Adenovirus-Mediated Overexpression of Uncoupling Protein-2 in Pancreatic Islets of Zucker Diabetic Rats Increases Oxidative Activity and Improves β-Cell Function

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The discovery of uncoupling protein (UCP)-2, a ubiquitously expressed protein homologous to UCP-1, has raised the possibility that energy balance of cells might be regulated in tissues other than brown adipocytes. In normal pancreatic islets, UCP-2 is upregulated by leptin and is low in leptin-resistant islets of ZDF rats. To determine whether UCP-2 does, in fact, have uncoupling activity and, if so, whether such activity would favorably influence the abnormalities in leptin-unresponsive UCP-2–underexpressing islets of diabetic ZDF rats, we transferred the UCP-2 gene to the islets of diabetic ZDF rats and lean (+/+) ZDF control rats. Although ATP was reduced by 23% in both groups of islets, the ATP:ADP ratio increased by 42 and 141%, respectively. [^{3}H]palmitate oxidation was increased by 50%, and [^{3}H]glucose oxidation was 42–63% higher. Preproinsulin mRNA was 2.9-fold above control levels, and glucose-stimulated insulin secretion, which was negligible in control ZDF rat islets, was improved in UCP-2–overexpressing islets. The high fat content of the islets was not reduced, however. We conclude that UCP-2 has uncoupling function when overexpressed in leptin-insensitive islets and that its overexpression corrects the underexpression of the insulin gene and ameliorates glucose-stimulated insulin secretion, possibly by increasing the ATP:ADP ratio. ***Diabetes*** **48:1020–1025, 1999**

Recent evidence indicates that pancreatic islet cells have the capacity to store small amounts of fat (1). It is postulated that this fat is required for normal β-cell function, inasmuch as its depletion prevents insulin secretion in response to nutrient secretagogues (2,3). The level of intracellular fat appears to be controlled by leptin, which limits triglyceride (TG) accumulation by stimulating the oxidation of excess fatty acids (4). Because of a Gln 269 → Pro mutation in the extracellular domain of all leptin receptor OB-R isoforms (5,6), ZDF rats are completely insensitive to the lipopenic action of leptin. Because β-oxidation is the only avenue available to β-cells for reduction of intracellular fat content, the lack of leptin activity in their islets is reflected by an increase in intracellular islet fat ranging from 10 to 100 times the normal (1). When the wild-type OB-Rb is transgenically overexpressed in such islets, leptin sensitivity is restored and exposure to leptin reduces TG content to normal (1). These findings have led to the conclusion that intracellular TG homeostasis of islets (and perhaps other tissues) is regulated by leptin (7), and that loss of this regulatory function leads to β-cell dysfunction secondary to overaccumulation of fat or so-called lipotoxicity (8).

Leptin has been shown to upregulate expression of genes encoding enzymes of fatty acid oxidation, such as carnitine palmitoyl transferase (CPT)-1 and acyl CoA oxidase (ACO) (9). Because the leptin-induced increase in fatty acid oxidation is accompanied by weight loss that exceeds the weight loss in pair-fed controls (10), a thermogenic effect of the hormone has been proposed (11,12). This concept has been reinforced by demonstration that hyperleptinemia increases uncoupling protein (UCP)-2 mRNA in tissues of hyperleptinemic rats (9,13). It is therefore presumed that leptin action via its receptor, OB-R, upregulates both the machinery required to oxidize excess long-chain fatty acids and also the capacity to dissipate the energy thereby produced.

If this is correct, it seems likely that a UCP is an important component of this system that controls intracellular TG homeostasis. In normal cells, we envision the following sequence: a surfeit of free fatty acids (FFAs) upregulates UCP-2, increasing β-oxidation of fatty acids by reducing ATP levels. After oxidation of the excess of fatty acids, UCP-2 returns to its normal level, thus allowing coupled oxidation to resume. If this hypothesis is correct, overexpression of UCP-2 in pancreatic islets of OB-R–defective ZDF rats should increase the rate of oxidative metabolism without increasing ATP levels. The following study was designed to test this hypothesis.

RESEARCH DESIGN AND METHODS

Experimental animals. Homozygous (*fa/fa*) ZDF rats were bred in our laboratory from ZDF/drt-*fa* (F10) rats purchased from R. Peterson (University of Indi-

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ACO, acyl CoA oxidase; Ad, adenovirus; β-gal, β-galactosidase; CMV, cytomegalovirus; CPT, carnitine palmitoyl transferase; FFA, free fatty acid; K_{ATP}, ATP-sensitive K^+ channel; KRBB, Krebs-Ringer bicarbonate buffer; PBS, phosphate-buffered saline; PCR, polymerase chain reaction; RT, reverse transcriptase; TG, triglyceride; UCP, uncoupling protein.

ana School of Medicine, Indianapolis, IN). We used 10-week-old male ZDF (*fa/fa*) rats for the experiments. Rats were on standard food (Harlan/Teklad 4% 7001; Madison, WI) ad libitum and had free access to water. Before each experiment a blood sample from the tail vein was collected in capillary tubes coated with EDTA. Plasma glucose was measured by the glucose oxidase method using the glucose analyzer II (Beckman, Brea, CA). Plasma glucose levels >200 mg/dl were considered diagnostic of diabetes. All animals used in this study had diabetes, with plasma glucose levels ranging from 352 to 438 mg/dl.

Overexpression of UCP-2 in fat-laden islets. A cDNA encoding the full-length human UCP-2 protein (927 bp encoding 309 amino acids) was ligated as a *Hind* III/*Bam* HI fragment into similarly restricted pACCMV.pLpA (14) and used to prepare a recombinant adenovirus, AdCMV-UCP-2, using previously described methods (15). To facilitate gene delivery to islets of obese and diabetic rats, pancreases of 10-week-old male ZDF (*fa/fa*) rats were perfused with 1×10^{12} plaque-forming units (pfu) of AdCMV-UCP-2 or, as a control, the same amount of virus containing the bacterial β-galactosidase gene, AdCMV-β-gal (16), in Krebs-Ringer bicarbonate buffer (KRBB) with 4.5% dextran T70, 1% bovine serum albumin, 5.6 mmol/l glucose, and 5 mmol/l each of sodium pyruvate, sodium glutamate, and sodium fumarate. Pancreatic islets were then isolated and maintained for 3 days in suspension culture in 60-mm petri dishes at 37°C in a humidified atmosphere of 5% CO_2 and 95% air, as described previously (17). The culture medium consisted of RPMI 1640 supplemented with 10% fetal bovine serum, 1% penicillin and streptomycin, and 8 mmol/l glucose.

Immunoblotting. For immunoblotting, ~100 islets were isolated from ZDF (*fa/fa*) rat pancreases that had been perfused with AdCMV-UCP-2 or AdCMV-β-gal and cultured for 3 days. Total islet protein was directly denatured by boiling in SDS-PAGE sample buffer, followed by resolution with 10% SDS-PAGE. After electrophoresis, the protein was transferred onto polyvinylidene difluoride membrane (Millipore, Bedford, MA). Membranes were then treated with rabbit anti-UCP-2 antibody obtained from Alpha Diagnostic International (San Antonio, TX). After washing the blots, bound primary antibody was detected by reaction with anti-rabbit immunoglobulin G antibody-peroxidase conjugate. Antibody complexes were visualized using a Super Signal-CL Kit (Pierce, Rockford, IL), as described elsewhere (18).

Reverse transcriptase–polymerase chain reaction. Total RNA from 100–200 islets perfused with either AdCMV-UCP-2 or AdCMV-β-gal was extracted using TRIzol Reagent (Life Technologies, Gaithersburg, MD). After treating with deoxyribonuclease I (Life Technologies), first-strand cDNA was prepared with 1 µg of RNA mixed with 200 U of reverse transcriptase (RT) (Clontech, Palo Alto, CA). First-strand cDNA was polymerase chain reaction (PCR)-amplified by using specific oligonucleotide pairs for UCP-2 (5′-AACAGTTCTACACCAAGGGC and 5′-AGCATGTAAGGGCACAGTG) and preproinsulin (5′-TGCCCGGGCTTTTGTCAAAC and 5′-CTCCAGTGCCAA GGTCTGAA). The conditions of PCR were as follows: denaturation for 45 s at 92°C, 45 s at 55°C, and elongation for 1 min at 72°C with either 30 cycles (UCP-2) or 22 cycles (preproinsulin). PCR products were subjected to electrophoresis on 1.2% agarose gel and were quantified by Southern blot analyses by means of gene-specific ^{32}P-labeled probes for UCP-2 (5′-GTCATCTGTCATGAGGTTGGCTTTCAGGAG) or preproinsulin (5′-ACACACCAGGTACAGAGCCTCCACCAGGTG). As a control for RNA quality and quantity, β-actin mRNA was amplified from all samples using oligonucleotides as described previously (19).

Perifusion of cultured islets. Islets isolated from ZDF (*fa/fa*) rat pancreases that had been perfused with AdCMV-UCP-2 or AdCMV-β-gal were cultured for 3 days and used for perifusion experiments. Islets were collected under a stereoscopic microscope, washed twice with KRBB-HEPES (pH 7.4, 3 mmol/l glucose), and loaded into a 13-mm chamber containing an 8-µm nylon membrane filter (Millipore). Islets were perifused with buffer containing either 3 mmol/l glucose as basal concentration, with 15-min periods of 23 mmol/l glucose, or 20 mmol/l arginine. Flow rate was 0.8 ml/min (17). Effluent fractions were collected at 2-min intervals and stored at –20°C before insulin assay. Immunoreactive insulin was determined by radioimmunoassay using charcoal separation as described previously (20).

TG content of islets. Islets isolated from ZDF (*fa/fa*) rat pancreases that had been perfused with AdCMV-UCP-2 or AdCMV-β-gal were cultured for 3 days and used for measurements of TG content. Groups of 100 islets were washed twice with phosphate-buffered saline (PBS) (pH 7.4) and sonicated in a high-salt buffer (2 mol/l NaCl, 2 mmol/l EDTA, and 50 mmol/l sodium phosphate buffer, pH 7.4). The homogenate was aliquoted, and its TG concentration was then measured by using a Triglyceride (GPO-Trinder) Kit (Sigma, St. Louis, MO) as described previously (1).

Measurements of glucose usage, glucose oxidation, lactate formation, and glycogen levels. For studies of glucose usage, 100 islets were selected at random and washed twice with Hanks' buffer. Reactions were started by adding islets to Hanks' buffer containing either 8 or 23 mmol/l glucose plus [5-^{3}H]glucose and incubated for 1 h at room temperature. The glucose usage by the islets was determined by measuring the production of ^3H_2O in reaction solution as described previously (17).

For studies of oxidation, triplicate aliquots of 100 islets each were used. Islets suspended in 100 µl of KRBB-HEPES were added to the incubation medium, which contained either 8 or 23 mmol/l [U-^{14}C]glucose in KRBB-HEPES. This was sealed into a 20-ml liquid scintillation vial (previously gassed with 95% O_2, 5% CO_2) and shaken for 15 min or 2 days (100 oscillations/min) in a 37°C water bath. Islet metabolism was terminated by injection of 0.1 ml of 6% (wt/vol) $HClO_4$ into the cup, after which 0.3 ml of benzethonium hydroxide (1.0 mol/l) was injected into the vial to collect $^{14}CO_2$. After a further 3 h of shaking, the cup was removed and the ^{14}C content of the vial was measured by liquid scintillation counting. Control incubations lacking islets were run with each series.

Medium was collected from the foregoing experiments for measurement of [^{14}C]lactate and $^{14}CO_2$, using the methods of Goodwin et al. (21). Glycogen levels in islets were measured by the method of Newgard et al. (22).

Measurements of 9,10-[^{3}H]palmitate oxidation by islets. Oxidation and esterification of palmitic acid in islets were determined as described previously (1). Briefly, groups of 100 islets isolated from ZDF (*fa/fa*) rat pancreases that had been perfused in advance with AdCMV-UCP-2 or AdCMV-β-gal were incubated in a culture medium containing 1 mmol/l of palmitic acid and 9,10-[^{3}H]palmitate for 2 days at 37°C. As an index for palmitate oxidation, the amount of radioactive ^3H_2O released in the culture medium was measured.

Measurement of total ATP and ADP contents. For studies of ATP and ADP, 3-day cultures of islets isolated from ZDF (*fa/fa*) rat pancreases pre-perfused with either AdCMV-UCP2 or AdCMV-β-gal were washed twice with ice-cold PBS (pH 7.4) and homogenized in ice-cold PBS on ice with a motorized pestle homogenizer. An equal volume of ice-cold trichloroacetic acid (12%) was then added to precipitate cellular proteins. Homogenates were clarified by centrifugation at 4°C for 5 min at 5,000 rpm. Through the use of a diagnostic kit from Sigma, the total amount of ATP in the protein-free supernatant was measured by quantifying the consumption of NADH in the phosphoglycerate kinase–glyceraldehyde phosphate dehydrogenase coupled reaction in which ADP and 3-phosphoglycerate were used as substrates (23,24). For ADP assays, one-half portion of the supernatant was incubated with phosphoenolpyruvate as well as pyruvate kinase to convert ADP into ATP, as described earlier (25). After the reactions, total resulting ATP was measured as described above. The other portion of supernatant was directly used for assays of ATP originally present in the supernatant. The ADP content was calculated by difference.

RESULTS

Effect of AdCMV-UCP-2 perfusion on UCP-2 expression. To determine whether AdCMV-UCP-2 perfusion in ZDF rat islets increased the level of UCP-2 expression, we semiquantitated UCP-2 mRNA by RT-PCR. In three separate experiments, the average UCP-2 mRNA level was 20 times that of islets that had been perfused with AdCMV-β-gal, indicating successful transfer and expression of the gene. A representative blot is shown in Fig. 1*A*. In three immunoblots, there was a fivefold increase in UCP-2 protein, evidence that its mRNA was being translated. A representative immunoblot is shown in Fig. 1*B*.

Effect of UCP-2 overexpression on ATP and ADP in islets. Although it is assumed that UCP-2 is a UCP because of its 59% amino acid identity to UCP-1, this has not yet been established directly. Based on the assumption that overexpression of a UCP should reduce ATP levels, we compared ATP in AdCMV-UCP-2– and AdCMV-β-gal–perfused islets of both *fa/fa* and +/+ islets. There was a 25% reduction of ATP in the UCP-2–overexpressing islets from each group (Table 1). However, ADP was reduced to an even greater degree, particularly in +/+ islets, raising the ATP:ADP ratio by 42% in *fa/fa* rat islets and 141% in +/+ rat islets.

Effect of UCP-2 overexpression on palmitate and glucose metabolism. Normally, oxidative metabolism raises ATP, which feeds back negatively to reduce the rate of oxidative metabolism. Consequently, we reasoned that if overexpression of UCP-2 actually uncoupled oxidation from ATP generation, the oxidative metabolism of glucose and long-chain fatty acids would be increased in islets deprived of this negative feedback. We therefore compared [^{3}H]palmitate oxidation, [^{3}H]glucose usage, glucose oxidation, and lactate production over a 2-day period in islets perfused with AdCMV-UCP-2 or AdCMV-β-gal. As seen in Fig. 2*A*, there was a 50% increase in oxidation of 1 mmol/l palmitate ($P < 0.01$)

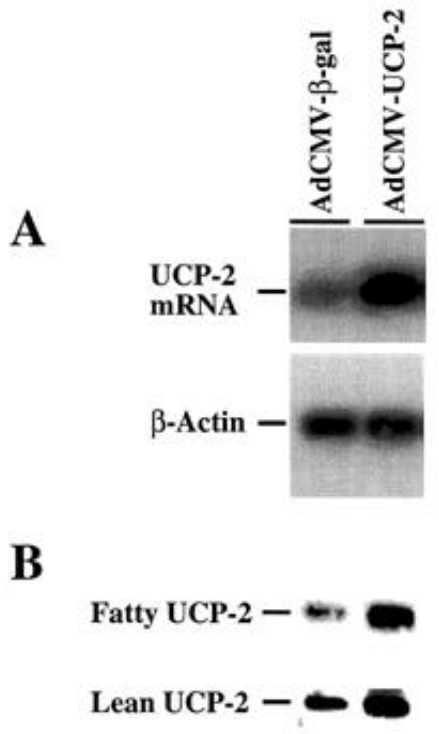

FIG. 1. Overexpression of UCP-2 in the islets of obese ZDF (*fa/fa*) rats. *A*: UCP-2 mRNA in ZDF rat islets cultured for 3 days immediately after perfusion with recombinant adenovirus carrying either AdCMV-UCP-2 or AdCMV-β-gal (*Escherichia coli lacZ* gene). UCP-2 transcripts were amplified by RT-PCR. *B*: Immunoblotting of total lysates from the foregoing cultured islets of fatty rats and islets of normal lean (+/+) ZDF rats with a polyclonal antibody to the COOH-terminal domain of UCP-2.

and a 63% increase in glucose oxidation at 8 mmol/l ($P <$ 0.01) and 42% at 23 mmol/l ($P < 0.01$) (Fig. 2*B* and *C*). Glucose usage at 8 mmol/l glucose was 44% higher in UCP-2–overexpressing islets, and at 23 mmol/l it was 76% higher. Lactate production (Fig. 2*D*) was significantly different at the 8 mmol/l, but not at the 23 mmol/l, glucose concentration. Glycogen content was unchanged (Fig. 2*E*).

Effect of UCP-2 overexpression on preproinsulin gene expression. An inability of β-cells to increase insulin gene expression in response to an increased demand for insulin secretion coincides with β-cell decompensation and the onset of hyperglycemia (26). To determine whether UCP-2 overexpression would improve insulin gene expression, we compared preproinsulin mRNA in islets isolated from pancreases perfused with AdCMV-UCP-2 or AdCMV-β-gal. Preproinsulin mRNA was 2.9-fold greater in UCP-2–overexpressing islets (Fig. 3*A*).

Effect of UCP-2 overexpression on β-cell function. The insulin response to glucose is absent in diabetic ZDF rats (27). To determine the effect of UCP-2 overexpression on β-cell dysfunction, we perifused cultured islets from pancreases that had been perfused earlier with AdCMV-UCP-2 or AdCMV-β-gal and determined their insulin responses to 23 mmol/l glucose and 20 mmol/l L-arginine. The glucose-stimulated insulin increment was negligible in AdCMV-β-gal–perfused control islets, while in the UCP-2–overexpressing islets, it was ~70% above the baseline (Fig. 3*B*). Arginine-stimulated insulin secretion, which is not reduced in diabetic ZDF rat islets (27), was the same in both groups (Fig. 3*B*).

TABLE 1
Effects of UCP-2 overexpression on the steady-state levels of total intracellular ATP and ADP in the islets of ZDF (*fa/fa*) and (+/+) rats

	ATP (nmol/50 islets)	ADP (nmol/50 islets)	ATP:ADP ratio
Zucker fatty (*fa/fa*)			
AdCMV-β-gal	5.240 ± 0.665	3.709 ± 0.233	1.413 ± 0.117
AdCMV-UCP-2	4.080 ± 0.475*	2.050 ± 0.125*	2.001 ± 0.213*
Zucker lean (+/+)			
AdCMV-β-gal	2.749 ± 0.118	3.199 ± 0.056	0.859 ± 0.174
AdCMV-UCP-2	2.105 ± 0.137*	1.015 ± 0.095*	2.074 ± 0.232*

Data are means ± SE. Experiments were performed with 10 different pools of islets isolated from eight pancreases perfused (for those from Zucker fatty rats) or incubated (for those from lean rats) with either AdCMV-UCP-2 or AdCMV-β-gal. *$P < 0.01$ vs. AdCMV-β-gal.

Effect of UCP-2 overexpression on islet TG content. To determine whether the increase in palmitate oxidation was sufficient to reduce the elevated TG content of the ZDF rat islets, we compared the fat content of UCP-2–overexpressing islets with AdCMV-β-gal–perfused control islets. The fat content was 368.5 ± 24 and 348 ± 16 ng/islet, respectively (NS).

DISCUSSION

The discovery of proteins homologous with UCP-1 (28) has filled an important theoretical gap in our understanding of energy balance by raising the possibility that it is controlled by UCPs expressed in cells other than brown adipocytes. The demonstration that chronic hyperleptinemia upregulates UCP-2 mRNA in white adipocytes of normal rats implied that the adipocyte hormone regulates a novel intracellular mechanism for preventing overaccumulation of fat (4); in rats made lipopenic by adenoviral transfer of the leptin gene, body fat disappeared within 7 days (10) without any increase in plasma FFAs or β-hydroxybutyric acid and without ketonuria (4). A marked increase in adipocytes of the mRNA encoding the enzymes of FFA oxidation, CPT-1 and ACO (9), suggested that the FFAs had been oxidized in situ, i.e., without exiting the adipocytes, while the rapid aketonuric weight loss in excess of pair-fed controls implied dissipation of the energy generated by the β-oxidation. The leptin-induced elevation of UCP-2 mRNA, in concert with the upregulation of mRNA of CPT-1 and ACO in adipocytes and in pancreatic islets (9), was consistent with this hypothesis.

With leptin now imputed to regulate intracellular fat homeostasis (29), the fat overload of islets of obese ZDF (1) rats could be attributed to a mutated leptin receptor OB-R (5,6). This genotype is associated with excess islet fat due to underexpression of islet CPT-1, ACO, and UCP-2 and overexpression of lipogenic enzymes, acetyl CoA carboxylase, fatty acid synthase (30), fatty acetyl CoA synthetase, and glycerophosphate-acyl transferase (9). If OB-Rb is overexpressed in ZDF rat islets, the fat content of the islets is reduced to normal by leptin treatment (31) and the other phenotypic abnormalities of diabetic β-cells are concomitantly repaired (32).

In this study, we overexpressed UCP-2 in ZDF rat islets. UCP-2 may be the most distal protein in the putative leptin-regulated pathway of intracellular fat homeostasis. Even

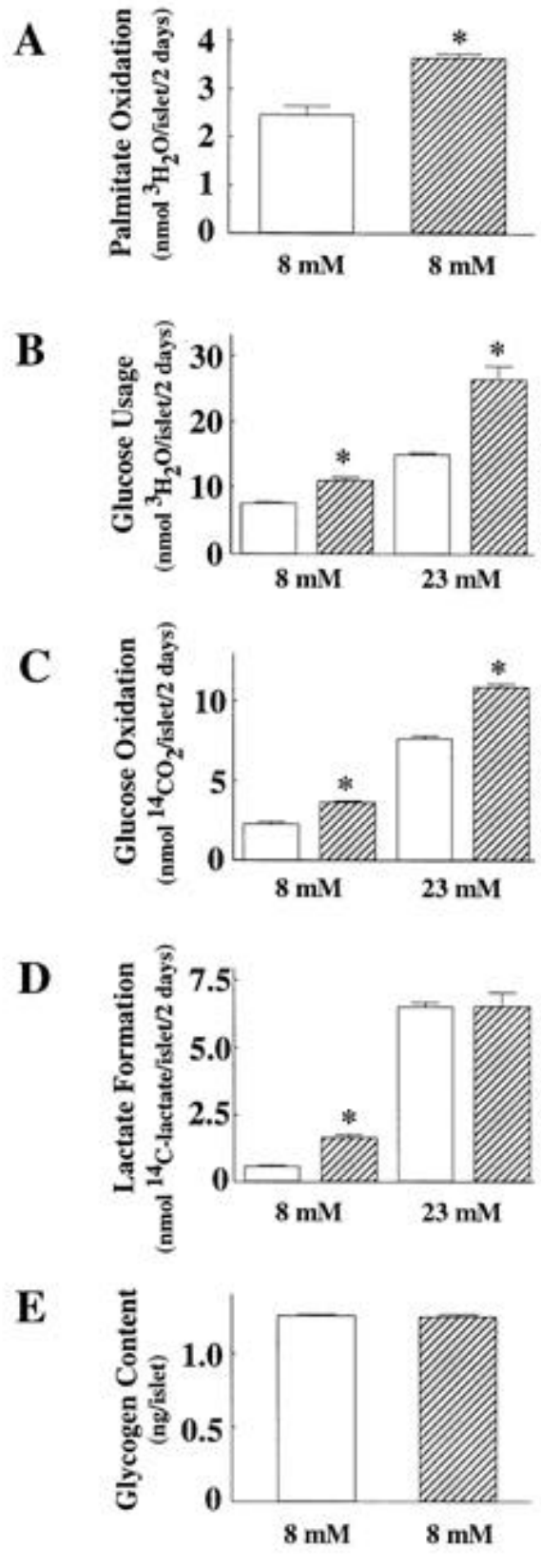

FIG. 2. Effects of UCP-2 overexpression on [^{3}H]palmitate oxidation (*A*), [^{3}H]glucose usage (*B*), [U-^{14}C]glucose oxidation (*C*), [^{14}C]lactate formation in the islets of ZDF (*fa/fa*) rats (*D*), and glycogen content (*E*). Islets had been perfused with AdCMV-UCP-2 (▨) or AdCMV-β-gal (□), isolated, and cultured as described in Fig. 1. Bars represent means ± SE of six to seven experiments. **P* < 0.01 vs. AdCMV-β-gal values.

though no attempt was made to upregulate the enzymes of FFA and glucose oxidation, when measured over a 2-day period, oxidation of [^{3}H]palmitate rose 50% above β-gal control levels, while oxidation of 8 and 23 mmol/l glucose increased by 63 and 42%, respectively (both $P < 0.01$). At the 8 mmol/l glucose concentration, lactate production was significantly higher in islets with UCP-2 overexpression, but at 23 mmol/l glucose, there was no difference. Islet fat was not reduced during the 2 days of culture; given the relatively modest increase in the rate of palmitate oxidation, this is hardly surprising.

Nevertheless, there was dramatic improvement in insulin gene expression and in glucose-stimulated insulin secretion, a function that is lost in ZDF rats coincident with the onset of hyperglycemia. This improvement was unexpected, given the apparent link between fat content and β-cell function and the lack of measurable reduction in islet fat.

While the mechanism of restored glucose-stimulated insulin secretion cannot be deduced from these studies, the results clearly demonstrate that UCP-2 overexpression enhances glucose utilization over a 2-day period, although no such enhancement was detectable in a 15-min experiment performed because of the improvement in first-phase insulin secretion (data not shown). Perhaps, by increasing glucose metabolism by reducing the ATP levels, UCP-2 overexpression lowered the level of certain metabolic products of glucose that are otherwise high in islets of diabetic ZDF rats (Y.L., unpublished observations); these products may reduce β-cell sensitivity to acute changes in extracellular glucose, a concept first proposed by Malaisse and Sener (33).

It is of interest that improved glucose-stimulated insulin secretion and insulin gene expression is achieved in ZDF rat islets despite a net decrease in intracellular ATP levels. The fall in ATP levels observed in the current study may be explained by the uncoupling of oxidative metabolism and ATP production. However, the ATP:ADP ratio rose substantially, consistent with a commonly accepted model of glucose signaling in which a rise in ATP:ADP ratio induced by glucose is thought to trigger insulin secretion via inhibition of ATP-sensitive K^+ channels (K_{ATP}) and subsequent activation of voltage-gated Ca^{2+} channels (34–38). In addition, while expression of UCP-2 could decrease mitochondrial ATP production, it should not affect ATP production from glycolysis (nor ADP consumption). Because there is growing evidence that the pool of ATP produced by glycolytic flux rather than oxidation of fuels may be the critical acute signal for insulin secretion (39,40), the increase in glucose usage induced by UCP-2 overexpression may explain both the increase in the ATP:ADP ratio and the improved glucose sensing in ZDF rat islets. This model may also help to explain the apparent discrepancy between the results of the current study and previous work using chemical uncouplers of ATP synthesis, which have been shown to block glucose-stimulated insulin secretion (41). With regard to this point, it is also important to note that our work focused on islets from ZDF rats, while previous studies with chemical uncouplers were performed in normal islets. Note that both the ATP level and the ATP:ADP ratio are approximately doubled in ZDF rat islets compared with lean control rat islets (Table 1). Thus, the high rates of insulin secretion under basal conditions in ZDF rat islets (27) (Fig. 3) and the loss of glucose sensing may be explained by the chronically elevated ATP:ADP ratio and attendant constitutive

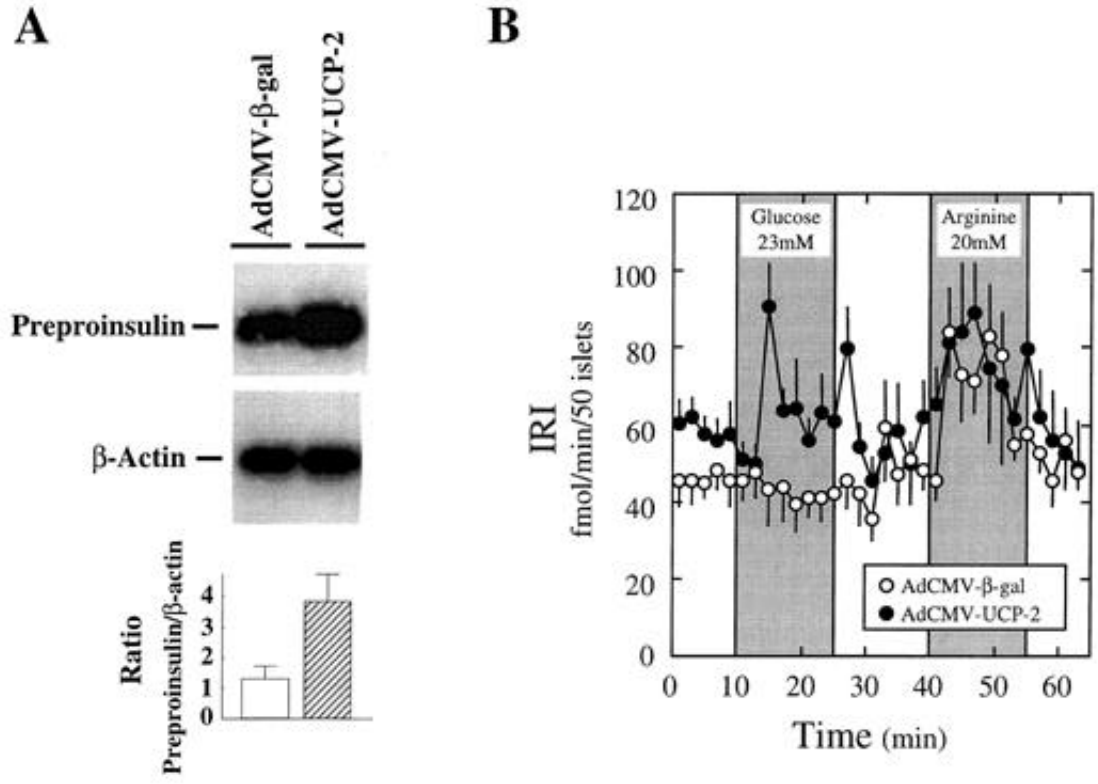

FIG. 3. Effects of perfusion with AdCMV-UCP-2 or AdCMV-β-gal on the levels of preproinsulin mRNA semiquantitated by RT-PCR and normalized for β-actin mRNA (*A*) and the glucose and arginine-stimulated insulin secretion during perifusion (*B*). Error bars indicate SDs (n = 5).

inhibition of the K_{ATP} channel complex. By lowering ATP levels in ZDF rat islets via UCP-2 expression, we may have eliminated chronic inhibition of K_{ATP} channels, allowing changes in the ATP:ADP ratio caused by glycolytic flux to exert a regulatory effect.

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