Genetic Manipulations of Fatty Acid Metabolism in β-Cells Are Associated With Dysregulated Insulin Secretion

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Triacylglyceride (TG) accumulation in pancreatic β-cells is associated with impaired insulin secretion, which is called lipotoxicity. To gain a better understanding of the pathophysiology of lipotoxicity, we generated three models of dysregulated fatty acid metabolism in β-cells. The overexpression of sterol regulatory element binding protein-1c induced lipogenic genes and TG accumulation. Under these conditions, we observed a decrease in glucose oxidation and upregulation of uncoupling protein-2, which might be causally related to the decreased glucose-stimulated insulin secretion. The overexpression of AMP-activated protein kinase was accompanied by decreased lipogenesis, increased fatty acid oxidation, and decreased glucose oxidation; insulin secretions to glucose and depolarization stimuli were decreased, probably because of the decrease in glucose oxidation and cellular insulin content. It was notable that the secretory response to palmitate was blunted, which would suggest a role of the fatty acid synthesis pathway, but not its oxidative pathway in palmitate-stimulated insulin secretion. Finally, we studied islets of *PPAR*-$\gamma^{+/-}$ mice that had increased insulin sensitivity and low TG content in white adipose tissue, skeletal muscle, and liver. On a high-fat diet, glucose-stimulated insulin secretion was decreased in association with increased TG content in the islets, which might be mediated through the elevated serum free fatty acid levels and their passive transport into β-cells. These results revealed some aspects about the mechanisms by which alterations of fatty acid metabolism affect β-cell functions. *Diabetes* 51 (Suppl. 3): S414–S420, 2002

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ACC, acetyl-CoA carboxylase; AICAR, 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside; AMPK, AMP-activated protein kinase; AMPK/CA, constitutive active form of AMPK; CPT-1, carnitine palmitoyltransferase-1; HNF, hepatocyte nuclear factor; K_{ATP} channel, ATP-sensitive potassium channel; L-PK, liver-type pyruvate kinase; PPAR, peroxisome proliferator–activated receptor; RXR, retinoid X receptor; SRE, sterol regulatory element; SREBP, sterol regulatory element binding protein; TG, triacylglyceride; TZD, thiazolidinedione; UCP, uncoupling protein.

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It has been widely known that the accumulation of triacylglycerides (TGs) in pancreatic β-cells is associated with dysfunction of the cells, including impaired insulin secretory response to glucose and lipoapoptosis (1–3). The mechanisms by which TG accumulation leads to these dysfunctions, however, have remained largely elusive. In the present study, we established three models of dysregulated metabolism of fatty acids in β-cells and studied their effects on cell functions, especially insulin secretion, as follows: *1*) overexpression of sterol regulatory element binding protein (SREBP)-1c (a master transcription factor for lipogenesis [4–7]) in β-cells to reconstitute lipotoxicity with a genetically determined cause; *2*) overexpression of AMP-activated protein kinase (AMPK; a metabolic master switch for energy utilization [8,9]) in β-cells to dissect its role in insulin secretion in relation to glucose and lipid metabolism; and *3*) β-cells with a moderately decreased activity of peroxisome proliferator–activated receptor (PPAR)-γ from *PPAR*-$\gamma^{+/-}$ mice to better understand the role of PPAR-γ in the regulation of lipid distribution to multiple organs and the influence on β-cells (10–12).

ROLE OF SREBPs IN LIPID METABOLISM

SREBPs are comprised of three forms, SREBP-1a, -1c, and -2, which belong to the basic helix-loop-helix leucine zipper transcription factors (5). SREBP-1a and -1c are encoded by a single gene through the use of alternative transcription start sites and differ in the first exon. SREBP-2 arises from a separate gene. Although all three forms of SREBP contribute to lipid biosynthesis, SREBP-1a and -1c are preferentially important for fatty acid and TG synthesis, whereas SREBP-2 is important for cholesterol synthesis (13). SREBP-1a has a longer NH_2-terminal acidic activation domain than SREBP-1c, rendering the 1a isoform much more active in stimulating transcription (14). In addition to their roles in transcription, SREBPs have been implicated in enhancing adipocyte differentiation (7). In one study, homozygous knockout of the *SREBP-1* gene in mice resulted in 50–85% of embryonic lethality, but the surviving mice appeared normal with unchanged amounts of white adipose tissue and mRNA levels for lipogenic enzymes, lipoprotein lipase, and leptin (15). These results suggest that SREBP-2 can compensate, at least in part, for SREBP-1 in the regulation

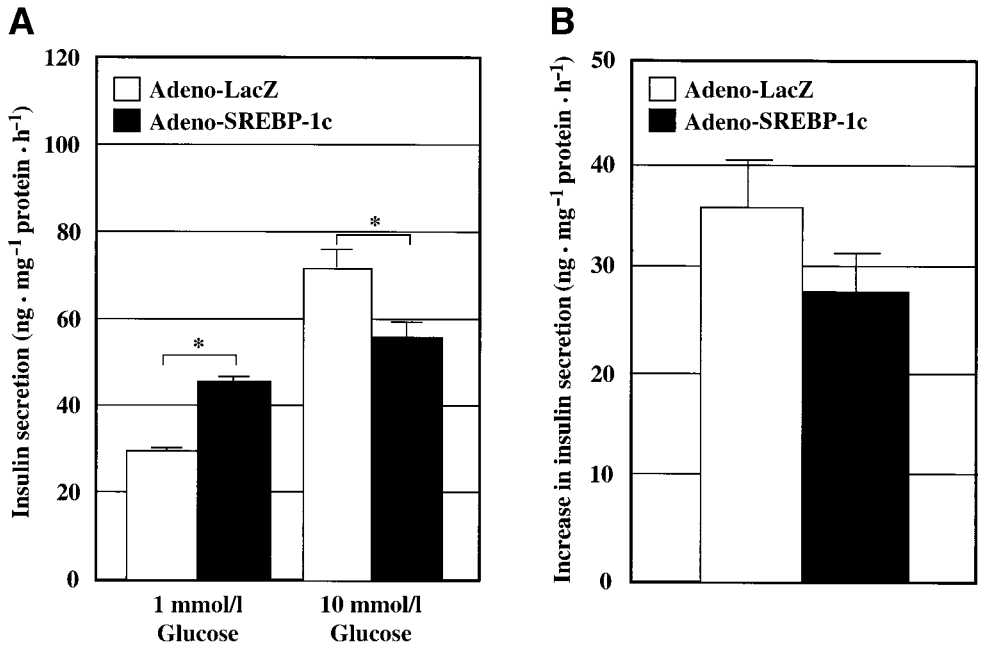

FIG. 1. *A*: Increased insulin secretion at 1 mmol/l glucose and decreased insulin secretion at 10 mmol/l glucose in INS-1 cells overexpressing SREBP-1c. *B*: Preserved potentiation of glucose-stimulated insulin secretion by palmitate in INS-1 cells overexpressing SREBP-1c. Increase by 1 mmol/l palmitate in insulin secretion at 10 mmol/l glucose is indicated. Values are expressed as means ± SE (n = 6). *P < 0.01.

of lipid synthesis. The transgenic overexpression of SREBP-1a in liver, driven by the phospho*enol*pyruvate carboxykinase promoter, developed into progressive and massive enlargement of the liver owing to the engorgement of the hepatocytes with TGs and cholesterol, accompanied by markedly elevated mRNA levels for acetyl-CoA carboxylase, fatty acid synthase, and stearoyl-CoA desaturase-1 (16). In β-cells, although all three forms of SREBPs were expressed, the amounts were small and barely detectable by RT-PCR in islets of Zucker lean wild-type rats (17).

DEFECTIVE GLUCOSE-STIMULATED INSULIN SECRETION IN β-CELLS OVEREXPRESSING SREBP-1c

To generate a β-cell−specific primary lipotoxicity model, we overexpressed a constitutively active form of SREBP-1c (1-403 aa) in the insulin-secreting cell line INS-1 via an adenoviral vector (7,18). The overexpression evoked a marked increase in the transcriptional activity of a reporter gene driven by the sterol regulatory element (SRE) cognitive sequences and resulted in a 60% increase in TG content at 48 h after the adenoviral infection at 10 m.o.i. Indeed, the expression of lipogenic enzymes, such as ATP-citrate lyase, acetyl-CoA carboxylase, acyl-CoA synthase, fatty acid synthase, and glycerol-phosphate acyltransferase, were markedly increased; on the other hand, the expression of enzymes for fatty acid oxidation, such as acetyl-CoA oxidase and acetyl-CoA dehydrogenase, was almost unchanged as compared to control cells infected with an adenoviral vector harboring the LacZ cDNA. Under these conditions, cellular insulin content was slightly decreased, but transcriptional activity of the pre-proinsulin gene, as measured with a luciferase reporter gene assay, was not affected.

We next studied the insulin secretory characteristics. The basal insulin secretion in the presence of 1 mmol/l glucose was increased by 50%, whereas the enhancement in insulin secretion in response to 10 mmol/l glucose was decreased by 70% as compared to controls (Fig. 1*A*). The insulin response to depolarizing stimuli such as 50 mmol/l KCl and 5 μmol/l glibenclamide was decreased by 35 and 13%, respectively. The effect of palmitate on initiating insulin secretion in the presence of 1 mmol/l glucose and potentiating insulin secretion in response to 10 mmol/l glucose was relatively preserved in cells overexpressing SREBP-1c as compared to controls (Fig. 1*B*). Consistent with the reduced secretory response to glucose, [6-^{14}C]glucose oxidation was decreased by 20% in cells overexpressing SREBP-1c. We then measured cellular contents of ATP and ADP. The ATP/ADP ratio in the presence of 10 mmol/l glucose was significantly decreased as compared to controls, a finding that supports the reduced catabolism of glucose. Thus, these impairments in glucose utilization may have affected the ATP-sensitive potassium (K_{ATP}) channel−dependent pathway of the glucose-stimulated insulin secretion in this model.

Interestingly, the expression of uncoupling protein (UCP)-2, which dissipates mitochondrial electrochemical potential to heat, thereby costing ATP generation (19), was increased twofold at both mRNA and protein levels. In agreement with this observation, SRE has been found in the promoter region of the *UCP-2* gene (20). It has also been reported that oleic acid and linolenic acid activate the transcription of the *UCP-2* gene (21,22). Thus, the upregulation of UCP-2 directly through the SRE by SREBP-1c, and/or indirectly through generation of specific fatty acids, is another plausible mechanism by which lipotoxicity causes the reduced responsiveness of insulin secretion to glucose. This hypothesis would be verifiable under experimental conditions where UCP-2 activity is constant despite changes in lipid amounts in β-cells. Such conditions are realized, for example, in islets of UCP-2

knockout mice. Indeed, the knockout of the *UCP-2* gene has been shown to lead to elevation of the ATP/ADP ratio and insulin secretion of islets in response to glucose and to reduce diabetes severity in *ob/ob* mice (23). Reducing the increased TG content in β-cells is another rational and straightforward modality for alleviating lipotoxicity, in addition to suppressing the expression of UCP-2. For this purpose, activation of AMPK in β-cells may have a potential beneficial effect, as it leads to the suppression of lipogenesis and the promotion of fatty acid oxidation, as is discussed below.

AMPK AS A FUEL SENSOR

AMPK is a key regulator of glucose and lipid metabolism, and its significance as one of the major metabolic switches under varying energy supply conditions has been increasingly understood (8). AMPK is a heterotrimeric serine/threonine kinase comprised of α, β, and γ subunits. The catalytic activity resides in the α subunit, which has two isoforms, α1 and α2. AMP is the most potent regulator of AMPK activity, the mechanisms of which seem to be multiple and include a direct allosteric activation by AMP, activation by AMP of the upstream kinase (AMPK kinase), and stabilization of the AMPK/AMP complex against protein phosphatase activities (8). Hypoxia, cellular stress, low pH, osmotic shock, and contraction are among the major factors that increase the cellular AMP concentration and, consequently, AMPK activity. Recently, the biguanide metformin has been reported to activate AMPK in hepatocytes and skeletal muscle (24), which is the emerging rationale behind its use as a hypoglycemic agent. The "adipocytekine" leptin has also been reported to activate AMPK activity in skeletal muscle through a direct peripheral action and through sympathetic nerve activation via its action on the hypothalamus (25). In liver, AMPK has been known to phosphorylate acetyl-CoA carboxylase (ACC) and 3-hydroxy-3-methylglutaryl-CoA reductase, which suppresses their enzyme activities followed by a decrease in fatty acid and cholesterol synthesis. Decreases in the malonyl-CoA level subsequent to the suppression of ACC activity leads to less suppression by malonyl-CoA of carnitine palmitoyltransferase-1 (CPT-1) activity and thus increased fatty acid β-oxidation. In skeletal muscle, in addition to the effect of increasing β-oxidation, AMPK activation plays a role in enhancing glucose uptake via translocation of GLUT4 to the plasma membrane (26). The glucose uptake effect is not blocked by wortmannin, an inhibitor of phosphatidylinositol 3-kinase that is located downstream of insulin receptors and insulin receptor substrates (27). Activation of both fatty acid β-oxidation and glucose utilization is thought to be crucial for skeletal muscle to meet its energy demand during contraction.

ROLES OF AMPK IN β-CELLS

In contrast to the case in liver and skeletal muscle, the role of AMPK in β-cell functions is less clear. Glucose stimulation of β-cells has been associated with a reduction in the AMP/ATP ratio and decreased AMPK activity (28). Activation of AMPK by 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR) at 200 μmol/l was reported to inhibit glucose-stimulated upregulation of liver-type pyruvate kinase (L-PK) promoter activity and pre-proinsulin promoter activity in MIN6 cells (29). Regarding insulin secretion, diverse effects have been observed with 1 mmol/l AICAR; it acutely (within 5 min) enhances 10 mmol/l glucose-stimulated insulin secretion, but inhibits it in the later phase by ~50% (28). The effect of AICAR on insulin secretion may also be affected by glucose concentrations. Thus, in the presence of 1 mmol/l AICAR, insulin secretion was inhibited by a high glucose concentration, whereas it was enhanced by a low glucose concentration in rat islets (28). The effects of AICAR seemed to be dependent on the culture conditions, including culture periods and glucose concentrations.

To further investigate the role of AMPK in β-cells, we overexpressed AMPK activity by infecting INS-1 cells with an adenoviral vector harboring a *constitutively active form of the rat AMPK-α1 subunit (adeno-AMPK/CA)*. This same construction of the adenoviral vector has been reported by others to be successful in overexpressing AMPK activity in hepatocytes (30). In INS-1 cells, the expression level of the intrinsic α1 subunit was greater than that of the α2 subunit. At 72 h after infection with adeno-AMPK/CA at 10 m.o.i., INS-1 cells abundantly expressed the truncated form of AMPK-α1 and showed an approximate fourfold increase in AMPK activity. Under these conditions, we observed major changes in metabolic parameters. First, the TG content of the cells was reduced to ~50% of that of control cells infected with adeno-LacZ. This reduction was accompanied by increased fatty acid oxidation. The fold increase in fatty acid oxidation was more evident in the presence of a high rather than a low glucose concentration. These results suggested that the overexpression of AMPK/CA phosphorylated ACC and consequently suppressed its activity. The decrease in ACC activity and subsequent decrease in malonyl-CoA level were assumed to suppress lipogenesis and simultaneously enhance fatty acid β-oxidation through activation of CPT-1. Another major change was a decrease in glucose oxidation. The oxidation of [6-^{14}C]glucose was decreased by ~40% at 10 mmol/l glucose and by ~30% at 1 mmol/l glucose in INS-1 cells overexpressing AMPK/CA. The overall metabolic flux of substrates was thus set to spare glucose usage, suppress lipogenesis, and promote combustion of the stored lipid. This mode of energy utilization seems to fit in with AMPK's role as a fuel sensor and its increased activity when cells or organs are exposed to starvation.

Then what about insulin biosynthesis and secretion under these conditions? Cellular insulin content was decreased by ~30% in AMPK/CA-overexpressing cells. These results were consistent with the finding that blockade of AMPK activity with antibody (microinjection of anti–AMPK-α2 antibody into the cytosol and nucleus) evoked a marked increase in insulin gene promoter activity (29), suggesting that AMPK activity negatively regulated insulin gene transcription in the normal state. We next examined insulin secretory responses to glucose, depolarization, and fatty acid stimuli. Basal insulin secretion at 1 mmol/l glucose was moderately reduced by AMPK/CA overexpression, whereas the response to 10 mmol/l glucose was substantially decreased (Fig. 2*A*). The impairment of secretion could be explained, at least in part, by the decreases in glucose oxidation and insulin content. Insulin

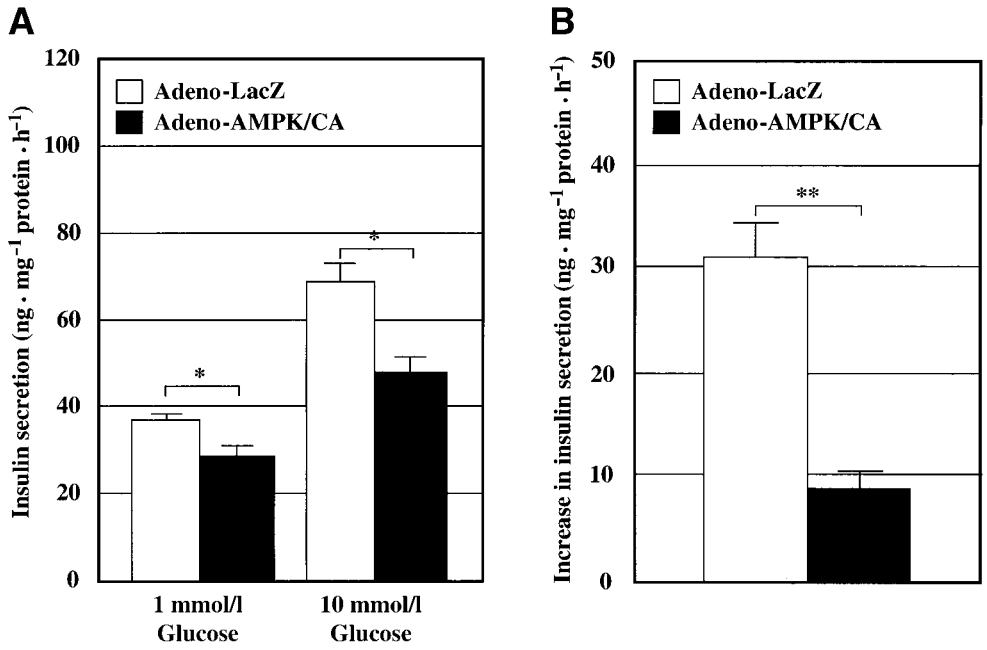

FIG. 2. *A*: Decreased insulin secretion at 1 and 10 mmol/l glucose in INS-1 cells overexpressing AMPK/CA. *B*: Severely blunted potentiation of glucose-stimulated insulin secretion by palmitate in INS-1 cells overexpressing AMPK/CA. Increase by 1 mmol/l palmitate in insulin secretion at 10 mmol/l glucose is indicated. Values are expressed as means ± SE (n = 6). *P < 0.5; **P < 0.01.

secretion was decreased by ~60% in response to cytosolic [Ca^{2+}] elevation, which was induced by the plasma membrane depolarization with 250 μmol/l diazoxide and 30 mmol/l KCl. In this case of a nonfuel secretagogue, the reduction in cellular insulin content may have been primarily responsible for the decrease in insulin secretion. The addition of glucose stimulation over the diazoxide/KCl-induced insulin secretion has been used to study the role of the K_{ATP} channel−independent pathway of glucose-induced insulin secretion (31). This pathway was almost totally abrogated in INS-1 cells overexpressing AMPK/CA. These results suggest that the generation of mediator(s) for the pathway was under the control of AMPK and was suppressed beneath a certain threshold level in these cells. Because AICAR has been reported to suppress the expression of hepatocyte nuclear factor (HNF)-4α, its downstream transcription factors, and enzymes for glucose metabolism such as GLUT2, aldolase B, and L-PK (32), we examined the expression levels of HNF family proteins. The expression of HNF-4α was decreased by ~50% as compared to controls, and that of HNF-3β was also decreased by ~30%. However, the expression levels of HNF-1α and -1β were not changed. Although the expression patterns of these HNF proteins under activated AMPK conditions were not identical in hepatocytes and β-cells, these results suggest the importance of HNF-4α as a physiological target of AMPK and possibly as a molecular link between altered AMPK activity and the pathophysiology of type 2 diabetes. Finally, we observed that palmitate-stimulated insulin secretion was severely blunted in the AMPK/CA-overexpressing cells at both 1 and 10 mmol/l glucose (Fig. 2*B*). One possible interpretation of these findings is that palmitate was preferentially utilized for β-oxidation and was unable to be directed toward lipogenesis. This would result in a decreased generation of lipid-related messenger molecules, which are normally derived from the intermediary metabolites of the lipogenic pathway. Candidates for these molecules may include long-chain acyl-CoAs (33,34). In summary, diverse effects of AMPK overexpression on metabolism were revealed, and the abnormalities described above were assumed to cause the impairments of insulin secretion to various stimuli.

ROLE OF PPAR-γ IN ADIPOCYTE DIFFERENTIATION AND AS A THRIFTY GENE

PPAR-γ is a ligand-activated transcription factor that belongs to the nuclear hormone receptor superfamily and forms a heterodimer with a retinoid X receptor (RXR) (10,35). PPAR-γ1 is expressed in many tissues, including skeletal muscle, whereas the splice variant PPAR-γ2 is mainly expressed in white and brown adipose tissue. Expression of PPAR-γ has also been demonstrated in human pancreatic islet cells (36). Agonist-induced activation of PPAR-γ/RXR is known to increase insulin sensitivity (37). Furthermore, the synthetic ligands of PPAR-γ, the thiazolidinediones (TZDs), which have the ability to directly bind and activate PPAR-γ and stimulate adipocyte differentiation (10), are used clinically to reduce insulin resistance and hyperglycemia in type 2 diabetes. In addition, TZDs have been shown to restore the reduced β-cell secretory response to an oscillatory glucose infusion (38). Several in vivo and in vitro studies have confirmed that treatment with TZDs improves pancreatic islet morphology and β-cell function. Thus, TZDs have been shown to ameliorate pancreatic islet hyperplasia, β-cell hypertrophy, and deranged α-cell distribution in Zucker fatty rats (39). TZDs also reduce islet TG content in correlation with improved glucose- and arginine-stimulated insulin secretion (40). Decreased expression of GLUT2 and altered morphology, such as degranulation, glycogen infiltration,

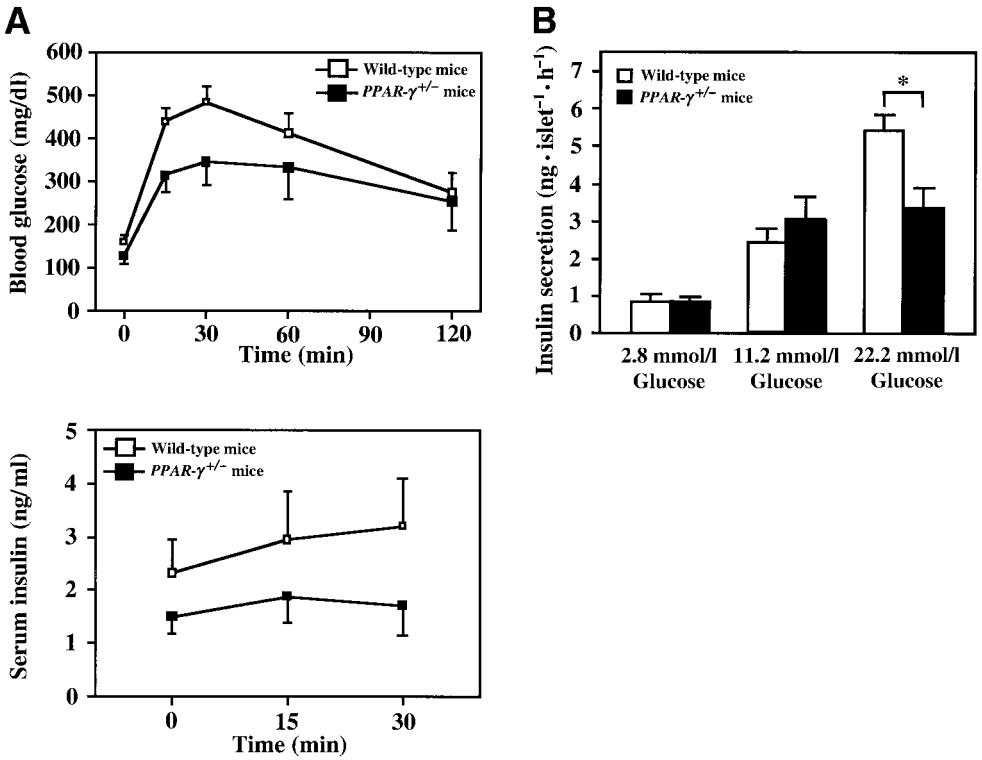

FIG. 3. *A*: Glucose tolerance test after 15 weeks on a high-fat diet in wild-type mice and *PPAR*-$\gamma^{+/-}$ mice. Blood glucose (*upper panel*) and serum insulin levels (*lower panel*) were measured at the indicated time points. Values are expressed as means ± SE (n = 7). *B*: Decreased insulin secretion in *PPAR*-$\gamma^{+/-}$ mice at 22.2 mmol/l glucose. Rate of insulin secretion during a static incubation of islets from wild-type and *PPAR*-$\gamma^{+/-}$ mice are shown. Values are expressed as means ± SE (n = 4). *$P < 0.05$.

and swelling of mitochondria, are also ameliorated by TZD treatment (41). A Pro12Ala polymorphism in human PPAR-γ2, which moderately reduces the transcriptional activity of PPAR-γ, has been shown to confer resistance to type 2 diabetes (42). Moreover, although the Ala12 variant of PPAR-γ has been reported to be associated with a reduced risk for the development of diabetes, it has also been shown to be a positive risk factor for insufficient insulin secretion and disease severity as measured by HbA_{1c} in individuals with type 2 diabetes (43). These results suggest a potential significance of PPAR-γ not only for insulin action, but also for insulin secretion.

Type 2 diabetes is considered to be a polygenic disease aggravated by environmental factors, such as low physical activity or a hypercaloric high-fat diet. Free fatty acids are thought to represent an important factor linking excess fat mass to type 2 diabetes. In addition, free fatty acids have been shown to influence both insulin secretion (44) and insulin action (45). According to the lipotoxicity hypothesis, chronic exposure to elevated levels of free fatty acids impairs β-cell function and is often accompanied by increased islet TG content. PPAR-γ represents a key mediator between adipocyte differentiation, free fatty acid metabolism, and glucose homeostasis. In β-cells, the metabolism of fatty acids is tightly coupled to glucose-stimulated insulin secretion by enzymes that, at least in adipose tissue, are under the transcriptional control of PPAR-γ (2).

β-Cells sense glucose through its metabolism and the resulting increase in the ATP/ADP ratio, which closes the K_{ATP} channels and leads to plasma membrane depolarization, the influx of Ca^{2+}, and, finally, insulin secretion. A deficiency of UCP-2 is beneficial for suppressing the dissipation of glucose-induced mitochondrial potential, thereby conferring resistance to diabetes, with increased insulin secretion (23). Long-term exposure to fatty acids uncouples the mitochondrial potential in association with increased expression of UCP-2 in β-cells (19). In addition, TZDs upregulate UCP-2 expression in skeletal muscle and white and brown adipose tissue (46,47). These results led us to examine whether PPAR-γ plays a role in insulin secretion through modifications of lipid metabolism and energy dissipation in β-cells.

DECREASED GLUCOSE-STIMULATED INSULIN SECRETION AND INCREASED TG CONTENT IN *PPAR*-$\gamma^{+/-}$ ISLETS ON A HIGH-FAT DIET

In *PPAR*-$\gamma^{+/-}$ mice, adipocyte hypertrophy and the development of insulin resistance in conjunction with a high-fat diet are partially prevented (12). These paradoxical findings may be attributable to a relative preservation of leptin effects caused by partial release of leptin expression by the loss of one *PPAR*-γ allele, which normally inhibits leptin transcription. This situation should also apply to humans, as the risk for development of diabetes is reduced in the population with Pro12Ala polymorphism in PPAR-γ2 (43), which moderately reduces the transcriptional activity of PPAR-γ.

We noticed that serum insulin levels at fasting and after a glucose tolerance test were reduced in *PPAR*-$\gamma^{+/-}$ mice as compared to wild-type mice given a high-fat diet (Fig. 3*A*) (12). Although this observation might be linked to the ameliorated insulin sensitivity, it could also be caused by

the primary secretory disturbance to glucose in β-cells. To address this issue, we isolated islets of comparable size and carried out static incubation experiments. In the presence of 2.8 or 11.1 mmol/l glucose, insulin secretion in islets from *PPAR*-$\gamma^{+/-}$ mice was similar to that from wild-type mice. However, it was reduced by 20% ($P < 0.01$) in the presence of 2.8 mmol/l glucose plus 50 mmol/l KCl, and reduced by 40% ($P < 0.05$) in the presence of 22.2 mmol/l glucose (Fig. 3*B*), although neither the number of cells per islet nor the β-cell mass assessed by immunostaining of the pancreas was different between *PPAR*-$\gamma^{+/-}$ and wild-type mice. Thus we concluded that although *PPAR*-$\gamma^{+/-}$ mice secreted less insulin than wild-type mice on high-fat diet, *PPAR*-$\gamma^{+/-}$ mice exhibited better glucose tolerance because of increased insulin sensitivity as compared to wild-type mice.

We have shown that moderate reduction of PPAR-γ activity in *PPAR*-$\gamma^{+/-}$ mice decreased TG contents of white adipose tissue, skeletal muscle, and liver, probably because of the increase in leptin expression, increase in fatty acid combustion, and decrease in lipogenesis, which ameliorate high-fat diet–induced obesity and insulin resistance (48). Moreover, it has been reported that TZDs lower islet TG content and restore β-cell function in Zucker diabetic fatty rats (40). It is possible that changes in lipid metabolism regulated by PPAR-γ may also influence TG content in islets. In fact, in contrast to the situation in white adipose tissue, skeletal muscle, and liver, the TG content of *PPAR*-$\gamma^{+/-}$ islets was increased. We observed that the expression level of PPAR-γ in islets was considerably less than that in white adipose tissue, skeletal muscle, and liver and that serum TG and free fatty acid levels were elevated in *PPAR*-$\gamma^{+/-}$ mice. Thus, one explanation for the increased TG content in *PPAR*-$\gamma^{+/-}$ islets is a passive influx of free fatty acids and their conversion to TG. The low expression level of PPAR-γ in islets was unable to sufficiently oxidize the lipid to attain net reduction of the TG store. Alternatively, because leptin has been found to reduce insulin secretion in vitro (49), the increased serum leptin level in *PPAR*-$\gamma^{+/-}$ mice may affect insulin secretion on the high-fat diet. In muscle/liver from *PPAR*-$\gamma^{+/-}$ mice, molecules involved in energy dissipation such as UCP-2 were augmented (48). Increased expression of UCP2 may reduce insulin secretion because of a decrease in mitochondrial electrochemical potential and subsequent islet ATP levels. However, UCP-2 expression in *PPAR*-$\gamma^{+/-}$ islets was comparable to that in wild-type islets and was thus unrelated to the decreased insulin secretion.

Thus, moderate reduction of PPAR-γ activity as seen in *PPAR*-$\gamma^{+/-}$ mice affects lipid metabolism on high-fat diet. Although the reduction ameliorated adipocyte hypertrophy and insulin resistance, it deteriorated β-cell secretory function, the mechanisms of which included lipotoxicity specifically directed to β-cells.

CONCLUSIONS

β-Cells are exposed to an increasing threat of lipotoxicity in times of feast and sedentary lifestyle. We have described three models of the dysregulated fatty acid metabolism, and these results have brought us some insight into the phenomenon. Briefly, *1*) SREBP-1c or the downstream genes regulated by it are candidate genes for primary β-cell dysfunction in insulin secretion, especially when they were upregulated genetically or secondarily by environmental factors. *2*) Overexpression of UCP-2 may be a key event in linking lipid accumulation to impaired insulin secretion to glucose. *3*) AMPK activation may, at least in a restricted situation, be a useful strategy for shutting down the lipotoxic effects. On the other hand, excess reduction in lipid storage is a factor that deteriorates insulin secretion, and therefore the optimal level should be sought. *4*) Moderately reduced PPAR-γ activity was associated with reduced insulin secretory response to glucose, which involved a lipotoxic mechanism. Elucidating its precise mechanisms and establishing novel therapeutic strategies to release β-cells from the lipotoxic burden are our present challenges.

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