

Potential Role of Peroxisome Proliferator-Activated Receptor- α in the Modulation of Glucose-Stimulated Insulin Secretion

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In this review, we discuss the influence of peroxisome proliferator-activated receptor (PPAR)- α on islet insulin secretion and develop the hypothesis that modulation of PPAR- α function may be important for the regulation of compensatory insulin secretion. We have attempted to analyze the role of PPAR- α -linked fatty acid metabolism in islet function in health and in insulin-resistant states linked to lifestyle factors, in particular pregnancy and a diet inappropriately high in saturated fat. We have emphasized the potential for both actions of PPAR- α on insulin sensitivity that may be relayed systemically to the islet, leading to modulation of the insulin response in accordance with changes in insulin sensitivity, and direct effects of PPAR- α action on the islet itself. Finally, we have developed the concept that compensatory insulin secretion may have a function not only in glucohomeostasis but also in liporegulation. Thus, augmented insulin secretion may reflect a requirement for lipid lowering as well as for increased glucose disposal and is perceived to aim to compensate for impaired suppression of islet lipid delivery by insulin. This introduces the possibility of a continuum between liporegulation with islet compensation and lipodysregulation leading to islet decompensation in the development of type 2 diabetes. *Diabetes* 53 (Suppl. 1): S71-S81, 2004

Deranged islet lipid metabolism contributes to β -cell failure, particularly when associated with intracellular accumulation of fatty acids (FAs) and fatty acyl-CoA (1) and/or engorgement with triacylglycerol (TAG) (2,3) secondary to increased TAG and/or FA delivery. Similarly, chronic overexposure of islets to high glucose concentrations impairs β -cell func-

tion (4,5). Lipotoxicity and glucotoxicity share many common features, and FA and glucose metabolism are intimately linked through their ability to act as competing oxidative substrates (6). Analysis of the relative contribution of elevated glucose and FA in the etiology of impaired β -cell function has led to the suggestion that neither hyperglycemia alone nor elevated circulating FAs alone are necessarily detrimental to β -cell function (4). Problems arise when both glucose and FA concentrations are concomitantly high. Thus, whereas an elevation in FA without hyperglycemia is matched by increased FA oxidation, a combination of excess glucose and FAs is proposed to result in the diversion of FAs from oxidation toward the formation of extramitochondrial lipid-derived signaling molecules, which initially modify insulin secretion but ultimately cause β -cell death. Implicit in this proposal is the concept that appropriate control of the fate of incoming lipids is critical to β -cell function (4). In this review, we extend this concept and develop the hypothesis that insulin hypersecretion linked to altered β -cell lipid handling is in part aimed to compensate for impaired suppression of islet lipid delivery by insulin.

Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors that belong to the nuclear hormone receptor superfamily. PPARs are transcriptionally active only after heterodimerization with the 9-*cis* retinoic acid-activated retinoid X receptor (RXR). The PPAR-RXR heterodimer binds to a specific recognition site, termed a PPAR response element (PPRE), located in the regulatory region of target genes. In the absence of ligand, the heterodimer forms high-affinity complexes with co-repressor proteins, which prevent access to the target gene promoter. Ligand binding produces a conformational change, resulting in dissociation of repressors, allowing the heterodimer to bind to the PPRE (7,8). At least two of the three PPAR family members, namely PPAR- γ and PPAR- α , exert profound effects on lipid supply, lipid storage, and the capacity for FA oxidation of individual tissues and thus whole-body lipid handling, in particular the preferential direction of available lipids toward specific tissues. For example, PPAR- γ activation directs lipid toward adipose tissue and PPAR- α activation predominantly directs lipid toward liver. In this review, we discuss the influence of PPAR- α on islet insulin secretion and develop the hypothesis that modulation of PPAR- α function may be important for the regulation of compensatory insulin secretion.

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ACL, ATP-citrate lyase; ΔB , area under the glucose curve after glucose challenge; ΔI , area under the insulin curve after glucose challenge; FA, fatty acid; FACS, fluorescence-activated cell sorter; GSIS, glucose-stimulated insulin secretion; K_{ATP} , ATP-sensitive potassium; PDC, pyruvate dehydrogenase complex; PDK, pyruvate dehydrogenase kinase; PPAR, peroxisome proliferator-activated receptor; PPRE, PPAR response element; RXR, retinoid X receptor; TAG, triacylglycerol.

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PPAR- α AND LIPID METABOLISM

PPAR- α is expressed in a wide range of tissues, including pancreatic islets (9,10). Natural ligands for the PPAR- α receptor include unsaturated FAs and their derivatives, including polyunsaturated FAs 8(S)-hydroxyeicosatetraenoic acid, 8(S)-hydroxyeicosapentaenoic acid, and leukotriene B4 (11). Synthetic ligands for PPAR- α include fibrates, for example clofibrate, fenofibrate, and WY14,643, which enhance the expression of genes involved in FA uptake and oxidation (12) including acyl-CoA dehydrogenase and muscle-type carnitine palmitoyltransferase-I (13,14). It has been suggested that PPAR- α may act as a general sensor of overall tissue lipid supply. Thus, the main physiological function of PPAR- α appears to reside in the regulation of lipid uptake and oxidation, contrasting with that of PPAR- γ , which promotes uptake but subsequent storage of lipid as TAG in adipose tissue. Selective PPAR- α ligands increase lipoprotein lipase expression in the liver (15) but suppress lipoprotein lipase catalytic activity in cardiac myocytes (16). Thus, the response to PPAR- α activation involves preferential targeting of lipoproteins toward the liver. A key role for PPAR- α in mediating some of the long-term adaptations to prolonged starvation has been demonstrated using the PPAR- α null mouse (17–19). Despite suppression of insulin levels and increases in FA supply, PPAR- α -deficient mice exhibit an impaired ability to adequately upregulate hepatic FA oxidation in response to fasting (17–19). Because hepatic FA uptake reflects increased FA delivery, an increased proportion of incoming FAs are re-esterified, and intracellular lipid derivatives, including TAG, accumulate (17,19,20). Impaired hepatic clearance of incoming FAs via oxidation is indicated by abnormally low ketone body concentrations in the fasting state (18,20). Interestingly, PPAR- α also modulates amino acid metabolism (21). Amino acid degradation significantly contributes to hepatic ATP production in the fed state (22). Analysis of gene expression in livers of control and PPAR- α null mice revealed an overall increase in the gene expression of enzymes involved in amino acid degradation as a result of PPAR- α deficiency (21). Thus, increased signaling via PPAR- α in starvation may coordinate a switch toward enhanced hepatic FA oxidation and a decline in the relative contribution of amino acid oxidation to hepatic ATP production, thereby sparing amino acids for glucose production and reducing the requirement for muscle proteolysis.

EFFECTS OF LOWERED PPAR- α SIGNALING ON INSULIN SECRETION

Glucose regulation of insulin release is mediated by metabolic signals generated secondary to increased glucose metabolism (23). Pancreatic β -cells express GLUT2, which permits rapid glucose uptake in proportion to the extracellular glucose concentration; however, low islet expression of hexokinases I, II, and III results in a low glycolytic flux and low [ATP]-to-[ADP] ratios at basal glucose levels (<2.5 mmol/l). At higher glucose concentrations, β -cells phosphorylate glucose via glucokinase (hexokinase IV). A sigmoidal relationship exists between glucokinase reaction velocity and glucose concentration (substrate concentration at half-maximal reaction [$S_{0.5}$]-8 mmol/l). Because glucokinase is not inhibited by glucose-6-phosphate, there

is a proportional increase in the cytoplasmic glucose-6-phosphate concentration when the glucose concentration increases, with maximal sensitivity to changes in extracellular glucose around the physiological plasma glucose level. Thus, the rate of β -cell glycolysis increases in concert with rises in blood glucose. Because pancreatic β -cells express low lactate dehydrogenase levels, glycolytically generated pyruvate preferentially enters the mitochondria for further metabolism.

Tight coupling between glycolysis and mitochondrial oxidation is crucial for distal steps in stimulus-secretion coupling (24,25). Oxidative metabolism via the pyruvate dehydrogenase complex (PDC) and the tricarboxylic acid cycle generates triggers for glucose-stimulated insulin secretion (GSIS). An increase in the [ATP]-to-[ADP] ratio leads to the closure of ATP-sensitive potassium (K_{ATP}) channels, plasma membrane depolarization, opening of voltage-gated calcium channels, and a resultant increase in cytosolic Ca^{2+} , which triggers the exocytosis of insulin. A second, less well defined, pathway occurs independently of K_{ATP} channels (23,26,27). Increased influx of glucose carbon into the tricarboxylic acid cycle via pyruvate carboxylase (anaplerosis) leads to increased production of intermediates (e.g., malate, citrate, and glutamate) that leave the mitochondria (cataplerosis) and, through poorly understood mechanisms, stimulate insulin release. Cataplerosis via citrate and ATP-citrate lyase (ACL) is a major metabolic pathway for pancreatic β -cell activation for insulin secretion (28). The importance of flux through ACL, delineated through the use of the ACL inhibitors (28), may reside in the production of cytoplasmic oxaloacetate, which allows citrate/pyruvate cycling and cytosolic NADPH production (29). In addition, the second product of the cytoplasmic ACL reaction, acetyl-CoA, acts as a precursor for the regulatory molecule malonyl-CoA, as well as other acyl-CoAs and acyl-CoA derivatives, or cholesterol, an important membrane component. Finally, in rat pancreatic β -cells, cAMP acts synergistically with the glucose metabolic signaling pathway for stimulation of insulin release (30). This may arise via phosphorylation of proteins involved in the exocytosis of secretory granules (31,32).

As well as acute effects on islet function, glucose has long-term actions on islet gene expression (33,34). An optimal glucose concentration is required for maintenance of insulin secretory competence, and chronic exposure to either lowered (<6 mmol/l) or elevated (20 mmol/l) glucose is detrimental to insulin secretion upon further acute glucose stimulation (34). In particular, exposure of β -cells to supraphysiological glucose levels for several days leads to a 60–80% reduction in PPAR- α mRNA expression (5) (Fig. 1). This is associated with decreased mRNA expression of acyl-CoA dehydrogenase, which catalyzes the limiting step of peroxisomal β -oxidation, and uncoupling protein 2, which is important for uncoupled mitochondrial lipid oxidation favoring accelerated FA disposal. Attesting to the potential pathophysiological impact of suppression of islet PPAR- α expression by high glucose, hyperglycemia in vivo in the 90% partial pancreatectomy rat model is also associated with a reduction in islet PPAR- α expression levels (35). Hence chronic exposure to high glucose affects β -cell lipid metabolism, such that the capacity for PPAR-

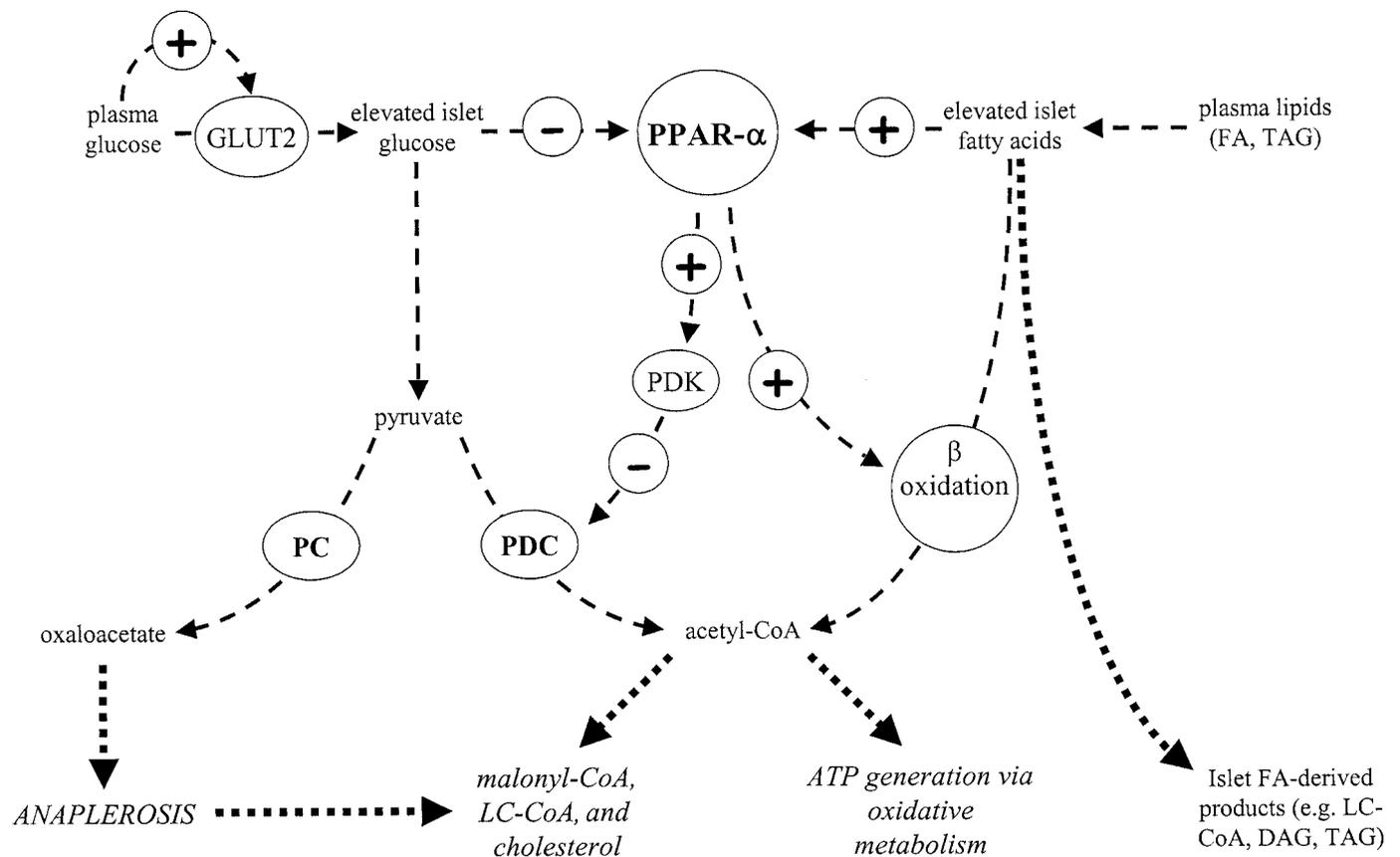


FIG. 1. Stimulus-secretion coupling of insulin secretion in relation to the reciprocal control of islet PPAR- α expression and signaling by glucose and FAs. + indicates stimulation; - indicates inhibition. Broad dotted lines indicate the predominant metabolic pathways involved in modulation of GSIS. DAG, diglyceride (diacylglycerol); LC-CoA, long-chain acyl-CoA; PC, pyruvate carboxylase.

α -linked oxidation of incoming lipids is diminished. An analysis of 24-h glucose-regulated gene expression (28,34) in fluorescence-activated cell sorter (FACS)-purified β -cells cultured at 3 vs. 10 mmol/l glucose demonstrated that gene expression of GLUT2 was increased, whereas gene expression of the inactivating kinase of PDC (pyruvate dehydrogenase kinase [PDK]) was suppressed after culture with 10 mmol/l glucose: the PDK isoform was not specified. Furthermore, multiple genes involved in cataplerosis, including ACL, showed higher expression at 10 mmol/l glucose compared with 3 mmol/l glucose. The expression of PDK4, one of three PDKs expressed in rat islets, is linked to signaling via PPAR- α (36). Hence, appropriate restraint of PPAR- α signaling by glucose may be important for maintaining glucose responsiveness of islet insulin secretion, and these data support the concept that coordination of pyruvate flux via PDC and pyruvate carboxylase is important for competent islet insulin secretory function.

Although steady-state glucose levels are similar, plasma insulin concentrations are 2.2-fold higher in 24-h fasted PPAR- α null mice compared with the wild-type controls (20). Furthermore, both the insulin-to-glucose concentration ratio and the product of steady-state fasting glucose levels and steady-state fasting insulin levels are increased in 24-h-fasted PPAR- α null mice compared with age-matched wild-type controls (20). These observations indicate that the absence of signaling via PPAR- α , possibly by impairing clearance of intracellular lipids via oxidation,

enhances insulin secretion at basal glucose concentrations in the fasted state. We have undertaken preliminary studies that demonstrate that administration of WY14,643 for the last 24 h of the 48-h starvation period does not greatly affect GSIS after an intravenous glucose challenge (M.J.H., N.D. Smith, M.C.S., unpublished data). It is thus possible that PPAR- α activation during the fed-to-fasted transition participates in the overall suppression of GSIS elicited by fasting because of a loss via oxidation of a FA-derived lipid moiety that is critical for insulin secretion (37,38). It is also possible that, as in liver, PPAR- α activation suppresses amino acid degradation in the islet. Glutaminolysis plays a key role in amino acid-stimulated insulin secretion (39), and the involvement of glutamate as an intracellular signal in the islets of Langerhans is gaining credence (25,40). Thus, absence of islet PPAR- α signaling in the fasted state could contribute to increased amino acid-stimulated insulin secretion, which could potentially contribute to elevated basal insulin secretion in PPAR- α null mice in vivo in the fasted state.

EFFECTS OF INCREASED PPAR- α SIGNALING ON INSULIN SECRETION

Adenovirus-mediated overexpression of PPAR- α in INS-1 cells increased palmitate oxidation in concert with increased expression of catalase (a marker of peroxisomal proliferation) and carnitine palmitoyltransferase-I and increased cellular TAG content. Under these conditions,

both basal insulin secretion and GSIS were suppressed. By contrast, chronic exposure of intact islets to high exogenous FA levels, which enhances endogenous PPAR- α expression (41) and PPAR- α -linked genes (42,43) in rat islets and pancreatic β -cells (Fig. 1), elevates insulin secretion at low glucose (43–46). The effect of chronic exposure to FAs to enhance islet PPAR- α expression is in marked contrast with the effect of high glucose to suppress β -cell PPAR- α expression (Fig. 1). Whether islet PPAR- α expression is enhanced or suppressed in response to concomitant chronic exposure to high glucose and high lipid concentrations (“glucolipotoxicity”) (4) will presumably reflect which fuel is more dominant under the precise pathophysiological condition studied. We have previously analyzed the potential role of PPAR- α signaling in the regulation of basal insulin secretion in batch-incubated islets from normal insulin-sensitive rats with free access to a standard high-carbohydrate low-fat diet (36). It would be predicted that under these conditions, the islet metabolic profile would be such that glucose would be dominant. Exposure to the PPAR- α agonist WY14,643 *in vivo* in the fed state significantly increased islet PPAR- α protein expression (36), consolidating previous studies showing that culture of normal islets with bezafibrate increases PPAR- α mRNA expression (41). Consistent with the effect of PPAR- α deficiency to elicit fasting hyperinsulinemia *in vivo* (20), PPAR- α activation *in vivo* induced a significant decline in plasma insulin concentrations in the postabsorptive state when glucose concentrations are relatively low (36). Because relative hypoinsulinemia was not accompanied by postabsorptive hyperglycemia, the insulin-to-glucose ratio was \sim 60% lower in postabsorptive WY14,643-treated rats compared with the untreated group. However, exposure of fed rats to WY14,643 *in vivo* modestly enhanced, rather than suppressed, insulin secretion at 2 mmol/l glucose in subsequently isolated islets in batch incubations (36), although this effect was not observed with perfused islets (47). These findings raise the possibility that circulating hormones and/or endogenous metabolites interact with an effect of increased islet PPAR- α expression to modulate basal insulin secretion.

Although prolonged (72-h) exposure to elevated FAs leads to elevated insulin secretion at low glucose that is not further enhanced when glucose concentrations are elevated (43), rats maintained on a standard low-fat high-carbohydrate diet and treated with WY14,643 for 24 h *in vivo* do not show impaired GSIS *in vivo* (47). Furthermore, islets prepared from such rats do not show impaired GSIS in either batch islet incubations (36) or islet perfusions (47,48). These observations further support the hypothesis that continuous exposure of the islet to ambient glucose concentrations in the high-physiological range can oppose the islet response to agonist-induced PPAR- α activation. In addition, they suggest that altered (enhanced) signaling via PPAR- α has a limited effect on insulin secretion in insulin-sensitive individuals.

β -CELL FUNCTION AT LOW GLUCOSE IN PREGNANCY

Pregnancy leads to adaptive changes in β -cell function, including a lowering of the threshold for GSIS, thought to be mediated by the placental lactogens and prolactin (49,50), which allows the pancreatic β -cells to secrete

more insulin under essentially normal blood glucose concentrations (51). Lowering of the islet glucose threshold is associated with increased expression of GLUT2 (49), increased glucokinase and hexokinase activities (49), increased cAMP levels at both mid-glucose (6.8 mmol/l) and high glucose (16.8 mmol/l), and increased islet glucose utilization and oxidation rates compared with those of control islets (49). The islet metabolic profile during pregnancy, with increased GLUT2 and glucose oxidation, is reminiscent of the glucose-induced transcriptome in FACS-purified rat β -cells (34), with coordinated glucose-regulated gene expression. Hence, although pregnancy is not characterized by hyperglycemia, the placental lactogens and/or prolactin appear to allow a phenotypic shift similar to that induced by elevated glucose to enhance the triggering and amplifying pathways of glucose-induced insulin release, but at a lower extracellular glucose level. Pregnancy-induced increases in β -cell glucose sensing and responsiveness are observed *ex vivo* (48,49,52) as well as *in vivo* (48,53–55), demonstrating that the pregnancy-induced insulin secretory adaptations are predominantly due to a persistent change in islet phenotype.

Pregnancy is characterized by elevated maternal circulating TAG levels (56) due, in part, to enhanced lipolysis (57–59), which provides additional FAs for VLDL production (60,61). Pregnancy is also associated with an accelerated response to starvation, with elevated FA and ketone body concentrations even after short-term food withdrawal (62,63). Because fasting-induced ketogenesis is greatly attenuated in PPAR- α null mice (18,20), the accelerated ketogenic response to starvation in mid-to-late pregnancy may reflect accelerated upregulation of hepatic PPAR- α -linked FA oxidation. Mid-to-late pregnancy is not associated with increased glycemia (20,54,55,62), and, therefore, islets are not simultaneously exposed to both high glucose and elevated lipid concentrations. Hence, glucolipotoxicity (4) is not normally an issue for islet secretory function in pregnancy.

Treatment of pregnant rats with fenofibrate for 1–2 days from day 16 of pregnancy (term is 23 days) decreases maternal hypertriglyceridemia (64). We extended this observation to investigate the effects of PPAR- α activation *in vivo* by WY14,643 on basal insulin secretion at day 15 of pregnancy in the rat in relation to changes in maternal plasma TAG concentrations (48). *In vivo*, treatment with the PPAR- α agonist WY14,643 reversed both pregnancy-induced hypertriglyceridemia and an effect of pregnancy to increase basal insulin concentrations (48). In contrast to the situation *in vivo*, WY14,643 treatment of 15-day pregnant rats significantly increased basal insulin release rates by perfused islets *in vitro* (48). This suggests that effects of antecedent PPAR- α activation to lower insulin levels in the immediately postabsorptive state are not due to a stable decrease in the capacity for insulin secretion at low glucose concentrations, but rather that systemic factors dominate the modulation of basal insulin secretion.

IMPACT OF PPAR- α ON PHYSIOLOGICAL INSULIN RESISTANCE AND COMPENSATORY GSIS IN PREGNANCY

Insulin resistance reflects a diminished response to insulin in target tissues (in particular, muscle, liver, and adipose

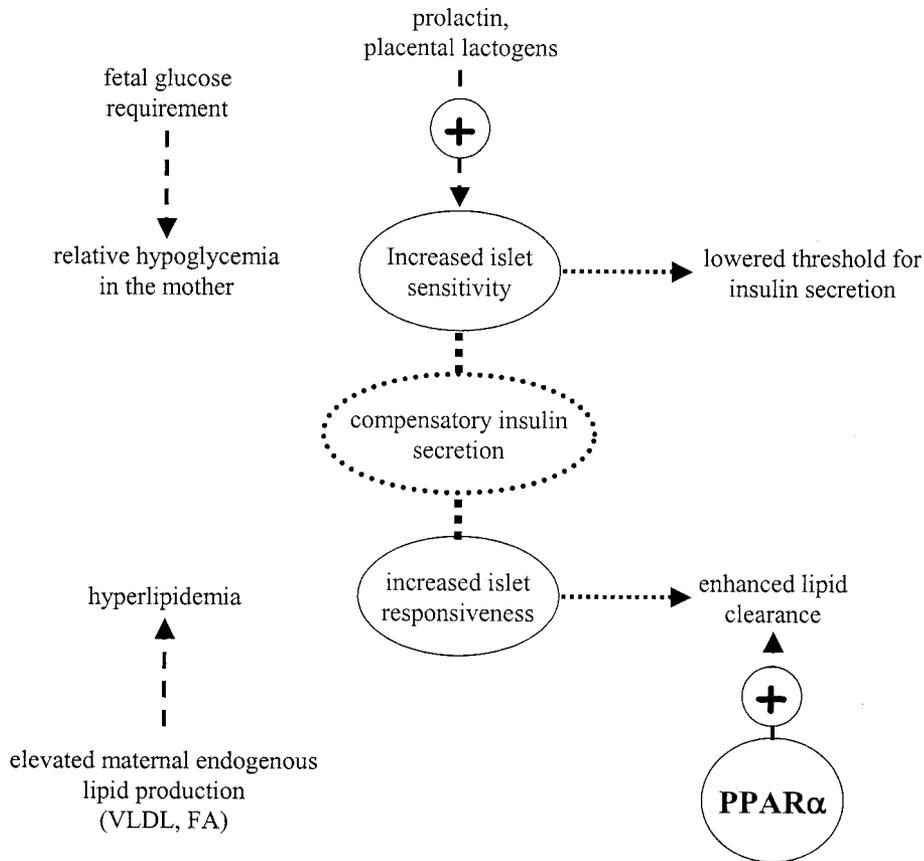


FIG. 2. Adaptive changes in pancreatic β -cell function in late pregnancy. + indicates stimulation.

tissue) and can predict the development of type 2 diabetes (65). In healthy nondiabetic individuals, a regulated negative feedback loop allows compensation for physiological changes in insulin sensitivity by inverse changes in pancreatic β -cell insulin secretion in relation to the degree of glucose tolerance (66,67). However, β -cell compensation for insulin resistance may be inadequate during the development of type 2 diabetes and insufficient to maintain blood glucose levels within the normal physiological range (68–70). The mechanisms that underlie the feedback loop between pancreatic β -cell function and insulin sensitivity remain incompletely understood, and the causes of impaired pancreatic β -cell compensation in type 2 diabetes are undelineated.

Pregnancy is characterized by a progressive state of maternal insulin resistance with respect to glucose disposal (48,62,71,72). In a healthy pregnancy, the development of insulin resistance is accompanied by reciprocal increases in insulin secretion (48,73) (Fig. 2). At day 15 of pregnancy in the rat, there is a close positive linear relationship between the acute insulin response to intravenous glucose challenge and the insulin resistance index, the product of the areas under the glucose (ΔG) and insulin (ΔI) curves after glucose challenge (48). Lowered fasting glucose concentrations in pregnancy indicate that relative hyperglycemia is not the critical signal by which peripheral insulin resistance is communicated to the pancreatic β -cell to elicit islet compensation in pregnancy. Insulin, in addition to its glucoregulatory action, exerts important actions to suppress adipose-tissue lipolysis (74) and hepatic VLDL secretion (75). Thus, the possibility is raised that augmented maternal pancreatic β -cell function

during late pregnancy may be vested in liporegulation. To examine this, we analyzed the relationship between maternal insulin resistance with respect to lipid homeostasis and compensatory GSIS in pregnancy. The insulin resistance index was significantly increased at day 15 of pregnancy compared with unmated rats (48). In conjunction with its lipid-lowering action, with complete normalization of maternal plasma TAG concentrations to nonpregnant levels, PPAR- α activation significantly improved insulin sensitivity in the pregnant group because of a marked (55%) lowering of ΔI (48). There was no increase in ΔG or significant impairment of the rate of glucose disposal (k) after WY14,643 treatment (48). The positive linear relationship between the acute insulin response to intravenous glucose challenge and the insulin resistance index was retained after WY14,643 treatment (48). These findings demonstrate that pregnancy-induced compensatory insulin secretion in vivo is independent of, but complementary to, the β -cell-targeted effects of pregnancy to increase the glucose sensitivity of insulin secretion. The effects of pregnancy to sensitize insulin secretion to glucose and to enhance glucose responsiveness of insulin secretion were retained in perfused islets from WY14,643-treated 15-day pregnant rats. Hence, it seems likely that PPAR- α activation in vivo in pregnancy either generates a circulating factor(s) that acutely suppresses GSIS or removes a circulating factor(s) that allows compensatory GSIS in vivo (but which is not required for augmented β -cell function in vitro). The latter may include increased systemic TAG lipid delivery to the islet. If this is so, it is implied that the development of maternal insulin resistance and compensatory insulin secretion in pregnancy

are both linked to altered maternal lipid handling (which can be improved by PPAR- α activation). Thus, compensatory hypersecretion of insulin during pregnancy may be geared primarily to normalization of maternal lipid fuel production through suppression of hepatic VLDL secretion and adipose-tissue lipolysis, with improved glucose handling, in part, due to improved lipid handling.

Higher rates of glucose oxidation in islets of pregnant rats imply that both enhanced GLUT2 expression and the suppression of PDK expression participate in triggering insulin release via an increased [ATP]-to-[ADP] ratio, closure of K_{ATP} channels, membrane depolarization, opening of voltage-dependent calcium channels, and activation of calcium-calmodulin-dependent effector proteins for exocytosis. However, it is possible that compensatory insulin hyper-responsiveness, linked to a perceived increased requirement for lipid lowering, may be more closely linked to the less well-defined cataplerotic signaling pathway. In INS1 cells, sensitization to low glucose induced by chronic exposure to high glucose is accompanied by an induction of lipogenic genes, including acetyl-CoA carboxylase and FA synthase, and increased conversion of glucose carbon into lipids (76), allowing amplification of the glucose signal. A similar lipogenic adaptation of islets in pregnancy would introduce the potential for specific modulation by PPAR- α of glucose conversion into lipids distal to the glycolytic pathway. Furthermore, *in vitro* with perfused islets, the absence of exogenous lipid would allow increased flux from glucose to lipid via the endogenous *de novo* synthetic pathway, thereby augmenting GSIS.

PPAR- α AND COMPENSATORY INSULIN SECRETION IN RESPONSE TO A HIGH-FAT DIET: A STATE OF ENVIRONMENTALLY INDUCED INSULIN RESISTANCE

In individuals with type 2 diabetes, insulin-stimulated glucose uptake is decreased by 30–40% compared with nondiabetic controls (77). Muscle is a primary site of insulin-stimulated glucose disposal (78,79). In muscle, impaired insulin-stimulated glucose uptake may be associated with defects in lipid oxidation due either to suppressed lipid oxidation (80) or to lipid oversupply relative to oxidation (81). A correlation between intramyocellular TAG content and insulin resistance has been revealed by nuclear magnetic resonance spectroscopy (82), and intramyocellular diacylglycerol accumulation has been correlated with translocation and activation of specific protein kinases, including PKC θ and IKK β (83–88). Serine phosphorylation of the insulin receptor or insulin receptor substrate 1 or 2 has been linked to lipid-induced inhibition of insulin signaling (87–90).

It would be anticipated that increased PPAR- α -linked FA oxidation, through increasing the clearance of intracellular lipid, would reverse lipid-induced insulin resistance, and this is observed in several animal models of lipid oversupply, including the high saturated fat-fed rat (91). Similarly, long-term (14-week) administration of fenofibrate as part of a high-fat diet fed to C57BL/6 mice reverses basal hyperinsulinemia and hyperglycemia (92). However, PPAR- α null mice appear to be protected from high-fat diet-induced insulin resistance, most likely because of the development of increased adiposity (i.e., TAG

storage in adipose tissue) (93). PPAR- α is necessary for the lipogenic action of hyperleptinemia (94), which is induced by high-fat feeding in both wild-type and PPAR- α null mice (93).

The development of peripheral insulin resistance induced by 4 weeks of high-saturated fat feeding is observed in conjunction with compensatory insulin hypersecretion *in vivo* such that glucose tolerance is affected little (47). The effect of high-saturated fat feeding to enhance GSIS was retained in perfused islets (47), eliminating acute influences of circulating factors, including systemic lipid delivery to the islet, and implicating a stable change in islet function. Treatment of high fat-fed rats with WY14,643 for 24 h *in vivo* reversed insulin hypersecretion *in vivo* without impairing glucose tolerance, suggesting that improved insulin action diminished the requirement for compensatory insulin secretion (47). The effect of PPAR- α activation to normalize insulin hypersecretion in the high-saturated fat-fed rat is again compatible with a role for compensatory insulin secretion to promote lipid lowering (Fig. 3). Attenuation of compensatory insulin secretion by PPAR- α activation in the high-saturated fat-fed rat reiterates that found in response to PPAR- α activation in pregnancy. However, unlike pregnancy, high-fat feeding would not be predicted to enhance the endogenous pathway of islet lipid synthesis from glucose (in the liver, for example, high-saturated fat feeding has a reverse effect on lipogenic gene expression and lipogenic flux [data not shown]). Moreover, the effect of antecedent PPAR- α activation to reverse the effect of high-fat feeding to enhance insulin secretion was also observed in perfused islets from high fat-fed rats, whereas this is not the case in pregnancy. These data appear to demonstrate that activation of PPAR- α *in vivo* can oppose insulin hypersecretion elicited by high-fat feeding, at least in part via stable effects exerted on the islet. Islets were perfused in the absence of exogenous lipid. It seems reasonable to suggest that insulin hypersecretion by isolated islets from high fat-fed rats requires continued lipid delivery, whereas, possibly because of additional adaptations (e.g., an enhanced capacity for lipid synthesis *de novo* from glucose), this is not the case in pregnancy.

HIGH-FAT FEEDING ELICITS GLUCOSE INTOLERANCE IN PREGNANCY BECAUSE OF INADEQUATE β -CELL COMPENSATION

As outlined above, a failure of the pancreatic β -cells to compensate for insulin resistance is critical to the pathology of type 2 diabetes (70), and impaired glucose tolerance is an established precursor of type 2 diabetes (95). A study comparing β -cell function between women who experienced transient diabetes during pregnancy (gestational diabetes) and healthy women after pregnancy demonstrated similar insulin responses in both groups (96–98). Although this could be taken to represent normal β -cell responses, analysis of insulin responses according to the degree of insulin resistance revealed that the women who had experienced gestational diabetes had inappropriately low insulin levels (70). Current evidence suggests that gestational diabetes occurs in women who cannot secrete sufficient insulin to compensate adequately for the reduction in insulin sensitivity that normally occurs during the

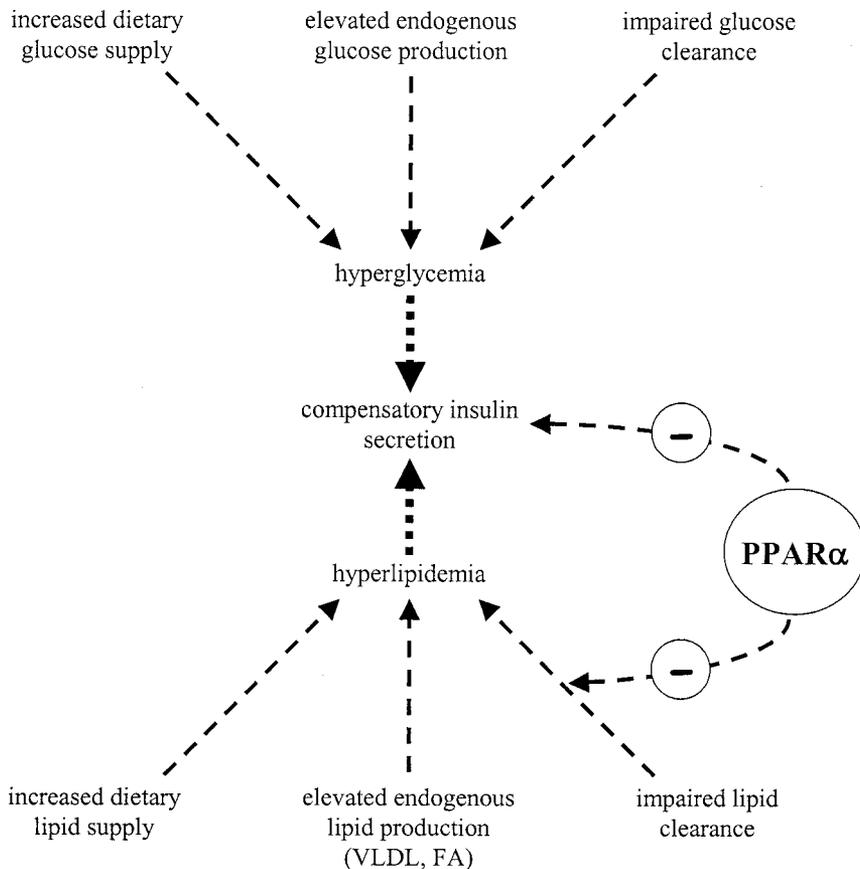


FIG. 3. Compensatory insulin secretion within the context of liporegulation. This scheme aims to highlight the dual actions of insulin to normalize both elevated glycemia and hyperlipidemia. In this scheme, we emphasize the requirement for compensatory insulin secretion to clear lipids in situations such as late pregnancy, where endogenous lipid production is enhanced, and high-saturated fat feeding, where dietary lipid delivery is increased.

third trimester of pregnancy (99). Thus, the compensatory feedback loop appears to be defective, leading to incomplete β -cell compensation and thus hyperglycemia in individuals with a predisposition toward the development of type 2 diabetes or gestational diabetes. We observed that compensatory insulin hypersecretion *in vivo* was reduced by PPAR- α activation in both pregnancy-induced (48) and diet-induced (high-fat feeding) (47) insulin resistance. We therefore examined whether modulation of the insulin secretory response to glucose by high-fat feeding in the insulin-resistant pregnant state resembled that found in the relatively insulin-sensitive unmated state and to what extent it could be modulated via PPAR- α activation.

For this study, pregnant rats placed on the high-saturated fat diet at the onset of pregnancy were sampled at day 19 of gestation. High-saturated fat feeding elicited further increases in GSIS in pregnancy (Fig. 4A); however, whereas pregnancy-induced insulin hypersecretion maintained normal glucose tolerance in low fat/high carbohydrate-fed 19-day pregnant rats, this was not the case for high fat-fed 19-day pregnant rats, in which k values were suppressed (Fig. 4B). Hence, β -cell compensation in high fat-fed 19-day pregnant rats is inadequate to maintain normal rates of glucose disposal, mimicking the situation in gestational diabetes. However, ratios of plasma insulin to blood glucose concentration in pregnant rats at intervals after the glucose challenge were unaffected by high saturated-fat feeding (Fig. 4C), suggesting that the level of insulin secretion was appropriate for the level of glycemia. The development of impaired glucose tolerance in high fat-fed pregnant rats may therefore reflect a failure to correct for the increased level of lipid input. This supports

the concept of a compensatory feedback loop between insulin secretion and “lipid + glucose” disposal, rather than glucose disposal alone (Fig. 3). Teleologically, use of lipid as an additional signal of a requirement for compensatory insulin resistance may be appropriate during pregnancy because of the variable drain on maternal glucose imposed by fetal demand.

PPAR- α activation lowered GSIS in high fat-fed 19-day pregnant rats (Fig. 4A); however, clear effects of high-fat feeding to increase GSIS persisted after WY14,643 treatment because GSIS was higher in WY4,643-treated high fat-fed pregnant rats than in WY4,643-treated low fat-fed pregnant rats (Fig. 5A). However, plasma insulin-to-blood glucose ratios at intervals after the glucose challenge were higher in WY4,643-treated high fat-fed pregnant rats than in WY4,643-treated low fat-fed pregnant rats (Fig. 5C), implying that although WY14,643 treatment lowers insulin secretion in pregnant rats, this effect is opposed by dietary provision of lipids. This could reflect lipid overload because of the combined effects of pregnancy and dietary lipid (which respectively elevate endogenous [hepatic] TAG production and adipose-tissue lipolysis and increase lipid influx from the gut), such that PPAR- α activation cannot compensate. The effects of high-saturated fat feeding to lower glucose clearance during pregnancy were not reversed by PPAR- α activation (Fig. 4B), although rates of glucose disposal were maintained by much lower levels of insulin (Fig. 4A). Nevertheless, rates of glucose disappearance in WY4,643-treated high fat-fed pregnant rats did not differ significantly from those of WY4,643-treated low fat-fed pregnant rats (Fig. 5B). Hence, PPAR- α activation in both high fat-fed and low fat-fed pregnant

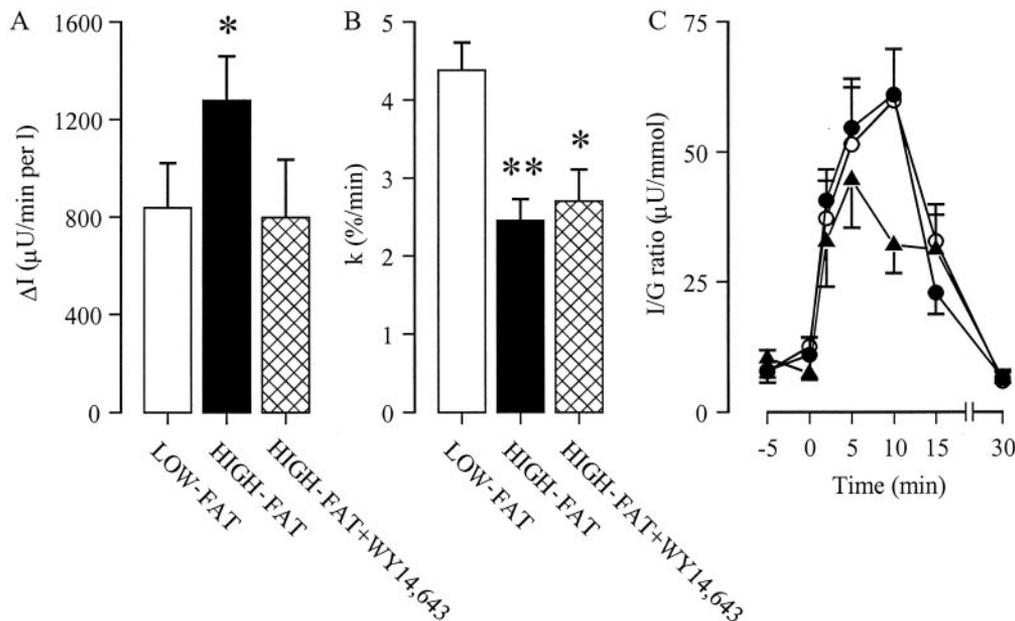


FIG. 4. Effects of high-saturated fat feeding on insulin secretion and glucose disposal during pregnancy. Further details are provided in the text. Glucose was administered as an intravenous bolus (0.5 g glucose/kg body wt) to control low fat/high carbohydrate-fed 19-day pregnant rats (LOW-FAT), high saturated fat-fed 19-day pregnant rats (HIGH-FAT), or high fat-fed 19-day pregnant rats administered WY14,643 (50 mg/kg body wt) at 24 h before sampling. Studies were undertaken in conscious unrestrained rats in the postabsorptive state. Blood samples were drawn at intervals for measurement of plasma insulin using a commercial kit. Insulin responses during the glucose tolerance test were used for calculation of the incremental plasma insulin values integrated over the 30-min period after the injection of glucose (ΔI), shown in *A*. Rates of glucose disappearance (k), calculated from the slopes of the regression lines obtained with log-transformed glucose values from 2 to 15 min after glucose administration and expressed as $\%/ \text{min}$, are shown in *B*. Plasma insulin-to-blood glucose (I/G) ratios are shown in *C*. Results are means \pm SE for seven LOW-FAT pregnant rats, eight HIGH-FAT pregnant rats, or eight WY14,643-treated HIGH-FAT pregnant rats. Statistically significant differences from LOW-FAT pregnant rats are indicated as follows: * $P < 0.05$; ** $P < 0.01$.

rats causes a shift in insulin sensitivity that reduces the requirement for β -cell compensation, but the required degree of β -cell compensation depends on the lipid content of the diet.

CONCLUSIONS

In this review, we have attempted to analyze the role of PPAR- α -linked FA metabolism in relation to islet function in health and in insulin-resistant states linked to lifestyle factors (namely pregnancy and high-saturated fat feed-

ing). We have emphasized the potential for both actions of PPAR- α on insulin sensitivity that may be relayed systemically to the islet (leading to modulation of the insulin response in accordance with changes in insulin sensitivity) and direct effects of PPAR- α action on the islet itself. Finally, we have developed the concept that compensatory insulin secretion may have a function in not only glucose regulation but also in liporegulation and that augmented insulin secretion may reflect a requirement for lipid lowering as well as for increased glucose disposal. This

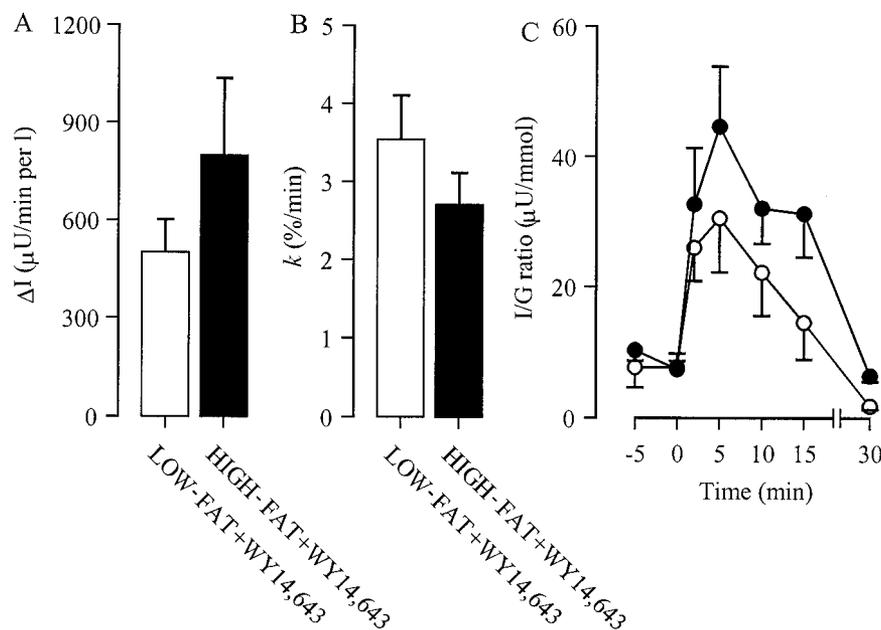


FIG. 5. Effects of PPAR- α activation on insulin secretion and glucose disposal in control or high fat-fed pregnant rats. Glucose was administered as an intravenous bolus (0.5 g glucose/kg body wt) to control low fat/high carbohydrate-fed 19-day pregnant rats administered WY14,643 at 24 h before sampling (LOW-FAT+WY14,643) or 19-day pregnant high fat-fed rats administered WY14,643 at 24 h before sampling (HIGH-FAT+WY14,643). Incremental insulin values (ΔI) are shown in *A*, rates of glucose disappearance (k) are shown in *B*, and plasma insulin-to-blood glucose (I/G) ratios are shown in *C*. Details are provided in the legend to Fig. 4. Results are means \pm SE for five WY14,643-treated pregnant rats or eight WY14,643-treated high fat-fed pregnant rats. I/G ratio, plasma insulin-to-blood glucose ratio.

introduces the possibility of a continuum between liporegulation with islet compensation and lipodysregulation leading to islet decompensation in the development of type 2 diabetes.

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This review is dedicated to the memory of our friend J. Denis McGarry, who was an inspirational researcher in this area.

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