Uncoupling Protein 2 Knockout Mice Have Enhanced Insulin Secretory Capacity After a High-Fat Diet

Jamie W. Joseph, Vasiliy Koshkin, Chen-Yu Zhang, Jing Wang, Bradford B. Lowell, Catherine B. Chan, and Michael B. Wheeler

Uncoupling protein 2 (UCP2) may act as an important regulator of insulin secretion. In this study, β-cell function in UCP2-deficient mice was examined after a 45% high-fat diet (HFD) to assess its role during the development of diet-induced type 2 diabetes. HFD-fed UCP2 (-/-) mice have lower fasting blood glucose and elevated insulin levels when compared with wild-type (WT) mice. UCP2 (-/-) mice also have enhanced β-cell glucose sensitivity compared with WT mice after HFD, a result that is due in part to the deterioration of glucose responsiveness in WT mice. HFD-fed UCP2 (-/-) mice have increased insulin secretory capacity as a result of increased pancreatic β-cell mass and insulin content per islet. Islets from WT mice exposed to 0.5 mmol/l palmitate for 48 h have significantly reduced mitochondrial membrane potential, ATP concentrations, and glucose responsiveness compared with UCP2 (-/-) islets, suggesting that elevated UCP2 in WT mice increases proton leak and decreases mitochondrial ATP production. Highly increased carnitine palmitoyl transferase-1 gene expression in UCP2 (-/-) mice is suggestive of enhanced fatty acid oxidizing capacity, particularly after HFD stress. These results further establish UCP2 as a component in glucose sensing and suggest a possible new aspect of UCP2 function during the progression of type 2 diabetes. Diabetes 51:3211–3219, 2002

Oxidative metabolism in the β-cell produces NADH and FADH₂, which donate electrons to the electron transport chain, leading to the generation of a proton-motive force that drives ATP production catalyzed by ATP synthase. The production of mitochondrial ATP is critical for glucose-stimulated insulin secretion (GSIS) (1,2). Given the importance of oxidative metabolism in β-cell glucose sensing and insulin secretion, it is important to identify the key proteins that regulate mitochondrial ATP production. Uncoupling proteins (UCPs) (3,4), of which UCP2 is the only member found in islets thus far (5), are thought to fulfill such a role. These proteins are localized to the inner mitochondrial membrane and have a high degree of homology (3,4).

The functions of UCP2 in any tissue, including islets, have not been established. Data from several laboratories support a role for UCP2 as a “typical” uncoupler (4,6,7) that modulates the efficiency of ATP production (3,4). We hypothesized that by regulating β-cell energy levels, insulin secretion would be enhanced in the absence of UCP2 (8), a concept verified by the demonstration of enhanced insulin secretion in UCP2 knockout (-/-) mice. The induction of UCP2 by high-fat diet (HFD) (9) or free fatty acid (FFA) exposure (10) may contribute to so-called lipotoxicity, wherein GSIS is suppressed. Alternatively, induction of UCP2 may be part of a cellular lipid detoxification effort (11), along with fat-metabolizing enzymes such as carnitine palmitoyl transferase-1 (CPT-1). A third possibility is that UCP2, by dissipating the mitochondrial proton-motive force, decreases reactive oxygen species (ROS), thereby promoting cell survival under a stress such as HFD. The latter is supported by data from the UCP2 knockout mouse, which has elevated ROS formation when exposed to a pathogen (12). Although each outcome is theoretically plausible, the actual contribution of UCP2 to islet dysfunction after HFD is unknown. The UCP2 (-/-) mouse provides a unique model for investigating these possibilities. The present data demonstrate that HFD UCP2 (-/-) mice maintain superior pancreatic glucose responsiveness over wild-type (WT) mice in vivo and in situ at least partly caused by an expanded β-cell secretory capacity.

RESEARCH DESIGN AND METHODS

Animals. Male and female UCP2 (WT) or (-/-) mice were used in the present study and were bred from lines generated previously, as described by us (8). At 4 months of age, half of the mice were placed on either a control diet (Rodent Diet #8664; Harlan Teklad, Madison, WI) or an HFD (HD12451, Research Diets, New Brunswick, NJ). The fat source was lard and composed 45% of the total calories. The mice remained on the HFD or control diet for 4.5 months.

Blood and plasma measurements. Blood glucose levels were measured using a Lifescan Elite glucose meter (Lifescan, Toronto, ON, Canada). Plasma insulin levels were determined using a rat insulin enzyme-linked immunosorbent assay kit (Crystal Chem, Chicago, IL). Plasma glucagon levels were measured by radioimmunoassay (Linco, St. Charles, MO). Plasma FFAs were measured using a NEFA C FFA kit (Wako Chemicals USA, Richmond, VA). Plasma triglycerides were measured using a BioScaner 2000 Triglyceride system (Polymer Technologies Systems, Indianapolis, IN). Intraperitoneal glucose tolerance tests (IPGTTs) and insulin tolerance tests (ITTs) were performed as previously described (8). The area under the curve (AUC) analysis represents the AUC independent of basal values.
Pancratic islet measurements. Pancreatic islets were isolated as previ-
ously described (8). RNA was extracted from cultured islets using TRIzol
reagent (Gibco BRL) following the manufacturer’s instructions. Pancreatic
insulin and glucagon contents were determined as previously described
(13,14).

Immunocytochemistry. Pancreatic tissues were fixed in Bouin’s fixative (pH 7.2)
for 45 min and embedded in paraaffin. Sections were prepared at three
levels of the pancreas separated by 100 μm. For insulin staining, the primary
antibody was guinea pig anti-insulin (1:1,000; RA Pederson, Vancouver, BC,
Canada) and the secondary was rabbit anti-guinea pig peroxidase (1:100;
antibody was guinea pig anti-insulin (1:1,000; RA Pederson, Vancouver, BC,
Canada) and the secondary was rabbit anti-guinea pig peroxidase (1:100;
antibody was guinea pig anti-insulin (1:1,000; RA Pederson, Vancouver, BC,
Canada) and the secondary was rabbit anti-guinea pig peroxidase (1:100;
antibody was guinea pig anti-insulin (1:1,000; RA Pederson, Vancouver, BC,
Canada) and the secondary was rabbit anti-guinea pig peroxidase (1:100;
antibody was guinea pig anti-insulin (1:1,000; RA Pederson, Vancouver, BC,
Canada) and the secondary was rabbit anti-guinea pig peroxidase (1:100;
antibody was guinea pig anti-insulin (1:1,000; RA Pederson, Vancouver, BC,
Canada) and the secondary was rabbit anti-guinea pig peroxidase (1:100;
antibody was guinea pig anti-insulin (1:1,000; RA Pederson, Vancouver, BC,
Canada) and the secondary was rabbit anti-guinea pig peroxidase (1:100;
antibody was guinea pig anti-insulin (1:1,000; RA Pederson, Vancouver, BC,
Canada) and the secondary was rabbit anti-guinea pig peroxidase (1:100;
antibody was guinea pig anti-insulin (1:1,000; RA Pederson, Vancouver, BC,
Canada) and the secondary was rabbit anti-guinea pig peroxidase (1:100;
antibody was guinea pig anti-insulin (1:1,000; RA Pederson, Vancouver, BC,
Canada) and the secondary was rabbit anti-guinea pig peroxidase (1:100;
antibody was guinea pig anti-insulin (1:1,000; RA Pederson, Vancouver, BC,
Canada) and the secondary was rabbit anti-guinea pig peroxidase (1:100;
antibody was guinea pig anti-insulin (1:1,000; RA Pederson, Vancouver, BC,
Canada) and the secondary was rabbit anti-guinea pig peroxidase (1:100;
antibody was guinea pig anti-insulin (1:1,000; RA Pederson, Vancouver, BC,
Canada) and the secondary was rabbit anti-guinea pig peroxidase (1:100;
antibody was guinea pig anti-insulin (1:1,000; RA Pederson, Vancouver, BC,
Canada) and the secondary was rabbit anti-guinea pig peroxidase (1:100;
antibody was guinea pig anti-insulin (1:1,000; RA Pederson, Vancouver, BC,
Canada) and the secondary was rabbit anti-guinea pig peroxidase (1:100;
antibody was guinea pig anti-insulin (1:1,000; RA Pederson, Vancouver, BC,
Canada) and the secondary was rabbit anti-guinea pig peroxidase (1:100;
antibody was guinea pig anti-insulin (1:1,000; RA Pederson, Vancouver, BC,
Canada) and the secondary was rabbit anti-guinea pig peroxidase (1:100;
antibody was guinea pig anti-insulin (1:1,000; RA Pederson, Vancouver, BC,
Canada) and the secondary was rabbit anti-guinea pig peroxidase (1:100;
antibody was guinea pig anti-insulin (1:1,000; RA Pederson, Vancouver, BC,
Canada) and the secondary was rabbit anti-guinea pig peroxidase (1:100;
antibody was guinea pig anti-insulin (1:1,000; RA Pederson, Vancouver, BC,
Canada) and the secondary was rabbit anti-guinea pig peroxidase (1:100;
antibody was guinea pig anti-insulin (1:1,000; RA Pederson, Vancouver, BC,
Canada) and the secondary was rabbit anti-guinea pig peroxidase (1:100;
antibody was guinea pig anti-insulin (1:1,000; RA Pederson, Vancouver, BC,
Canada) and the secondary was rabbit anti-guinea pig peroxidase (1:100;
antibody was guinea pig anti-insulin (1:1,000; RA Pederson, Vancouver, BC,
Canada) and the secondary was rabbit anti-guinea pig peroxidase (1:100;
antibody was guinea pig anti-insulin (1:1,000; RA Pederson, Vancouver, BC,
Canada) and the secondary was rabbit anti-guinea pig peroxidase (1:100;
antibody was guinea pig anti-insulin (1:1,000; RA Pederson, Vancouver, BC,
Canada) and the secondary was rabbit anti-guinea pig peroxidase (1:100;
antibody was guinea pig anti-insulin (1:1,000; RA Pederson, Vancouver, BC,
Canada) and the secondary was rabbit anti-guinea pig peroxidase (1:100;
antibody was guinea pig anti-insulin (1:1,000; RA Pederson, Vancouver, BC,
Canada) and the secondary was rabbit anti-guinea pig peroxidase (1:100;
antibody was guinea pig anti-insulin (1:1,000; RA Pederson, Vancouver, BC,
Canada) and the secondary was rabbit anti-guinea pig peroxidase (1:100;
antibody was guinea pig anti-insulin (1:1,000; RA Pederson, Vancouver, BC,
Canada) and the secondary was rabbit anti-guinea pig peroxidase (1:100;
antibody was guinea pig anti-insulin (1:1,000; RA Pederson, Vancouver, BC,
Canada) and the secondary was rabbit anti-guinea pig peroxidase (1:100;
antibody was guinea pig anti-insulin (1:1,000; RA Pederson, Vancouver, BC,
Canada) and the secondary was rabbit anti-guinea pig peroxidase (1:100;
antibody was guinea pig anti-insulin (1:1,000; RA Pederson, Vancouver, BC,
Canada) and the secondary was rabbit anti-guinea pig peroxidase (1:100;
antibody was guinea pig anti-insulin (1:1,000; RA Pederson, Vancouver, BC,
Canada) and the secondary was rabbit anti-guinea pig peroxidase (1:100;
antibody was guinea pig anti-insulin (1:1,000; RA Pederson, Vancouver, BC,
Canada) and the secondary was rabbit anti-guinea pig peroxidase (1:100;
reported (8) (Fig. 1C). HFD fasting plasma insulin concentrations rose significantly in both WT \( (P < 0.001) \) and UCP2 \((-/-)\) mice \( (P < 0.01) \) compared with the control diet–fed animals. However, the increase in insulin secretion was significantly \( (P < 0.01) \) higher in HFD UCP2 \((-/-)\) mice compared with HFD WT mice (Fig. 1C).

Similar results were found with fed insulin values (Fig. 1D).

Control diet fasting plasma FFAs were not different between UCP2 \((-/-)\) mice and WT mice. HFD WT mice had significantly \( (P < 0.05) \) higher fasting FFA concentrations than their control diet counterparts; however, UCP2

---

### TABLE 2

<table>
<thead>
<tr>
<th>Treatment</th>
<th>WT</th>
<th>UCP2 ((-/-))</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female body weight (g) ( (n = 25) )</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control diet</td>
<td>20.8 ± 0.4</td>
<td>19.9 ± 0.4</td>
<td>NS</td>
</tr>
<tr>
<td>HFD</td>
<td>23.0 ± 0.5*</td>
<td>23.5 ± 0.6*</td>
<td>NS</td>
</tr>
<tr>
<td>Female net weight gain (g)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control to HFD</td>
<td>2.5 ± 0.5</td>
<td>3.7 ± 0.6</td>
<td>( P &lt; 0.05 )</td>
</tr>
<tr>
<td>Male body weight (g) ( (n = 25) )</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control diet</td>
<td>24.7 ± 0.6</td>
<td>25.8 ± 0.6</td>
<td>NS</td>
</tr>
<tr>
<td>HFD</td>
<td>34.7 ± 1.4†</td>
<td>38.5 ± 1.6†</td>
<td>NS</td>
</tr>
<tr>
<td>Male net weight gain (g)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control to HFD</td>
<td>9.8 ± 1.1</td>
<td>12.7 ± 1.5</td>
<td>( P &lt; 0.05 )</td>
</tr>
<tr>
<td>Fasting plasma FFA ( (mEq/l) (n = 20) )</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control diet</td>
<td>1.80 ± 0.19</td>
<td>1.74 ± 0.20</td>
<td>NS</td>
</tr>
<tr>
<td>HFD</td>
<td>2.15 ± 0.17*</td>
<td>1.73 ± 0.11</td>
<td>( P &lt; 0.05 )</td>
</tr>
<tr>
<td>Fed plasma FFA ( (mEq/l) (n = 20) )</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control diet</td>
<td>0.82 ± 0.05</td>
<td>0.88 ± 0.04</td>
<td>NS</td>
</tr>
<tr>
<td>HFD</td>
<td>0.99 ± 0.09*</td>
<td>1.08 ± 0.1*</td>
<td>NS</td>
</tr>
<tr>
<td>Fed plasma triglyceride ( (mg/dl) (n = 20) )</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control diet</td>
<td>72.2 ± 4.5</td>
<td>75.2 ± 3.9</td>
<td>NS</td>
</tr>
<tr>
<td>HFD</td>
<td>167.8 ± 17.7†</td>
<td>89.5 ± 6.1</td>
<td>( P &lt; 0.001 )</td>
</tr>
<tr>
<td>Fed plasma glucagon ( (pg/ml) (n = 20) )</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control diet</td>
<td>34.2 ± 3.3</td>
<td>38.5 ± 4.5</td>
<td>NS</td>
</tr>
<tr>
<td>HFD</td>
<td>48.7 ± 3.4‡</td>
<td>46.9 ± 4.3*</td>
<td>NS</td>
</tr>
<tr>
<td>Pancreatic insulin content ( (ng insulin/mg protein) (n = 10) )</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control diet</td>
<td>14.5 ± 2.8</td>
<td>14.0 ± 2.2</td>
<td>NS</td>
</tr>
<tr>
<td>HFD</td>
<td>12.2 ± 1.3</td>
<td>55.6 ± 8.9†</td>
<td>( P &lt; 0.001 )</td>
</tr>
<tr>
<td>Pancreatic glucagon content ( (ng glucagon/mg protein) (n = 10) )</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control diet</td>
<td>3.1 ± 0.4</td>
<td>3.3 ± 0.5</td>
<td>NS</td>
</tr>
<tr>
<td>HFD</td>
<td>3.3 ± 0.6</td>
<td>3.1 ± 0.4</td>
<td>NS</td>
</tr>
</tbody>
</table>

\*\( P < 0.05 \), †\( P < 0.001 \), ‡\( P < 0.01 \) comparing control diet with HFD weight and significance column comparing HFD WT with HFD UCP2 \((-/-)\) mice. NS, not significant.
(-/-) mice did not (Table 2). Fed plasma FFA concentrations of control diet–fed mice were not different; however, HFD-fed plasma FFAs were significantly elevated in both groups. Plasma triglycerides were not significantly different from each other in control diet–fed mice. However, HFD plasma triglycerides were only significantly elevated in HFD WT mice compared with control diet mice (Table 2). Immune reactive plasma glucagon levels in the control diet and HFD mice were not different between the two groups. HFD WT and UCP2 (-/-) mice had significantly increased plasma glucagon concentrations (P < 0.05; Table 2).

Blood glucose and insulin concentrations after an intraperitoneal glucose load. Mice were assessed for glucose tolerance after an IPGTT. UCP2 (-/-) mice had significantly (P < 0.05 to P < 0.001) lower blood glucose concentrations at 10, 20, and 30 min after glucose load than WT mice on HFD (Fig. 2A). The AUC for the blood glucose responses of control diet mice was significantly lower for UCP2 (-/-) mice than WT mice (277.8 ± 15.8 mmol·l⁻¹·120 min⁻¹ vs. 406.1 ± 13.5 mmol·l⁻¹·120 min⁻¹, respectively; P < 0.001). Of importance, for HFD mice, all time points from 10 to 120 min after the glucose load were significantly (P < 0.01) lower for UCP2 (-/-) mice compared with WT mice (Fig. 2B). HFD UCP2 (-/-) mice had a 32% lower AUC than WT mice (526.1 ± 53.6 mmol·l⁻¹·120 min⁻¹ vs. 789.1 ± 55.6 mmol·l⁻¹·120 min⁻¹, respectively; P < 0.05).

The plasma insulin response was also assessed during the IPGTT. Insulin concentrations were measured at 0, 10, 30, and 120 min after glucose challenge. Control diet UCP2 (-/-) insulin responses were significantly elevated at 0, 10, and 30 min compared with control diet WT mice (Fig. 2C). Control diet UCP2 (-/-) mice had significantly higher AUC for 0–30 min than WT mice (24.3 ± 1.6 vs. 14.5 ± 1.2 ng insulin/30 min, respectively; P < 0.05). A similar trend was found in the HFD animals, but the peak insulin response was further exaggerated in the UCP2 (-/-) mice (Fig. 2D). For insulin, UCP2 (-/-) mice had a twofold higher AUC for 0–30 min than WT mice (42.2 ± 6.0 vs. 21.4 ± 2.3 insulin/30 min, respectively; P < 0.05).

ITT. Given that UCP2 (-/-) mice had markedly elevated plasma insulin levels, one might expect that they could develop insulin resistance, particularly when fed an HFD. To test this possibility and whether changes in the glucose response to the IPGTT were due to changes in insulin sensitivity, we performed an ITT. Control diet mice showed no difference in their ITT responses (Fig. 2E). However, HFD WT mice were significantly more insulin-resistant than UCP2 (-/-) mice (Fig. 2F).

In situ pancreatic insulin secretion. For assessing β-cell glucose responsiveness in control diet and HFD animals, glucose was presented to the isolated pancreas as a gradient (1.4–17.4 mmol/l; Fig. 3). The control diet UCP2 (-/-) mice showed a significant increase in their glucose sensitivity when compared with control mice (Fig. 3A). The HFD-fed WT mice had a rightward shift in their glucose sensitivity as compared with control diet WT mice (Fig. 3A and B); however, this did not occur in the UCP2 (-/-) mice.

Islet morphology. On control diet, pancreatic insulin content was not different between WT and UCP2 (-/-) mice (Table 2). On the HFD, UCP2 (-/-) mice had significantly higher pancreatic insulin content (P < 0.001; Table 2) and islet insulin content corrected for cell number
(P < 0.01) than WT mice (Fig. 4A). The glucagon content of the pancreas was not different for control diet or HFD mice (Table 2). To examine the possible mechanism of the increased insulin content, we performed β-cell apoptosis, replication, and islet morphometric analyses. The relative β-cell area of the pancreas was significantly higher in both control diet– and HFD-fed UCP2 (−/−) mice (Figs. 4B and 5A and B). HFD UCP2 (−/−) mice had a 2.3-fold higher relative β-cell area compared with HFD control diet mice. There was also an increase in islet number (Fig. 4C) as well as a significant increase in average size of the islets in HFD UCP2 (−/−) mice (Fig. 4D). Apoptosis was two times higher in control diet UCP2 (−/−) mice (P < 0.05; Figs. 4E and 5C and D). After the HFD, apoptosis significantly increased in both WT and UCP2 (−/−) mice (P < 0.001 and P < 0.05, respectively). The percentage of BrdU-positive β-cells was significantly elevated in control diet UCP2 (−/−) mice (Figs. 4F and 5E and F). HFD significantly increased the percentage of BrdU-positive cells in UCP2 (−/−) mice as compared with control diet UCP2 (−/−) mice as well as HFD WT mice.

**Short-term effects of palmitic acid on islet function.** To examine further the effects of the HFD in mice, we implemented a 48-h in vitro 0.5 mmol/l palmitic acid incubation of islets from either WT or UCP2 (−/−) mice. Before and after FFA treatment, there was no significant difference in islet insulin content among the groups. The WT mice showed elevated basal but attenuated GSIS, whereas UCP2 (−/−) mouse islets showed elevated basal and enhanced GSIS (Fig. 6A and B following palmitate treatment). Islet ATP levels and mitochondrial membrane potential were significantly (P < 0.05) reduced in palmitate-treated WT mouse islets as compared with control islets, whereas UCP2 (−/−) mouse islets showed no significant reduction in ATP levels (Fig. 6C) or mitochondrial membrane potential (Fig. 6D).

**Quantification of gene expression.** To assess the effect of HFD on UCP2 and CPT-1 gene expression in islet RNA, we performed real-time PCR. No detectable UCP2 expression was found in UCP2 (−/−) mice. HFD WT mice, however, had a 6.8-fold increase in UCP2 mRNA compared with their control diet WT counterparts (Table 3). CPT-1 is a key enzyme involved in transporting fatty acids into the mitochondria for β-oxidation and can act, in some situations, to remove lipid accumulation in cells. CPT-1 gene expression was also significantly increased in both WT (P < 0.05) and UCP2 (−/−) mice (P < 0.01) after HFD, but the level of mRNA expression was higher in the UCP2 (−/−) mice (P < 0.05; Table 3).

**DISCUSSION**

The detrimental effects of a long-term HFD on β-cell function and insulin sensitivity in mice have been clearly demonstrated and can lead to type 2 diabetes (22,23). The present study focused on comparing the effects of a 4.5-month HFD on glucose homeostasis and pancreatic...
islet function in WT and UCP2 (−/−) mice. Previous studies have shown that the loss of expression of UCP2 improved glucose tolerance in ob/ob mice; therefore, we hypothesized that UCP2 (−/−) mice would likewise tolerate HFD better than WT mice. We have shown that giving mice an HFD led to elevated plasma triglyceride and fasting FFA concentrations in WT mice, as has been shown in other models of rodent fed diets with elevated fat content (24–26).

As expected, administration of HFD to WT mice was associated with a number of metabolic perturbations, notably insulin resistance and glucose intolerance (Fig. 2). HFD WT mice were more insulin-resistant than HFD UCP2 (−/−) mice. The lack of insulin resistance seen in HFD UCP2 (−/−) mice could be secondary to improved control of diabetes. Although plasma insulin concentrations of HFD WT mice increased in both fasting and postglucose states, the additional secretion was insufficient to maintain a normal glucose profile after an IPGTT. Despite this, HFD UCP2 (−/−) mice maintained lower blood glucose levels than HFD WT mice in both fasting and fed states. It seems that UCP2 (−/−) mice achieved better glucose homeostasis at least partly by a compensatory increase in insulin secretion (Fig. 2D). Therefore, the present data are consistent with a protective effect of low UCP2 on insulin secretion capacity, even when stressed by an HFD. The enhancement seems to be due, in part, to an effect on first-phase insulin secretion in UCP2 (−/−) mice (Fig. 2D). This is further supported in the perfused pancreas model, which shows the maintenance or a small enhancement of glucose sensitivity (Fig. 3).

One mechanism by which pancreatic islet function was preserved in the UCP2 (−/−) mice on HFD was an increase in their insulin secretory capacity. Islet insulin content was significantly higher in HFD UCP2 (−/−) mice than in HFD WT mice. Thus, despite overall higher secretion in vivo, the UCP2 (−/−) mice are able to maintain a sufficient insulin-secretory response when subjected to a long-term HFD. Saturated FFA (the major FFA in lard) inhibits insulin biosynthesis in vitro (27–31).

The total β-cell mass of HFD UCP2 (−/−) mice was also increased; this likely allowed HFD UCP2 (−/−) mice to maintain glucose responsiveness, whereas this did not occur in WT mice. Glucose has been proposed to be one stimulus for increasing pancreatic β-cell mass. Short-term hyperglycemia can stimulate β-cell proliferation, decrease apoptosis, and promote β-cell neogenesis (32–35). The number of functionally intact β-cells in the islet is important for the development, course, and outcome of diabetes. The total β-cell mass reflects the balance between the renewal and loss of these cells (36). Several studies in vitro have so far confirmed the mitogenic action of a high extracellular glucose concentration (32–35,37). However, there is no explanation of how glucose stimulates these changes or how β-cells lose this ability during chronic hyperglycemia. The current data suggest that hyperglycemia per se is not essential for stimulating β-cell replication or proliferation. Instead, enhanced glucose metabolism permitted by higher levels of coupled oxidative phosphorylation may be the stimulus for β-cell proliferation and neogenesis. Thus, the expression of UCP2 may limit the ability of the β-cell to compensate for increasing insulin resistance by increasing β-cell mass. The mechanism by which UCP2 may perform this task is to limit the production of a metabolic stimulus, such as ATP, for increasing β-cell mass. ATP, for example, could be working through
the mammalian target of rapamycin to stimulate β-cell proliferation (38). Work is currently under way in our laboratory to explore further this potential relationship.

CPT-1 activity is critical to promoting FFA acid oxidation in cells; moreover, its expression is induced in β-cells by FFA exposure (39). Preventing transport of FFAs to the mitochondrial matrix by use of CPT-1 inhibitors prevents the inhibition in GSIS observed after exposure of islets to long-chain FFAs (28). Although gene expression levels do not necessarily equate to functional activity, it is of interest to point out that UCP2 (−/−) mice fed control diet had elevated CPT-1 mRNA expression compared with WT islets. After HFD, the difference increased. The implication of this finding is that islets from UCP2 (−/−) mice may be able to oxidize greater amounts of FFA compared with WT mice. This would prevent accumulation of islet triglyceride, which is a key marker of lipotoxicity (40). CPT-1 induction could be the dominant mechanism by which islets from UCP2 (−/−) mice maintain their high level of functionality under stress. Alternatively, it could be a complementary mechanism to our primary hypothesis: that maintenance of higher levels of ATP (whether via glucose or fat metabolism) is key to enhanced β-cell glucose responsiveness and proliferative capacity. Additional studies, such as the measurement of the ATP/ADP ratio and a comprehensive assessment of glucose and FFA metabolism, are required to quantify the relative contributions to islet health from these two possible outcomes in UCP2 (−/−) mice. It is also important to note that UCP2 and CPT-1 are coordinately upregulated by FFA in WT mice, yet the upregulation of CPT-1 alone does not prevent the loss of β-cell function. Moreover, UCP2 is downregulated in β-cells exposed to high glucose, whereas CPT-1 expression remains unchanged (41); therefore, simply examining the ratio between the two entities does not predict the outcome on β-cell metabolism.

The concept of lipotoxicity in β-cells is attractive as an explanation for the loss of GSIS in obesity-induced diabetes. However, evidence is accumulating that fats exert most of their deleterious effects on islets only when glucose is also elevated; this concept is termed “glucolipotoxicity” (42). Thus, only in hyperglycemic rats did HFD cause a loss of GSIS concomitant with induction of UCP2 (43). Our current results are similar because UCP2 (−/−) mice that maintained fasting euglycemia were also resistant to HFD. It is interesting that in the Briaud et al. study (43), HFD did not increase islet triglyceride, suggesting that fat accumulation is not a necessary consequence of exposure of islets to high fat.

As a consequence of its ability to dissipate the proton-motive force, UCP2 is predicted to reduce the generation of ROS (44). This hypothesis is supported by data showing that macrophages from UCP2 (−/−) mice produce more ROS when challenged with infection (12) and that UCP2 is activated by ROS in mitochondria from MIN6 cells (7).
increase in ROS can lead to β-cell apoptosis (45); therefore, the presence of elevated UCP2 may reduce ROS production by dissipating the excess energy. This excessive energy metabolism, as seen with lipid exposure, may induce multiple mechanisms that lead to cell damage, such as ceramide formation (46) and apoptosis (47) in islets. At the outset, it was unclear whether the positive effects of the lack of UCP2 on insulin secretion would be negated by the putative greater increase in apoptosis in HF2 UCP2 (–/−) mice. However, this was not the case, at least in the time frame studied here. These results also suggest that inhibition and not activation of UCP2 could be an effective means of improving β-cell function in individuals with type 2 diabetes. Physiologically, an advantage of increasing the expression of UCP2 may be to prevent long-term effects of excessive fat expression often associated with increasing ROS production; however, as we have shown, UCP2 negatively impacts insulin secretion and the compensatory increase in pancreatic β-cell mass.

In conclusion, UCP2 (–/−) mice have enhanced β-cell responsiveness to a glucose challenge in vivo after an HF2. These mice also have preserved islet sensitivity to glucose, enhanced insulin content, and increased β-cell mass after an HF2. These factors seem to contribute to an increased insulin response to glucose in vivo that produces improvements in glucose tolerance in the face of insulin resistance.

ACKNOWLEDGMENTS

This work was supported by operating grants from the Canadian Institutes of Health Research (CIHR) to M.B.W. and C.B.C. (MOP 12898 and MOP 85464). J.W.J. was supported by the Banting Best Diabetes Center studentship award and by a CIHR doctoral award. M.B.W. is supported by a CIHR investigator award. C.-Y.Z. and B.B.L. are supported by research grants from the National Institutes of Health and the American Diabetes Association.

We thank Dr. Sandy Der for assistance with real-time PCR studies.

REFERENCES

32. Boileau-Lee HC, Skelly RH, Chester MW, McGarry JD, Rhodes CJ: Chronic exposure to free fatty acid reduces pancreatic beta cell insulin content by increasing basal insulin secretion that is not compensated for by a corresponding increase in proinsulin biosynthesis transcription. J Clin Invest 101:1094–1101, 1998