Mechanisms of the free fatty acid-induced increase in hepatic glucose production

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Lam, Tony K. T., André Carpentier, Gary F. Lewis, Gérald van de Werve, I. George Fantus, and Adria Giacca. Mechanisms of the free fatty acid-induced increase in hepatic glucose production. Am J Physiol Endocrinol Metab 284: E863–E873, 2003; 10.1152/ajpendo.00033.2003.—The associations between obesity, insulin resistance, and type 2 diabetes mellitus are well documented. Free fatty acids (FFA), which are often elevated in obesity, have been implicated as an important link in these associations. Contrary to muscle glucose metabolism, the effects of FFA on hepatic glucose metabolism and the associated mechanisms have not been extensively investigated. It is still controversial whether FFA have substantial effects on hepatic glucose production, and the mechanisms responsible for these putative effects remain unknown. We review recent progress in this area and try to clarify controversial issues regarding the mechanisms responsible for the FFA-induced increase in hepatic glucose production in the postabsorptive state and during hyperinsulinemia.

FREE FATTY ACIDS (FFA), which are often elevated in obese individuals (6, 15, 29, 70), have been implicated as an important causative link in the associations between obesity, insulin resistance, and type 2 diabetes mellitus (11, 48, 56, 73, 82). Elevated plasma FFA clearly impair the ability of insulin to stimulate muscle glucose uptake (45, 98). This effect is associated with a reduction in insulin receptor substrate (IRS)-1-associated phosphatidylinositol (PI) 3-kinase activity, an important step in the insulin-signaling cascade that has been shown to be inhibited by FFA-induced PKC activation (42, 53). In contrast, although a number of studies have indicated that elevated plasma FFA and high-fat diets can increase postabsorptive (referred to throughout this manuscript as “basal”) hepatic glucose production (HGP) (7, 14, 67, 68, 113) and induce hepatic insulin resistance (i.e., reduce the ability of insulin to suppress HGP) (9, 13, 51, 67, 68, 74, 91, 97, 102, 111, 125), the magnitude of these effects and the mechanisms involved remain controversial.

Because elevated HGP and hepatic insulin resistance have been well documented in individuals with type 2 diabetes (12, 40, 85), studies that examine the effects of FFA on hepatic glucose metabolism are essential in understanding the pathogenesis of insulin resistance in type 2 diabetes. In this brief review, the physiological effects of FFA on hepatic glucose metabolism and the putative mechanisms of these effects will be discussed.

FFA AND BASAL HGP

FFA and Gluconeogenesis

Glucose is produced by glycogenolysis and gluconeogenesis in the liver. FFA increase hepatic gluconeogenesis both in vitro and in vivo (11, 20, 127). FFA stimulation of gluconeogenesis has been attributed to the production of 1) acetyl-CoA derived from FFA oxidation, which allosterically activates pyruvate carboxylase, 2) NADH, which is used for the formation of glyceraldehyde 3-phosphate from 1,3-bisphosphoglycerate, and 3) ATP, which is used as an energy source. Additionally, increased levels of citrate (product of acetyl-CoA derived from FFA oxidation and oxaloacetate) and citrate-induced inhibition of phosphofructokinase 1 were observed in the perfused rat liver and in isolated hepatocytes exposed to FFA (81, 87, 126). We have also observed that infusion of Intralipid + heparin, a standard method of elevating plasma FFA in vivo, increases hepatic citrate content (68).

Two secondary pathways that could provide a source for increased gluconeogenesis in the presence of FFA elevation have been proposed in recent years: 1) the glyoxylate pathway and 2) the xylulose 5-phosphate
pathway. 1) Through the glyoxylate pathway, free acetic acid, which increases with elevated FFA oxidation, is converted to succinate (112). An increase in succinate could lead to elevation of glucose through the tricarboxylic acid cycle and the gluconeogenic pathway. 2) FFA oxidation has been shown to decrease xylulose 5-phosphate (product of 6-phosphogluconate and NADP) because of a reduction of the NADP/NADPH ratio (76). Xylulose 5-phosphate is an activator of phosphofructokinase 1 (PFK-1) and an inhibitor of fructose-1,6-bisphosphatase (FBP-1). This is because xylulose 5-phosphate activates protein phosphatase 2A, which dephosphorylates the bifunctional enzyme (fructose 6-phosphate, 2-kinase: fructose-2,6-phosphate bisphosphatase), thus leading to increased Fru 2,6-bisphosphate (activator of PFK-1 and inhibitor of FBP-1) (76).

Hepatic Autoregulation

An increase in gluconeogenesis induced by FFA does not necessarily lead to increased HGP (23, 25, 99). Previous studies have suggested that a compensatory reduction in glycogenolysis prevents an FFA-induced increase in gluconeogenesis from elevated HGP (23, 25, 99). This is defined as “hepatic autoregulation.” To date, hepatic autoregulation has been attributed to both extrahepatic and intrahepatic mechanisms. The extrahepatic mechanism consists of the inhibition of glycogenolysis caused by the FFA-induced increase in insulin secretion and levels, which provide autoregulation of HGP in the presence of elevated FFA (73). The intrahepatic mechanism (independent of insulin) consists of the activation of glycogen synthase and inactivation of glycogen phosphorylase by activated glucose 6-phosphate from gluconeogenesis (47, 122, 133) and the inhibition of glycogen phosphorylase by ATP derived from FFA oxidation (33, 99).

Previous studies in overnight-fasted humans suggested that an elevation of plasma FFA, achieved by Intralipid + heparin infusion, did not have a significant effect in increasing basal HGP (14, 20), although FFA did significantly increase gluconeogenesis (14, 99). When endogenous insulin secretion was inhibited by somatostatin, and exogenous insulin was infused intravenously to maintain plasma insulin concentrations at basal levels (pancreatic clamp), FFA increased HGP in some studies (14) but not in others (99). FFA were also found to increase HGP during insulinopenia achieved by somatostatin infusion without insulin replacement (7, 35). These studies suggest that, in overnight-fasted humans, the stimulatory effect of FFA on HGP in the basal state is counteracted by an increase in insulin secretion. The increase in insulin secretion could lead to an increase in portal insulin concentration, which would decrease hepatic glycolysis and provide autoregulation of HGP through extrahepatic autoregulatory mechanisms (73). The fact that in some studies FFA did not increase HGP during a pancreatic clamp (20, 99) at basal insulin levels suggests that hepatic autoregulation in humans can also occur through intrahepatic mechanisms.

Similar to humans, in overnight-fasted dogs an elevation of FFA did not have significant effects on basal HGP (97). Also, elevated FFA increased gluconeogenesis but did not increase HGP when insulin was clamped at basal levels (23). In the latter study, the FFA-induced increase in basal HGP was prevented because glycogenolysis decreased, which compensated for the FFA-induced increase in gluconeogenesis (i.e., hepatic autoregulation). This hepatic autoregulation was not due to small changes in portal insulin concentrations induced by FFA-stimulated insulin secretion, because the basal insulin and glucose levels were clamped (23). These data also suggest that intrahepatic autoregulatory mechanisms come into play in the presence of elevated FFA, thereby reducing the tendency for elevated FFA to stimulate HGP. All of the above data concerning hepatic autoregulation were derived from acute or relatively short-term experiments. It is not known whether long-term exposure of the liver to elevated FFA is similarly associated with compensatory autoregulation.

Breakdown of Hepatic Autoregulation

There are conditions in which a breakdown of autoregulation has been described. Song et al. (113) have shown that, in overnight-fasted/liver glycogen-depleted rats, high-fat diet increased basal HGP (i.e., there was a breakdown of hepatic autoregulation) in the presence of elevated plasma insulin levels. However, in 5-h-fasted rats, lowering plasma FFA levels with acipimox did not affect basal HGP (72). The difference in results between the overnight-fasted and 5-h-fasted rats may be due to the fact that, after overnight fasting, glycogenolysis is limited by glycogen depletion (72, 113) and may not further decrease to provide hepatic autoregulation of basal HGP in the presence of FFA-stimulated gluconeogenesis (20). Thus the latter would lead to an FFA-induced increase in basal HGP. In fact, we have demonstrated that prolonged elevation of FFA increases basal HGP despite increased insulin secretion and higher insulin levels in overnight-fasted rats (68), which also suggests a breakdown of hepatic autoregulation in the presence of liver glycogen depletion.

Liver glycogen depletion may not be the only reason for the breakdown of hepatic autoregulation in our studies. Our data suggest that FFA may induce hepatic insulin resistance in the basal state in overnight-fasted rats, because fasting insulin and glucose levels were elevated and hepatic PKC-δ translocation was induced (67, 68). Hepatic insulin resistance could have increased basal HGP by decreasing the ability of insulin to suppress glycogenolysis. Another potential mechanism that we have described in the breakdown of hepatic autoregulation in the presence of elevated FFA is that FFA may have an allosteric stimulatory effect on glucose-6-phosphatase (67), which would lead to an
increase in basal HGP, since this enzyme catalyzes the final step leading to glucose production from both gluconeogenesis and glycogenolysis.

In type 2 diabetic individuals, inhibition of adipose tissue lipolysis achieved by nicotinic acid administration reduced plasma FFA concentration (12), thereby decreasing gluconeogenesis and HGP. HGP decreased because glycogenolysis did not increase, suggesting a breakdown in hepatic autoregulation (12). It is still unclear why hepatic autoregulation does not operate effectively in type 2 diabetes. However, impairment in insulin secretion and hepatic insulin resistance are present in type 2 diabetic individuals and likely account for a substantial part of this defect in autoregulation. In addition, as discussed above, liver glycogen has an important role in hepatic autoregulation, and liver glycogen content is reduced in type 2 diabetes (77).

Summary

FFA increase gluconeogenesis in the liver, but under usual basal physiological conditions, hepatic autoregulation prevents FFA from increasing HGP. In type 2 diabetic individuals, hepatic autoregulation appears to be defective, which leads to increased HGP in the presence of chronic FFA elevation. Further studies are needed to clarify the extrahepatic and intrahepatic mechanisms that have a role in hepatic autoregulation in the presence of elevated FFA and the reason for its dysfunction in conditions such as type 2 diabetes. The factors that could have a role in the breakdown of autoregulation, especially after prolonged exposure to FFA, are FFA-induced hepatic insulin resistance and allosteric stimulation of glucose-6-phosphatase activity. In addition to hepatic insulin resistance, breakdown of autoregulation appears to be favored by impaired insulin secretion and depletion of liver glycogen content, which are all important pathophysiological features of type 2 diabetes.

FFA AND HEPATIC INSULIN ACTION

Hepatic Insulin Signaling

Insulin binds to its receptor at the surface of the hepatocytes to initiate its action through a cascade of signaling molecules (1, 103, 104, 116). Upon insulin binding to its receptor, the insulin receptor tyrosine kinase is activated, which results in receptor autophosphorylation. The activated insulin receptor tyrosine kinase also phosphorylates insulin receptor substrates (IRSs), which include IRS-1 through IRS-4 and Shc. Phosphorylated IRS-1 through -4 mainly activate the PI 3-kinase pathway, and Shc mainly activates the Grb2/Sos and MAP kinase pathway (116). IRS-2 has been shown to have an important role in hepatic insulin action (100). IRS-2 knockout mice have impaired hepatic insulin signaling (i.e., impaired PI 3-kinase activity or impaired insulin suppression of HGP) and develop type 2 diabetes from lack of a compensatory increase in β-cell insulin secretion (IRS-2 is also lost in the β-cell) (64, 128). Similar to the IRS-2 knockout mouse, loss of hepatic insulin signaling is very likely responsible for the failure of insulin to suppress HGP in liver-specific insulin receptor knockout mice (84). This strongly supports the notion that insulin has a direct hepatic effect in suppressing HGP in vivo.

Previous studies have demonstrated that insulin acts directly at the liver to suppress HGP by acutely inhibiting glycogenolysis (110). Insulin also indirectly suppresses HGP via its peripheral actions of reducing the level of gluconeogenic precursors and FFA (39, 74, 97, 111). The reduction of FFA diminishes gluconeogenesis and leads to diversion of glycogenolytic flow to lactate output rather than glucose output from the liver (110). Insulin can suppress HGP indirectly also via its action of suppressing glucagon secretion. This action physiologically is dependent on the intrapancreatic insulin levels achieved by endogenous insulin secretion (105), whereas with exogenous insulin administration, as in insulin-treated type 1 diabetes, glucagon suppression is dependent on the peripheral insulin levels (39). In addition, the chronic effect of insulin in suppressing HGP consists of direct inhibition of the gene transcription of gluconeogenic enzymes (84).

Lipid and Hepatic Insulin Resistance

A large number of studies have demonstrated that an elevation of FFA, achieved by Intralipid + heparin infusion, increases HGP during euglycemic hyperinsulinemic clamp experiments (9, 13, 67, 68, 74, 97, 102, 111, 125) and that high-fat diet impairs the ability of insulin to suppress HGP (51, 91). The increase of HGP could be attributed partly to breakdown of hepatic autoregulation, as discussed above, because a breakdown of autoregulation is very likely facilitated under hyperinsulinemic clamp conditions, presumably because, during hyperinsulinemia, glycogenolysis is already maximally suppressed. Alternatively or in addition, FFA may decrease the ability of insulin to suppress HGP (i.e., induce hepatic insulin resistance) by impairing hepatic insulin action (signaling), as suggested by recent studies. A-ZIP/F-1 fatless mice (60), which express a dominant negative protein named A-ZIP/F-1 in adipocyte tissue that prevents DNA binding and inactivates members of the C/EBP and JUN families of B-ZIP transcription factors, resulting in virtually no white adipose tissue and a reduced amount of inactive brown adipose tissue in the mice (86), and mice with liver lipoprotein lipase overexpression (59) have increased liver triglyceride content and decreased hepatic IRS-2-associated PI 3-kinase activity (i.e., decreased hepatic insulin signaling). Also, high-fat feeding in rats decreased hepatic IRS-2-associated PI 3-kinase activity (106).

Long-Chain Fatty Acyl-CoA, Ceramide, Diacylglycerol, and Triglyceride Accumulation

Hepatic insulin resistance induced by high-fat feeding in rats is not ameliorated by etomoxir, which blocks fatty acid oxidation (91). Also, in our studies, the time
course of FFA-induced hepatic insulin resistance appeared to be different from that of the increase in hepatic fatty acid oxidation (68), suggesting that the fat inhibition of insulin signaling is mediated by factors that are not dependent on fatty acid oxidation. Both of these studies suggest that the fatty acid esterification pathway, rather than fatty acid oxidation, could be implicated in hepatic insulin resistance. In states of energy excess (increased availability of FFA, glucose, and insulin), malonyl-CoA increases because of stimulation of acetyl-CoA carboxylase (ACC) by glucose and insulin and because of increased citrate, which provides cytosolic acetyl-CoA and activates ACC. Malonyl-CoA is an allosteric inhibitor of carnitine palmitoyltransferase I (CPT I), the enzyme required to transport FFA into the mitochondria, where β-oxidation takes place (83). Thus CPT I inhibition prevents further FFA oxidation and results in accumulation of cytosolic long-chain fatty acyl-CoA (LCFA-CoA) (19) and diversion of their metabolism toward esterification rather than oxidation (101). The diversion to the esterification pathway would lead to accumulation of diacylglycerol (DAG), triglycerides (TG), and ceramides (Fig. 1).

Cytosolic TG (the end product of the fatty acid esterification pathway) are a positive marker for hepatic insulin resistance (65). Previous studies have shown that hepatic insulin resistance is reversed when hepatic TG content is reduced by 1) administration of the fat-derived hormone “adiponectin” into lipatrophic mice (lipatrophic mice have elevated hepatic TG content and hepatic insulin resistance) (131), 2) fat transplantation into A-ZIP/F-1 fatless mice, which have elevated hepatic TG content and hepatic insulin resistance (60), and/or 3) administration of an AMP-activated protein kinase (AMPK) activator, 5-aminoimidazole-4-carboxamide-1-B-D-ribofuranoside (AICAR), to high fat-fed rats (51).

LCFA-CoA and DAG stimulate PKC (109). We have shown that an elevation of plasma FFA increases hepatic PKC-δ plasma membrane translocation (68), probably through an accumulation of hepatic LCFA-CoA and/or DAG, along the fatty acid esterification pathway. In addition to the formation of DAG from LCFA-CoA, LCFA-CoA can form ceramides through de novo synthesis (Fig. 1). Ceramides have been implicated in fat-induced insulin resistance (17, 43, 108), but the effects of ceramides on insulin action in vivo remain unclear. Turinsky et al. (121) observed that ceramide levels are increased in the liver of insulin-resistant obese vs. lean Zucker rats. However, liver ceramides did not increase in safflower oil-fed insulin-resistant rats (90), and muscle ceramides did not increase in Intralipid + heparin-induced muscle insulin resistance in humans (53). This is possibly due to the fact that unsaturated fatty acids (such as those found in safflower oil and Intralipid) contribute less to the synthesis of ceramides than the saturated fatty acid palmitate (108).

AMPK is the fuel sensor of mammalian cells (44) and is activated in response to exercise, hypoxia, or prolonged starvation due to an increased AMP/ATP ratio (57). AMPK has recently been implicated in hepatic insulin sensitivity (51) and regulates hepatic gluconeogenesis and glucose production (26, 130). Adiponectin, an AMPK activator, was found to increase hepatic FFA oxidation and reduce hepatic gluconeogenic gene expression and HGP (26, 130). AICAR, also an AMPK activator, was found to prevent high-fat diet-induced hepatic insulin resistance, and this was associated with inhibition of hepatic cytosolic TG accumulation (51). These studies further confirm the notion that accumulation of lipid esterification products has an important role in lipid-induced hepatic insulin resistance. To our knowledge, it is still unclear whether high-fat diet or FFA, being a source of ATP, impair hepatic AMPK activity, since studies gave conflicting results. Short-term incubation of cardiac myocytes (50) and rat skeletal muscle (92) with palmitate increased or did not change AMPK activity, respectively. Short-term incubation of rat liver-purified AMPK with palmitoyl-CoA increased AMPK activity (18), but prolonged incubation of cardiac myocytes with palmitate decreased AMPK activity (50).

**PKC**

Early evidence has implicated PKC in insulin resistance (123), and more recently some novel and classical PKC (DAG-sensitive) isoforms have been implicated in lipid-induced insulin resistance (42, 53, 54, 107). PKC is known to phosphorylate the serine/threonine residues of the insulin receptor, and such phosphorylation reduces insulin receptor tyrosine kinase activity and insulin action (16, 21, 117). Also, PKC phosphorylates IRS, which becomes an inhibitor of the insulin receptor tyrosine kinase activity (30, 55, 96). It is known that FFA or high-fat diet-induced muscle insulin resistance is associated with PKC-δ, -ε, -δ, and -βII plasma membrane translocation (42, 53, 107). However, the role of hepatic PKC in FFA-induced hepatic insulin resistance is unclear.

Hepatic PKC activity was found to be greater in obese hypertriglyceridemic diabetic rats than in lean rats (95). Obese diabetic rats and obese subjects with type 2 diabetes have higher hepatic membrane-associated PKC-α, -ε, and -ζ than controls (27). Normalization of circulating glucose levels in obese diabetic rats did not result in reduction of hepatic membrane PKC.
Oxidative Stress, Hexosamine Pathway, Ceramide, iNOS, and IKKβ

New emerging biochemical pathways have been implicated in FFA-induced insulin resistance. Our preliminary studies show that N-acetyl-l-cysteine (NAC), an antioxidant, prevents FFA-induced hepatic insulin resistance in vivo in rats (34), suggesting that oxidative stress may have a role in FFA-induced hepatic insulin resistance. Reactive oxygen species (ROS) are highly reactive molecules that inflict oxidative damage, also referred to as “oxidative stress.” It is known that FFA increase oxidative stress by increasing ROS via their mitochondrial oxidation, via peroxidation reactions, and via the hexosamine biosynthetic pathway (HBP) (45, 80, 93, 120). The HBP has been suggested to play a role in FFA-induced insulin resistance in muscle (45). It has been proposed that, in a number of tissues, ROS can increase the flow through the HBP by inhibiting glyceraldehyde-3-phosphate dehydrogenase (32), whereas flux through the HBP may increase ROS through glycosylation reactions (46, 124). Indeed, we have previously shown that the antioxidant NAC prevents glucosamine-induced insulin resistance in rat adipocytes (22). Because we (68) and others (42, 53) have demonstrated that FFA induce PKC translocation, and both ROS (114) and glucosamine (41, 46, 62, 63) are known to stimulate PKC, and in particular PKC-δ, PKC may be the common mediator for FFA/glucosamine/ROS-induced insulin resistance. On the other hand, PKC activation can lead to ROS formation (52), and ROS can directly induce insulin resistance (10, 118, 119). However, no studies, to our knowledge, have investigated the HBP and ROS in relationship to the effects of FFA on hepatic insulin action. On the basis of our preliminary in vivo data supporting NAC prevention of hepatic insulin resistance in the presence of elevated FFA (34), our current working hypothesis is that both increased hepatic ROS levels and PKC-δ translocation (68) have a role in FFA-induced hepatic insulin resistance.

Oxidative stress also activates sphingomyelinase (3, 79) and thus increases ceramides, breakdown products of sphingomyelin, which in turn can cause peripheral insulin resistance (17, 43). The mechanisms responsible for ceramide-induced muscle insulin resistance appear to be mediated by PKB (43, 108). Conversely, ceramides increase the mitochondrial production of ROS (3, 38, 79). Both ROS and ceramides induce inducible nitric oxide synthase, which has recently been implicated in fat-induced muscle insulin resistance (94). As discussed above, however, the effects of ceramides on hepatic and muscle insulin action in vivo are still controversial.

Another important player that has recently been implicated in lipid-induced insulin resistance is IkB kinase-β (IKKβ), which activates nuclear factor κB (NF-κB) through its release from IkB-α after phosphorylation of the latter. IKKβ can phosphorylate insulin-signaling molecules (i.e., insulin receptor and IRS/Shc) on serine/threonine residues. Salicylates, inhibitors of IKKβ, have been found to prevent downregulation of hepatic and muscle insulin receptor tyrosine phosphorylation in insulin-resistant Zucker fa/fa rats (134) and in fat-infused normal rats (61). Interestingly, PKC-δ is known to be an activator of IKK (115), and FFA-induced muscle insulin resistance was found to be associated with increased muscle PKC-δ and -βII plasma membrane translocation and decreased muscle IkB-α (because of degradation subsequent to its phosphorylation by IKKβ) (53), although the association between PKC-δ and -βII translocation and IkB-α remains to be clarified. Taken together, these data suggest that FFA induce muscle insulin resistance through a PKC/IKKβ-dependent pathway, but the role of IKKβ in FFA-induced hepatic insulin resistance remains to be determined.

Hepatic Glucokinase and Glucose-6-Phosphatase

We have reviewed some of the potential mechanisms responsible for FFA-induced hepatic insulin resistance. Nevertheless, the rate-limiting site of the FFA-induced impairment in insulin action on HGP remains unclear. In our overnight-fasted/hepatic glycogen-depleted rat model, a mild increase of plasma insulin content, suggesting that factors other than hyperglycemia were responsible for this finding. These factors may include elevated FFA and TG levels, which were not measured in that study (27).

We have shown that an elevation of FFA induces translocation of the novel PKC-δ isoform from the cytosolic to the membrane compartment in rat liver, and this effect is associated with FFA-induced hepatic insulin resistance (68). Other investigators have shown that FFA promotes translocation of PKC from the cytosol to the plasma membrane in hepatocytes (31). Thus PKC, and in particular PKC-δ, may be a key mediator of FFA-induced hepatic insulin resistance, possibly through impairment of hepatic IRS-associated PI 3-kinase activity (Fig. 2).
ing the ability of insulin to increase this flux by impairing the ability of insulin to increase this flux (67). Short-term elevation of FFA decreased insulin’s ability to suppress HGP by impairing the ability of insulin to increase this flux (67). These data suggest that FFA induce hepatic insulin resistance at the site of glucokinase. We speculate that FFA decrease insulin-induced glucokinase translocation (an effect that is possibly mediated by PKC-δ) (67), because insulin-potentiated glucose-induced glucokinase translocation and palmitic acid partially counteracted glucose-induced glucokinase translocation in hepatocytes (2). However, high-fat feeding induced hepatic insulin resistance at the site of glucose-6-phosphatase in 5-h-fasted rats (91). Differences in duration of fasting and in liver glycogen content could be the main reasons for the difference in observations between the two studies (67). Nonetheless, the interconversion between glucose and glucose 6-phosphate was found to be the major site of lipid-induced hepatic insulin resistance in both studies.

Summary

It is well established that FFA induce hepatic insulin resistance, but the detailed mechanisms remain to be elucidated. On the basis of studies that have been described, hepatic PKC-δ may be an important signaling mechanism of FFA-induced hepatic insulin resistance. The rate-limiting site of impairment of hepatic insulin action by the FFA-activated signaling mechanisms appears to be at the level of the interconversion between glucose and glucose 6-phosphate.

CHRONIC EFFECTS OF LIPID ON Glucose PRODUCTION

In animal studies, high-fat feeding increased basal HGP (113) and decreased the ability of insulin to suppress HGP (51, 91). Liver-specific insulin receptor knockout mice have increased expression of gluconeogenic enzymes, such as phosphoenolpyruvate carboxykinase and glucose-6-phosphatase (also a glycolytic enzyme), and have decreased expression of glycolytic enzymes such as glucokinase and pyruvate kinase (84). The ability of insulin to regulate hepatic gene expression appears to be dependent on PI 3-kinase activity, since it has been shown that insulin suppresses gluconeogenic enzymes through a PI 3-kinase-dependent pathway (37). The downstream effects of this pathway possibly consist of phosphorylation of the forkhead transcription factor (88, 89) and the peroxisome proliferator-activated receptor coactivator, or PGC-1 (28, 132). Thus insulin chronically suppresses HGP by increasing glycolytic and decreasing gluconeogenic enzymes, and one of the major chronic effects of FFA and high-fat feeding on hepatic enzyme expression/activities can be indirect and consist of the FFA-induced impairment of the action of insulin on these enzymes. The latter concept is supported by recent studies in which high-fat feeding decreased hepatic IRS-2-associated PI 3-kinase activity (106), and visceral fat was found to impair insulin action on the gene expression of hepatic gluconeogenic enzymes (8).

Peroxisome Proliferator-Activated Receptor and Sterol Regulatory Element-Binding Protein

In addition to indirectly influencing gene expression by inducing insulin resistance, FFA can directly influence gene expression via modulating the activity of transcription factors, including peroxisome proliferator-activated receptors (PPAR) and sterol regulatory element-binding protein (SREBP)-1 (24). PPARα is expressed abundantly in the liver and to a much lesser extent in muscle (5). Polysaturated fatty acids are strong activators of PPARα, and it has been shown previously that a high unsaturated fat diet increases PPARα gene expression in the liver (58). Most studies that have examined SREBP-1 were done on liver tissue, since SREBP-1 regulates genes that are involved in lipogenesis [e.g., ACC and fatty acid synthase (FAS)], which takes place mainly in the liver and to a lesser extent in the adipocyte. Polysaturated fatty acids suppress the expression of SREBP-1 (both SREBP-1a and -1c) by decreasing SREBP-1 mRNA stability (129).

In the liver, FFA affect gene expression of enzymes involved in peroxisomal and mitochondrial FFA oxidation (mainly through PPARα) and hepatic lipogenesis (mainly through SREBP-1) (24, 129). The overall effect is that of limiting hepatic TG accumulation as an adaptation to the excess of FFA supply. This could account for increased FFA oxidation and decreased accumulation of TG. Polysaturated fatty acids suppress hepatic lipogenic genes through the SREBP-1 transcription factor (24). This effect is paralleled by a significant decrease in the hepatic production of malonyl-CoA (24). The decrease in malonyl-CoA production can be explained by the fact that polysaturated fatty acids repress the gene expression of ACC, which is SREBP-1 dependent (24). This decrease in malonyl-CoA not only reduces the rate of accumulation of TG but can also facilitate FFA oxidation by releasing the malonyl-CoA inhibition of CPT I. Also, the reduction in rate of accumulation of TG can be due in part to a decrease in FAS expression induced by polysaturated fatty acids (24). This effect is likely mediated by SREBP-1, since recent findings indicate that FAS expression is mediated by SREBP-1 (69). It is known that LCFA-CoA can inhibit the expression of some lipogenic enzymes, such as FAS, by binding and inhibiting hepatocyte nuclear factor (HNF)-4α; however, the importance of this mechanism in the reduction of TG accumulation is not known (24). It is also possible that the regulation of HNF-4α by FFA is indirect, being mediated by AMPK (71).

Recently, we have demonstrated that fatty acids with different degrees of unsaturation induce hepatic insulin resistance to a different extent (66). Interestingly, polysaturated fatty acids (soybean oil) induce
less hepatic insulin resistance than saturated (lard oil) or monounsaturated (oleate) fatty acids (66). Previous studies have shown that, unlike polyunsaturated fatty acids, saturated or monounsaturated fatty acids have no suppressive effect on the expression of the lipogenic enzymes ACC and FAS in the liver (129). This is due to the fact that polyunsaturated, but not saturated or monounsaturated, fat suppresses the expression of SREBP-1 transcription factors for lipogenic gene expression in the liver (129). The repression of ACC by polyunsaturated fat leads to greater FFA oxidation (because lower malonyl-CoA, the product of ACC, relieves the inhibition of CPT 1), thus altering the partitioning of FFA metabolism toward oxidation rather than esterification to TG (75). The additional repression of FAS by polyunsaturated fatty acids could also contribute to lowered TG accumulation through reduced de novo lipogenesis.

Our current working hypothesis is that polyunsaturated fatty acids induce less hepatic insulin resistance than saturated or monounsaturated fatty acids because LCFA-CoA, DAG, and/or TG accumulates to a lesser extent with polyunsaturated than with saturated or monounsaturated fatty acids. It is also tempting to speculate that hepatic PKC-δ will be activated to a smaller extent by polyunsaturated fatty acids than by saturated or monounsaturated fatty acids because LCFA-CoA and DAG will be less accumulated. This remains to be proven.

The elevation of FFA directly influences the gene expression/protein levels of enzymes involved not only in lipid but also in hepatic glucose metabolism. The gene expression of the gluconeogenic enzyme phosphoenolpyruvate carboxykinase (PEPCK) is increased by FFA, and PPAR response elements have been shown on the PEPCK gene in 3T3-F442 adipocytes and FAO hepatoma cells (4). Glucose-6-phosphatase gene expression and protein levels were increased by prolonged elevation of FFA in vivo, and the effect of FFA appears to be PPAR dependent (78). LCFA-CoA may inhibit glycolytic enzymes such as pyruvate kinase through inhibition of HNF (24), and polyunsaturated fatty acids may also inhibit glycolytic enzymes by decreasing SREBP-1 (24). Therefore, in the presence of FFA elevation, PPAR activation would limit hepatic TG accumulation but increase gluconeogenesis. On the other hand, SREBP and HNF inhibition would decrease TG accumulation and increase glycolytic enzymes. These dual effects are not completely understood. NF-κB is a regulator for the gluconeogenic enzyme FBP-1 (49), and elevated hepatic lipid peroxidation has been shown to increase oxidative stress and NF-κB (36). It is also known that a high-fat diet increases FBP-1 protein levels (113). Thus it is tempting to speculate that chronic elevation of FFA may increase hepatic gluconeogenesis through an oxidative stress/NF-κB-induced increase in FBP-1 expression/activity. In addition to ROS and NF-κB, it is well known that PKC, HBP, and AMPK all have effects on gene transcription. However, the role of these mediators in the effect of FFA on genes that regulate HGP has yet to be determined.

Summary

It is clear that chronic elevation of FFA affects gene expression and protein levels of enzymes that are involved in hepatic lipid and glucose metabolism. It is still unclear, however, how these molecular events affect the physiology of lipid and glucose metabolism in the liver. Further genetic, molecular, and pharmacological manipulations of these enzymes in the intact animal are required to determine the overall impact induced by a chronic elevation of FFA.

CONCLUSIONS

It is becoming increasingly clear that FFA have diabetogenic effects in the liver and that the mechanisms responsible for such diabetogenic effects are complex and incompletely defined. A number of candidate molecules and pathways have recently been proposed to mediate the deleterious effects of FFA on hepatic insulin sensitivity and HGP, but the relative importance of these factors is currently not clear. It also remains an open question how much of a role chronically elevated FFA have in initially causing hepatic insulin resistance and sustaining the elevated rates of HGP that characterize established type 2 diabetes. Although there are similarities between the cellular effects of FFA in liver and other insulin-sensitive tissues such as muscle, there are also important differences. Pathways elucidated in one tissue cannot necessarily be generalized and extrapolated to the liver. Future research directed at identifying pharmaceutical targets that inhibit or diminish the deleterious effect of elevated FFA on glucose metabolism in the liver could prove useful for the treatment of hepatic insulin resistance in obesity-associated type 2 diabetes.

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