Beta Cell Failure in Diet-Induced Obese mice stratified according to body weight gain: secretory dysfunction and altered islet lipid metabolism without steatosis or reduced beta cell mass

Marie-Line Peyot¹, Emilie Pepin¹, Julien Lamontagne¹, Martin G. Latour¹, Bader Zarrouki ¹, Roxane Lussier¹, Marco Pineda¹, Thomas L. Jetton², S.R. Murthy Madiraju¹, Erik Joly¹ and Marc Prentki¹,³

Montreal Diabetes Research Center and CRCHUM, Montreal, QC¹; Diabetes and Metabolism, University of Vermont College of Medicine, Burlington, VT²; Departments of Nutrition and Biochemistry, University of Montreal, QC³.

Correspondence:
Marie-Line Peyot, PhD
E-mail: marie-line.peyot@crchum.qc.ca

Running title: β-cell dysfunction in diet-induced obese mice

Additional information for this article can be found in an online appendix at http://diabetes.diabetesjournals.org

Submitted 30 September 2009 and accepted 30 May 2010.

This is an uncopyedited electronic version of an article accepted for publication in Diabetes. The American Diabetes Association, publisher of Diabetes, is not responsible for any errors or omissions in this version of the manuscript or any version derived from it by third parties. The definitive publisher-authenticated version will be available in a future issue of Diabetes in print and online at http://diabetes.diabetesjournals.org.
**Objective**- C57Bl/6 mice develop obesity and mild hyperglycemia when fed a high fat diet (HFD). Although diet-induced obesity (DIO) is a widely studied model of type 2 diabetes, little is known about β-cell failure in these mice.

**Research designs and methods**- DIO mice were separated in two groups according to body weight gain: low- and high-HFD-responders (LDR and HDR). We examined whether mild hyperglycemia in HDR mice is due to reduced β-cell mass or function, and studied islet metabolism and signalling.

**Results**- HDR mice were more obese, hyperinsulinemic, insulin resistant, hyperglycemic and showed a more altered plasma lipid profile than LDR. LDR mice largely compensated insulin resistance, whereas HDR showed perturbed glucose homeostasis. Neither LDR nor HDR mice showed reduced β-cell mass, altered islet glucose metabolism and triglyceride deposition. Insulin secretion in response to glucose, KCl and arginine was impaired in LDR and almost abolished in HDR islets. Palmitate partially restored glucose- and KCl-stimulated secretion. The glucose induced rise in ATP was reduced in both DIO groups, and the glucose-induced rise in Ca\(^{2+}\) was reduced in HDR islets relatively to LDR. Glucose-stimulated lipolysis was decreased in LDR and HDR islets, whereas fat oxidation was increased in HDR islets only. Fatty acid esterification processes were markedly diminished and free cholesterol accumulated in HDR islets.

**Conclusions**- β-cell failure in HDR mice is not due to reduced β-cell mass and glucose metabolism or steatosis but to a secretory dysfunction that is possibly due to altered ATP/Ca\(^{2+}\) and lipid signalling, and free cholesterol deposition.

While insulin resistance is a common feature in most obese subjects, insulin secretion is increased to compensate for its reduced action and normoglycemia is maintained (1; 2). In obese type 2 diabetes (T2D) subjects, however, β-cell compensation fails due to marked impairment of glucose-stimulated insulin secretion (GSIS), often with reduced β-cell mass (2). The relationship between β-cell function and mass as causative factors in β-cell failure and diabetes progression is debated, with emphasis on the relevance of “functional β-cell mass” rather than total mass (2). Increased adiposity leads to elevated circulating free fatty acids (FFA) and triglycerides (TG), and *in vitro* and *in vivo* studies have indicated a causative role for dyslipidemia in insulin resistance (1; 3). Although FFA are necessary for the amplification of GSIS, their excess supply may also have a role in β-cell failure (4), as prolonged elevation of FFA levels both *in vivo* and *in vitro* cause β-cell dysfunction (5; 6) and, at least *in vitro*, apoptosis (7).

At least part of the β-cell compensation to insulin resistance is due to an increase in β-cell mass (4). Either long-term high fat diet (HFD) (8) or a short-term lipid infusion (9) can result in increased β-cell mass without augmentation of GSIS, indicating that β-cell function and mass are not necessarily linked. Rodent studies have indicated that HFD leads to increased β-cell mass (8), which is also observed in normoglycemic obese individuals (10). Unclear at present is the dynamics between the factors driving compensatory increase in β-cell mass and function, and those
β-cell dysfunction in diet-induced obese mice

reducing them through the various stages of T2D development, particularly as FFA may do both. Genetic islet susceptibility may be a critical determinant of these dynamics, both in humans and animal models (4; 11; 12).

Even though studies employing genetically modified models (e.g. Zucker Diabetic Fatty (ZDF) rats, db/db mice) have helped in understanding some of these pathological processes (13-16), several of these models are of extreme nature, with rapid development of pronounced T2D. These models, therefore, differ from human obesity-linked T2D, which usually develops more gradually. In an attempt to gain insight into the basis of β-cell failure in a mild model of diabetes, we recently developed a new model of T2D, the 60% pancreatectomized obese hyperlipidemic Zucker Fatty (ZF) rat (14). In this model, severe β-cell dysfunction was found without any evidence of a falling β-cell mass or islet steatosis (14). More detailed examination of the pancreatectomized ZF rat islets showed marked depletion of insulin stores and altered glycerolipid metabolism (14). The ZF rat, as opposed to the ZDF, however, does not have genetic predisposition to diabetes, as it maintains normoglycemia despite severe obesity-related insulin resistance (4). The diet-induced obese (DIO) C57BL/6 mouse gradually develops hyperglycemia (17). This suggests that DIO islets are unable to fully compensate for the obesity-related insulin resistance, as occurs in human T2D.

In the present study we investigated β-cell dysfunction in DIO mice stratified into two groups according to the effect of HFD on body weight: the low responders to HFD (LDR) were less obese, developed intermediate severity insulin resistance and had only mild impairment in glycemia. The high responders to HFD (HDR) were more obese, insulin resistant and hyperinsulinemic and were clearly hyperglycemic. Thus, the LDR and HDR groups allowed for analysis and comparison of islet β-cell mass and function in response to different levels of insulin resistance with corresponding very mild perturbation of glucose homeostasis and overt but mild hyperglycemia, respectively. When extended to obese humans, these two groups correspond to the pre-diabetes and early diabetes situations.

RESEARCH DESIGN AND METHODS

Animals and diets. Five-week-old male C57BL/6 mice on a pure genetic background were purchased from Charles River Laboratories (St-Constant, QC, Canada). We verified by RT-PCR analysis that this mouse strain does not harbour a mutation in the nicotinamide nucleotide transhydrogenase gene found in the C57BL/6J strain (18) (data not shown). They were housed 3-4 per cage on a 12-h light/dark cycle at 21°C with free access to water and standard diet (Teklad Global 18% protein rodent diet; Harlan Teklad, Madison, WI, 15% fat by energy). One week after arrival, mice were fed with either the standard or HFD (Bio-Ser Diet#F3282, Frenchtown, NJ, 60% fat by energy) for 8 weeks. Body weight and energy intake were measured weekly. For the energy intake measurement, the mice were placed in individual cages. Fed blood glucose was determined by a portable glucometer (Accu-check Advantage, Roche, Indianapolis, IN). All procedures were approved by the Institutional Committee for the Protection of Animals at the Centre Hospitalier de l’Université de Montréal.

Plasma parameters. Blood was collected between 8:00 and 10:00 A.M. in fed mice. Plasma insulin and proinsulin were measured by radioimmunoassay (Linco Research, St. Charles, MO) and by ELISA (proinsulin mouse kit, Alpco Diagnostics, Salem, NH), respectively. Plasma non-esterified fatty acids (NEFA) were assessed using the NEFA C kit (Wako Chemical, Osaka, Japan). Plasma TG was determined with the GPO Trinder kit.
β-cell dysfunction in diet-induced obese mice

Glucose homeostasis, insulin action and secretion in vivo. Two-hour hyperinsulinemic euglycemic clamp (HIEC) studies were performed in 2h-fasted anesthetised ND, LDR and HDR mice after 8 weeks on the experimental diets (19). An intravenous glucose tolerance test (IVGTT) (0.5 g/kg) was conducted in 16h-overnight fasted mice (20). An oral glucose tolerance test (OGTT) (1g/kg) was also made in 6h-fasted mice (20). Blood glucose was measured using a portable meter and plasma insulin using the UltraSensitive mouse Insulin ELISA Kit (Alpco Diagnostics).

β-cell proliferation and mass. These parameters were measured as described (21).

Islet isolation and culture. Islets were isolated as described (22) and kept in culture at 3 mmol/L glucose for 2h in RPMI medium supplemented with 10% FBS (complete RPMI) at 37°C, for recovery (23).

Insulin secretion ex vivo. For static incubations, batches of 10 islets each, were distributed in 12-well plates and pre-incubated for 45 min at 37°C in Krebs-Ringer bicarbonate containing 10 mmol/L HEPES (pH 7.4) (KRBH), 0.5% defatted BSA (d-BSA) and 3 mmol/L glucose. They were then incubated for 1h in KRBH/0.5% d-BSA at 3, 8 and 16 mmol/L glucose or with 35 mmol/L KCl, in the presence or absence of 0.25 mmol/L palmitate. After 1h, media were kept for insulin measurements by radioimmunoassay. Islet insulin and proinsulin contents were measured following acid-ethanol (0.2 mmol/L HCl in 75% ethanol) extraction by radioimmunoassay and ELISA, respectively. For perfusion experiments, batches of 120 cultured islets each were placed in perfusion chambers at 37°C and perfused at a flow rate of 1ml/min for 30 min in KRBH/0.5% d-BSA at 3 mmol/L glucose prior to stimulation with 16 mmol/L glucose for 60 min, and then with 10 mmol/L arginine for 10 min. Islets were then perifused at 3 mmol/L glucose for 30 min.

Intracellular calcium. Ca$^{2+}$ was measured using a modified version of a procedure previously described (24). Dispersed-islet cells from 350-500 freshly isolated islets were plated on 42 mm coverslip and incubated at 37°C overnight in complete RPMI at 11 mmol/L glucose. Cells loaded with Fluo-4 AM calcium indicator were perifused with KRBH/1% d-BSA, 2.5 mmol/L probenecid, 0.2 mmol/L sulfipyrazone, 0.1 mmol/L IBMX and 3 or 16 mmol/L glucose or 3 mmol/L glucose + 35 mmol/L KCl. Computational analysis was done using measurements every 3 s. An average of 25 cells per coverslip was analyzed.

Intracellular ATP content. Batches of 50 islets cultured and pre-incubated as described for insulin secretion experiments were incubated for 15 min in KRBH/0.07% d-BSA at 3 or 16 mmol/L glucose. At the end of the incubation, ATP was extracted from islets and assay as described (22).

Islet triglyceride, cholesterol and protein contents. TG, cholesterol and protein contents were measured in batches of 100, 40 and 20 freshly isolated islets, respectively (23; 25).

Lipid metabolism. For lipolysis, 60 islets per determination were used (23). Fatty acid (FA) oxidation and esterification into cholesterol ester were measured in batches of 50 islets (14). NEFA content and FA esterification into TG in 50 islets were determined as described (26).

Glucose metabolism. Islet glucose usage and oxidation were measured as described (22).

Quantitative RT-PCR. see supplemental research design and methods in the online appendix which is available at http://diabetes.diabetesjournals.org.

Statistical analysis. Data are expressed as means ± SE. Statistical significance was calculated by one-way or two-way ANOVA with Bonferroni, Tukey or Dunnett post hoc
testing as indicated. A $P$ value of <0.05 was considered significant.

**RESULTS**

**Metabolic characteristics of LDR and HDR mice.** For mechanistic analyses, pooling mice with markedly different characteristics (for example little weight gain, no insulin resistance and normoglycemia with animals with high weight gain, insulin resistance and hyperglycemia), as has been done in the vast majority of studies so far, can be misleading. We reasoned that this caveat can be dealt by stratifying mice in two groups (LDR and HDR) (as described below) that correspond on one hand to lower weight gain and mild perturbation in glucose homeostasis, and on the other hand to higher weight gain and overt but mild hyperglycemia. Based on the heterogeneous weight response to diet (approximately 12 g range for DIO mice housed by group of 3-4/cage or individually) (Fig. 1A), DIO mice were segregated into two groups after 7.5 weeks on HFD: LDR mice, representing 40% of the DIO mice and weighing 33-38.9 g (lower 6 g range) with an average fed glycemia of 8.6±0.1 mmol/L, and HDR mice, representing 52% of the DIO mice and weighing 39-45 g (higher 6 g range) with an average glycemia of 9.4±0.1 mmol/L (Fig. 1B). DIO outlier mice weighing less than 33g (around 4% of total DIO mice) and more than 45g (around 4%) were excluded from this study (Fig. 1A). The control group fed with the standard diet (normal diet, ND) had an average weight of 28.3±0.2 g and a glycemia of 7.5±0.1 mmol/L (Fig. 1B). Difference in body weight in DIO mice was already present after 1 week under HFD and steadily increased during the 8 weeks of the study (Fig. 1C). The considerably larger weight of HDR mice can be accounted at least in part to an increased energy intake (106.8±1.4 vs. 98.3±1.4 Kcal/week in LDR mice, Fig. 1D, inset). Table 1 shows that over 8 weeks HFD-fed LDR and HDR mice, respectively, gained approximately 2-3 and 3-4 times more weight compared to ND mice. LDR mice had a very slightly raised glycemia in the fed state (Fig. 1B). The 2h-fasting blood glucose of LDR mice was not significantly increased (Fig. 2A). In contrast the HDR group showed a more prominent hyperglycemia than LDR mice. Blood glucose in HDR mice was increased in awake fed mice (9.4±0.1 vs. 7.5±0.1 mmol/L in ND mice, Fig. 1B) and in 2h-fasted anaesthetized mice (11.0±0.9 vs. 5.7±0.5 mmol/L in ND mice, Fig. 2A). Glucose levels in 6h-fasted HDR mice versus ND mice (11.6±0.5 vs. 7.4±0.3 mmol/L) was elevated, and the LDR group showed intermediate values (9.8±0.4 mmol/L) (Suppl. Fig. 1A and C). Glycemia in overnight 16h-fasted HDR mice with anaesthesia was slightly elevated, without reaching statistical significance (8.3±0.3 vs. 7.0±0.4 mmol/L, Fig. 2E). Glucose values at time 60 min during an IVGTT (Fig. 2E) and time 120 min during an OGTT (Suppl. Fig. 1A and D) remained markedly elevated in HDR, in contrast to the LDR mice. Fasting and fed insulinaemia were 3-4 fold higher in the HDR compared to ND mice, whereas in LDR mice fed insulinaemia was unchanged and fasting insulinaemia was increased by twofold (not statistically significant) (Table. 1). Fed proinsulinaemia was higher in HDR mice in comparison to ND, and the proinsulin to insulin ratio was unchanged in LDR and HDR mice versus controls. Of the measured lipid parameters, when compared to ND mice, fed plasma cholesterol was significantly increased by 70% in HDR mice, fasting plasma FFA were 2-fold higher in HDR mice and fed plasma TG was 30% decreased in both DIO groups (Table. 1).

**LDR and HDR mice are insulin-resistant, glucose-intolerant and have a decreased first phase insulin secretion in response to glucose in vivo.** A hyperinsulinemic-euglycemic clamp was made in 2h-fasted ND and DIO mice (Fig. 2A-D). Both the glucose
infusion rate (Fig. 2C) required to maintain glycemia at ~7.2 mmol/L (Fig. 2B) and the insulin sensitivity (M/I) index (Fig. 2D) were decreased by ~50% and ~80% in LDR and HDR mice, respectively, indicating that HDR mice are more insulin resistant than LDR. To measure GSIS in vivo, overnight-fasted DIO and ND mice were subjected to an IVGTT (Fig. 2E-I). Glucose clearance was reduced in HDR mice (Fig. 2E), indicating a decreased glucose tolerance as shown by a 2-fold increase in the area under the curve (AUC) for glycemia (Fig. 2G). LDR mice tended to be glucose intolerant but without reaching statistical significance (Fig. 2E and G). Similar observation was made using the OGTT test (Suppl. Fig. 1A and B). Calculation of the AUC for insulinemia during the first 15 min of the IVGTT, subtracting basal values, indicated that first phase GSIS is reduced by 66 and 76% in the LDR and HDR groups, respectively (Fig. 2F and H). However, the AUC for the second phase GSIS of both DIO mice was not different from control mice (Fig. 2F and I).

Impaired insulin secretion in isolated islets from LDR and HDR mice. We next examined GSIS in presence or absence of exogenous FFA in isolated islets. In ND mice, glucose from 3 to 16 mmol/L increased insulin secretion by 2.6 fold (Fig. 3A). The glucose effect in both DIO groups was reduced, and was almost completely blunted in HDR mice (Fig. 3A and D). LDR and HDR mice also showed defective KCl-induced insulin secretion (KSIS), the reduction being more pronounced in the HDR group (Fig. 3C). Exogenous palmitate enhanced GSIS in the 3 groups (Fig. 3B) and partially restored GSIS, as evaluated by the difference between high and low glucose values (Fig. 3D), as well as KSIS (Fig. 3C), in both DIO groups. First phase GSIS was reduced by 47 and 65% in LDR and HDR perifused islets, respectively (Fig. 3E-F). HDR mice also showed a 5-fold decrease in second phase GSIS (Fig. 3G) and in secretion in response to arginine (Fig. 3H).

Lack of decreased pancreatic β-cell mass and islet insulin stores in DIO islets. HDR mice showed a 1.7- and 2-fold increase in β-cell mass (Fig. 4A) and proliferation (Fig. 4B), respectively. There was a trend for β-cell mass and proliferation to be increased in the LDR group. Islet protein contents were increased by 24 and 44% in LDR and HDR mice, respectively (Fig. 4C). Total insulin content per islet was similar in all groups (Fig. 4D), however islet content corrected per protein content decreased by 20 and 24% in LDR and HDR islets, respectively (Fig. 4E). The islet proinsulin content per protein was similar in all groups (Fig. 4F).

Islet glucose metabolism is unchanged in DIO mice. Previous work in T2D rodent models has suggested a role for altered glucose metabolism in β-cell dysfunction (27; 28). Fig. 4 indicates that impaired GSIS in LDR and HDR mice cannot be ascribed to altered glucose metabolism, since glucose usage (Fig. 4G) and oxidation (Fig. 4H) were unchanged.

Decreased glucose-stimulated ATP content in DIO islets and altered Ca²⁺ signaling in HDR islet cells versus LDR. ND mice showed a 40 % increase in intracellular ATP content in response to high glucose, whereas both DIO groups displayed a reduced response (Fig. 4I). The rise in Ca²⁺ in response to glucose was markedly larger in LDR versus ND islet cells (Fig. 4J). However, the HDR group did not show such potentiation that likely reflects a compensation phenomenon.

Altered lipid partitioning, reduced glycerolipid/fatty acid cycling and free cholesterol deposition in HDR islets. Islet steatosis was proposed to contribute to β-cell failure (29). However, there was no accumulation of TG in DIO islets (Fig. 5A). Work from our (13; 14; 22; 23) and other groups (30-32) documented a role of β-cell
lipolysis and GL/FFA cycling in insulin secretion. Lipolysis, evaluated by glycerol release, was increased 2-fold by elevated glucose in ND islets (Fig. 5B), and this increase was abolished in DIO islets. Fatty acid partitioning (FA oxidation and esterification) was studied in DIO islets since it provides lipid signals for secretion (33). FA oxidation was similar in both ND and LDR islets (Fig. 5C). However, FA oxidation was increased by 60% in HDR islets. HDR islets had a 70% higher level of NEFA (an index of long-chain acyl-CoA content) at 3 mmol/l glucose (Fig. 5D) consistent with the elevated FA oxidation at low glucose (Fig. 5C). At 16 mmol/l glucose, intracellular NEFA remained not significantly different in all groups (Fig. 5D). Glucose-stimulated palmitate esterification into TG was reduced by about 25% in LDR and HDR islets (Fig. 5E). An altered lipid esterification process in DIO islets was also evident from the observed reduction in palmitate esterification into cholesterol esters in islets from both DIO groups (Fig. 5F). This was associated with a 90% and 60% increase in free cholesterol in HDR versus ND and LDR islets, respectively (Fig. 5G).

Expression of key transcriptional factors, exocytosis proteins and enzymes of glucose and lipid metabolism. A panel of mRNA levels was measured to gain insight into the basis of β-cell (de)compensation. Only the mRNA levels of insulin-2, UCP-2 (uncoupling protein-2), SCD-1 (stearoyl-CoA desaturase-1), ACC-2 (acetyl-CoA carboxylase-2) and ATGL (adipose triglyceride lipase) significantly changed in DIO islets (Suppl. Fig. 2A-D). Insulin-2 mRNA was increased in both DIO groups, reaching significance only in HDR islets (Suppl. Fig. 2B). Increased expression of UCP-2 in the β-cell is thought to play a role in reducing reactive oxygen species production but the role of UCP2 in mitochondrial uncoupling or changes in GSIS is debated (34; 35). UCP-2 mRNA level was increased by about 40% in both LDR and HDR islets (Suppl. Fig.2C). A 56% increase in ATGL expression, an enzyme involved in triglyceride hydrolysis, was detected in HDR islets (Suppl. Fig. 2C), that may reflect a compensation phenomenon. In accordance with the increased FFA oxidation in HDR islets (Fig. 5C) and the decreased FA esterification in TG in DIO islets (Fig. 5E), ACC-2 mRNA was decreased by 45% in HDR islets and SCD-1 mRNA was reduced in DIO islets (Suppl. Fig. 2D). Thus, SCD-1 is involved in the desaturation of FFA and is important for FA esterification (36) whereas ACC-2 negatively regulates FFA oxidation via the production of malonyl-CoA (37).

DISCUSSION

According to body weight response to HFD, we stratified C57Bl/6 DIO mice in two groups. HDR mice are markedly obese, hyperinsulinemic, insulin resistant, glucose intolerant, and show an altered plasma lipid profile in association with overt but mild hyperglycemia (Table. 2). LDR mice have an “intermediate” phenotype and show relatively successful compensation since they are barely glucose intolerant and only very slightly hyperglycaemic. Thus, defining these two groups allows the study of the basis of β-cell compensation and failure in two groups of obese mice with the same genetic background and under identical experimental conditions. The HDR group and its comparison with LDR and ND mice also allows insight into the causal factors of β-cell failure to sustain the increase demand of insulin in the face of elevated insulin resistance. Importantly, study of HDR and LDR mice may distinguish the alteration in the β-cell that are related to coping with the HFD load and obesity situation from those that may cause β-cell maladaptation to the rising insulin resistance with resulting dysfunction and mild
β-cell dysfunction in diet-induced obese mice

hyperglycemia (the equivalent of prediabetes and early diabetes phases in humans).

What is the basis of the β-cell failure to insufficiently compensate for the higher insulin resistance in HDR mice? The results allow the exclusion of several factors (Table. 2): decreased β-cell glucose metabolism, islet steatosis, reduced β-cell mass and proliferation, and depletion of insulin stores since the latter was not significantly decreased per islet and reduced by only 30% if expressed per islet protein in a context where pancreatic β-cell mass increased twofold. However it cannot be discounted that failure to promote even greater than twofold compensatory increase in β-cell mass, in part contributed to β-cell failure in the HDR group. Significant β-cell "dedifferentiation" is also unlikely to be involved in β-cell failure of HDR mice since they were markedly hyperinsulinemic, the islet mRNA expression of a large number of genes related to the phenotype of a well differentiated β-cell (PDX-1, MAF-A, Nkx6.1, Glut2, GK, PC, preproinsulin, Suppl. Fig. 2A) was unchanged, and islet glucose oxidation and utilization remained unaltered.

The results point to a role of alterations in the classical ATP/Ca²⁺ signalling and lipid amplification pathways as well as free cholesterol deposition, in the failure of the HDR β-cells to compensate for the marked insulin resistance (Fig. 6). Thus, LDR islets displayed an amplified Ca²⁺ response to glucose that was not apparent in the HDR group, together with reduced rise in ATP content at high glucose, the latter, however, being also reduced in LDR islets. The reason for the enhanced Ca²⁺ response in the LDR group that should favour compensating insulin secretion remains to be determined. Furthermore, LDR and HDR mice showed altered lipid metabolism and signalling that may explain reduced glucose-, KCl- and arginine-induced insulin secretion. Thus, exogenous palmitate partially restored GSIS and KSIS in islets of both DIO groups. Importantly, the differences that were found between the compensating prediabetic LDR and decompensating (here defined as insufficient compensation) HDR groups were: enhanced fat oxidation and elevated intracellular NEFA at basal glucose in HDR islets, in association with an increased free cholesterol content, as well as a more pronounced reduction of FA esterification into cholesterol esters and SCD-1 expression (Table. 2). Our previous work indicated that both the balance of fat oxidation versus esterification, β-cell lipolysis and GL/FFA cycling play a role in GSIS (13; 14). As shown in Fig. 6, in HDR mice, impaired glucose-stimulated lipolysis and FA esterification should reduce the production of lipid signalling molecules such as diacylglycerol (33), that has been linked to the activation of C-kinase enzymes (38) and the exocytic protein Munc13-1 (39), whereas enhanced fat oxidation should simultaneously cause their removal. Increased FA oxidation should also reduce FA-CoA availability for cholesterol ester formation (a protective mechanism against the toxic effects of excess cholesterol) (40) leading to free cholesterol accumulation.

Alteration in β-cell cholesterol metabolism has emerged as a factor contributing to β-cell dysfunction as evidenced in a number of in vitro and in vivo studies (25; 41; 42). Interestingly, total plasma cholesterol and islet free cholesterol deposition occurred only in the HDR group, illustrating the interest of stratification of DIO groups. Cholesterol is abundant in lipid rafts that are implicated in exocytosis (43) and it can be hypothesized that such modification may contribute to the observed reduction in insulin secretion in response to all classes of secretagogues. Consistent with this view, Collins et al (44) observed reduced β-cell exocytosis elicited by short depolarization in DIO mice.
Comparison of in vitro and in vivo insulin secretion of DIO mice indicated that the secretory defect is more prominent in vitro. The nature of the factors allowing compensatory increased (but not sufficient) insulin secretion with hyperinsulinemia in vivo remains to be known and may include FFA as suggested by our in vitro work, enhanced incretin signalling, reduced levels of counter-regulatory hormones and neural pathways (45).

In conclusion, HDR mice fail to fully compensate for the prominent insulin resistance that exacerbates the demand for insulin to maintain euglycemia. HDR islets have a defect in β-cell secretory function in response to glucose and non-fuel stimuli that is possibly caused by: 1) a lack of compensatory increase in ATP/Ca\(^{2+}\) signalling; 2) free cholesterol accumulation; 3) impaired lipid signalling due to a metabolic shift with altered lipid partitioning and GL/FFA cycling. We propose that all three factors contribute to the inappropriate capacity to maintain sufficient secretion in the face of insulin resistance. Additional work is required to define in further details the biochemical basis of β-cell failure in HDR DIO mice.

Author contributions: M-L.P. researched data, contributed to discussion, wrote manuscript, reviewed/edited manuscript. E.P. researched data, contributed to discussion. J.L. researched data. M.G.L. researched data, contributed to discussion. B.Z. researched data. R.L. researched data. M.P. researched data. T.L.J. researched data. S.R.M.M. contributed to discussion. E.J. contributed to discussion. M.P. contributed to discussion, wrote manuscript, reviewed/edited manuscript.

ACKNOWLEDGMENTS
This work was supported by grants from the Canadian Diabetes Association, the Canadian Institute of Health Research and Génome Québec/Canada, (to MP and MM) and the US National Institutes of Health (to TLJ). MP is the recipient of a Canadian Chair in Diabetes and Metabolism. JL is supported by graduate studentships from the Fonds de Recherche en Santé du Québec and EP was supported by a graduate studentship from association du Diabète du Québec. BZ is supported by a post-doctoral fellowship from Merck Frosst. We thank Drs Christopher J Nolan (ANU Medical School, The Canberra Hospital, Australia), Barbara Corkey (Obesity Research Center, Department of Medicine, Boston University School of Medicine, USA) and Vincent Poitout (Montréal Diabetes Research Center, CRCHUM and Departments of Medicine, Nutrition and Biochemistry, University of Montréal, Canada) for critical review of the manuscript.

REFERENCES
β-cell dysfunction in diet-induced obese mice


18. Wong N, Blair AR, Morahan G, Andrikopoulos S: The deletion variant of nicotinamide nucleotide transhydrogenase (Nnt) does not affect insulin secretion or glucose tolerance. *Endocrinology* 151:96-102, 2010

β-cell dysfunction in diet-induced obese mice


40. Busch AK, Gurisik E, Cordery DV, Sudlow M, Denyer GS, Laybutt DR, Hughes WE, Biden TJ: Increased fatty acid desaturation and enhanced expression of stearoyl coenzyme A desaturase protects pancreatic beta-cells from lipoapoptosis. *Diabetes* 54:2917-2924, 2005
FIG. 1. Body weight, energy intake and glycemia of C57Bl/6 mice fed normal (ND) or high fat diet (HFD) diet for 8 weeks. DIO mice were classified after 7.5 weeks on HFD as low- and high-HFD-responders (LDR and HDR) following body weight (BW) determinations. (A) Distribution plots of body weight at 7.5 weeks of ND or HFD of mice housed 3-4 per cage (ND, LDR and HDR) or individually (NDi and DIOi). Encircled symbol corresponds to DIO mice excluded from the study. (B) Fed glycemia and body weight of ND, LDR and HDR mice after 7.5 weeks of dietary treatment. (C) Weight and (D) energy intake curves of ND, LDR and HDR mice during 8 weeks of normal or HF diets. Inset (D) Mean of energy intake per week per group. (A) Means ± SE of 52 mice for ND, LDR and HDR, of 13 and 33 mice for NDi and DIOi, respectively. (B) and (C) Means ± SE of 136, 117, 148 mice for the ND, LDR and HDR groups, respectively. (D) Means ± SE of 13, 14, 17 mice for the ND, LDR and HDR groups, respectively. ND versus LDR or HDR, **P<0.01, ***P<0.001; LDR versus HDR, §P<0.05, §§P<0.01, §§§P<0.001; one-way (B) or two-way ANOVA (C-D), Bonferroni post hoc test.
FIG. 2. DIO mice are insulin-resistant, glucose intolerant and have defective first phase GSIS. (A-B) Hyperinsulinemic euglycemic clamp (HIEC) in 2h-fast fed anesthetised mice. (A) Arterial glucose levels at time 0 of the clamp; (B) glucose levels during the clamp. The dashed line corresponds to the clamped glucose value at ~7.2 mmol/L. (C) Glucose infusion rate during the last 30 min of the 2h HIEC (GIR) and (D) insulin sensitivity index (M/I) calculated as the glucose infusion rate (M) divided by the average insulinemia during the last 30 min of the clamp (I). M/I index is expressed as µmol.kg⁻¹.min⁻¹ glucose infused per pmol/L insulin. (E-I) Intravenous glucose tolerance test (IVGTT). Plasma glucose (E) and insulin (F) were measured at times 0, 3, 5, 15, 30, 45 and 60 min in response to a glucose challenge (iv; 0.5g/kg) in 16h-overnight fasted anesthetised mice; (G) Area under the curves (AUC) of the 0-60 min glycemic responses; (H) First phase insulin response to glucose (Glc) is the AUC of the 0-15 min insulinemic response; (I) Second phase insulin response to glucose is the AUC of the 15-60 min insulinemic response. Means ± SE of 5-8 animals per group. ND versus LDR or HDR: *P<0.05, **P<0.01, ***P<0.001; LDR versus HDR: $P<0.05, §§P<0.01, §§§P<0.001; one-way (A, C-D, G-I) or two-way ANOVA (B, E-F), with Bonferroni post hoc test.
β-cell dysfunction in diet-induced obese mice

**FIG. 3.** Isolated islets from DIO mice show defective glucose-, KCl- and arginine-induced insulin release. Insulin secretion was measured in freshly isolated islets from ND, LDR and HDR mice. Group of 10 islets were incubated 1h in KRBH at 3, 8 or 16 mmol/L glucose (G; A and D) ± 0.25 mmol/L palmitate/0.5% d-BSA (Pal; B and D) or 3 mmol/L glucose ± 35 mmol/L KCl (C) ± 0.25 mmol/L palmitate/0.5% d-BSA (Pal; C). Means ± SE are of 14-16 determinations from islets of 21-22 animals per group in five (A-B, D) and three (C) separate experiments. (E-H) Insulin secretion by islets perfused at 3 mmol/L glucose (3G) or 16 mmol/L glucose (16G) with or without 10 mmol/L arginine (Arg). Area under the curve (AUC) for insulin over the first 15 min (F) and from 15-60 min (G) following glucose concentration increased from 3 to 16 mmol/L. AUC for maximal insulin secretion was determined from 60-80 min after arginine injection (H). Means ± SE are of 6 ND, 7 LDR and 7 HDR. Insulin release in the media was determined after the 1h incubation or at the different time points of the perifusion experiment. *P<0.05, **P<0.01, ***P<0.001 ND versus LDR or HDR; #P<0.05, ##P<0.01, ###P<0.001 versus 3 mmol/L glucose (A) plus palmitate (B) or plus KCl (C) or versus the value in absence of palmitate (D) for the same group (ND, LDR, HDR), one-way ANOVA-Bonferroni’s multiple comparison test.
FIG. 4. β-cell mass and proliferation, islet cell cytosolic free calcium, ATP, proinsulin, insulin and protein contents, and glucose metabolism of DIO mice. (A) β-cell mass and (B) proliferation as indicated by the number of Ki-67 positive β-cells/islet. Means ± SE of 6 animals for ND and LDR and 7 for HDR. ND versus LDR or HDR, *P<0.05. One-way ANOVA with Dunnett’s post hoc test. (C) Protein, (D-E) insulin expressed per islet (D) or per islet protein content (E) and (F) total islet proinsulin contents. Means ± SE of 83, 35 and 9 animals per group for (C), (D-E) and (F), respectively. ND versus LDR or HDR, *P<0.05, **P<0.01 and ***P<0.001; LDR versus HDR, §§P<0.01. One-way ANOVA with Bonferroni post hoc test. Glucose utilization (G) and oxidation (H) were measured in islets incubated in KRBH at 3, 8 and 16 mmol/L glucose (G) with [U-14C]glucose and [5-3H]glucose. Means ± SE of 13-15 determinations from islets of 9 ND, 8 LDR and 10 HDR mice in three separate experiments. ###P<0.001 versus 3 mmol/L glucose for the same group. One-way ANOVA-Bonferroni’s multiple comparison test. (I) Total ATP content was determined in islets incubated for 15 min in KRBH at 3 or 16 mmol/L glucose (G). Means ± SE of 13-15 determinations from islets of 9 ND, 9 LDR and 9 HDR mice in three separate experiments. #P<0.05 versus 3 mmol/L glucose for the same group. One-way ANOVA-Bonferroni’s multiple comparison test. (J) Cytosolic free calcium was measured by confocal microscopy using Fluo-4 AM dye in dispersed-islet cells of ND, LDR and HDR mice. Cells were perifused for 3 min at 3 mmol/L glucose, then at 16 mmol/L glucose for 10 min and finally at 3 mmol/L glucose + 35 mmol/L KCl for 2 min. Fluorescence level is expressed in arbitrary units. For clarity purposes, the mean only of 6 independent experiments from 6 mice per group is represented. Of note, SE was no more than 15% at all time. One-way ANOVA with repeated measures, Tukey post test. ***P<0.001 ND versus LDR and §§§P<0.001 LDR versus HDR.
FIG. 5. Altered lipid partitioning, lipolysis and glycerolipid/FA cycling in DIO islets. (A) Islet TG content. Means ± SE of 10-12 animals per group. (B) Glycerol release, as an index of lipolysis, was determined in islets incubated for 3h in KRBH at 3 and 16 mmol/L glucose (G). Means ± SE of 19-20 determinations from islets of 17-18 mice per group in four separate experiments. Islets were incubated in KRBH at 3 and 16 mmol/L glucose with [9,10-3H]palmitate to assess (C) FFA oxidation, (D) total intracellular labelled non-esterified FFA (NEFA), and (E) fatty acid esterification into triglycerides (TG) and (F) cholesterol esters (CE). Means ± SE of 9-12 determinations from islets of 14-18 mice per group in three (C) or four (D-F) separate experiments. (G) Total cholesterol (TC) and free cholesterol (FC) fractions extracted from ND, LDR and HDR islets. Means ± SE of 10-15 animals per group. LDR or HDR versus ND: *P<0.05, **P<0.01, ***P<0.001; LDR versus HDR: §P<0.05; #P<0.05, ##P<0.01, ###P<0.001 versus 3 mmol/L glucose for the same islet group. One-way ANOVA-Bonferroni’s multiple comparison test.
FIG. 6. Model depicting the possible mechanisms of -cell failure to compensate for insulin resistance in HDR mice. In normal mice, an elevation of glucose leads to an increase in ATP/ADP ratio and intracellular Ca$^{2+}$ and a decrease in fatty acid oxidation which allows long chain-acyl-CoA (FA-CoA) availability for glycerolipid/fatty acid (GL/FA) cycling which produces lipid signalling molecules (LSM), such as diacylglycerol, necessary for insulin secretion. Enhanced GL/FA cycling is an “on” signal for insulin secretion that contributes to the amplification arm of GSIS. Enhanced fat oxidation is an “off” signal for insulin secretion because it removes molecules from the cycle (46). In HDR mice, to adapt to the elevation of circulating FFA and post-prandial glucose and to prevent -cell glucolipotoxicity and steatosis, fatty acid esterification processes and lipolysis are simultaneously decreased in association with enhanced FFA oxidation. The increase in FA oxidation, in parallel to depleting LSM, reduces FA-CoA availability for cholesterol ester (CE) synthesis, contributing with elevated blood cholesterol to free cholesterol accumulation and -cell dysfunction with reduced secretion. Besides the amplification arm of GSIS, the classical triggering ATP/Ca$^{2+}$ pathway is also affected in HDR versus LDR mice due to reduced glucose stimulated ATP production and a lack of compensatory increase in the rise in Ca$^{2+}$ promoted by high glucose. GPR40 activation by FFA may contribute to maintain high level of secretion and hyperinsulinemia. However, in the absence of insufficient insulin secretion for the demand due to the marked insulin resistance, HDR mice become hyperglycaemic. Black arrows indicated a difference between DIO group (LDR and HDR) and ND group. Striped arrows indicated a difference between HDR and LDR groups.
TABLE 1.
Metabolic parameters of C57Bl/6 mice fed with a normal or high-fat diet for 8 weeks.

<table>
<thead>
<tr>
<th></th>
<th>FED</th>
<th>Fasted</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ND</td>
<td>LDR</td>
<td>HDR</td>
<td>ND</td>
</tr>
<tr>
<td>Weight gain (g)</td>
<td>8.5±0.2 (62)</td>
<td>15.5±0.2*** (60)</td>
<td>20.4±0.2***,€€€ (72)</td>
<td>5.5±1.0 (5)</td>
</tr>
<tr>
<td>Insulin (pmol/L)</td>
<td>252±24 (59)</td>
<td>266±20 (58)</td>
<td>658±54***,€€€ (67)</td>
<td>133±87 (5)</td>
</tr>
<tr>
<td>ProIns (pmol/L)</td>
<td>18.5±2.3 (8)</td>
<td>23.7±1.9 (9)</td>
<td>31.8±4.0* (9)</td>
<td>ND</td>
</tr>
<tr>
<td>ProIns/Ins (%)</td>
<td>7.8±1.3 (8)</td>
<td>9.4±1.4 (9)</td>
<td>5.5±0.9€ (9)</td>
<td>ND</td>
</tr>
<tr>
<td>FFA (mmol/L)</td>
<td>0.60±0.04 (33)</td>
<td>0.55±0.03 (33)</td>
<td>0.58±0.03 (38)</td>
<td>0.77±0.06 (5)</td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>0.41±0.02 (33)</td>
<td>0.32±0.01*** (34)</td>
<td>0.33±0.01*** (38)</td>
<td>0.36±0.04 (5)</td>
</tr>
<tr>
<td>TC (mmol/L)</td>
<td>1.50±0.07 (8)</td>
<td>1.50±0.14 (8)</td>
<td>2.58±0.21***,€ (8)</td>
<td>ND</td>
</tr>
</tbody>
</table>

Plasma triglyceride (TG), free fatty acids (FFA), total cholesterol (TC), proinsulin (ProIns) and insulin (Ins) were determined in anesthetized overnight fasted and/or fed male mice on a high-fat diet (LDR and HDR) or a standard chow diet (ND) for 8 weeks. Delta body weight represents the weight gain of mice since the introduction of diets (chow or HFD) at 6 weeks of age. Means ± SE of n animals as indicated in parentheses. LDR or HDR versus ND: *P<0.05, **P<0.01, ***P<0.001; HDR versus LDR: €P<0.05, €€P<0.01, €€€P<0.001. One-way ANOVA-Bonferroni’s multiple comparison test. ND, not determined.
TABLE 2.
Summary of the metabolic and islet β-cell parameters obtained in C57Bl/6 male mice fed a high-fat diet for 8 weeks. TG, triglyceride, CE, cholesterol esters. Horizontal arrow, unchanged versus normal diet fed animals; arrows in parenthesis indicate a clear trend that did not reach statistical significance.

<table>
<thead>
<tr>
<th>Metabolic parameters</th>
<th>LDR</th>
<th>HDR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight gain</td>
<td>↑</td>
<td>↑↑</td>
</tr>
<tr>
<td>Insulin resistance</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Insulinemia</td>
<td>(↑)</td>
<td>↑</td>
</tr>
<tr>
<td>Proinsulinemia/insulinemia</td>
<td>↔</td>
<td>↔</td>
</tr>
<tr>
<td>OGGT glucose tolerance</td>
<td>(↓)</td>
<td>↓</td>
</tr>
<tr>
<td>ivGTT glucose tolerance</td>
<td>(↓)</td>
<td>↓</td>
</tr>
<tr>
<td>ivGTT/1st phase GSIS</td>
<td>↓</td>
<td>↓↓</td>
</tr>
<tr>
<td>ivGTT/2nd phase GSIS</td>
<td>↔</td>
<td>↔</td>
</tr>
<tr>
<td>Fasting FFA</td>
<td>(↑)</td>
<td>↑</td>
</tr>
<tr>
<td>Fed total cholesterol</td>
<td>(↑)</td>
<td>↑</td>
</tr>
<tr>
<td>Fed glycemia</td>
<td>↑</td>
<td>↑↑</td>
</tr>
<tr>
<td>2h-fasted glycemia</td>
<td>(↑)</td>
<td>↑</td>
</tr>
<tr>
<td>6h-fasted glycemia</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>16h-fasted glycemia</td>
<td>↔</td>
<td>(↑)</td>
</tr>
<tr>
<td>ivGTT, 1h glucose</td>
<td>↔</td>
<td>↑</td>
</tr>
<tr>
<td>OGGT, 2h glucose</td>
<td>(↑)</td>
<td>↑</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Islet β-cell parameters</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>β-cell mass</td>
<td>(↑)</td>
<td>↑</td>
</tr>
<tr>
<td>β-cell proliferation</td>
<td>(↑)</td>
<td>↑</td>
</tr>
<tr>
<td>Protein content/islet</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Insulin content/islet</td>
<td>↔</td>
<td>↔</td>
</tr>
<tr>
<td>Proinsulin content/islet protein</td>
<td>↔</td>
<td>↔</td>
</tr>
<tr>
<td>Glucose-induced insulin secretion</td>
<td>↓</td>
<td>↓↓</td>
</tr>
<tr>
<td>KCl-induced insulin secretion</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>Glucose usage/oxidation</td>
<td>↔</td>
<td>↔</td>
</tr>
<tr>
<td>TG content</td>
<td>↔</td>
<td>↔</td>
</tr>
<tr>
<td>Free cholesterol content</td>
<td>↔</td>
<td>↑</td>
</tr>
<tr>
<td>Glucose-stimulated lipolysis</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>Glucose effect on NEFA content</td>
<td>(↓)</td>
<td>↓</td>
</tr>
<tr>
<td>Glucose effect on FA esterif. in TG</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>FA esterification in CE</td>
<td>↓</td>
<td>↓↓</td>
</tr>
<tr>
<td>FA oxidation</td>
<td>↔</td>
<td>↑</td>
</tr>
<tr>
<td>Glucose-induced ATP</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>Glucose-induced Ca²⁺</td>
<td>↑</td>
<td>↔</td>
</tr>
<tr>
<td>KCl-induced Ca²⁺</td>
<td>↔</td>
<td>↔</td>
</tr>
</tbody>
</table>