in S_I (269, 274, 345), it is critical to take this effect into account in interpreting any FFA-mediated change in insulin secretion. The FFA effect on insulin clearance (345) should also be taken into account, as this is expected to decrease, in part, the need for insulin secretion. Finally, when examining the *in vivo* data on the action of prolonged elevation of plasma FFAs, one has to keep in mind that iv infusion of triglyceride emulsion could also modulate autonomic nervous system activity, which can in turn affect both insulin sensitivity and insulin secretion (402).

The paper of Boden *et al.* (346) was the first to study the effect of prolonged elevation of plasma FFAs on insulin secretion in humans. These investigators found higher absolute insulin secretion during a 48-h hyperglycemic clamp with concurrent iv infusion of Intralipid plus heparin. They did not, however, examine insulin secretion in relationship to the FFA-mediated change in S_I, which was reduced with infusion of Intralipid plus heparin in this study. In contrast to the above findings, Paolisso *et al.* (357) reported that a 6-h elevation of plasma FFAs results in an absolute increase in GSIS, as assessed by the first phase insulin response to an iv glucose tolerance test, but that prolonged FFA elevation for 24 h results in an absolute reduction in the acute insulin response to glucose. The impairment in GSIS was reversible, implicating a functional defect. The same group also showed, in first-degree relatives of patients with type 2 diabetes in whom FFAs were lowered for 1 wk using the nicotinic acid analog acipimox, that there was a 50% increase in first phase insulin secretion during an iv glucose tolerance test (403).

Our group (345) assessed insulin secretion in lean, healthy men after an iv infusion of Intralipid and heparin resulting in a 2-fold elevation of fasting plasma FFAs. We found that acute elevation of FFAs for 1.5 h before a two-step hyperglycemic clamp of 4-h duration resulted in elevated GSIS and reduced S_I without a change in DI, showing that, acutely, the β-cell precisely compensates for the FFA-induced reduction in insulin sensitivity. In contrast, the FFA-mediated potentiating effect on GSIS was completely lost after 48 h of elevation of plasma FFA, and there was a concomitant significant decrease in insulin sensitivity, and consequently, a significantly lower DI. In other words, prolonged elevation of FFAs disables the β-cell's ability to appropriately increase its secretion in response to a reduction in S_I. We also found that obese nondiabetic subjects had an absolute reduction of

insulin secretion after prolonged elevation of plasma FFA, whereas diabetic subjects displayed a slight but significant absolute increase in insulin secretion (404). These findings suggest that individuals at risk for developing type 2 diabetes may be more susceptible to FFA-mediated β-cell desensitization than healthy insulin-sensitive individuals, but that those who already have type 2 diabetes may have no further FFA-induced deterioration in β-cell function. Our findings in humans are supported by similar findings in rats (405). In addition, our studies in rats suggest that the type of fatty acid is an important determinant of the effect of prolonged plasma FFA on GSIS. Both oleate and Intralipid/heparin (405), but not lard oil/heparin (406), decreased the absolute rate of GSIS at high glucose concentrations, with a much greater impairing effect of oleate than Intralipid and heparin. At low glucose concentrations, Intralipid/heparin actually increased insulin secretion, which is consistent with other studies in rats (402). In our studies, all types of fat appeared to disable the β-cell-compensatory response to FFA-induced insulin resistance (the latter is less in rats than in humans, probably accounting for the findings of a small absolute reduction of GSIS by Intralipid in rats but not in humans), perhaps to a different extent (406). Furthermore, our studies suggest that prediabetic rat models of type 2 diabetes (407), and perhaps also type 1 diabetes (408), may be particularly susceptible to the fat-induced impairment of GSIS. As to the mechanism of this effect, our preliminary experiments (in collaboration with Dr. I. G. Fantus) in normal rats suggest that oxidative stress may be involved, as iv infusion of NAC, an antioxidant, prevented the decrease in GSIS induced by prolonged oleate or Intralipid/heparin infusion (409).

In contrast to the impairing effect of a prolonged elevation of FFAs on GSIS, we recently failed to demonstrate a similar effect on arginine-stimulated insulin secretion (379). These findings are in keeping with *in vitro* studies that have suggested that the impairment of β-cell insulin secretion mediated by prolonged exposure to FFAs may be specific for GSIS (368, 383, 410, 411). Because arginine is believed to stimulate insulin secretion distal in the insulin secretion cascade of events, primarily by inducing depolarization of the β-cell membrane (412, 413), the absence of a significant effect, combined with our previous observation of a FFA-induced reduction of GSIS, would suggest that prolonged FFA exposure may alter GSIS primarily by interfering with the metabolism of glucose, leaving relatively intact the exocytotic machinery.

D. Summary of the effects of FFAs on insulin secretion

From the above discussion, the following conclusions can be drawn: 1) The effects of fatty acids on β-cells are complex and probably involve multiple direct metabolic interactions as well as delayed modulation of gene expression, resulting in time-dependent differential effects on insulin secretion *in vitro*. 2) In states of caloric deprivation, fatty acids have an important role in maintaining basal insulin secretion, but whether this regulation is independent of FFA-mediated change in insulin sensitivity has yet to be answered. 3) Acute elevation or lowering of plasma FFAs clearly results in potentiation and blunting, respectively, of GSIS *in vivo*. 4) The bulk of evidence both from *in vitro* and *in vivo* studies suggests that chronic exposure of β-cells to elevated fatty acids results in blunting of GSIS, particularly when insulin secretion is appropriately assessed in relation to concomitant changes in insulin sensitivity. Furthermore, based on our results in humans, it is possible that individuals at risk for developing type 2 diabetes may be more susceptible to the β-cell lipotoxic effect of fatty acids. FFAs, therefore, appear to be an important link between obesity, insulin resistance, fat intolerance, and the development of β-cell dysfunction and type 2 diabetes. The challenge for investigators is to better define the molecular basis for the β-cell lipotoxic or glucolipotoxic effect, and to further delineate the metabolic phenotypes and genetic factors that interact with fatty acids *in vivo*, placing individuals at risk of developing β-cell dysfunction.

VIII. Inhibition of Fatty Acid Flux from Adipose Tissue: Is it Effective in Ameliorating the Manifestations of IRS and Type 2 Diabetes?

Theoretically, a sustained reduction in FFA flux from adipose tissue would be predicted to result in improvement in the metabolic abnormalities discussed throughout this review. Therapies that directly or indirectly improve insulin sensitivity, such as weight reduction, exercise, oral hypoglycemic agents, and insulin, are indeed associated with a reduction in FFAs and improvement in many of the metabolic disturbances of IRS and type 2 diabetes. It has not, however, been possible to prove the link between FFA reduction and improvement in these other parameters in response to such therapies, due to the multiple metabolic effects of such therapies. Drugs that target adipose tissue lipolysis *per se* have been associated with only partial and inconsistent clinical success, as discussed below, partly due to their inability to produce a sustained reduction in plasma FFAs over a prolonged period of time.

The agent that has been used most frequently to investigate the metabolic and clinical effects of reducing fatty acids is the antilipolytic, long-acting nicotinic acid analog, acipimox (414, 415). Acute administration of acipimox has been shown by numerous investigators to reduce plasma FFAs, fatty acid oxidation, and gluconeogenesis and to increase glucose oxidation rates, with some but not all studies showing suppression of endogenous glucose production, increased insulin-mediated suppression of glucose production, and insulin-mediated glucose uptake (416–428). In addition, large VLDL particle (VLDL1) production has been shown to be reduced (240), as LDL particle size shifted from the smaller, dense particles to larger particles, a change that may be associated with less atherogenicity (429), and insulin secretion was potentiated with 1-wk acipimox treatment (403). There is a rebound elevation of FFAs that occurs with longer-term acipimox treatment, which may limit its potential therapeutic benefit (419, 423, 430, 431). Diabetic patients treated with acipimox have shown variable but generally disappointing clinical improvement in glycemic control (419, 432–436), whereas the triglyceride-lowering and high density

lipoprotein-raising effects of acipimox in hyperlipidemic patients have been more impressive (433–441). Acipimox has also been used with some success to reduce LDL in patients with hypercholesterolemia (442) and combined hyperlipidemia (443–445).

We speculate that drugs whose principal mechanism of action is to inhibit adipose tissue lipolysis are unlikely to prove totally effective in ameliorating the metabolic disturbances associated with IRS and type 2 diabetes. Firstly, they are destined to produce a rebound increase in adipocyte triglyceride lipolysis due to the mass effect of greater adipocyte triglyceride stores that occurs secondary to the drug-induced inhibition of lipolysis. Secondly, they fail to correct the fundamental defect of insulin-mediated fatty acid re-esterification in adipose tissue and are therefore unlikely to effectively reduce the postprandial diversion of FFAs from adipose tissue to other tissues of the body. On the other hand, agents such as PPARγ activators that overcome insulin resistance of adipose tissue by improving adipocyte FFA esterification are postulated to more effectively reduce the deleterious metabolic effects of fat dysregulation. Unfortunately, the trade-off of therapies designed to improve FFA uptake and deposition in adipose tissue and to limit FFA efflux may be weight gain, unless accompanied by a reduction in total daily calorie intake and/or an increase in energy expenditure. Possibly the only truly effective therapies will be those designed to reduce positive net energy balance. At present, however, the most important of these therapies are lifestyle changes.

IX. Summary

Dysregulation of fat metabolism occurs very early in the development of insulin resistance and well before the onset of hyperglycemia in type 2 diabetes. The mechanism for this dysregulation remains to be determined; however, there are suggestions that it might be related to decreased oxidative or fat oxidative capacity (154), with a tendency toward a positive energy balance and tissue triglyceride accumulation. There is general agreement that elevated FFA flux from an expanded adipose tissue to nonadipose tissues has a deleterious effect on insulin regulation of carbohydrate metabolism, is an important cause of the hypertriglyceridemia of IRS and type 2 diabetes, aggravates cytosolic triglyceride accumulation in nonadipose tissues, and may have other direct adverse effects, such as effects on endothelium, myocardium, and cell proliferation. More controversial is the role of chronic elevation of FFAs on pancreatic β-cell function and the role of fatty acids in the conversion of compensated insulin resistance to type 2 diabetes, but the bulk of evidence suggests that they may play a role.

There is little question that abnormal fatty acid metabolism is an important component of IRS and type 2 diabetes. A major question that remains to be answered is precisely how important a role fatty acids play in the cross-talk between adipose tissue and extraadipocyte insulin-sensitive and insulin-secretory tissues. Are fatty acids the dominant signal between these tissues, or will other factors such as peptides and cytokines prove to play a more important role?

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