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Disordered Lipid Metabolism and the Pathogenesis of Insulin Resistance

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Savage DB, Petersen KF, Shulman GI. Disordered Lipid Metabolism and the Pathogenesis of Insulin Resistance. *Physiol Rev* 87: 507–520, 2007; doi:10.1152/physrev.00024.2006.—Although abnormal glucose metabolism defines type 2 diabetes mellitus (T2DM) and accounts for many of its symptoms and complications, efforts to understand the pathogenesis of T2DM are increasingly focused on disordered lipid metabolism. Here we review recent human studies exploring the mechanistic links between disorders of fatty acid/lipid metabolism and insulin resistance. As "mouse models of insulin resistance" were comprehensively reviewed in *Physiological Reviews* by Nandi et al. in 2004, we will concentrate on human studies involving the use of isotopes and/or magnetic resonance spectroscopy, occasionally drawing on mouse models which provide additional mechanistic insight.

I. INTRODUCTION

Insulin resistance is a key element in the pathogenesis of the metabolic syndrome and type 2 diabetes mellitus (T2DM), both of which have reached epidemic proportions worldwide (131). Although the pathogenesis of T2DM remains poorly understood, most investigators agree on the following.

1) Insulin resistance, which can be defined as a state of reduced responsiveness to normal circulating levels of insulin, plays a major role in the development of T2DM. This conclusion is based on the following observations: *a*) cross-sectional studies demonstrating the consistent presence of insulin resistance in patients with T2DM (42, 60), *b*) the presence of insulin resistance in nondiabetic offspring of patients with T2DM (119), *c*) prospective studies demonstrating the usefulness of insulin resistance as a predictive marker of the future development of T2DM (60, 119), and *d*) prevention of diabetes by insulin-sensitizing agents (18, 39).

2) Early in the disease, β-cells secrete sufficient insulin to compensate for insulin resistance and maintain euglycemia. Ultimately, however, relative or absolute insulin deficiency supervenes precipitating hyperglycemia and overt diabetes. β-Cell dysfunction is therefore a sine qua non of the diabetic state but need not be the primary abnormality (10).

3) T2DM is a heterogeneous cluster of conditions rather than a uniform entity. The spectrum includes individuals with maturity-onset diabetes of the young or MODY, manifesting predominantly β-cell dysfunction caused by mutations in genes involved in β-cell function (32), and people with Donohue's syndrome due to insulin receptor mutations, whose phenotype is dominated by insulin resistance (57).

The current rise in prevalence of T2DM and the metabolic syndrome is believed to be a result of increasingly

sedentary life-styles combined with ready access to energy-rich food sources in genetically susceptible individuals. Healthy humans respond to positive energy balance primarily by storing excess energy as triglyceride in adipose tissue. While this response enables humans to cope efficiently with fluctuating energy supplies, it predisposes persistently over-nourished individuals to weight gain and ultimately obesity. It also appears to induce lipid accumulation in "ectopic sites," such as the liver and skeletal muscle, and possibly in pancreatic β-cells and kidney (104, 117). The simple explanation is that in obese states, energy intake exceeds the storage capacity of adipose tissue leading to energy "overflow" to ectopic sites (25, 104). This notion is supported by the almost universal finding of ectopic lipid accumulation in mice and humans with generalized lipodystrophy,[1] an extreme example of limited adipose tissue storage capacity in the face of excess calorie ingestion (food intake tends to be increased in subjects with generalized lipodystrophy secondary to hypoleptinemia). One way to reduce ectopic lipid deposition in lipodystrophic mice is to transplant adipose tissue from wild-type mice, a procedure which dramatically improved insulin sensitivity (38, 53). Another way in which ectopic lipid deposits can be reduced in lipodystrophic mice and humans is by replacing leptin, an anorexogenic adipocyte-derived hormone (74, 102). This leads to a significant reduction in energy intake and dramatic improvements in insulin-stimulated liver and muscle carbohydrate metabolism (84). The notion of energy intake exceeding adipose tissue storage capacity is further supported by the finding that weight loss induced by large-scale liposuction fails to improve the metabolic status of obese humans (54), in effect this procedure simply reduces adipose tissue storage capacity in the face of unchanged energy intake and could potentially exacerbate lipid accumulation in liver and skeletal muscle. On the other hand, relatively small reductions in weight due to dieting and/or exercise can substantially improve insulin sensitivity (81, 82, 112). These observations, together with a growing awareness of the molecular interplay between lipid and carbohydrate metabolism, have led to what might be termed a "lipocentric" view of the pathogenesis of insulin resistance and T2DM.

Here, after describing the use of magnetic resonance spectroscopy in humans, we begin by very briefly reviewing human studies utilizing this technique in combination with stable isotope measurements to determine the key rate-controlling steps in insulin-stimulated glucose disposal in muscle and the effects of insulin on hepatic glucose production in normal volunteers. We then consider abnormalities in these processes observed in insulin-resistant type 2 diabetics and in insulin-resistant offspring of type 2 diabetics, before going on to consider studies exploring the notion that "ectopic" lipid accumulation and disorders of fatty acid-lipid metabolism might cause these abnormalities in insulin action. We also review recent insights into the mechanisms of ectopic lipid accumulation and briefly allude to mechanistic insights into the pathogenesis of T2DM obtained from studies using thiazolidinediones. Finally, we consider the potential impact of inflammatory pathways on the insulin signaling cascade and the notion that inflammation in adipose tissue may be involved in inducing systemic insulin resistance in obese states. As the latter has been recently reviewed by Wellen and Hotamisligil (121), it is not covered in detail here.

A. Basic Principles of Magnetic Resonance Spectroscopy in Muscle and Liver

The basic principles of magnetic resonance spectroscopy (MRS) have been described in a number of reviews (91, 95, 107). In short, some nuclei possess magnetic properties (referred to as the magnetic moment or "spin"). Within a strong, static magnetic field generated by a nuclear magnetic resonance (NMR) spectrometer, the nuclei spin around their own axis with a characteristic frequency to align with or against the magnetic field. Stimulation of nuclei by an additional oscillating magnetic field at their frequency of precession transiently swings these nuclei out of alignment. Return to the low energy-state within the static magnetic field is associated with emission of energy in the form of radiowaves that are detected by a receiver coil. Under standard experimental conditions, resonant waves from various nuclei are superimposed, generating a picture of oscillating amplitudes in an intensity versus time display (free induction decay, FID). Fourier transformation is used to convert the FID into a display of signal intensities versus frequencies, thereby enabling one to distinguish compounds with characteristic peak frequencies. The area under the particular peak corresponds to its tissue concentration. This result can be converted into molar terms by comparison with data obtained from a phantom containing a known amount of that compound. It can also be compared with the area under the peak of an intrinsic compound with a known concentration, e.g., water peak in muscle.

Despite a low natural abundance of 1.1%, ^{13}C can be used to measure hepatic glycogen and muscle glycogen. ^{13}C spectroscopy can also be used to trace ^{13}C incorporation into glycogen during infusion or ingestion of 1-^{13}C-enriched glucose, which can increase the sensitivity of the method by up to 100-fold. Sequential infusions of ^{13}C-enriched and unlabeled glucose (^{13}C pulse-^{12}C chase ex-

[1] The lipodystrophic syndromes encompass a rare group of conditions characterized by partial or complete absence of adipose tissue. The disorders may be genetic or acquired and are further classified according to the anatomic distribution of the lipodystrophy.

periments) have facilitated measurements of rates of glycogen synthesis and simultaneous glycogenolysis in humans. In these experiments, the increment in total hepatic glycogen over time during infusion of [1-^{13}C]glucose gives the flux through glycogen synthase. The [1-^{13}C]glucose infusate is then switched to an unenriched glucose infusate. To obtain an estimate of glycogenolysis, the change in [1-^{13}C]glycogen concentration is compared with the predicted increment, assuming constant flux through glycogen synthase and no glycogen breakdown. Glycogenolysis can then be estimated from the difference between predicted and observed glycogen concentrations. The ratio of glycogen breakdown to glycogen synthesis provides relative rates of glycogen turnover (83, 88, 89, 105). ^{13}C spectroscopy has recently also been used to trace the incorporation of infused 2-^{13}C-labeled acetate into glutamate in skeletal muscle and brain during a constant [2-^{13}C]acetate infusion. The rate of incorporation reflects tricarboxylic acid (TCA) cycle activity providing an index of mitochondrial function in muscle (or brain) (79).

Proton (^{1}H) MRS is now widely used to measure hepatic and muscle triglycerides (TG). One of the major problems with direct measurements of TG in muscle biopsies is the need to carefully dissect off fat surrounding the myotubules. Fortunately, in vivo MRS can identify two sets of resonances from methylene and methyl protons of TG acyl chains within muscle, shifted in frequency from each other by 0.2 ppm. It turns out that these signals originate from two distinct compartments, namely, an extramyocellular adipocyte pool and intramyocellular TG. Magnetic susceptibility differences between compartments and the geometric arrangement of the tissue in musculature might cause the observed frequency shift. This technique has been well validated against biochemical TG measurements (111).

Phosphorus (^{31}P) MRS can be used to measure the rate of ATP synthesis by direct observation of ^{31}P-magnetization transfer between P_i and ATP. The steady-state intramyocellular P_i magnetization is measured in the presence of selective irradiation of the γ-resonance of ATP and then compared with the equilibrium P_i magnetization in a control spectrum (without irradiation of γ-ATP) (59, 79). To date, this technique has been used to measure the rate of ATP synthesis in skeletal muscle in humans.

II. INSULIN RESISTANCE AND GLUCOSE METABOLISM

A. Muscle

How do healthy individuals dispose of glucose loads? Ingested glucose can either be oxidized or stored as glycogen, or to a lesser extent as fat (via de novo lipogenesis). Early studies using indirect calorimetry in combination with femoral vein catheterization and the euglycemic-insulin clamp suggested that nonoxidative glucose metabolism was the major pathway for glucose disposal in healthy subjects (27, 28). Ex vivo glycogen measurements in sequential muscle biopsy studies taken in the presence of high plasma glucose concentrations (peak 20 mM) suggested that over half of an infused glucose load was stored as muscle glycogen (8, 73). ^{13}C MRS provided the first opportunity to directly assess small sequential changes in muscle glycogen concentration during hyperglycemic-hyperinsulinemic clamps (106). Indirect calorimetry was used concurrently to calculate whole body nonoxidative glucose disposal. These data suggested that during a hyperglycemic-hyperinsulinemic clamp, skeletal muscle accounts for the vast majority of glucose uptake in normal humans and that over 80% of this glucose is then stored as muscle glycogen (106). Direct ^{13}C MRS measurements of muscle glycogen have also been undertaken in healthy subjects following standard meals. In this situation, muscle glycogen synthesis accounts for ~30% of the ingested glucose (123). Muscle glycogen concentrations peak at ~100 mM around 5 h after a meal, declining thereafter (20, 115).

How is this altered in insulin-resistant type 2 diabetics and in insulin-resistant diabetic offspring? Baseline muscle glycogen concentrations were ~30% lower in type 2 diabetics than in matched controls (20, 106), and the rate of glycogen synthesis in skeletal muscle was ~50% lower in diabetic subjects than in normal volunteers during hyperglycemic-hyperinsulinemic clamps (106). Postprandial increments in muscle glycogen were also significantly lower than those in normal volunteers (20). First-degree relatives of type 2 diabetics have a ~40% lifetime risk of developing diabetes (56). Insulin resistance is the best predictor of the development of diabetes in these offspring (60, 119) and probably plays an important role in its pathogenesis. Baseline muscle glycogen concentrations (~70 mM) were similar to those of healthy controls, but insulin-stimulated rates of muscle glycogen synthesis were reduced by 63% in these individuals (78).

What are the rate-controlling steps in glucose disposal? Under the influence of insulin, glucose is transported into myocytes via GLUT4 (glucose transporter 4), where it is phosphorylated by hexokinase. Glucose-6-phosphate is then either utilized in the glycolytic pathway or incorporated into glycogen by glycogen synthase: extracellular glucose → intracellular glucose → glucose-6-phosphate → glycogen.

^{13}C and ^{31}P MRS were used together to monitor both intracellular glucose-6-phosphate concentration and intramuscular glycogen synthesis during hyperinsulinemic-hyperglycemic clamps (94). Glucose-6-phosphate is an intermediate between glucose transport into the cell and its subsequent phosphorylation by hexokinase, and glycogen synthesis. The fact that the increment in glucose-6-

phosphate concentration was significantly reduced in type 2 diabetics suggested that glucose transport and/or phosphorylation was the rate-controlling step in insulin-stimulated glucose disposal in skeletal muscle rather than glycogen synthase (94). Similar observations were also made in lean insulin-resistant offspring of type 2 diabetics (93) and in nondiabetic obese women (body mass index 33 ± 1 kg/m^2) (82), suggesting that this defect precedes the development of T2DM and is common to insulin-resistant offspring of type 2 diabetics and obese subjects; both states significantly increase the risk of developing T2DM. Glucose transport in skeletal muscle is largely mediated by a specific insulin-responsive glucose transporter, known as GLUT4, whereas glucose phosphorylation is catalyzed by hexokinase. To determine which of these two steps was defective, a novel ^{13}C MRS method was used to assess intracellular free glucose in muscle (21), the idea being that if hexokinase were rate-controlling in insulin-resistant type 2 diabetics intracellular glucose concentrations should increase substantially (>2 mM). The fact that intracellular glucose concentrations in skeletal muscle from type 2 diabetics (during a hyperinsulinemic-hyperglycemic clamp) were 1/25 what they would have been if hexokinase were the primary rate-controlling enzyme suggested that glucose transport was rate-controlling as opposed to hexokinase (21). Taken together, these data indicate that glucose transport into muscle is the rate-controlling step for insulin-stimulated muscle glycogen synthesis in patients with insulin-resistant T2DM. They also suggest that this defect precedes the development of T2DM in lean offspring of type 2 diabetics and in obese adults. Of course, insulin-stimulated glucose transport is itself contingent upon a series of steps culminating in GLUT4 translocation from an endosomal compartment to the plasma membrane.

B. Liver

The liver plays a pivotal role in maintaining energy homeostasis during fed-fasting transitions. While peripheral tissues (predominantly skeletal muscle) account for the majority of postprandial insulin-stimulated glucose disposal, the liver also plays a key role in buffering ingested carbohydrate by suppressing hepatic glucose output and stimulating glucose deposition as liver glycogen (27). In the fasting state, hepatic glycogen stores are rapidly mobilized to maintain circulating glucose concentrations. It is, however, important to appreciate that hepatic glycogenolysis (GL) contributes only ~50% of endogenous glucose production (EGP) in the first 6–12 h of a fast [i.e., gluconeogenesis (GNG) accounts for the remaining 50% of EGP during the early phase of fasting], and as little as 4% 46–64 h into a fast (89). During prolonged fasting, fat oxidation and ketone bodies also make a substantial contribution toward meeting whole body energy requirements.

Hepatic glucose output is suppressed within 30 min after an oral glucose load, and the liver takes up glucose to replenish glycogen stores (27). Taylor et al. (114) observed that liver glycogen concentrations increased from 207 ± 22 mM after an overnight fast to a peak of 316 ± 19 mM 5 h after a liquid mixed meal (representing ~19% of the carbohydrate content of the meal). The data are consistent with a splanchnic balance study (47) and an independent MRS study (5).

1. What determines net hepatic glycogen turnover?

Net glycogen synthesis is directly regulated by two enzymes, glycogen synthase and glycogen phosphorylase. A combination of ^{13}C MRS and ^{13}C glucose pulse-^{12}C glucose chase techniques were used to demonstrate that glycogen synthesis and glycogenolysis occur simultaneously in the liver, i.e., glycogen cycling (26, 62, 83, 88, 105). This technique was also used to assess the relative impact of glucose and insulin on glycogen turnover under hypoglucagonemic conditions (83). With the use of this ^{13}C MRS approach, it was shown that hyperglycemia inhibits net hepatic glycogenolysis primarily by inhibiting glycogen phosphorylase flux, whereas hyperinsulinemia inhibited net hepatic glycogenolysis primarily by stimulating glycogen synthase flux. Neither hyperlgycemia nor hyperinsulinemia alone was sufficient to promote net hepatic glycogen synthesis, and only by combining both substrate and hormonal signals was substantial net hepatic glycogen synthesis achieved. The net rate of glycogen synthesis depends on portal vein insulin, requiring concentrations in the 130–170 pM range for half-maximal stimulation of glycogen synthesis (88). Furthermore, under basal insulin concentrations, the presence and absence of glucagon was shown to have a profound effect on regulating net hepatic glycogen synthesis (88).

2. What happens in insulin-resistant T2DM?

It is well established that fasting hyperglycemia is related to increased rates of endogenous glucose production (16, 27, 35). This phenomenon could be a consequence of increased gluconeogenesis and/or increased glycogenolysis. Magnussen et al. (63) measured rates of net hepatic glycogenolysis in poorly controlled type 2 diabetics (mean hemoglobin A1c 12 ± 1%). Gluconeogenesis was simultaneously calculated as the difference between rates of net hepatic glycogenolysis and whole body glucose production. Despite pair feeding, baseline liver glycogen concentration was reduced in the diabetic subjects (131 ± 20 vs. 282 ± 60 mmol/l liver), demonstrating that in addition to having defects in insulin-stimulated muscle glycogen synthesis (106) these patients also have a defect in liver glycogen synthesis, which contributes to

postprandial hyperglycemia. This reduction in net hepatic glycogen synthesis was associated with lower rates of net hepatic glycogenolysis and an ~60% increase in gluconeogenesis. More importantly, the 25% increase in rates of endogenous glucose production observed in these poorly controlled type 2 diabetic subjects could entirely be attributed to this increased rate of gluconeogenesis (Fig. 1) (63).

III. FATTY ACID/LIPID-INDUCED INSULIN RESISTANCE

A. Muscle (Fig. 2*A*)

Lipid infusions designed to increase plasma fatty acid concentrations impair both oral glucose tolerance (33) and insulin-stimulated glucose disposal in humans (11, 50, 90). Furthermore, the fall in insulin sensitivity during such clamp procedures only occurs 3–5 h after elevations in fatty acid concentrations (10) and is related to the degree of fatty acid elevation (6), in keeping with the idea that fatty acid metabolite accumulation in skeletal muscle and liver is responsible for this phenomenon. Randle et al. (85) originally showed that fatty acids compete with glucose for substrate oxidation in isolated rat heart muscle and rat diaphragm muscle. They speculated that an increase in fat oxidation might be responsible for insulin resistance. According to their proposal, increased fatty acid oxidation would cause an increase in the mitochondrial acetyl CoA:CoA and NADH:NAD^+ ratios with subsequent inactivation of pyruvate dehydrogenase. This in turn would induce a rise in intracellular citrate levels, leading to inhibition of phosphofructokinase and glucose-6-phosphate accumulation. As glucose-6-phosphate inhibits its hexokinase activity, this would result in intracellular glucose accumulation and decreased glucose uptake. A series of studies has recently challenged this mechanism (13, 31, 41, 90). Nonesterified fatty acid levels in healthy subjects were maintained at either high or low levels during hyperinsulinemic-euglycemic clamps. Maintaining high free fatty acid levels for 5 h caused the expected reduction in insulin sensitivity as assessed by glucose uptake, glucose oxidation, and glycogen synthesis in skeletal muscle, just as had been observed in type 2 diabetics and their insulin-resistant offspring. However, rather than increasing intracellular glucose-6-phosphate levels, as predicted by the Randle mechanism (85), increasing plasma fatty acid concentrations reduced intracellular glucose-6-phosphate levels (90). This was consistent with what had been observed in patients with T2DM (93). These findings are in contrast to those of Boden et al. (13) who observed increased glucose-6-phosphate concentrations during similar increases in plasma fatty acid concentrations that can likely be attributed to hydrolysis of muscle glycogen to glucose-6-phosphate due to warm ischemia during the muscle biopsy procedure.

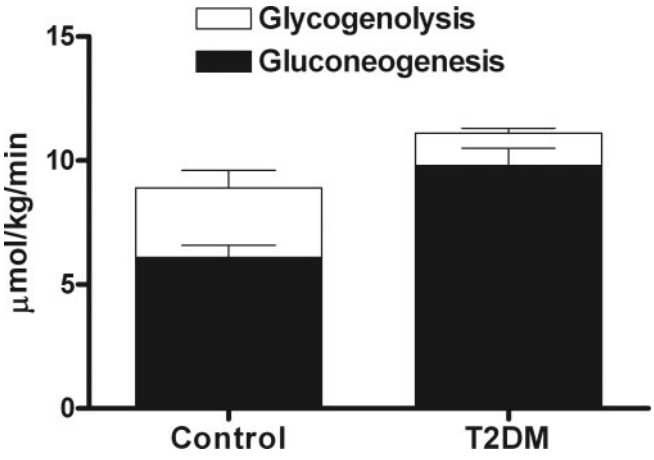

FIG. 1. Relative contributions of gluconeogenesis and glycogenolysis to hepatic glucose production in controls versus type 2 diabetics (T2DM). [Derived from Magnusson et al. (63).]

Fatty acid infusion could conceivably have direct effects on GLUT4 activity, or it could alter insulin-regulated GLUT4 trafficking between intracellular compartments and the cell membrane. To explore the latter possibility, insulin signaling intermediates were examined in skeletal muscle biopsies from subjects exposed to high fatty acid levels for 5 h before and during hyperinsulinemic-euglycemic clamps (31). Glucose oxidation and glycogen synthesis were 50–60% lower following the lipid infusion than with the glycerol (control) infusion and were associated with an ~90% decrease in the increment in intramuscular glucose-6-phosphate concentration, implying diminished glucose transport or phosphorylation activity. The fact that intracellular glucose concentrations were significantly lower in the lipid infusion studies compared with those during glycerol infusion implied that glucose transport was the rate-controlling step. Insulin receptor substrate 1 (IRS-1)-associated phosphoinositol (PI) 3-kinase activity was significantly reduced under these conditions (31). Subsequent rodent and human studies suggested that this might be a consequence of serine phosphorylation of IRS-1 (31, 41, 70, 129). An important and as yet unanswered element in this proposed mechanism for insulin resistance is the precise nature of the lipid moiety responsible for fatty acid-induced insulin resistance. Although TG accumulation in skeletal muscle and liver clearly correlates with insulin resistance, TG are generally perceived to be metabolically inert associates of more favored candidates which include long-chain acyl-coenzyme A (LCCoAs), diacylglycerol (DAG) (46, 129), and ceramides (1). Studies by Yu et al. (129) have been able to disassociate lipid-induced insulin resistance from any increases in intramuscular TG or ceramide content, suggesting that these lipid metabolites are not the trigger

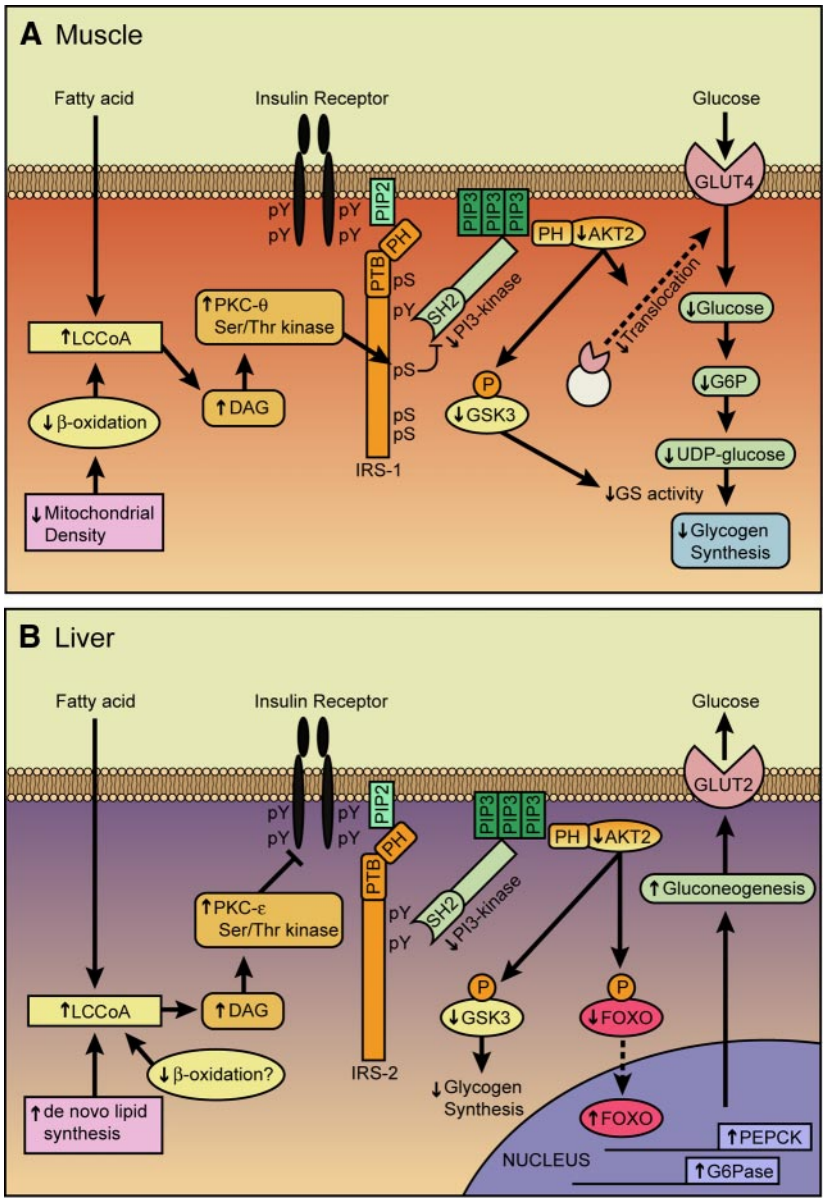

FIG. 2. Mechanism of fatty acid-induced insulin resistance in skeletal muscle (*A*) and liver (*B*). *A*: muscle. Increases in intramyocellular LCCoA and DAG, due to increased fatty acid delivery and/or decreased mitochondrial fatty acid oxidation, trigger a serine/threonine kinase (Ser/Thr) cascade initiated by nPKCs and possibly involving IKK-β and/or JNK-1. This ultimately induces serine/threonine phosphorylation of critical IRS-1 sites in muscle, thereby inhibiting IRS-1 tyrosine phosphorylation and activation of PI 3-kinase, resulting in reduced insulin-stimulated muscle glucose transport and diminished muscle glycogen synthesis. *B*: liver. Increases in intracellular DAG, due to increased lipogenesis and/or decreased mitochondrial fatty acid oxidation activate PKC-ϵ, which binds to and inactivates the insulin receptor kinase resulting in reduced insulin-stimulated IRS-1 and IRS-2 tyrosine phosphorylation. This in turn results in reduced insulin activation of PI 3-kinase and AKT2. Reduced AKT2 activation results in lower GSK3 phosphorylation and lower FOXO phosphorylation, which in turn results in lower insulin-stimulated liver glycogen synthesis and decreased suppression of hepatic gluconeogenesis, respectively. DAG, diacylglycerol; FOXO, forkhead box protein O; GLUT, glucose transporter; G6P, glucose 6-phosphate; GSK3, glycogen synthase kinase-3; IRS, insulin receptor substrate; IKK-β, IkB kinase-β; JNK-1, Jun kinase-1; LCCoA, long-chain acylcoenzyme A; nPKCs, novel protein kinase Cs; PEPCK, phospho*enol*pyruvate carboxykinase; PI 3-kinase, phosphoinositol 3-kinase; PTB, phosphotyrosine binding domain; PH, pleckstrin homology domain; SH2, src homology domain. [Adapted from Morino et al. (70a), with permission from The American Diabetes Association.]

in mediating fat-induced insulin resistance in skeletal muscle.[2] The fact that mitochondrial acyl-CoA:glycerol-*sn*-3-phosphate acyltransferase 1 (mtGPAT1) knockout mice, which have elevated LCCoAs but reduced DAG and TG in liver, have improved hepatic insulin sensitivity suggests DAG may be a better candidate than LCCoA in mediating fat-induced insulin resistance in liver (72). Protein kinase C (PKC) is a serine/threonine kinase known to be activated by DAGs and might account for the link between lipid accumulation and serine phosphorylation of IRS-1 in rodents (41, 99, 100). In keeping with these rodent studies, Itani et al. (46) noted that DAG accumulation in human muscle during lipid/heparin infusions was associated with increased PKC-β11 and PKC-δ activity. If this hypothesis is true, perturbations that result in accumulation of LCCoAs, DAGs, or other fatty acid derivatives within muscle and liver, either through increased delivery and/or decreased metabolism, ought to induce insulin resistance (104).

B. Liver (Fig. 2*B*)

The effects of elevated fatty acid concentrations on hepatic glucose metabolism are less clear. In the presence of postabsorptive insulin concentrations, plasma fatty acid elevation increased EGP during somatostatin-insulin

[2] In contrast to our data, Summers et al. (110) recently argued that ceramides may be a key factor in the pathogenesis of insulin resistance. This is largely based on in vitro data.

clamps (14, 34), but not after an overnight fast (14, 22). These apparent discrepancies may be caused by fatty acid-induced insulin secretion counterbalancing the stimulatory effect of fatty acids on EGP, and/or hepatic autoregulation,[3] preventing a net increase in hepatic glucose output despite increased gluconeogenesis. Roden et al. (92) combined measurements of EGP using D-[6,6-^{2}H]glucose with measurements of GNG derived from ^{2}H enrichments in carbons 2 and 5 of blood glucose after D_2O ingestion during short-term intralipid/heparin infusions. Plasma fatty acid elevation induced insulin secretion sufficiently to prevent any change in plasma glucose concentrations. However, if plasma insulin concentrations were maintained at fasting peripheral concentrations by a combined somatostatin-insulin infusion, fatty acid exposure significantly increased plasma glucose primarily by increasing gluconeogenesis. Boden et al. (12) lowered fasting plasma fatty acid concentrations by administering nicotinic acid and then increased fatty acids by withdrawing nicotinic acid (induces a rebound increase in fatty acids) whilst measuring GNG and GL. They concluded that increased fatty acids stimulated GNG, whereas reduced fatty acid concentrations inhibited GNG.

The mechanisms by which fatty acids induce GNG remain poorly understood. Postulates have included activation of pyruvate carboxylase (secondary to increases in acetyl CoA) (2) and increased availability of NADH and ATP (128). Roden et al. (92) excluded the possibility that elevated glycerol, a gluconeogenic substrate which is released with fatty acids during lipolysis, could have stimulated GNG by matching plasma glycerol levels during lipid/heparin infusion with a glycerol infusion in controls.

Studies of hepatic insulin signaling have for obvious reasons largely been confined to animals. PKC-ϵ translocation to the plasma membrane and activity is increased in rats with isolated hepatic steatosis and hepatic insulin resistance following 3 days of high fat feeding (96), suggesting that PKC-ϵ may be an important mediator of fat-induced insulin resistance in liver. This notion is further supported by recent data showing that antisense oligonucleotide (ASO)-mediated inhibition of PKC-ϵ reverses hepatic insulin resistance in this rat model of nonalcoholic fatty liver disease (96a). PKC-ϵ has also been shown to be activated in the liver in patients with T2DM (23).

IV. ECTOPIC LIPID ACCUMULATION

MRS measurements of intramyocellular lipids (IMCL) correlate more closely with insulin resistance than any other commonly measured indices including body mass index (BMI), waist-hip ratios, or total body fat (58, 67). Nonalcoholic steatohepatitis is also increasingly recognized as a component of the insulin resistance or metabolic syndrome (64, 76, 101). Lipid accumulation in ectopic sites can occur in three ways: increased uptake of fatty acids, increased synthesis within the tissue involved, and/or reduced fatty acid oxidation/disposal (104). The relative contribution of these factors to ectopic lipid accumulation may well vary in different physiological states and in different tissues. Simplistically, obesity and lipodystrophy would appear to be associated with ectopic lipid accumulation predominantly due to excess lipid influx/synthesis in the liver and muscle, whereas in lean elderly subjects (79) and lean insulin-resistant offspring of type 2 diabetics (80), impaired mitochondrial fatty acid oxidation may play a major role in this process.

A. Adipose Tissue Disorders: Obesity/Lipodystrophy

Obesity is a very common cause of insulin resistance and a major risk factor for the development of T2DM. Interestingly, lipid accumulation in muscle and liver is characteristic of obesity and what might be considered its opposite extreme, lipodystrophy (40, 81, 84). As alluded to above, it is clear that lipid/heparin infusions which increase plasma fatty acids can lead to lipid accumulation in skeletal muscle, and short-term high-fat feeding elevates liver TG in rats (96); in both cases, insulin resistance ensues. Mice overexpressing lipoprotein lipase (LPL) in muscle or liver also accumulate lipid in the respective tissues and manifest selective muscle or hepatic insulin resistance (51). Conversely, lowering plasma fatty acids for 1 wk with acipimox in subjects with T2DM reduced intramuscular long-chain acyl CoAs and improved insulin sensitivity (4). Increased fatty acid concentrations are often said to be typical of obesity (15), most likely due to increased fatty acid release from an expanded fat mass, providing a convenient link between obesity and ectopic lipid accumulation. Whilst suppression of fatty acid levels by insulin is consistently impaired in insulin-resistant obese subjects and at least in some forms of lipodystrophy (118), fasting plasma fatty acid levels are not always elevated in insulin-resistant obese subjects (82), nor are they typically elevated in lipodystrophics (97) [one needs to bear in mind that since TG concentrations are typically increased in lipodystrophy (37), ex vivo hydrolysis is likely to produce falsely elevated fatty acid measurements if samples are not appropriately collected and stored; one also needs to consider the fact that the normal range for fasting plasma fatty acids is particularly wide (of the order of 290–720 μM)]. The notion that obesity and lipodystrophy are associated with persistently elevated plasma fatty acids, which in turn result in lipid accumu-

[3] Autoregulation refers to the tendency of hepatic glycogenolysis to compensate for increased gluconeogenesis, thereby preventing an increase in HGP.

lation in muscle and liver, is therefore probably overly simplistic. What is more likely is that chronic imbalances between energy delivery/uptake and oxidation ultimately result in excess intracellular lipid accumulation, both at the whole body level and in individual organs or tissues (104). Kelley and co-workers (48, 109) have proposed the idea that metabolic inflexibility i.e., an impaired capacity to appropriately switch between "fed" (predominantly carbohydrate oxidation) and "fasting" (predominantly lipid oxidation) metabolism, typifies obesity and insulin resistance. This mismatching between energy supply and demand may over time contribute to the accumulation of ectopic lipid. Adipose tissue plays a major role in maintaining metabolic flexibility by buffering the postprandial influx of fatty acids, a function which is disturbed in both obesity and lipodystrophy (36).

Peroxisome proliferator-activated receptor gamma (PPAR-γ), a nuclear hormone receptor predominantly expressed in adipocytes, appears to be a key player in the ability of adipose tissue to buffer fatty acid influx. Humans with dominant negative loss-of-function mutations in the ligand-binding domain of PPAR-γ manifest a stereotyped form of partial lipodystrophy and impaired postprandial fatty acid trapping (98). They also have fatty livers and are severely insulin resistant. Thiazolidinediones are commercially available PPAR-γ agonists which substantially improve insulin sensitivity despite promoting weight gain. Whilst their precise mode of action remains unclear, they seem to improve the ability of adipose tissue to buffer fatty acids (113), a phenomenon which is likely to be a key component of their insulin sensitizing properties (55, 65). Thiazolidinediones also improve hepatic steatosis (66) and hepatic insulin sensitivity (3). Interestingly, they do not consistently lower muscle TG (66), nor is muscle TG increased in humans with loss-of-function mutations in PPAR-γ (98); observations which further suggest that intramyocellular TG is not the trigger for fat-induced insulin resistance but rather serves as a surrogate marker for the intracellular lipid metabolite that triggers insulin resistance under certain circumstances as previously discussed. However, thiazolidinediones have been shown to lower intramuscular LCCoAs in rodents which was associated with improvement in muscle insulin sensitivity (52).

Recent work employing stable isotopes to track the fate of ingested nutrients suggests that lipid accumulation in the liver is a result of both fatty acid re-esterification and de novo lipogenesis (30). One interesting hypothesis suggests that hyperinsulinemia could be an important driving force for lipogenesis in the liver (and muscle) by increasing sterol regulatory element-binding protein 1c (SREBP1c) expression (103). SREBP1c is a key transcriptional regulator of de novo lipogenesis (17).

B. Oxidative Disorders: Mitochondrial Dysfunction

Together with obesity, aging is a very common cause of insulin resistance, and like obesity, it too is characterized by lipid accumulation in muscle and liver (24, 79). While an increasingly sedentary life-style and the associated relative increase in fat mass almost certainly contributes to aging-induced insulin resistance, Petersen et al. (79) documented insulin resistance in elderly subjects matched to young adult controls in terms of fat mass, lean body mass, and activity. Furthermore, the ability of insulin to suppress lipolysis as measured by glycerol turnover was similar to that seen in young controls, suggesting that adipose tissue dysfunction was unlikely to be a major determinant of the observed increase in liver and muscle TG in their study. To measure mitochondrial oxidative phosphorylation activity in skeletal muscle, MRS was used to monitor ^{13}C labeling of carbon atoms in glutamate during an intravenous infusion of [2-^{13}C]acetate, providing a direct measure of TCA cycle flux. Rates of ATP synthesis were measured using ^{31}P MRS by the direct observation of ^{31}P-magnetization transfer between P_i and ATP. Both of these measurements of mitochondrial function in muscle were impaired in the lean elderly insulin-resistant subjects. Similar defects in mitochondrial function in muscle were found in lean insulin-resistant offspring of type 2 diabetics, who also have elevated intramyocellular TG (5a, 80). In the case of the elderly, this is likely to be a consequence of acquired mitochondrial mutations, a phenomenon known to occur with aging (68), whereas in the insulin-resistant offspring, it is more likely that the reduction in mitochondrial oxidative phosphorylation is a primary genetic defect. Insulin-resistant diabetic offspring were also found to have a lower ratio of oxidative type 1 to glycolytic type 2 muscle fibers in an independent study (80). Enzyme defects have also been described in isolated mitochondria derived from human skeletal muscle biopsies from type 2 diabetics (49). Mitochondria in skeletal muscle appear to be compartmentalized at a subcellular level, and one report suggests that there is a disproportionately large reduction of electron transport chain activity in the subsarcolemmal mitochondrial fraction (the other fraction being the intermyofibrillar fraction which generates energy for muscle contraction) in nondiabetic obese subjects and in obese type 2 diabetics (87). Subsarcolemmal mitochondria are believed to be crucial for fatty acid oxidation, glucose transport, and propagation of insulin signaling as well as several other energy-requiring processes at the cell surface (44, 87). Interestingly, the reduction in mitochondrial electron transport chain activity did not appear to be entirely attributable to a reduction in mitochondrial mass, suggesting that total cellular mitochondrial activity reflects both mitochondrial mass and the enzyme activity within the mitochondria; in other words, some mitochon-

dria may not function optimally (87). Together, these data suggest that alterations in nuclear-encoded genes that regulate mitochondrial biogenesis such as PPAR-γ coactivator 1α (PGC-1α), AMP kinase (7, 132), and calmodulin IV kinase (125) may form the genetic basis for inheritance of at least some forms of T2DM. They also suggest that genes involved in regulating both mitochondrial enzyme activity and mitochondrial mass may be involved in the pathogenesis of T2DM. This notion is further supported by two microarray studies that revealed a reduction in PGC-1α responsive transcripts in patients with T2DM and their first-degree relatives (69, 77). PGC-1α is a key regulator of mitochondrial biogenesis (126). However, despite demonstrating that the reduction in mitochondrial function seen in insulin-resistant diabetic offspring is due to reduced mitochondrial content (assessed by electron microscopy), Morino et al. (70) did not find any differences in PGC-1 mRNA or protein expression. These data suggest that the previously reported reduction in PGC-1 may be a secondary phenomenon. The primary factors responsible for the reduced mitochondrial content in insulin-resistant diabetic offspring remain unknown (70).

Finally, one needs to remain cognizant of the fact that, to date, inherited mitochondrial disorders have tended to produce pleiotropic manifestations (108), often without diabetes. In those cases where diabetes is a feature, β-cell dysfunction tends to be the primary abnormality (61, 108). One example where insulin resistance also seems to be a feature is Fredreich's ataxia, although insulin sensitivity has still to be carefully evaluated in this model (86). In our view, the mitochondrial dysfunction seen in the elderly, lean insulin-resistant diabetic offspring, and insulin-resistant type 2 diabetics is probably milder than that observed in disorders where β-cell dysfunction predominates. An important remaining question is whether mitochondrial defects, be they inherited or acquired, cause the increases in intramyocellular lipid and insulin resistance, or are they secondary in nature.

V. LINKS AMONG DISORDERED LIPID METABOLISM, INFLAMMATION, AND INSULIN RESISTANCE

While lipid accumulation in muscle and liver may be sufficient to explain the development of insulin resistance in obese subjects, obesity is also associated with a systemic chronic inflammatory response characterized by altered cytokine production and activation of inflammatory signaling pathways (122); recent reports have linked this inflammatory response to the development of insulin resistance. Almost all of these data have, to date, come from animal studies and have recently been comprehensively reviewed (121), so they are not covered in detail here. To summarize, the major findings in humans include the following.

Increased plasma inflammatory markers [e.g., C-reactive protein (CRP)] and altered plasma adipokine concentrations have been detected in some (121), but not all, insulin-resistant states. In particular, plasma levels of adiponectin, resistin, interleukin-6, tumor necrosis factor-α, and leptin were unchanged in both lean insulin-resistant offspring of type 2 diabetics (80) and in lean insulin-resistant elderly subjects (79) compared with insulin-sensitive controls, suggesting that inflammatory changes are unlikely to be the primary abnormality in these groups of subjects. Moderate weight loss in obese insulin-resistant subjects also failed to significantly alter adipokine levels despite significant improvements in hepatic insulin sensitivity and normalization of fasting plasma glucose concentrations (81).

Significantly increased numbers of macrophages accumulate in adipose tissue in obese states (120, 127). Macrophages are found in the stromovascular fraction of adipose tissue in humans and appear to make a substantial contribution to gene expression within adipose tissue. These cells are derived from bone marrow precursors and appear to infiltrate adipose tissue in greater numbers in obese states. Whether this inflammatory infiltrate is responsible for the development of insulin resistance is not yet clear, although Xu et al. (127) did suggest that the increase in inflammatory gene expression within adipose tissue preceded the dramatic increase in plasma insulin levels noted in high-fat fed mice. They also reported downregulation of these macrophage-derived genes in response to treatment with an insulin-sensitizing PPAR-γ agonist (rosiglitazone) (127). Subsequent studies in humans have suggested that the inflammatory cell infiltrate [as reflected by mRNA levels of a macrophage marker (CD68)] correlates with insulin sensitivity more closely than with BMI and that thiazolidinediones reduce CD68 mRNA levels and improve insulin sensitivity (29). Surgically induced weight loss (bariatric) also reduces macrophage infiltration in adipose tissue of morbidly obese subjects and improves insulin sensitivity (19).

In addition to PKC, a number of serine/threonine kinases are activated in inflammatory states. For example, Yuan et al. (130) recently proposed that fatty acid-induced serine phosphorylation of IRS-1 might be mediated by IKB kinase-β (IKK-β). This hypothesis has been supported by a human study demonstrating that pharmacological inhibition of IKK-β activity by high-dose salicylate therapy (high doses of salicylate are required to inhibit IKK-β activity) reduced fasting hyperglycemia and basal hepatic glucose production and improved peripheral glucose uptake in patients with T2DM (45). Hotamisligil and coworkers (43) have identified another inflammatory serine kinase potentially involved in inducing serine phosphorylation of IRS-1, namely, Jun kinase 1 (JNK1). They reported increased JNK activity in obese rodents and human adipose tissue, as well as reduced adiposity and

improved insulin sensitivity in JNK1 knockout mice. The same group has also suggested that endoplasmic reticulum stress in adipocytes and hepatocytes may be involved in JNK activation in obese mice (75). Suppressor of cytokine signaling 3 (SOCS3) is another potential contributor to the links among obesity, inflammation, and insulin resistance (116). One particularly intriguing aspect of the SOCS3 hypothesis is that increases in SOCS3 expression have also been observed in the hypothalamus where SOCS3 may be involved in inducing leptin resistance (9). This notion provides a potentially unifying mechanism for both insulin and leptin resistance, states which frequently coexist in obese humans.

VI. CONCLUSIONS

The ability to noninvasively monitor biochemical changes in living subjects in real time has yielded a number of significant novel insights into the pathogenesis of insulin resistance and T2DM. Insulin resistance in skeletal muscle manifests primarily as a reduction in insulin-stimulated glycogen synthesis, which is in turn a consequence of reduced glucose transport. In the liver, insulin resistance seems to be somewhat paradoxically associated with a reduced ability of insulin signaling to inhibit glucose production, whereas insulin-stimulated lipogenesis is enhanced. Lipid accumulation within skeletal muscle is associated with serine phosphorylation on critical sites on IRS-1 and reduced tyrosine phosphorylation of IRS-1 (Fig. 2). This in turn inhibits binding and activation of PI 3-kinase. A number of different serine kinases could be responsible for serine phosphorylation of IRS-1. Candidates include members of the nPKC family, which may be activated by accumulation of lipid intermediates (particularly DAGs), as well as inflammatory intermediates such as IKKβ, JNK1, and tumor necrosis factor-α. The latter may be activated within adipose tissue in obese states. Lipid accumulation in skeletal muscle and liver may be a result of increased delivery/synthesis of fatty acids to/in these tissues in states in which energy intake exceeds adipose tissue storage capacity (as seen in obesity and lipodystrophy), or a consequence of either acquired or inherited mitochondrial dysfunction. As well as reinforcing the importance of life-style interventions in the management of T2DM, dietary restriction to limit the stress on energy stores and exercise to increase mitochondrial number and function, these novel ideas about the molecular pathogenesis of insulin resistance have provided several new therapeutic targets for the treatment and possible prevention of T2DM.

Studies in lean offspring of type 2 diabetics suggest that intramyocellular lipid accumulation and muscle insulin resistance precede the development of hepatic insulin resistance and T2DM (80). We propose that insulin resistance in skeletal muscle is the earliest event in the pathogenesis of T2DM in most patients. Muscle insulin resistance is in turn associated with peripheral and portal vein hyperinsulinemia which promotes hepatic steatosis, at least in part by inducing SREBP1c-mediated de novo lipogenesis and inhibiting fatty acid oxidation. Recent work by Wolfram et al. (124) suggests that hyperinsulinemia induces nuclear exclusion and inhibition of Foxa2, a regulator of fatty acid oxidation. In time, this leads to lipid accumulation in the liver, hepatic insulin resistance, and ultimately T2DM. Adipocyte dysfunction due to either obesity or lipodystrophy is associated with excessive and untimely delivery of fatty acids to the liver and skeletal muscle and probably contributes to insulin resistance in both organs, by altering the balance between fatty acid uptake/synthesis and disposal leading to increases in intracellular lipid content (104). Further human studies will be required to prove or disprove this theory and are also expected to continue to unveil novel therapeutic approaches for the treatment and prevention of insulin resistance and T2DM.

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