

# Role of ATP Production and Uncoupling Protein-2 in the Insulin Secretory Defect Induced by Chronic Exposure to High Glucose or Free Fatty Acids and Effects of Peroxisome Proliferator-Activated Receptor- $\gamma$ Inhibition

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In rat pancreatic islets chronically exposed to high glucose or high free fatty acid (FFA) levels, glucose-induced insulin release and mitochondrial glucose oxidation are impaired. These abnormalities are associated with high basal ATP levels but a decreased glucose-induced ATP production ( $\Delta$  of increment over baseline  $0.7 \pm 0.5$  or  $0.5 \pm 0.3$  pmol/islet in islets exposed to glucose or FFA vs.  $12.0 \pm 0.6$  in control islets,  $n = 3$ ;  $P < 0.01$ ) and, as a consequence, with an altered ATP/ADP ratio. To investigate further the mechanism of the impaired ATP formation, we measured in rat pancreatic islets glucose-stimulated pyruvate dehydrogenase (PDH) activity, a key enzyme for pyruvate metabolism and for the subsequent glucose oxidation through the Krebs cycle, and also the uncoupling protein-2 (UCP-2) content by Western blot. In islets exposed to high glucose or FFA, glucose-stimulated PDH activity was impaired and UCP-2 was overexpressed. Because UCP-2 expression is modulated by a peroxisome proliferator-activated receptor (PPAR)-dependent pathway, we measured PPAR- $\gamma$  contents by Western blot and the effects of a PPAR- $\gamma$  antagonist. PPAR- $\gamma$  levels were overexpressed in islets cultured with high FFA levels but unaffected in islets exposed to high glucose. In islets exposed to high FFA concentration, a PPAR- $\gamma$  antagonist was able to prevent UCP-2 overexpression and to restore insulin secretion and the ATP/ADP ratio. These data indicate that in rat pancreatic islets chronically exposed to high glucose or FFA, glucose-induced impairment of insulin secretion is associated with (and might be due to) altered mitochondrial function, which results in impaired glucose oxidation, overexpression of the UCP-2 protein, and a consequent decrease of ATP production. This alteration in FFA cultured islets is mediated by the PPAR- $\gamma$  pathway. *Diabetes* 51: 2749–2756, 2002

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BADGE, bisphenol A diglycidyl ether; FFA, free fatty acid; KRHB, Krebs Ringer HEPES buffer; PDH, pyruvate dehydrogenase; PPAR, peroxisome proliferator-activated receptor; UCP-2, uncoupling protein-2.

Patients with type 2 diabetes are characterized by a progressive decline of insulin secretion that becomes more severe with the increasing duration of the disease (1–4). In these patients, the mechanisms that cause the progressive  $\beta$ -cell failure are currently under investigation: the altered insulin secretory pattern depends, at least in part, on the negative influence of chronic high glucose (5–8) and/or high free fatty acid (FFA) (9–12) plasma concentrations (gluco- or lipotoxicity). These metabolites are believed to affect pancreatic  $\beta$ -cell function by chronic  $\beta$ -cell stimulation and consequent “desensitization” to glucose. However, the molecular mechanisms of glucose desensitization induced by hyperglycemia or hypertriglyceridemia are still unclear (13,14).

Rat pancreatic islets that are chronically exposed to high glucose or FFAs have an impaired glucose oxidation (15). Because glucose oxidation generates ATP and the rise of ATP and ATP/ADP ratio plays a central role in glucose-induced insulin release by causing  $K^+$ -ATP channel closure, membrane depolarization, increased calcium influx, and insulin granule exocytosis, we first measured ATP and ADP levels in islets that were chronically exposed to high glucose or FFAs. In addition to mitochondrial glucose oxidation, ATP synthesis and ATP/ADP ratio are regulated by uncoupling protein-2 (UCP-2) expression. UCP-2 is a member of a family of proteins that are located in the mitochondrial inner membrane and that act as proton channels to uncouple mitochondrial oxidative phosphorylation. By this mechanism, energy is wasted through heat, and cellular ATP synthesis is decreased. UCP-2 overexpression in rat pancreatic islets has already been shown to inhibit glucose-stimulated insulin secretion by decreasing ATP formation (16,17). Therefore, to investigate the role of two mechanisms involved in ATP synthesis in pancreatic islets that are chronically exposed to high glucose and FFAs, we studied the activity of pyruvate dehydrogenase (PDH), a key enzyme for pyruvate metabolism, and the subsequent glucose oxidation through the Krebs cycle and UCP-2 levels.

To study further the mechanisms implicated in the transcriptional regulation of the UCP-2 gene in pancreatic

islets cultured with high glucose or FFAs, we evaluated the role of peroxisome proliferator-activated receptor (PPAR)- $\gamma$  (18). In several tissues, this transcription factor has been shown to mediate the response of UCP-2 to fatty acids (19–23), because it has been found that fatty acids act as natural ligands for PPAR- $\gamma$  (24,25). Therefore, in pancreatic islets exposed to chronically high glucose and FFAs, we measured the PPAR- $\gamma$  protein expression and analyzed whether a PPAR- $\gamma$  antagonist was able to prevent the effects (26).

## RESEARCH DESIGN AND METHODS

**Materials.** Crude collagenase was obtained from Boehringer Mannheim (Mannheim, Germany). Culture medium CMRL-1066, heat-inactivated FCS, glutamine, and gentamicin were obtained from Gibco (Glasgow, U.K.). Bisphenol A diglycidyl ether (BADGE) was obtained from Fluka (Buchs, Switzerland). All other chemicals were of analytical grade.

**Islet preparation and culture conditions.** Pancreatic islets were isolated by the collagenase method from 200- to 250-g fed male Wistar rats that received an intraperitoneal injection of 0.2 ml of a 0.2% pilocarpine solution 2 h before being killed by decapitation. With this technique, 300–400 islets were isolated from each pancreas (27). Purified islets were first cultured overnight at 5.5 mmol/l glucose in CMRL-1066 medium and then either for 48 h at 5.5 or 16.7 mmol/l glucose or for 72 h in a culture medium containing 5.5 mmol/l glucose and 2% BSA (FFA-free) with or without 2 mmol/l long-chain fatty acids (oleate/palmitate 2:1), as described by Lee et al. (28). In a set of experiments, long-chain fatty acid concentration was 0.4 mmol/l. In some experiments, islets were cultured in the presence or absence of 100  $\mu$ mol/l BADGE, a synthetic antagonist for PPAR- $\gamma$ .

**Insulin secretion.** At the end of the culture period, islets were washed twice in Krebs Ringer HEPES buffer (KRHB; containing 115 mmol/l NaCl, 5.4 mmol/l KCl, 2.38 mmol/l CaCl<sub>2</sub>, 0.8 mmol/l MgSO<sub>4</sub>, 1 mmol/l Na<sub>2</sub>HPO<sub>4</sub>, 10 mmol/l HEPES, and 0.5% BSA [pH 7.35]). Groups of five purified islets were then incubated with glucose 2.8 or 16.7 mmol/l (30 min, 37°C), and then insulin was measured in the medium by radioimmunoassay. Results are expressed as insulin released in the medium (pg  $\cdot$  islet<sup>-1</sup>  $\cdot$  30 min<sup>-1</sup>) (29).

**Glucose oxidation.** Glucose oxidation was determined by measuring the <sup>14</sup>CO<sub>2</sub> formation from [U-<sup>14</sup>C]glucose (New England Nuclear, Boston, MA) (30). At the end of the culture period, islets were washed twice in KRHB. Groups of 15 islets were incubated in a plastic cup in 100  $\mu$ l of Krebs-Ringer bicarbonate buffer (118 mmol/l NaCl, 4.8 mmol/l KCl, 2.5 mmol/l CaCl<sub>2</sub>, 1.2 mmol/l MgSO<sub>4</sub>, 1.2 mmol/l KH<sub>2</sub>PO<sub>4</sub>, and 25 mmol/l NaHCO<sub>3</sub>) supplemented with 10 mmol/l HEPES (pH 7.4) and containing 3  $\mu$ Ci D-[U-<sup>14</sup>C]glucose (specific activity 302 mCi/mmol) plus nonradioactive glucose to a final concentration of either 2.8 or 16.7 mmol/l. The cups, suspended in standard 20-ml glass scintillation vials, were gassed with O<sub>2</sub>:CO<sub>2</sub> (95:5) and capped airtight. The vials were then shaken continuously at 37°C for 120 min. The islet metabolism was stopped by injecting 100  $\mu$ l of 0.05 mmol/l Antimycin A (dissolved in 70% ethanol) into the cup. This was immediately followed by an injection of 250  $\mu$ l of Hyamine hydroxide (New England Nuclear) into the vials. <sup>14</sup>CO<sub>2</sub> was liberated from the incubation medium by a subsequent injection into the cup of 100  $\mu$ l of 0.4 mmol/l Na<sub>2</sub>HPO<sub>4</sub> solution adjusted to pH 6.0. After 2 h at room temperature (to allow liberated <sup>14</sup>CO<sub>2</sub> to be trapped by the Hyamine hydroxide), the cup was removed, 10 ml of a scintillation fluid was added to each flask, and the radioactivity was measured in a liquid scintillation counter. The <sup>14</sup>CO<sub>2</sub> recovery (88.8  $\pm$  0.9%) was assessed as previously described (31).

**Measurements of adenine nucleotides.** Adenine nucleotides were measured according to Detimay et al. (32). At the end of the culture period, islets were washed twice in KRHB. Groups of five islets were preincubated for 30 min at 37°C in 675  $\mu$ l of KRHB with 2.8 or 16.7 mmol/l glucose. The incubation was stopped by the addition of 0.125 ml of TCA to a final concentration of 5%. The tubes were then mixed, placed on ice for 5 min, and centrifuged. For eliminating TCA, 400  $\mu$ l of supernatant was mixed with 1.5 ml of diethylether, and the ether phase containing TCA was removed. This procedure was repeated three times to ensure complete elimination of TCA. The extracts were then diluted with 0.4 ml of a buffer containing 20 mmol/l HEPES, 3 mmol/l MgCl<sub>2</sub>, and KOH as required to adjust pH to 7.75 (assay buffer). The diluted extracts were frozen at -70°C until assayed.

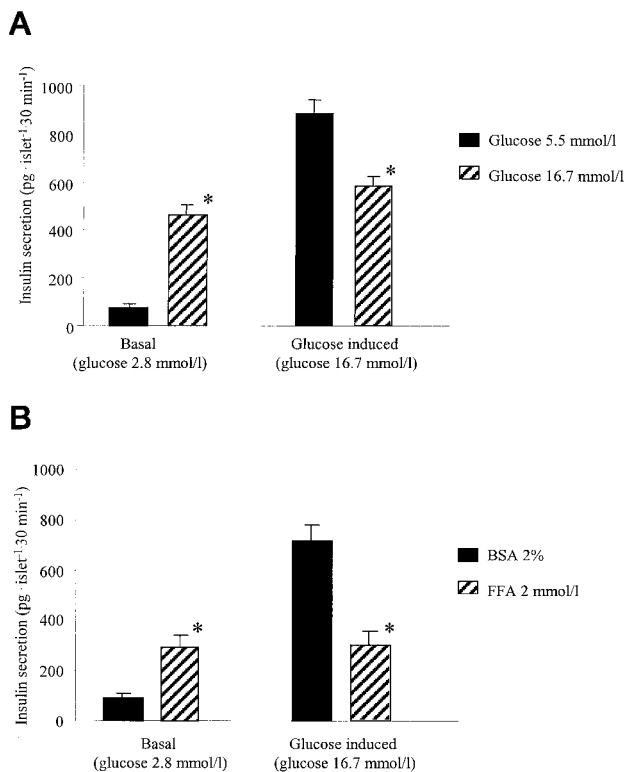
After appropriate sample dilution, ATP and ADP were measured in triplicate by a luminometric method (33). For measuring total ATP+ADP, ADP was first converted into ATP by mixing 100  $\mu$ l of the diluted extract with 300  $\mu$ l of the assay buffer supplemented with 1.5 mmol/l phosphoenolpyruvate and 2.3 units/ml pyruvate kinase and incubating at room temperature for 15

min. Samples with known ADP concentrations but without ATP were run in parallel as controls for complete transformation. ATP was measured by the addition of 100  $\mu$ l of an ATP monitoring reagent containing luciferase and luciferin (Sigma, St. Louis, MO). The emitted light was measured in a luminometer (Turner TD-20/20). For measuring only ATP, the same procedure was followed, except for the first incubation step, where pyruvate kinase was lacking. ADP levels were then calculated by subtracting ATP from the total ATP+ADP. Blanks and ATP standards were run through the entire procedure, including the extraction steps.

**Preparation of islet homogenate extracts and radiochemical assay of PDH activity.** Islet PDH complex was extracted and measured as previously described (34). With this method, we measured active, not total, PDH activity. At the end of the culture period, groups of 200 islets were washed twice in KRHB and then incubated with 16.7 mmol/l glucose (3 h, 37°C). After this period, islets were washed twice in PBS and homogenized in 300  $\mu$ l of homogenization buffer (50 mmol/l HEPES [pH 7.5], 0.2 mmol/l KCl, 3 mmol/l ethylenediaminetetraacetate, 5 mmol/l dithiothreitol, 0.1 mmol/LN<sup>+</sup>-*p*-tosyl-L-lysine chloromethyl ketone, 0.1 mg/ml trypsin inhibitor from egg white, 200 KIE/ml aprotinin, 2% rat serum, and 0.25% [vol/vol] Triton X-100). For extracting the PDH complex, islets were frozen-thawed three times and then passed 20 times through a 0.5-ml insulin syringe (Becton-Dickinson, Rutherford, NJ). Before measurement of PDH activity, islet extracts were left on ice for 30 min. Then, 50  $\mu$ l of extracts and 100  $\mu$ l of the enzyme reaction mixture (50 mmol/l HEPES [pH 7.5], 1 mmol/l MgCl<sub>2</sub>, 3 mmol/l NAD<sup>+</sup>, 0.4 mmol/l dithiothreitol, 0.1% vol/vol Triton X-100, 7.5 units/ml lipomaide dehydrogenase, EC 1.8.1.4, and 1.0 mmol/l pyruvate) were placed in 1.5-ml uncapped Eppendorf tubes inside a capped 20-ml scintillation vial containing 1.0 ml of KOH (1.2 mol/l). The reaction was started by adding 0.1  $\mu$ Ci of [1-<sup>14</sup>C]pyruvate (NEN, Boston, MA). After 20 min in a 37°C shaking water bath, the reaction was stopped by injecting into the Eppendorf tube 100  $\mu$ l of 15% of trichloroacetate. After an additional 2-h incubation at 37°C (to allow the liberated CO<sub>2</sub> to be absorbed by KOH), the Eppendorf tubes were removed, 5 ml of the scintillation fluid was added to each vial, and the radioactivity was measured. Blanks (lacking islets) containing 50  $\mu$ l of homogenization buffer were run with each series, and the averaged blank count was subtracted from sample counting. One unit of PDH complex activity was defined as the production of 1  $\mu$ mol CO<sub>2</sub>/min at 37°C. Activity was linear with time between 5 and 30 min and with the amount of islet extract from 5 to 20 islets.

**UCP-2 protein measurement.** The UCP-2 protein levels were measured by Western blot analysis as previously described (35). Briefly, at the end of the culture period, groups of 200 islets were washed twice in PBS and homogenized by sonication in SDS-PAGE sample buffer. Islet proteins were denatured by boiling for 3 min, and protein content was measured by the BCA protein assay kit (Pierce, Rockford, IL), using BSA as standard. Equivalent amounts of proteins were used in the different experimental groups. Homogenates (25  $\mu$ g of total cellular protein) were separated on 10% SDS-polyacrylamide gel (Mini-Protein; Bio-Rad) and electrophoretically transferred onto nitrocellulose membrane (Amersham Pharmacia Biotech, Buckinghamshire, U.K.). Blotting efficiency and the position of protein standards were assessed by Ponceau staining. Membranes were then washed at room temperature with PBS containing 1% nonfat dried milk and 0.2% Tween 20 (two changes within 30 min) and blocked with the same buffer containing 10% nonfat dried milk at room temperature for 1 h. After blocking, the membranes were washed two times, as described above, and incubated with a rabbit polyclonal anti-UCP-2 antibody (Alpha Diagnostic International, San Antonio, TX) at 1:4,000 dilution in blocking solution, 4°C, overnight. Membranes were then washed and blotted with a donkey anti-rabbit Ig peroxidase-linked whole antibody (Pierce) diluted 1:40,000 for 1 h at room temperature. Peroxidase activity was detected using a Super Signal-CL Kit (Pierce). As verified in each experiment by Ponceau red staining, the total amount of protein load was similar in each lane for the different culture conditions.

**PPAR- $\gamma$  protein measurement.** The protein levels were measured by Western blot analysis. As for UCP-2 protein measurement, islets were homogenized, separated on 10% SDS-polyacrylamide gel, and transferred electrophoretically onto nitrocellulose membranes. Membranes were then washed at room temperature with PBS containing 1% nonfat dried milk and 0.2% Tween 20 (two changes within 30 min) and blocked with the same buffer containing 10% nonfat dried milk at room temperature for 1 h. After blocking, the membranes were washed two times, as described above, and incubated with a rabbit polyclonal anti-PPAR- $\gamma$  antibody (Affinity Bioreagents, Golden, CO) at 1:4,000 dilution in blocking solution, 4°C, overnight. Membranes were then washed and blotted with a donkey anti-rabbit Ig peroxidase-linked whole antibody (Pierce) diluted 1:4,000, 1 h at room temperature. Peroxidase activity was detected using an enhanced chemiluminescence detection reagent (Amersham Pharmacia Biotech) for 1 min at room temperature.

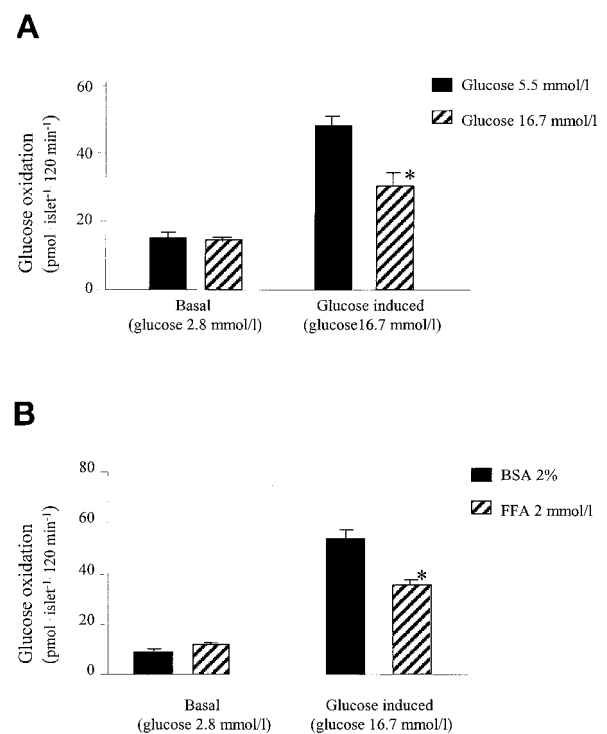


**FIG. 1. A:** Effect of preexposure to high glucose concentration (16.7 mmol/l) on insulin release from rat pancreatic islets. After a 48-h culture with either 5.5 or 16.7 mmol/l glucose, groups of five islets were transferred to Krebs-Ringer in the presence of 2.8 (basal) and 16.7 mmol/l (glucose-stimulated) glucose; 30 min later, insulin released in the medium was measured. **B:** Effect of preexposure to high FFA levels (2 mmol/l, oleate to palmitate, 2:1) on insulin release from rat pancreatic islets. After a 72-h culture at 5.5 mmol/l glucose with or without 2 mmol/l FFA, insulin release was measured in groups of five islets. In both panels, data are means  $\pm$  SE of five separate experiments; \* $P$  < 0.01 vs. control islets.

**Statistical analysis.** Data are mean  $\pm$  SE. Statistical significance was assessed by the Student's *t* test for unpaired comparison and two-tailed analysis.

## RESULTS

**Insulin release.** In control rat islets, basal insulin release (in the presence of a nonstimulatory glucose concentration, 2.8 mmol/l) was  $73 \pm 15$  pg  $\cdot$  islet<sup>-1</sup>  $\cdot$  30 min<sup>-1</sup> (mean  $\pm$  SE,  $n = 5$ ) and significantly increased in response to 16.7 mmol/l glucose ( $879 \pm 63$  pg  $\cdot$  islet<sup>-1</sup>  $\cdot$  30 min<sup>-1</sup>;  $n = 5$ ; Fig. 1). In islets preexposed for 48 h to 16.7 mmol/l glucose, as expected, basal insulin secretion was significantly increased ( $461 \pm 40$  pg  $\cdot$  islet<sup>-1</sup>  $\cdot$  30 min<sup>-1</sup>;  $n = 5$ ;  $P < 0.01$  vs. control) and glucose-stimulated insulin release was markedly reduced ( $567 \pm 43$  pg  $\cdot$  islet<sup>-1</sup>  $\cdot$  30 min<sup>-1</sup>;  $n = 5$ ;  $P < 0.01$ ; Fig. 1). Similar data were obtained in islets exposed to FFA (Fig. 1): basal insulin release was increased ( $292 \pm 48$  vs.  $89 \pm 22$  pg  $\cdot$  islet<sup>-1</sup>  $\cdot$  30 min<sup>-1</sup> in control islet;  $n = 5$ ;  $P < 0.01$ ) and glucose-stimulated insulin release was reduced ( $301 \pm 60$  vs.  $717 \pm 62$  pg  $\cdot$  islet<sup>-1</sup>  $\cdot$  30 min<sup>-1</sup> in control islet;  $n = 5$ ;  $P < 0.01$ ) in comparison with control islets. As requested by a reviewer, these data were also confirmed in islets cultured for 72 h with 0.4 mmol/l FFA: glucose-stimulated insulin release was reduced in comparison with control islets ( $389 \pm 78$  vs.  $899 \pm 109$  pg  $\cdot$  islet<sup>-1</sup>  $\cdot$  30 min<sup>-1</sup>;  $n = 5$ ;  $P < 0.01$ ); basal insulin secretion was increased ( $153 \pm 24$  vs.



**FIG. 2. A:** Effect of preexposure to high glucose concentration (16.7 mmol/l) on glucose oxidation in rat pancreatic islets. After a 48-h culture with either 5.5 or 16.7 mmol/l glucose, groups of 15 islets were incubated in 100  $\mu$ l of KRHB containing 10 mmol/l HEPES, 3  $\mu$ Ci D-[U-<sup>14</sup>C]glucose, and either 2.8 (basal) or 16.7 mmol/l (glucose-stimulated) glucose. Glucose oxidation was evaluated by measuring <sup>14</sup>CO<sub>2</sub> formation of from D-[U-<sup>14</sup>C]glucose. **B:** Effect of preexposure to high FFA levels (2 mmol/l, oleate to palmitate, 2:1) on glucose oxidation in rat pancreatic islets. After a 72-h culture at 5.5 mmol/l glucose with or without 2 mmol/l FFA, glucose oxidation was measured in groups of 15 islets as in A. In both panels, data are means  $\pm$  SE of four separate experiments; \* $P$  < 0.01 vs. control islets.

$78 \pm 18$  pg  $\cdot$  islet<sup>-1</sup>  $\cdot$  30 min<sup>-1</sup> in control islet;  $n = 5$ ;  $P < 0.05$ ).

**Glucose oxidation.** In response to the glucose concentration increase, glucose oxidation also increased in control islets. In contrast, in islets preexposed to high glucose, whereas glucose oxidation at 2.8 mmol/l glucose was similar to that of control islets ( $14.4 \pm 0.5$  vs.  $14.8 \pm 1.9$  pmol  $\cdot$  islet<sup>-1</sup>  $\cdot$  120 min<sup>-1</sup>, respectively;  $n = 4$ ), glucose oxidation was significantly reduced after stimulation with 16.7 mmol/l glucose ( $29.8 \pm 3.6$  vs.  $47.5 \pm 2.4$  pmol  $\cdot$  islet<sup>-1</sup>  $\cdot$  120 min<sup>-1</sup> in control islets;  $n = 4$ ;  $P < 0.01$ ; Fig. 2). Similar data were obtained in islets preexposed to 2 mmol/l FFA: glucose oxidation at 2.8 mmol/l was unchanged ( $11.5 \pm 1.2$  vs.  $8.9 \pm 1.4$  pmol  $\cdot$  islet<sup>-1</sup>  $\cdot$  120 min<sup>-1</sup> in control islet;  $n = 4$ ), but the value measured at 16.7 mmol/l glucose was significantly reduced in respect to control islets ( $35.0 \pm 2.2$  vs.  $53.7 \pm 3.7$  pmol  $\cdot$  islet<sup>-1</sup>  $\cdot$  120 min<sup>-1</sup>, respectively;  $n = 4$ ;  $P < 0.01$ ; Fig. 2).

**ATP and ADP content.** In response to glucose concentration increase (16.7 mmol/l), in control islets, ATP levels increased, ADP levels decreased, and, as a consequence, the ATP/ADP ratio clearly increased. In islets preexposed for 48 h to 16.7 mmol/l glucose, the basal (2.8 mmol/l glucose) ATP level was higher in respect to control islets ( $35.5 \pm 2.5$  vs.  $25.7 \pm 1.6$  pmol/islet, respectively;  $n = 3$ ;  $P < 0.05$ ). After stimulation with 16.7 mmol/l glucose, the ATP level did not further increase over baseline in high

**TABLE 1**  
Effects of pancreatic islet chronic exposure (48 h) to high glucose on ATP levels and the ATP/ADP ratio

Culture conditions	Glucose 2.8 mmol/l	Glucose 16.7 mmol/l	Δ Over basal
Glucose 5.5 mmol/l			
ATP (pmol/islet)	25.7 ± 1.7	37.7 ± 1.8‡	12.0 ± 0.6
ADP (pmol/islet)	5.5 ± 0.5	3.8 ± 0.7	
ATP/ADP ratio	4.8 ± 0.5	10.1 ± 1.4‡	
Glucose 16.7 mmol/l			
ATP (pmol/islet)	35.5 ± 2.5*	36.2 ± 2.3	0.7 ± 0.5†
ADP (pmol/islet)	7.9 ± 1.1	8.0 ± 1.0*	
ATP/ADP ratio	4.5 ± 1.1	4.5 ± 0.9*	

Data are mean ± SE of three separate experiments. \**P* < 0.05, †*P* < 0.01 vs. controls; ‡*P* < 0.05 vs. 2.8 mmol/l glucose.

glucose-exposed islets (Δ of increment over baseline: 0.7 ± 0.5 vs. 12.0 ± 0.6 pmol/islet in control islets; *n* = 3; *P* < 0.01). Therefore, in islets incubated for 48 h with high glucose, the ATP/ADP ratio in response to glucose stimulation was clearly reduced (4.5 ± 0.9 vs. 10.1 ± 1.4; *n* = 3; *P* < 0.05; Table 1). Also, in islets cultured for 72 h with high FFA, the basal level of ATP was increased in respect to control islets cultured without FFA (38.5 ± 1.9 vs. 30.7 ± 1.7 pmol/islet, respectively; *n* = 3; *P* < 0.05), the glucose-induced ATP raise was impaired (Δ of increment: 0.5 ± 0.3 pmol/islet in FFA exposed islets vs. 12.1 ± 1.5 pmol/islet in control islets; *n* = 3; *P* < 0.01), and the ATP/ADP ratio in response to high glucose was reduced (5.8 ± 0.8 vs. 13.8 ± 1.5 pmol/islet; *n* = 3; *P* < 0.01; Table 2).

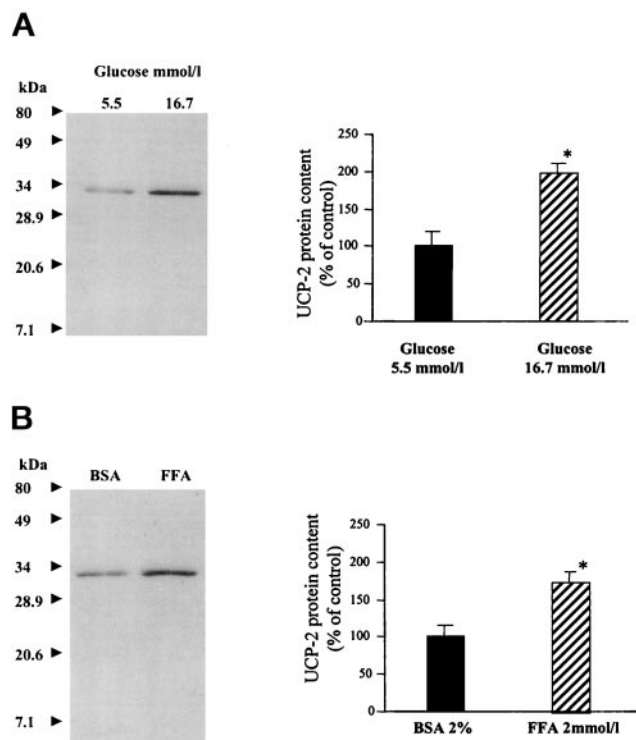
**PDH activity.** To study the mechanism responsible for the impaired glucose oxidation after exposure to high glucose or FFA, we measured PDH activity in the pancreatic islet homogenates. In islets exposed for 48 h to high glucose, the glucose-stimulated PDH activity was significantly decreased at ~54% of control islets (1.28 ± 0.08 vs. 2.38 ± 0.16 μU/islet, respectively; *n* = 4; *P* < 0.01). Similar results were obtained after 72 h exposure to FFAs: the islet PDH activity in response to glucose stimulation was greatly reduced, at ~45% of control islets (1.49 ± 0.22 vs. 3.29 ± 0.42 μU/islet, respectively; *n* = 4; *P* < 0.01).

**UCP-2 levels.** To investigate whether metabolic pathways other than glucose oxidation might play a role in modu-

**TABLE 2**  
Effects of pancreatic islet chronic exposure (72 h) to high FFA on ATP levels and the ATP/ADP ratio

Culture conditions	Glucose 2.8 mmol/l	Glucose 16.7 mmol/l	Δ Over basal
72 h			
BSA 2%, glucose 5.5 mmol/l			
ATP (pmol/islet)	30.7 ± 1.7	42.2 ± 2.0‡	12.1 ± 1.5
ADP (pmol/islet)	4.6 ± 0.3	3.1 ± 0.2‡	
ATP/ADP ratio	6.7 ± 0.5	13.8 ± 1.5‡	
FFA 2 mmol/l, glucose 5.5 mmol/l			
ATP (pmol/islet)	38.5 ± 1.9*	38.1 ± 0.8	0.5 ± 0.3†
ADP (pmol/islet)	6.5 ± 0.9	6.6 ± 1.0*	
ATP/ADP ratio	6.0 ± 0.9	5.8 ± 0.8*	

Data are mean ± SE of three separate experiments. \**P* < 0.05, †*P* < 0.01 vs. controls; ‡*P* < 0.05 vs. 2.8 mmol/l glucose.

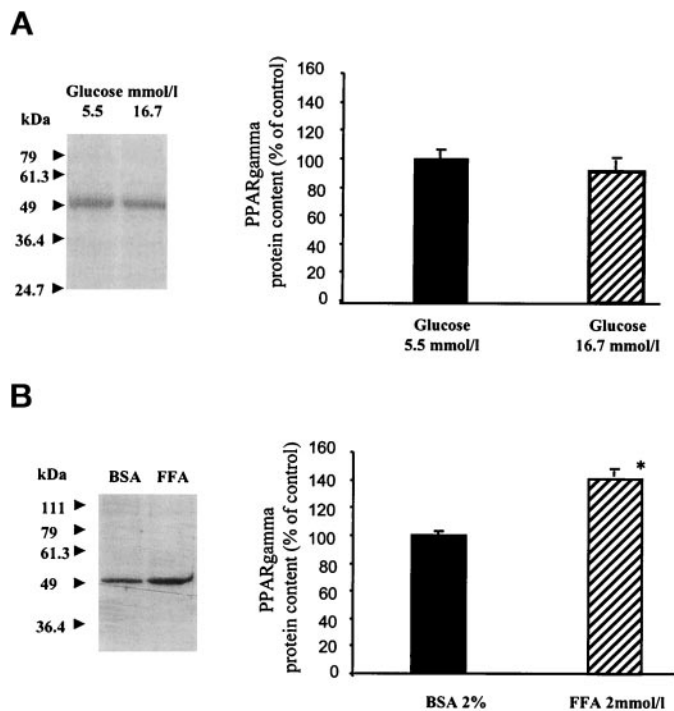


**FIG. 3. A:** Effect of preexposure to high glucose concentration (16.7 mmol/l) on UCP-2 protein content. After a 48-h culture with either 5.5 or 16.7 mmol/l glucose, islets were homogenized, 25 μg of protein was resolved by electrophoresis on 10% polyacrylamide gel, and UCP-2 protein content was measured by Western blot analysis. **B:** Effect of preexposure to high FFA levels (2 mmol/l, oleate to palmitate, 2:1) on UCP-2 protein levels. After a 72-h culture at 5.5 mmol/l glucose with or without 2 mmol/l FFA, UCP-2 protein content was measured by Western blot analysis as in A. In both panels, the left part shows a representative experiment, and the right part shows the means ± SE of four separate experiments; \**P* < 0.05 (A) and *P* < 0.01 (B) vs. control islets.

lating mitochondrial energy metabolism, we measured the UCP-2 protein level in islets exposed to either high glucose (48 h, 16.7 mmol/l) or FFA (72 h, 2 mmol/l). The UCP-2 protein content was significantly increased in islets exposed to high glucose for 48 h with respect to control islets (191 ± 29%; *n* = 4; *P* < 0.05). Similar data were obtained in islets preexposed to 2 mmol/l FFAs: also in these islets, the UCP-2 expression was significantly increased with respect to control islets (197 ± 13%; *n* = 4; *P* < 0.01). Protein recovery was similar in islets exposed for 48 h to either 5.5 or 16.7 mmol/l glucose or for 72 h with or without 2 mmol/l FFAs (Fig. 3).

**PPAR-γ levels.** To investigate whether chronic exposure to high glucose or FFA can modulate PPAR-γ protein levels, known transcription factors of UCP-2, we measured PPAR-γ expression in islets exposed to either high glucose (48 h, 16.7 mmol/l) or FFA (72 h, 2 mmol/l). In islets preexposed to 2 mmol/l FFAs, the PPAR-γ expression was significantly increased with respect to control islets (138 ± 6%; *n* = 3; *P* < 0.05). In contrast, PPAR-γ protein content was unchanged in islets exposed to high glucose for 48 h with respect to control islets (*n* = 4; Fig. 4).

**PPAR-γ inhibition.** To study the role of PPAR-γ in modulating UCP-2 expression and, as consequence, in impaired glucose-stimulated insulin secretion induced by chronic culture with high glucose and FFA levels, we analyzed whether PPAR-γ inhibition by synthetic antago-

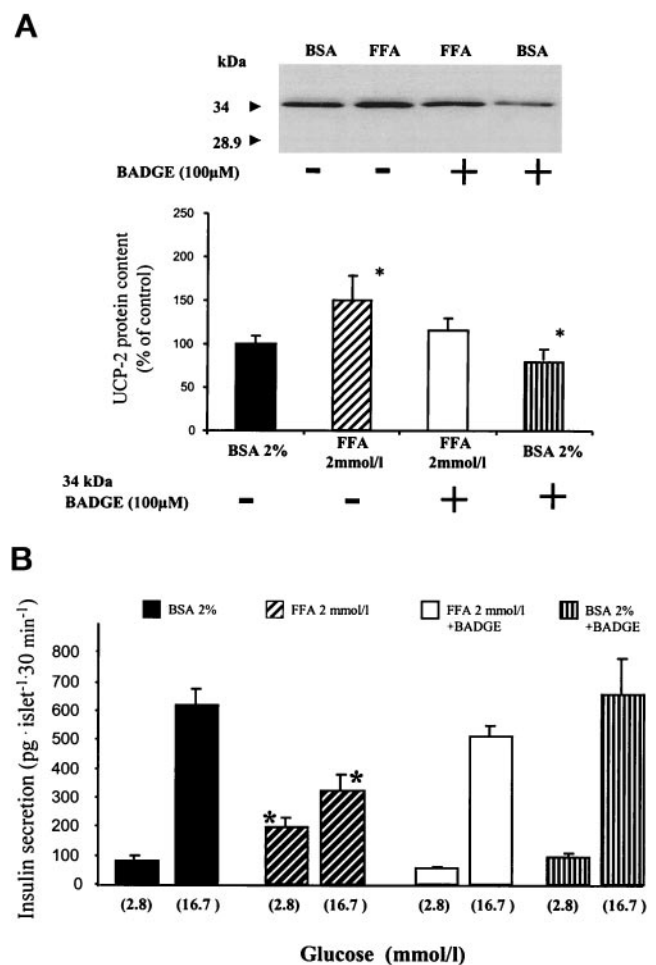


**FIG. 4.** *A:* Effect of preexposure to high glucose concentration (16.7 mmol/l) on PPAR- $\gamma$  protein content. After a 48-h culture with either 5.5 or 16.7 mmol/l glucose, islets were homogenized, 25  $\mu$ g of protein was resolved by electrophoresis on 10% polyacrylamide gel, and PPAR- $\gamma$  protein content was measured by Western blot analysis. *B:* Effect of preexposure to high FFA levels (2 mmol/l, oleate to palmitate, 2:1) on PPAR- $\gamma$  protein levels. After a 72-h culture at 5.5 mmol/l glucose with or without 2 mmol/l FFA, PPAR- $\gamma$  protein content was measured by Western blot analysis as in *A*. In both panels, the left part shows a representative experiment, and the right part shows the means  $\pm$  SE of four separate experiments;  $P = \text{NS}$  (*A*) and  $*P < 0.05$  (*B*) vs. control islets.

nist of PPAR- $\gamma$  was able to prevent the deleterious effects of gluco- and lipotoxicity. BADGE is a ligand for PPAR- $\gamma$  and can antagonize the ability of FFA agonist ligand to stimulate the transcriptional activity of PPAR- $\gamma$ . When islets were cultured for 72 h with or without FFA in the presence of BADGE, an inhibition of FFA-induced UCP-2 overexpression was observed; in addition, BADGE presence in control islets induced a reduction of UCP-2 protein levels ( $-28 \pm 7\%$ ;  $n = 3$ ;  $P < 0.05$ ; Fig. 5A).

In this condition, a reversal of insulin release pattern to controls was found. In fact, in islets cultured with FFA and BADGE for 72 h, glucose-stimulated insulin release was increased compared with islets cultured with FFA alone ( $510 \pm 19$  vs.  $323 \pm 18$   $\text{pg} \cdot \text{islet}^{-1} \cdot 30 \text{ min}^{-1}$ ;  $n = 4$ ;  $P < 0.05$ ) and basal insulin release was reduced ( $38.5 \pm 6.7$  vs.  $176 \pm 10$   $\text{pg} \cdot \text{islet}^{-1} \cdot 30 \text{ min}^{-1}$ ;  $n = 4$ ;  $P < 0.05$ ; Fig. 5B). Similar effects were obtained in islets cultured in the presence of 0.4 mmol/l FFA and BADGE: glucose-stimulated insulin secretion was increased compared with islets cultured with FFA alone ( $934 \pm 150$  vs.  $389 \pm 78$   $\text{pg} \cdot \text{islet}^{-1} \cdot 30 \text{ min}^{-1}$ ;  $n = 5$ ;  $P < 0.01$ ), and basal insulin secretion was reduced ( $86 \pm 13$  vs.  $153 \pm 24$   $\text{pg} \cdot \text{islet}^{-1} \cdot 30 \text{ min}^{-1}$ ;  $n = 5$ ;  $P < 0.01$ ). Accordingly, BADGE addition to culture medium containing FFAs induced an increase of ATP/ADP ratio (Table 3) and a recovery of PDH activity ( $2.64 \pm 0.21$  vs.  $1.20 \pm 0.13$   $\mu\text{U}/\text{islet}$ , respectively;  $n = 3$ ;  $P < 0.01$ ) with respect to FFA-exposed islets.

In contrast, in islets incubated for 48 h with 5.5 or 16.7



**FIG. 5.** *A:* Effect of BADGE on UCP-2 protein content in islets exposed to high FFA concentration (2 mmol/l, oleate to palmitate, 2:1). After a 72-h culture with 5.5 mmol/l glucose with or without FFA in the presence or absence 100  $\mu\text{M}$  BADGE, islets were homogenized, 25  $\mu\text{g}$  of protein was resolved by electrophoresis on 10% polyacrylamide gel, and UCP-2 protein content was measured by Western blot analysis. Upper panel shows a representative experiment, lower panel shows the mean  $\pm$  SE of three separate experiments;  $*P < 0.05$  vs. control islets. *B:* Effect of BADGE on insulin secretion in islets exposed to high FFA concentration (2 mmol/l, oleate to palmitate, 2:1). After a 72-h culture at 5.5 mmol/l glucose with or without 2 mmol/l FFA in the presence or absence of 100  $\mu\text{M}$  of BADGE, groups of five islets were transferred in Krebs-Ringer in the presence of 2.8 (basal) and 16.7 mmol/l (glucose-stimulated) glucose; 30 min later, insulin released in the medium was measured. Data are means  $\pm$  SE of four separate experiments.  $*P < 0.01$  vs. control islets and islets cultured with or without FFA plus BADGE.

mmol/l glucose in the presence or absence BADGE, PDH activity was unchanged (data not shown) and glucose-induced UCP-2 overexpression was unaffected, but BADGE presence in control islets induced a reduction of UCP-2 protein levels ( $-32 \pm 9\%$ ;  $n = 3$ ;  $P < 0.05$ ; Fig. 6).

## DISCUSSION

Our data indicate that the blunted insulin release as a result of chronic exposure of pancreatic islets to either high glucose or FFA levels (gluco- and lipotoxicity) is associated with a decrease in ATP synthesis as a result of the inability of  $\beta$ -cells to increase the ATP levels in response to glucose stimulation. We investigated the mechanism of the reduced ATP production and observed that both a defect in substrate supply to the Krebs cycle

TABLE 3  
Effects of pancreatic islet chronic exposure (72 h) to high FFA with or without BADGE on ATP levels and the ATP/ADP ratio

Culture conditions 72 h	Glucose 2.8 mmol/l	Glucose 16.7 mmol/l	Δ Over basal
BSA 2%, glucose 5.5 mmol/l			
ATP (pmol/islet)	32.4 ± 2.4	44.4 ± 3.1‡	11.9 ± 1.8
ADP (pmol/islet)	5.1 ± 0.5	3.4 ± 0.4‡	
ATP/ADP ratio	6.3 ± 0.6	13.1 ± 1.1§	
FFA 2 mmol/l, glucose 5.5 mmol/l			
ATP (pmol/islet)	38.8 ± 2.5	39.9 ± 1.7	1.15 ± 0.8†
ADP (pmol/islet)	5.9 ± 0.4	5.5 ± 0.6	
ATP/ADP ratio	6.6 ± 0.8	7.3 ± 0.8*	
FFA 2 mmol/l, glucose 5.5 mmol/l + BADGE 100 μmol/l			
ATP (pmol/islet)	42.5 ± 1.6	51.8 ± 1.8‡	9.3 ± 1.3
ADP (pmol/islet)	6.4 ± 0.5	4.6 ± 0.5‡	
ATP/ADP ratio	6.6 ± 0.7	11.2 ± 1.1‡	
BSA 2% + glucose 5.5 mmol/l BADGE 100 μmol/l			
ATP (pmol/islet)	43.2 ± 1.9	56.8 ± 2.0§	13.5 ± 1.9
ADP (pmol/islet)	6.4 ± 0.6	4.4 ± 0.4‡	
ATP/ADP ratio	6.8 ± 0.7	12.9 ± 0.9§	

Data are mean ± SE of four separate experiments. \**P* < 0.05, †*P* < 0.01 ‡*P* < 0.05, §*P* < 0.01 vs. 2.8 mmol/l glucose.

and UCP-2 protein overexpression could contribute to this abnormality. For the first pathway, we studied PDH, a key enzyme for pyruvate metabolism and for the subsequent glucose oxidation through the Krebs cycle. In fact, pyru-

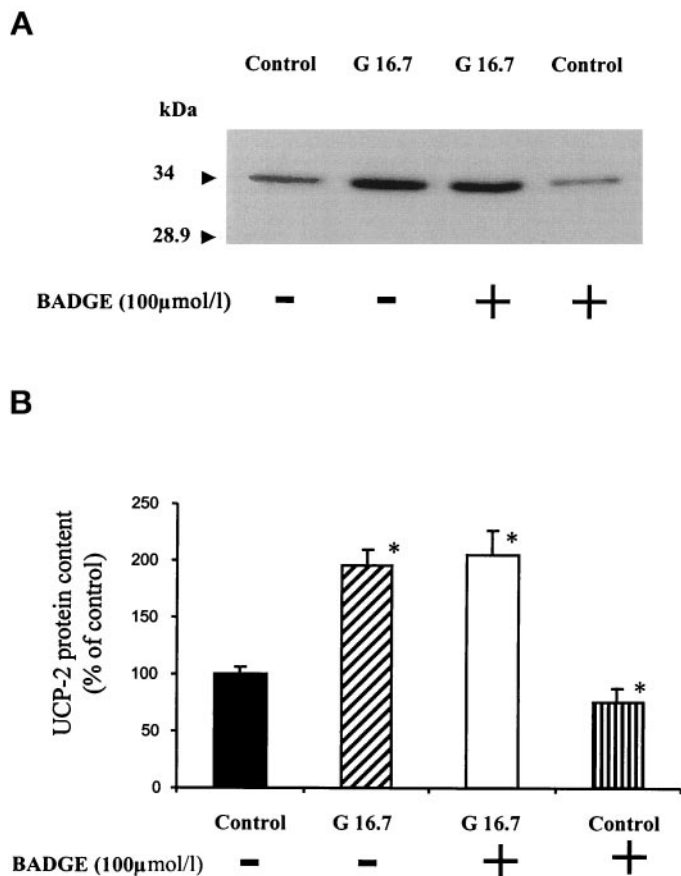


FIG. 6. Effect of BADGE on UCP-2 protein content in islets exposed to high glucose concentration (16.7 mmol/l). After a 48-h culture with either 5.5 or 16.7 mmol/l glucose in the presence or absence 100 μmol/l BADGE, islets were homogenized, 25 μg of protein was resolved by electrophoresis on 10% polyacrylamide gel, and UCP-2 protein content was measured by Western blot analysis. A: A representative experiment. B: The means ± SE of three separate experiments. \**P* < 0.05 vs. control islets.

vate, the final product of the glycolytic pathway, enters mitochondrial metabolism by decarboxylation to acetyl-CoA (a reaction that is catalyzed by PDH, an enzymatic complex of the mitochondrial inner membrane).

In islets exposed to high glucose or FFA levels, we found a significant decrease of glucose-induced PDH activity. This finding suggests that the reduced glucose oxidation observed in islets chronically exposed to high glucose or FFA might be due, at least in part, to the impaired PDH activity. Because in several tissues PDH activity has been demonstrated to be inhibited by high mitochondrial ATP levels (36), it is possible that also in our model the increased ATP availability (as a result of the substrate excess in the environment) could exert an adverse effect on PDH activity. It is also possible that high glucose or FFAs could decrease PDH activity either by increasing the PDH kinase activity (37) or by affecting the PDH protein expression (38).

Indirect evidence suggests that PDH could play a role in pancreatic islet desensitization induced by hyperglycemia. In fact, a decreased PDH activity has already been observed in vivo, in GK rats, a genetic model of type 2 diabetes (34). In this article, we first demonstrate a direct effect of chronic hyperglycemia on PDH activity and the association with a reduced ATP synthesis and impaired insulin secretion. In addition, we demonstrate that both chronic hyperglycemia and chronically high FFA levels reduce PDH activity in pancreatic islets and that this alteration could be due to similar mechanism: an overload fueled by increasing basal ATP levels could blunt glucose-induced PDH activity and could be responsible, at least in part, for impaired insulin secretion. Zhou et al. (39) reported a decrease of PDH activity in pancreatic islets exposed to high levels of FFA but suggested that this defect was related to the glucose-FFA cycle (Randle cycle) (40).

The second pathway that can contribute to the impaired ATP formation that we have observed in our model involves UCP-2 and the respiratory chain function. In fact, after prolonged culture with high glucose and FFA, we observed a UCP-2 protein upregulation. This increase

could be a protective response induced by high energetic fuels (glucose and FFA), resulting in basal ATP load. Because UCP-2 uncouples fuel oxidation from conversion of ADP to ATP, the increased UCP-2 expression may contribute to the altered glucose-induced ATP formation and the decreased insulin release that we observed at the end of chronic incubation with high glucose or FFA.

Recent data indicate that in tumoral  $\beta$ -cell line, chronic exposure to high FFA both reduced insulin secretion and increased UCP-2 levels by regulating glucose-induced ATP formation (41). In normal rat islets, overexpression of UCP-2 inhibits glucose-stimulated insulin secretion (16,17). In other reports, UCP-2 overexpression by reducing ATP levels but enhancing the ATP/ADP ratio restored insulin secretion in islets from ZDF rat.

Conflicting data have been reported in islets exposed to high glucose provided on UCP-2 expression: in islets from hyperglycemic 90% pancreatectomized rats, UCP-2 mRNA was increased (42); in contrast, in INS  $\beta$ -cell line, chronic exposure to high glucose reduced UCP-2 mRNA levels (43). Furthermore, we investigated the mechanism by which high glucose or FFA levels increase UCP-2 expression, and we observed that PPAR- $\gamma$  were to some extent involved. PPAR- $\gamma$  are nuclear transcription factors involved in several functions ongoing from cellular cycle control to the regulation of enzymes implicated in glucose and lipid metabolism (44,45).

In our study, we observed an increase of PPAR- $\gamma$  protein levels in islets exposed to high FFAs and the reversal effects induced by a PPAR- $\gamma$  antagonist. BADGE added to the culture medium was able to prevent UCP-2 protein increase and to restore both insulin secretion and the ATP/ADP ratio. In addition, in the presence of BADGE, we observed a decrease of UCP-2 expression; accordingly, in both control and FFA-treated islets, very high ATP levels further increased in response to glucose stimulation. These data could indicate that UCP-2 modulates the basal ATP content and that only when UCP-2 is upregulated, new ATP synthesis is impaired. In contrast, when UCP-2 was downregulated by PPAR- $\gamma$  antagonist, the basal ATP levels were high and further increased in response to glucose stimulation, preserving glucose-induced insulin secretion (46). In addition, our data show that BADGE is able to reverse the decrease of PDH activity. This effect could be explained by a direct effect of PPAR- $\gamma$  on PDH. Alternatively, BADGE could normalize lipid oxidation and then reduce acetyl-CoA levels and, by this mechanism, reverse PDH inhibition. This last view is supported by data obtained in ZDF rats, in which troglitazone, a PPAR- $\gamma$  agonist, is able to increase FFA oxidation (47). These data support the hypothesis that the FFA effects on  $\beta$ -cell function are mediated by PPAR- $\gamma$  transcription factors, whereas PPAR- $\gamma$  could not be involved in the glucose-induced  $\beta$ -cell dysfunction. In fact, PPAR- $\gamma$  levels were not altered by chronic hyperglycemia, and both the UCP-2 levels and PDH activity were not influenced by PPAR- $\gamma$  antagonist. The role of PPAR- $\gamma$  in pancreatic islets is still not clarified. Protective effects of PPAR- $\gamma$  on  $\beta$ -cells were reported in a genetic model of lipotoxicity (47,48). In contrast, after chronic hyperglycemia, an impaired islet function was associated with a PPAR- $\gamma$  RNA increase (42,49). In our report, hyperglycemia does not modify

PPAR- $\gamma$  protein levels, and this, with respect to previous reports, could be due to our relatively short exposure time or an impaired mRNA translation.

In conclusion, our data show that the chronic exposure to either high glucose or FFA levels impairs glucose-induced ATP formation through at least two different mechanisms. On the one hand, it decreases PDH activity and glucose oxidation through the Krebs cycle. On the other hand, it increases UCP-2 expression. It is conceivable that when an increased fuel influx through the metabolic pathways overcomes the oxidative capacity of pancreatic  $\beta$ -cells, large amounts of high-energy substrates might load the cells and impair their function (48,50). In this condition, the  $\beta$ -cell, like many other cell types, protects itself from fuel overload by decreasing glucose oxidation and increasing energy disposal by increased UCP-2 protein expression. This compensatory mechanism, by causing a decrease of ATP production, a key regulator of insulin secretion, however, impairs glucose-induced insulin release and further contributes to the long-term deleterious effects of excess substrate availability.

## REFERENCES

- Cavaghan MK, Ehrmann DA, Polonsky KS: Interactions between insulin resistance and insulin secretion in the development of glucose intolerance. *J Clin Invest* 106:329–333, 2000
- De Fronzo RA, Bonadonna RC, Ferrannini E: Pathogenesis of NIDDM: a balanced overview. *Diabetes Care* 15:318–368, 1992
- McGarry JD, Dobbins RL: Fatty acids, lipotoxicity and insulin secretion. *Diabetologia* 42:128–138, 1999
- Unger RH: Lipotoxicity in the pathogenesis of obesity-dependent NIDDM: genetic and clinical implications. *Diabetes* 44:863–870, 1995
- Grodsky GM, Bolaffi JL: Desensitization of the insulin-secreting beta cell. *J Cell Biochem* 48:3–11, 1992
- Leahy JL, Cooper HE, Deal DA, Weir GC: Chronic hyperglycemia is associated with impaired glucose influence on insulin secretion. *J Clin Invest* 77:908–915, 1986
- Hoening M, MacGregor LC, Matschinsky FM: In vitro exhaustion of pancreatic  $\beta$ -cells. *Am J Physiol* 250:E502–E511, 1986
- Purrello F, Vetri M, Gatta C, Gullo D, Vigneri R: Effects of high glucose on insulin secretion by isolated rat islets and purified B-cells and possible role of glycosylation. *Diabetes* 38:1417–1422, 1989
- Bjorklund A, Yaney G, McGarry JD, Weir G: Fatty acids and beta-cell function. *Diabetologia* 40 (Suppl. 3):B21–B26, 1997
- Paolesso G, Gambardella A, Amato L, Tortoriello R, D'Amore A, Varricchio M, D'Onofrio F: Opposite effects of short- and long-term fatty acid infusion on insulin secretion in healthy subjects. *Diabetologia* 38:1295–1299, 1995
- Hosokawa H, Corkey BE, Leahy JL: Beta-cell hypersensitivity to glucose following 24-h exposure of rat islets to fatty acids. *Diabetologia* 40:392–397, 1997
- Elks MN: Chronic perfusion of rat islets with palmitate suppresses glucose-stimulated insulin release. *Endocrinology* 133:208–214, 1993
- Sako Y, Grill VE: A 48-hour lipid infusion in the rat time-dependently inhibits glucose-induced insulin secretion and B cell oxidation through a process likely coupled to fatty acid oxidation. *Endocrinology* 127:1580–1589, 1990
- Anello M, Rabuazzo AM, Degano C, Caltabiano V, Patané G, Vigneri R, Purrello F: Fast reversibility of glucose-induced desensitization in rat pancreatic islets: evidence for an involvement of ionic fluxes. *Diabetes* 45:502–506, 1996
- Patané G, Piro S, Rabuazzo AM, Anello M, Vigneri R, Purrello F: Metformin restores insulin secretion altered by chronic exposure to free fatty acids or high glucose: a direct metformin effect on pancreatic beta-cells. *Diabetes* 49:735–740, 2000
- Chan CB, MacDonald PE, Saleh MC, Johns DC, Marban E, Wheeler MB: Overexpression of uncoupling protein 2 inhibits glucose-stimulated insulin secretion from rat islets. *Diabetes* 48:1482–1486, 1999
- Chan CB, De Leo D, Joseph JW, McQuaid TS, Ha XF, Xu F, Tsushima RG, Pennefather PS, Salapatek AM, Wheeler MB: Increased uncoupling protein-2 levels in beta-cells are associated with impaired glucose-stimulated insulin secretion: mechanism of action. *Diabetes* 50:1302–1310, 2001

18. Dubois M, Pattou F, Kerr-Conte J, Gmyr V, Vandewalle B, Desreumaux P, Auwerx J, Schoonjans K, Lefebvre J: Expression of peroxisome proliferator-activated receptor gamma (PPAR $\gamma$ ) in normal human pancreatic islet cells. *Diabetologia* 43:1165–1169, 2000
19. Aubert J, Champigny O, Saint-Marc P, Negrel R, Collins S, Ricquier D, Ailhaud G: Up-regulation of UCP-2 gene expression by PPAR agonists in preadipose and adipose cells. *Biochem Biophys Res Commun* 238:606–611, 1997
20. Shimabukuro M, Zhou YT, Lee Y, Unger RH: Induction of uncoupling protein-2 mRNA by troglitazone in the pancreatic islets of Zucker diabetic fatty rats. *Biochem Biophys Res Commun* 237:359–361, 1997
21. Nisoli E, Carruba MO, Tonello C, Macor C, Federspil G, Vettor R: Induction of fatty acid translocase/CD36, peroxisome proliferator-activated receptor- $\gamma$ 2, leptin, uncoupling proteins 2 and 3, and tumor necrosis factor-alpha gene expression in human subcutaneous fat by lipid infusion. *Diabetes* 49:319–324, 2000
22. Spiegelman BM, Flier JS: Adipogenesis and obesity: rounding out the big picture. *Cell* 87:377–389, 1996
23. Kelly LJ, Vicario PP, Thompson GM, Candelore MR, Doebber TW, Ventre J, Wu MS, Meurer R, Forrest MJ, Conner MW, Cascieri MA, Moller DE: Peroxisome proliferator-activated receptors gamma and alpha mediate in vivo regulation of uncoupling protein (UCP-1, UCP-2, UCP-3) gene expression. *Endocrinology* 139:4920–4927, 1998
24. Van Der Lee KA, Willensens PH, Van Der Vusse GJ, Van Bilsen M: Effects of fatty acids on uncoupling protein-2 expression in the rat heart. *FASEB J* 14:495–502, 2000
25. Green S, Wahli W: Peroxisome proliferator-activated receptors: finding the orphan a home. *Mol Cell Endocrinol* 100:149–153, 1994
26. Wright HM, Clish CB, Mikami T, Hauser S, Yanagi K, Hiramatsu R, Serhan CN, Spiegelman BM: A synthetic antagonist for the peroxisome proliferator-activated receptor gamma inhibits adipocyte differentiation. *J Biol Chem* 275:1873–1877, 2000
27. Purrello F, Buscema N, Rabuazzo AM, Caltabiano V, Forte F, Vinci C, Vetri M, Vigneri R: Glucose modulates glucose transporter affinity, glucokinase activity and secretory response in rat pancreatic  $\beta$ -cells. *Diabetes* 42:199–205, 1993
28. Lee Y, Hirose H, Ohneda M, Johnson JH, McGarry JD, Unger RH: Beta-cell lipotoxicity in the pathogenesis of non-insulin-dependent diabetes mellitus of obese rats: impairment in adipocyte-beta-cell relationships. *Proc Natl Acad Sci U S A* 91:10878–10882, 1994
29. Patane G, Piro S, Anello M, Rabuazzo AM, Vigneri R, Purrello F: Exposure to glibenclamide increases rat beta cells sensitivity to glucose. *Br J Pharmacol* 129:887–892, 2000
30. Rabuazzo AM, Buscema M, Caltabiano V, Anello M, Patané G, Vigneri R, Purrello F: Interleukin-1 $\beta$  inhibition of insulin release in rat pancreatic islet: possible involvement of G-proteins in the signal transduction pathway. *Diabetologia* 38:779–784, 1995
31. Schuit F, De Vos A, Farfari S, Moens K, Pipeleers D, Brun T, Prentki M: Metabolic fate of glucose in purified islet cells: glucose-regulated anaplerosis in beta cells. *J Biol Chem* 272:18572–18579, 1997
32. Detimary P, Jonas JC, Henquin JC: Stable and diffusible pools of nucleotides in pancreatic islet cells. *Endocrinology* 137:4671–4676, 1996
33. Hampp R: Luminometric method. In *Methods of Enzymatic Analysis*. Bergmeyer HU, Ed. Weinheim, Germany, Verlagsgesellschaft, p. 370–379, 1986
34. Zhou YP, Ostenson CG, Ling ZC, Grill V: Deficiency of pyruvate dehydrogenase activity in pancreatic islets of diabetic GK rats. *Endocrinology* 136:3546–3551, 1995
35. Wang MY, Shimabukuro M, Lee Y, Trinh KY, Chen JL, Newgard CB, Unger RH: Adenovirus-mediated overexpression of uncoupling protein-2 in pancreatic islets of Zucker diabetic rats increases oxidative activity and improves beta-cell function. *Diabetes* 48:1020–1025, 1999
36. Kobayashi K, Neely JR: Effects of ischemia and reperfusion on pyruvate dehydrogenase activity in isolated rat hearts. *J Mol Cell Cardiol* 15:359–367, 1983
37. Zhou YP, Grill VE: Palmitate-induced beta-cell insensitivity to glucose is coupled to decreased pyruvate dehydrogenase activity and enhanced kinase activity in rat pancreatic islets. *Diabetes* 44:394–399, 1995
38. MacDonald MJ, Kaysen JH, Moran SM, Pomije CE: Pyruvate dehydrogenase and pyruvate carboxylase: sites of pretranslational regulation by glucose of glucose-induced insulin release in pancreatic islets. *J Biol Chem* 266:22392–22397, 1991
39. Zhou YP, Berggren PO, Grill V: A fatty acid-induced decrease in pyruvate dehydrogenase activity is an important determinant of beta-cell dysfunction in the obese diabetic db/db mouse. *Diabetes* 45:580–586, 1996
40. Randle PJ, Kerbey AL, Espinal J: Mechanism decreasing glucose oxidation in diabetes and starvation: role of lipid fuels hormones. *Diabetes Metab Rev* 4:623–638, 1988
41. Lameloise N, Muzzin P, Prentki M, Assimakopoulos-Jeannet F: Uncoupling protein 2: a possible link between fatty acid excess and impaired glucose-induced insulin secretion. *Diabetes* 50:803–809, 2001
42. Weir GC, Laybutt DR, Kaneto H, Bonner-Weir S, Sharma A: Beta-cell adaptation and decompensation during the progression of diabetes. *Diabetes* 50 (Suppl. 1):S154–S159, 2001
43. Roduit R, Morin J, Masse F, Segal L, Roche E, Newgard CB, Assimakopoulos-Jeannet F, Prentki M: Glucose down-regulates the expression of the peroxisome proliferator-activated receptor-alpha gene in the pancreatic beta-cell. *J Biol Chem* 275:35799–35806, 2000
44. Fajas L, Debril MB, Auwerx J: Peroxisome proliferator-activated receptor-gamma: from adipogenesis to carcinogenesis. *J Mol Endocrinol* 27:1–9, 2001
45. Debril MB, Renaud JP, Fajas L, Auwerx J: The pleiotropic functions of peroxisome proliferator-activated receptor gamma. *J Mol Med* 79:30–47, 2001
46. Zhang CY, Baffy G, Perret P, Krauss S, Peroni O, Grujic D, Hagen T, Vidal-Puig AJ, Boss O, Kim YB, Zheng XX, Wheeler MB, Shulman GI, Chan CB, Lowell BB: Uncoupling protein-2 negatively regulates insulin secretion and is a major link between obesity, beta cell dysfunction, and type 2 diabetes. *Cell* 105:745–755, 2001
47. Shimabukuro M, Zhou YT, Lee Y, Unger RH: Troglitazone lowers islet fat and restores beta cell function of Zucker diabetic fatty rats. *J Biol Chem* 273:3547–3550, 1998
48. Buckingham RE, Al-Barazani KA, Toseland CD, Slaughter M, Connor SC, West A, Bond B, Turner NC, Clapham JC: Peroxisome proliferator-activated receptor-gamma agonist, rosiglitazone, protects against nephropathy and pancreatic islet abnormalities in Zucker fatty rats. *Diabetes* 47:1326–1334, 1998
49. Laybutt DR, Groff A, Kaneto H, Sharma A, Weir GC: Hyperglycemia-induced loss of beta cell differentiation is associated with altered expression of genes involved in lipid metabolism (Abstract). *Diabetes* 49 (Suppl. 1):A263, 2000
50. Zhou YT, Shimabukuro M, Lee Y, Koyama K, Higa M, Ferguson T, Unger RH: Enhanced de novo lipogenesis in the leptin-unresponsive pancreatic islets of prediabetic Zucker diabetic fatty rats: role in the pathogenesis of lipotoxic diabetes. *Diabetes* 47:1904–1908, 1998