Evidence is reviewed that free fatty acids (FFAs) are one important link between obesity and insulin resistance and NIDDM. First, plasma FFA levels are elevated in most obese subjects. Second, physiological elevations in plasma FFA concentrations inhibit insulin-stimulated peripheral glucose uptake in a dose-dependent manner in normal controls and in patients with NIDDM. Two possible mechanisms are identified: 1) a fat-related inhibition of glucose transport or phosphorylation, which appears after 3-4 h of fat infusion, and 2) a decrease in muscle glycogen synthase activity, which appears after 4-6 h of fat infusion. Third, FFAs stimulate insulin secretion in nondiabetic individuals. Some of this insulin is transmitted in the peripheral circulation and is able to compensate for FFA-mediated peripheral insulin resistance. FFA-mediated portal hyperinsulinemia counteracts the stimulation of FFAs on hepatic glucose production (HGP) and thus prevents hepatic glucose overproduction. We speculate that, in obese individuals who are genetically predisposed to develop NIDDM, FFAs will eventually fail to promote insulin secretion. The stimulatory effect of FFAs on HGP would then become unchecked, resulting in hyperglycemia. Hence, continuously elevated levels of plasma FFAs may play a key role in the pathogenesis of NIDDM in predisposed individuals by impairing peripheral glucose utilization and by promoting hepatic glucose overproduction. *Diabetes* 45:3-10, 1996

NIDDM affects between 5 and 20% of the population in Western industrialized societies (1) and is responsible for a significant amount of morbidity and mortality (2). Unfortunately, the pathogenesis of this disease remains incompletely understood despite decades of investigative efforts. It has recently been suggested that NIDDM may have more to do with abnormalities in fat than carbohydrate metabolism (3). Supporting this notion are the well-known facts that ~85% of patients with NIDDM in the United States are obese and that obesity is virtually always associated with insulin resistance, which is arguably the earliest detectable and dominant metabolic defect in patients with this disease (4,5). Moreover, there is evidence to suggest that the association between obesity and insulin resistance may be a cause and effect relationship. For instance, it has been shown in humans and in animals that weight gain decreased, while weight loss increased, insulin sensitivity and glucose tolerance (6-9). It remains uncertain, however, how obesity produces insulin resistance. One possible mechanism would be the generation of one or more metabolic messengers by the adipose tissue, which when released would inhibit insulin action on muscle and/or the liver. Here, we review the evidence, gained from studies in humans, that FFAs are likely candidates for such messengers. They are elevated in most obese individuals (10,11) primarily because of an increase in the rate of lipolysis from the expanded fat cell mass (12,13). Elevated plasma FFA concentrations produce peripheral and hepatic insulin resistance, which in normal subjects is compensated by FFA-induced potentiation of glucose stimulated insulin secretion. We propose that, in the development of NIDDM, FFAs fail to stimulate insulin secretion, which leaves hepatic and peripheral insulin resistance unchecked, resulting in hepatic overproduction and peripheral underutilization of glucose (Fig. 1).

**FFAS AND GLUCOSE UPTAKE IN MUSCLE**

**Historical review.** The concept that elevated blood levels of FFAs play a key role in the development of insulin resistance in obesity and NIDDM was first proposed by Randle et al. (14) more than 30 years ago. Based on their demonstration that the increased availability of FFAs decreased carbohydrate oxidation in isolated perfused rat hearts and hemidiaphragms, Randle et al. (14) proposed a glucose–fatty acid cycle. The key points of this cycle were: the increased availability of FFAs in blood produces an increase in intramuscular acetyl-CoA and citrate content; acetyl-CoA inhibits pyruvate dehydrogenase allosterically, and this in turn reduces glucose oxidation; citrate inhibits phosphofructokinase 1 and thus glycolysis itself, eventually resulting in the impairment of glucose uptake. The initial enthusiasm for this intriguing concept was dampened when several groups were unable to reproduce the inhibitory effects of fatty acids on glu-
FATTY ACIDS, INSULIN RESISTANCE, AND NIDDM

Cose uptake in rat skeletal muscle that Randle et al. had demonstrated in rat heart (15-18). More recently, several groups have reexamined glucose-fatty acid interactions in vivo, using indirect calorimetry (to determine rates of carbohydrate and fat oxidation) in combination with hyperinsulinemic clamping (to determine insulin sensitivity). Practically all of the groups found that raising plasma fatty acid concentrations increased fat oxidation and inhibited carbohydrate oxidation (19-25). Some also found inhibitory effects of fat on glucose uptake (19,20), but most did not (21-25). Hence, while the suppressive effect of fatty acids on carbohydrate oxidation was generally confirmed, it remained controversial whether fatty acids also inhibited insulin-stimulated glucose uptake (i.e., caused peripheral insulin resistance).

Recent development. It has recently been demonstrated in healthy volunteers that the fatty acid-mediated inhibition of insulin-stimulated carbohydrate oxidation occurred early (i.e., within 1-2 h), whereas the inhibition of glucose uptake developed only after ~4 h of fat infusion (26). Thus, insufficient time of fat plus insulin infusion (2 h in most studies) was the most likely reason why the inhibitory effect of fatty acids on glucose uptake was not found in many studies. It was also shown that FFAs inhibited glucose uptake in a dose-dependent fashion throughout the physiological range of plasma FFA concentrations (from ~50 to ~800 μmol/l) (27) (Fig. 2). Further, fatty acids have been demonstrated to inhibit insulin-stimulated glucose uptake in healthy subjects and patients with NIDDM (28). Three groups, who had previously failed to find inhibition of glucose uptake by fatty acids, had infused fat plus insulin together for only 2 h, which was not long enough to develop inhibition of glucose uptake (24,29,30).

Also of interest was the finding that, under conditions of comparable euglycemia and low plasma FFAs (<100 μmol/l), insulin-stimulated glucose uptake was 2 times higher in normal controls than in patients with NIDDM, indicating that FFAs could account for only a part of the insulin resistance in diabetic patients and that a major part, perhaps as much as 50%, was unrelated to fatty acids (27,28) (Fig. 3).

Hence, there is currently strong evidence, in normal as well as in diabetic subjects, that physiological elevations of plasma FFA levels lower peripheral insulin sensitivity dose-dependently. It is reasonable to assume, therefore, that chronically elevated plasma levels of FFA, perhaps together with FFAs released from intramuscular fat depots (31), are contributing to the insulin resistance commonly seen in obesity. It should also be pointed out that FFA-induced insulin resistance serves an important physiological role, preserving glucose for oxidation in the central nervous system when glucose is scarce, for instance, during fasting, prolonged exercise, or late pregnancy. In obesity, these same mechanisms can become counterproductive, inhibiting glucose utilization when there is no need to spare glucose.

Most obese individuals have normal glucose tolerance because their insulin resistance is matched by enhanced insulin secretion. The mechanism responsible for the increased insulin secretion in obesity is not clear, particularly in cases where blood glucose concentrations are not elevated. Hyperinsulinemia has been suggested to be, at least in part, a consequence of reduced insulin uptake by the liver, caused by exposure of the liver to elevated plasma FFA levels coming from enlarged intra-abdominal fat depots (32,33). Recent in vitro and in vivo studies from our laboratory, however, have failed to support the concept...
FIG. 2. Effect of plasma FFAs on insulin-stimulated glucose uptake. Euglycemic (-4.7 mmol/l) hyperinsulinemic (-420 pmol/l) clamping was performed in healthy volunteers for 6 h. High levels of plasma FFAs were produced by the infusion of triglycerides (4.3 mol/min) plus heparin (0.4 U·kg⁻¹·min⁻¹) (Δ, n = 4); intermediate plasma FFA levels, by infusion of triglycerides without heparin (○, n = 4); and low FFA levels, by infusion of saline alone (●, n = 6). Data shown are mean ± SE. The inhibition of insulin-stimulated glucose uptake became statistically significant ~3.5 h after the start of the lipid infusion. *P < 0.05; **P < 0.01, comparing high with low FFAs. From Boden et al. (27).

that hyperinsulinemia associated with central obesity is caused by FFA-mediated reduction in hepatic insulin clearance (34,27,28). Alternatively, it has been proposed that, in obesity, FFAs could produce both insulin resistance and a compensatory increase in insulin secretion (35).

FATTY ACIDS AND INSULIN SECRETION

The acute stimulation of insulin secretion by FFAs has been well established (36–40). It has recently been reported, however, that prolonged exposure to elevated concentrations of FFAs (for 48 h) produced a biphasic response: an initial stimulation (after 3 and 6 h) was followed later (after 24 and 48 h) by severe inhibition of insulin secretion from isolated islets or isolated perfused pancreas (41,42). These in vitro findings appeared to be incompatible with the notion that FFAs were the link between insulin resistance and compensatory insulin secretion. Instead, they suggested that increased FFA levels produced a second major pathogenetic defect (i.e., decreased insulin secretion). A recent study, however, showed that the in vivo effects of FFAs obtained in normal subjects were different from the in vitro effects obtained in animals. High plasma FFA concentrations were not only not associated with decreased insulin secretion, but actually increased insulin secretion rates for as long as 48 h (43) (Fig. 4). Glycerol, which was infused together with lipid, could not have been responsible for the increase in insulin secretion since glycerol is not an insulin secretagogue (44). Compared with glucose, FFAs were weak insulin secretagogues. Nevertheless, some of the insulin secreted into the portal circulation in response to FFAs was transmitted into the peripheral circulation and normalized the previously suppressed abnormal glucose utilization (43).

Obviously, obesity-related hyperlipidemia lasting for months or years may have different effects than hyperlipidemia lasting for 2 days. Moreover, the demonstration that a prolonged (48 h) elevation of plasma FFAs stimulated insulin secretion in normal subjects did not exclude the possibility that FFA-mediated insulin secretion may be reduced in patients with NIDDM or subjects who are genetically predisposed to develop NIDDM. The latter has been shown in animals. Basal insulin secretion from normal Wistar rat islets increased when cultured for 7 days in 2 mmol/l FFAs, while it decreased in islets from Zucker fatty rats that were predisposed to develop diabetes (35,45). These findings suggested that, in individuals predisposed to develop NIDDM, the FFA stimulation of insulin secretion may be reduced, compared with normal subjects. This reduction may result in uncompensated peripheral insulin resistance and, in addition, may have consequences with respect to hepatic glucose production (HGP).

FFAS AND HGP

There is good in vitro evidence to show that FFAs promote gluconeogenesis (46–48). The proposed mechanisms include increased production of ATP and NADH.
FIG. 4. Effect of the prolonged elevation of plasma FFAs on insulin secretory rates (ISRs). Lipid plus heparin or saline was infused for 48 h in 6 healthy volunteers during hyperglycemic (-8.8 mmol/l) clamping. Elevated plasma FFAs were associated with increased ISRs throughout the 48-h study. △, lipid; ○, saline. From Boden et al. (43).

FIG. 5. Effect of FFAs on plasma glucose and HGP. Triglyceride (Liposyn II, 0.5 and 1.5 ml/min or 2.15 and 4.3 pmol/min) plus heparin (0.4 U/min) were infused in 6 healthy volunteers during pancreatic clamping (somatostatin, 305 nmol/h; insulin, 0.33 pmol · kg⁻¹ · min⁻¹; glucagon, 0.25 ng · kg⁻¹ · min⁻¹). FFAs produced marked increases in HGP and plasma glucose concentrations. *P < 0.05; **P < 0.01 vs. saline controls. ○, lipid; ●, saline. From Boden and Jadali (59).

and the activation of pyruvate carboxylase by the acetyl-CoA that is generated via fatty acid oxidation (49,50). The available in vivo evidence is less strong. On one hand, Rebrin et al. (51) have recently reported a very strong relationship in conscious dogs between plasma FFAs and HGP at steady state and during dynamic insulin changes. On the other hand, the lowering of plasma FFAs with nicotinic acid or acipimox, a nicotinic acid analog, has been reported to decrease (52–54), to increase (55), or not to change (56) HGP. When plasma FFAs were raised
DIABETES, VOL. 46, JANUARY 1997

G. BODEN

FIG. 6. Effects of elevated plasma FFAs on rates of glucose uptake (GRd), glycogen synthesis (GS), and glycolysis (GLS) in 7 patients with NIDDM during hyperinsulinemic (-900 pmol/l) isoglycemic (-11 mmol/l) clamping and in 6 nondiabetic control subjects during euglycemic hyperinsulinemic (-500 pmol/l) clamping. Total length of bars represent insulin-stimulated GRd, GS, or GLS, set as 100%. The darker shaded parts of the bars represent insulin-stimulated GRd, GS, or GLS after 4 h of elevated plasma FFAs (-1,200 pmol/l in NIDDM, -600 pmol/l in controls). FFAs inhibited insulin-stimulated GRd, GS, and GLS similarly in patients with NIDDM and in normal control subjects, regardless of blood insulin and FFA levels.

during euglycemic- or hyperglycemic-hyperinsulinemic clamping in normal controls or patients with NIDDM, the insulin suppression of HGP was partially inhibited (24,27,55,57,58). Additional evidence in favor of a stimulatory effect of FFAs on HGP was obtained in overnight-fasted normal volunteers in whom plasma FFAs were raised (by infusing triglycerides and heparin), while insulin was clamped at basal concentrations (by the infusion of somatostatin and basal insulin replacement) (59). Under these conditions, HGP and plasma glucose levels rose dramatically (Fig. 5). On balance, the available human data suggest that FFAs increase HGP but that the extent of the increase is determined by the FFA-mediated stimulation of insulin secretion.

Mechanisms. The mechanism responsible for the inhibitory effect of fatty acids on carbohydrate oxidation, first demonstrated by Randle et al. (14) in rat hearts, has been confirmed in humans where fat infusion produced large increments in acetyl-CoA (43%) and in acetyl-CoA/free CoA (48%) (27) and the inhibition of pyruvate dehydrogenase in skeletal muscle (60).

It needs to be emphasized, however, that the inhibition of carbohydrate oxidation by FFAs alone does not affect insulin-stimulated glucose uptake since glucose uptake during lipid infusions remained unchanged for 3–4 h while carbohydrate oxidation was inhibited (26,27). An explanation for this phenomenon became apparent when it was found that for the initial 3–4 h FFAs had little or no effect on glycolytic flux and that the glucose carbons that could not be oxidized were shunted into lactate and alanine production (28).

Relevant to the understanding of the mechanisms responsible for the inhibitory effects of fatty acids on glucose uptake was the finding that fatty acids did not affect basal (i.e., insulin-independent) glucose uptake (59) or the glucose uptake stimulated by hyperglycemia (28). This indicated that fatty acids selectively inhibited glucose uptake stimulated by insulin.

In an attempt to localize the fat-induced defects on glucose uptake, glucose fluxes through all major pathways of intracellular glucose utilization were determined using noninvasive methods that have been recently validated (61). It was found that insulin stimulated rates of glucose uptake, glycogen synthesis, and glycolysis are inhibited to about the same extent (28) (Fig. 6). These results were most compatible with an FFA-induced defect in glucose transport or phosphorylation since the primary inhibitions of glycogen synthesis or glycolysis would be expected to result in disproportionately reduced rates of these pathways. The presence of a transport or a phosphorylation defect was also supported by another independent finding, namely, that glycogen synthase activity was normal in muscle biopsies taken 4 h after the start of fat infusion (i.e., at a time when glucose uptake was significantly inhibited) (27). On the other hand, 2 h later (i.e., between 4 and 6 h of fat infusion) plasma FFA concentrations >500 µmol/l caused a decrease in muscle glycogen synthase activity and an increase in muscle glucose-6-phosphate concentrations (27). Thus, there appeared to be at least two mechanisms by which FFAs inhibited insulin-stimulated glucose uptake: 1) by the inhibition of glucose transport or phosphorylation (after 3–4 h of fat infusion) and 2) by decreasing muscle glycogen synthase activity (after more than 4 h of fat infusion) (Fig. 7).

The cellular and molecular mechanisms responsible for these transport/phosphorylation and glycogen synthase defects are not known. Possibilities include the fatty acid–induced inhibition of insulin stimulated glucose transport via the accumulation of glucosamine pathway metabolites, including N-acetylglucosamine, N-acetylglucosamine-6-phosphate, or uridine-diphosphate N-acetylglucosamine-6-phosphate. A similar mechanism has been proposed by Marshall et al. (62) to explain the decrease in insulin sensitivity that occurs after hyperglycemia (glucose toxicity) (62–64). Alternatively, FFAs could interfere with GLUT4 gene expression in muscle and adipose tissue. This would explain the relatively long lag period of ~4 h needed for the development of the
transportation/phosphorylation defect. In support of this possibility, Long and Pekala have recently shown that several long-chain fatty acids decreased GLUT4 mRNA levels in fully differentiated 3T3-L1 cells by decreasing GLUT4 gene transcription and by destabilizing the GLUT4 message (65).

Another possibility to be considered would be fatty acid–induced changes in membrane fluidity. Insulin receptors are embedded in the lipid bilayer of plasma membranes, and there is evidence to suggest that altering the fatty acid content of membranes can alter insulin receptor accessibility, insulin binding, and action. Generally, it has been found that increasing polyunsaturated fatty acid content increased membrane fluidity, insulin binding, and action, whereas decreasing their content has the opposite effect (66–69). It is not known, however, whether 4 h of fat infusion (the time necessary to produce insulin resistance) can produce changes in membrane fatty acid composition of sufficient degree to inhibit insulin action.

TUMOR NECROSIS FACTOR (TNF)-α AND INSULIN RESISTANCE

It has recently been suggested that the cytokine TNF-α may play a pivotal role in the pathogenesis of peripheral insulin resistance in obesity. This hypothesis is supported by the observation that TNF-α is overexpressed in the adipose tissue of obese insulin-resistant rodents and humans and that the neutralization of TNF-α in fa/fa Zucker rats decreased insulin resistance and increased insulin-receptor tyrosine kinase activity in adipose tissue and muscle (70). Several possible mechanisms have been suggested by which TNF-α could produce insulin resistance in obese subjects. 1) TNF-α released from adipose tissue could produce insulin resistance in muscle, although this endocrine mode of action seems unlikely since TNF-α is undetectably low in the circulation (70). Supporting this notion, Hurel et al. (71) have recently reported that the neutralization of TNF-α with an antibody over a 4-week period had no effect on insulin sensitivity in obese patients with NIDDM. 2) TNF-α could inhibit insulin action through local (paracrine) action on muscle (31). And 3) TNF-α may act indirectly through another factor that is released into the circulation and inhibits insulin action on muscle. Inasmuch as TNF-α has been shown to increase lipolysis, this factor could well be FFAs; hence, the effect of TNF-α on glucose uptake may be mediated, at least in part, by fatty acids. This notion is supported by the observations that the neutralization of TNF-α in Zucker rats was associated not only with an increase in insulin sensitivity, but also with a decrease in plasma FFA levels (72) and that infusion of TNF-α in humans increased plasma FFAs (73). Clearly, the role of TNF-α as a link between human obesity and insulin resistance and the interrelationship between TNF-α and FFAs need to be explored further.

PHYSIOLOGICAL AND CLINICAL SIGNIFICANCE

The 4-h lag period between the rise in plasma FFAs and the onset of inhibition of insulin-stimulated glucose uptake most likely prevents insulin resistance in normal weight healthy individuals after eating a fat-rich meal as plasma fatty acid levels rarely remain elevated for that long. The situation is likely to be different in obese healthy individuals in whom fatty acid levels are persistently elevated. There, elevated plasma FFAs can be expected to cause peripheral insulin resistance. They also stimulate insulin secretion, which is critically important. The increased insulin secretion will not only compensate for the increased peripheral insulin resistance, but will also prevent FFAs from increasing HGP.

Obese individuals who are genetically predisposed to develop NIDDM may eventually lose their ability to increase insulin secretion in response to elevated plasma FFA levels. The FFA-induced stimulation of HGP would then become unchecked and peripheral underutilization, together with the hepatic overproduction of glucose, would result in postprandial (early) and fasting (late) hyperglycemia (Fig. 1). This could initiate a vicious cycle with hyperglycemia producing progressively more β-cell desensitization and more peripheral insulin resistance (74).

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