High-fat diet-induced β-cell proliferation occurs prior to insulin resistance in C57Bl/6J male mice

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β-CELL COMPENSATION IN RESPONSE TO OBESITY is observed in both humans and rodent models. Human autopsy studies have revealed that nondiabetic obese individuals have 50% greater β-cell mass compared with lean individuals, and pancreata from type 2 diabetes patients have diminished β-cell mass compared with nondiabetic BMI-matched individuals (7). In mice, high-fat diet (HFD) feeding leads to increased body weight and a corresponding expansion in β-cell mass via increased β-cell proliferation (19, 35, 43, 46). The β-cell response to HFD feeding in mice could enhance our understanding of the β-cell response to energy excess in humans and help us develop new strategies for augmenting β-cell mass in type 2 diabetes patients, but a key point is to discern early responses vs. compensatory responses that might occur after prolonged HFD feeding. The β-cell response to HFD feeding has been studied extensively; however, the data are difficult to interpret due to varying model systems and experimental designs. Studies vary in the β-cell characteristics measured, the composition and timing of diet, and mouse genotype, age, and, sex. Only by combining parallel measurements can a relationship between β-cell adaptations and the progression of diet-induced obesity be defined. The majority of studies utilize long-term HFD consumption, and the β-cell response is assessed after rodents have been on a HFD for anywhere from 2 to 12 mo. These studies demonstrated that long-term HFD consumption induces glucose intolerance, insulin resistance, and enhanced β-cell mass and proliferation (2, 6, 17, 26, 27, 32, 40, 44). Some studies have explored the acute consequences of overnutrition; for example, significant weight gain and hyperglycemia have been reported in mice after as little as 1 wk after beginning a diet high in fat (2, 34, 46). β-Cell mass expansion due to increased β-cell proliferation has been reported in response to only 1 wk of HFD feeding, and this was suggested to occur in the absence of insulin resistance (35). However, the value of the intraportal insulin tolerance test (IPITT), which was used in that study, to assess insulin resistance in mice is limited by the potentially confounding effect of counterregulatory responses (5, 26) and the very short half-life of insulin in mice (8). Therefore, in the current study, after the primary screen using IPITTs, we performed hyperinsulenic euglycemic clamps to assess insulin sensitivity with greater sensitivity as recommended by the Mouse Metabolic Phenotyping Center Consortium (5).

The current study was designed to assess both the onset and the magnitude of functional and morphological adaptations of β-cells in response to HFD feeding. To this end, starting at 8 wk of age, male C57Bl/6J mice were fed either a normal chow diet (CD) or a HFD for a duration of 11 wk and periodically examined for indications of insulin resistance, glucose intolerance, β-cell mass, and β-cell proliferation. We determined not only when but also the degree to which the parameters change in response to overnutrition. Here, we report that HFD induces β-cell proliferation as early as 3 days after diet initiation, the earliest this has been shown to occur. Furthermore, we found that enhanced β-cell proliferation induced by HFD feeding occurs in response to factors not initiated by HFD-induced insulin resistance (as determined by hyperinsulenic euglycemic clamp). A precise characterization of the natural progression of β-cell proliferation and insulin resistance is key to understanding the pathogenesis of type 2 diabetes and the regulation of compensatory β-cell proliferation in response to metabolic and nutritional cues.
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qRT-PCR, quantitative RT-PCR.

Fig. 1. Impaired glucose tolerance occurs within 1 wk of HFD feeding and persists throughout the study. A: high-fat diet (HFD)-fed mice (■) gain weight progressively over an 11-wk period. Weight of chow diet (CD)-fed mice (○) plateaus after week 5. B–F: glucose tolerance tests are shown for weeks 1 (B), 3 (C), 5 (D), 7 (E), and 11 (F). Data are shown as means ± SE [n = 6–9 for body weight and 3–6 for intraperitoneal insulin tolerance test (IPGTT)]. P values were calculated using the unpaired t-test, comparing the difference in weight between CD and HFD within the weekly time points. Significance for IPGTTs was calculated using the 2-way ANOVA with Sidek correction for multiple comparisons. *P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001; ****P ≤ 0.0001.
RESEARCH DESIGN AND METHODS

Experimental animals. With the exception of hyperinsulinemic eu-
glycemic clamp studies, all experiments were performed in the Vander-
bilt University facility. Male C57Bl/6J mice (Jackson Laboratory, Bar
Harbor, ME) were delivered to the Association for Assessment and
Accreditation of Laboratory Animal Care International-accredited Divi-
sion of Animal Care at Vanderbilt University at 7 wk of age. After 1 wk
of acclimatization, mice were weighed and randomly assigned to one of
two groups: 1) CD (11% kcal from fat, Lab Diet 5LJ5; Purina, St. Louis,
MO) and 2) HFD (60% kcal from fat; BioServ F3282, Frenchtown, NJ).
Mice were housed on a 12:12-h light-dark cycle, receiving water and food
ad libitum. Body weight was obtained prior to starting the diets and prior
to each metabolic measurement. Euthanasia was performed at time of
pancreatic dissection, using isoflurane until the mice were unconscious,
followed by cervical dislocation. These methods are consistent with the
Panel on Euthanasia of the American Veterinary Medical Association.
All procedures were conducted in accordance with Vanderbilt University
Division of Animal Care and Use Committee-approved protocols and
under the supervision of the Division of Animal Care. For hyperinsulin-
emic eu-glycemic clamps, male C57Bl/6J mice (Jackson Laboratory, Bar
Harbor, ME) were delivered to the Animal Facility of the Research
Center of the University of Montreal Hospital Centre (CRCHUM) at 7 wk
of age and the clamps performed at 8 wk of age. All procedures were
approved by the Institutional Committee for the Protection of Animals at
the CRCHUM.

Metabolic measurements. Intraperitoneal glucose tolerance tests
(IPGTT) and IPITT were performed as described previously (18). For
IPGTT, animals were fasted for 16 h. Fasting blood glucose was mea-
sured from 2 μl of tail vein blood with an Accuchek glucometer and
glucose test strips (Abbott Diabetes Care). Animals received an in-
traperitoneal (ip) injection of filter-sterilized glucose (2 mg dextrose/g body wt),
and blood glucose was measured at 15-, 30-, 60-, 90-, and 120-min intervals following injection; n = 3 (weeks 3, 7, and 9) or 6 (weeks 1, 5,
and 11). For IPITT, animals were fasted for 6 h. Fasting blood glucose
was measured as described above, and animals received an ip injection of
0.075 U/ml insulin (recombinant human insulin, no. I9278; Sigma-
Aldrich) in filter-sterilized 1X PBS at 0.1 ml/10 g body wt. Subsequent
changes in blood glucose were measured at 15-, 30-, 60-, 90-, and 120-
min intervals following injection; n = 3 (weeks 3, 7, and 9) or 6 (weeks 1, 5,
and 11). Plasma insulin assays were performed as described in (31), with the modification that blood was harvested from the saphen-
ous veins of 16-h-fasted animals using heparinized Natemol blood-
collecting tubes (Kimble Chase, Rockwood, TN).

Hyperinsulinemic eu-glycemic clamps. Two-hour hyperinsulinemic-
euglycemic clamps were performed in 4-h-fasted mice (n = 6 for CD
and 7 for HFD), as described previously (4). Briefly, following a 1-min
bolus insulin infusion (85 mU/kg; Humulin R), insulin was infused at 8
mU/kg·min⁻¹. Twenty percent dextrose was infused beginning 5 min
after the insulin infusion to clamp glycemia at ~120 mg/dl. Insulin levels
during the steady state were measured at 90 and 120 min using the
AlphaLISA kit. The insulin sensitivity index (M/I) was calculated as the
glucose infusion rate (GIR) divided by the average insulinemia during
the last 30 min of the clamp (1); n = 6 CD and 7 HFD.

Tissue preparation and histology. At euthanization, pancreata were
processed as described previously (14). Antibodies were guinea pig
anti-insulin (1:500; Dako, Carpenteria, CA), rabbit anti-Ki67 (1:500;
AbCam, Cambridge, MA), CY2-conjugated anti-guinea pig IgG (1:300;
Jackson Laboratories, Bar Harbor, ME), CY3-conjugated anti-rabbit
IgG (1:300, Jackson Laboratories), and horseradish peroxidase-
conjugated anti-guinea pig IgG (1:300, Jackson Laboratories).

β-Cell mass, β-cell proliferation, and β-cell death. Analysis and
quantification of β-cell mass was performed as described in (18). For β-
cell mass assessment, ~2% of each pancreas was immunolabeled
and analyzed (5–10 sections/animal, each separated by 250 μm).
Slides were scanned at ×20 magnification (Scan Bright field Scope
System; Aperio, Vista, CA), and an algorithm developed from a Genie
macro within Spectrum (Aperio) was used to identify β-cells and
other tissue (15); n = 3 (weeks 1, 3, 7, and 9 for CD and HFD), 5
(week 11 for CD), or 6 (week 5 for CD and HFD and week 11 for
HFD). β-Cell proliferation was determined by immunolabeling sec-
tions ~400 μm apart (~5 slides/animal) for insulin and Ki-67
(1:500; Dako) or insulin and phosphorylated histone H3 (1:200; Cell
Signaling Technology). For Ki-67 labeling, antigen retrieval consisted of microwaving slides for 14 min in 10 mM sodium citrate buffer;
n = 3 (weeks 3, 7, and 9 for CD and HFD), 4 (3 days for CD), 5 (3 days for HFD), or 6 (weeks 1, 5, and 11 for CD and HFD). For pH3 labeling, antigen retrieval was placed in TEG buffer at
pH 9.0 and microwaved on high power for 1 min and then 10% power
for 7.5 min. Nuclei were labeled with 1 μg/ml 4’,6’-diamidino-
2-phenylindole (DAPI, Molecular Probes, Grand Island, NY) and
mounted with Aqua-Mount (Thermo Scientific, Kalamazoo, MI); n =
3 for all time points and diets. Slides were scanned as above. At least

Fig. 2. Area under the curve (AUC) reveals that HFD causes the greatest
changes in late time points of IPGTTs. AUC was determined from 0 to 120 min
(4), 0 to 30 min (B), and 30 to 120 min (C). ○, CD; □, HFD. Data are shown
as means ± SE (n = 3–6). The unpaired t test was used for comparing CD and
HFD areas under the curve. **P ≤ 0.05, comparisons between diets (1); *P
comparision between CD weekly time points; tP comparision between HFD weekly
time points. **P ≤ 0.01; ***P ≤ 0.001; ****P ≤ 0.0001.

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5,000 insulin-positive cells/mouse were counted using MetaMorph software. Calculations were made by dividing the number of insulin/Ki-67 or insulin/ pHH3 co-localized cells by the total number of insulin-positive cells. To assess β-cell death, terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) was performed using the ApoAlert Kit (Clontech) according to the manufacturer’s instructions (16). For TUNEL assay, pancreata (3 sections/animal) from three CD- and three HFD-fed animals were analyzed at each of three time points (3 days, 1 wk, and 11 wk).

Quantitative RT-PCR. Islet RNA from 1-wk-treated mice was isolated, and quantitative RT-PCR (qRT-PCR) was performed as described previously (1). Primer sequences are listed in Table 1. Data are shown as 2^{−ΔΔCt} (24); n = 6.

Metabolomic analysis. Whole liver, epididymal fat, skeletal muscle (gastrocnemius, soleus, and plantaris muscles), bone (fibula and tibia), and plasma were collected from 9-wk-old C57Bl/6J mice either fed a HFD (n = 8) or maintained on a CD (n = 8) for 1 wk. Tissues were dissected, flash-frozen, and kept at −80°C before being shipped to Metabolon (Durham, NC) for metabolite analysis. Sample preparation, instrument analysis, and data processing analysis were performed by Metabolon, as detailed in previous publications (9, 27).

Statistical analysis and calculations. Data are shown as means ± SE (12). P values were calculated with either the two-tailed unpaired Student’s t-test or the two-way ANOVA with the Sidek correction for multiple comparisons as indicated. P values ≤0.05 were considered significant, and P values >0.05 were not reported. For metabolomic data shown in Fig. 5B, any missing values were assumed to be below the detection limit and were imputed with the minimum observed value. Following imputation and log transformation, P values were calculated using the Welch two-sample t-test, and estimates of the false discovery rate (q value) were calculated to account for multiple comparisons, as described previously (36).

RESULTS

Weight gain and impaired glucose tolerance in HFD-fed mice. To correlate the consumption of HFD with changes in body weight, glucose homeostasis, and β-cell growth, 8-wk-old male mice were either maintained on CD or switched to HFD. The average initial body weight for the CD group was identical to the HFD group (20.4 ± 2.1 vs. 20.5 ± 1.5 g, respectively). Mice from each diet were examined at 1, 3, 5, 7, 9, and 11 wk after the start of the study for body weight, glucose homeostasis, β-cell mass, and β-cell proliferation. HFD-fed mice weighed significantly more than CD-fed mice throughout the study, starting with an initial weight gain of 1.1 ± 0.9 g (P = 0.006) in the 1st wk compared with the static weight of the CD group (−0.7 ± 1.5 g, P = not significant) (Fig. 1A). The largest weight increase for both diets occurred between weeks 3 and 5, where CD-fed mice gained 3.9 ± 1.0 g (P = 0.001) and HFD-fed mice gained a similar 3.7 ± 1.1 g (P = 0.008). After week 5, the HFD group continued to gain weight, showing an 8.4 ± 1.5 g (P = 0.0001) increase over the next 6 wk, whereas body weights of CD-fed mice plateaued during this time frame with an insignificant increase in body weight of 1.6 ± 0.8 g.

Changes in glucose tolerance in response to both acute and long-term HFD consumption were tracked by performing an IPGTT every other week. As expected from previously published studies, glucose tolerance was already impaired after 1 wk of HFD and was consistently impaired throughout the study despite unchanged fasting blood glucose (Fig. 1, A–F). To depict subtle but significant changes in glucose tolerance as mice were maintained on HFD, we calculated the area under the curve for glucose (AUCglc) both between diets and between intradiet time points (Fig. 2, A–C). The AUCglc for HFD-fed mice increased gradually between weeks 1 and 9 and culminated with a highly significant increment in AUCglc from weeks 9 to 11 (P = 0.001); the AUCglc for CD-fed mice was relatively unchanged (Fig. 2A). The gradual increase in HFD-fed AUCglc between weeks 1 and 9 was due to an elevation in glucose during the first 30 min of the IPGTTs (Fig. 2B). In fact, the

![Fig. 3. Insulin tolerance was enhanced in HFD-fed mice in week 1 but worsened gradually over time. Insulin tolerance tests are shown for weeks 1 (A and D), 5 (B and E), and 11 (C and F). Results for each time point normalized to fasting blood glucose levels are shown in D–F. ○, CD; ■, HFD. Data are shown as means ± SE (n = 3–6); significance was calculated using 2-way ANOVA with Sidek correction for multiple comparisons. *P ≤ 0.05; **P < 0.01; ***P ≤ 0.001; ****P ≤ 0.0001.](http://ajpendo.physiology.org/)
last 90 min of the IPGTTs remained relatively consistent between intradiet time points, with the exception of week 11 in the HFD-fed group, when a large increase in AUC gluc occurs (Fig. 2C).

Insulin resistance is not apparent with short term HFD feeding, but impaired insulin tolerance develops with prolonged HFD feeding. The progression of impaired glucose tolerance in response to both acute and long-term HFD suggests impairments in insulin secretion and/or insulin sensitivity. First, we determined the effects of short- and long-term HFD feeding on insulin tolerance. Since injection of insulin can dramatically lower blood glucose levels, mice were fasted for only 6 h prior to insulin injection. Fasting blood glucose concentrations were not altered in response to short-term HFD feeding but were elevated with long-term HFD feeding (Fig. 3C). Interestingly, mice fed HFD for 1 wk exhibited a significantly enhanced decline in blood glucose during the latter time points of the IPITT (Fig. 3A), and this was still apparent after normalization to fasting blood glucose (Fig. 3D). In week 5, the blood glucose concentrations of the HFD-fed group were indistinguishable from controls (Fig. 3, B and E). After 11 wk of HFD feeding, we observed elevated blood glucose throughout the test relative to CD-fed mice (Fig. 3C). These data show that insulin tolerance in HFD-fed mice gradually diminished with duration of HFD, whereas the CD-fed mice maintained a consistent response throughout the study. However, after normalization to basal glucose concentrations, insulin tolerance in the long-term HFD-fed mice was only slightly compromised at the later time points (Fig. 3F). Similar to week 5, there was no significant difference between the diets at weeks 3, 7, and 9 (data not shown).

Plasma was assayed every other week for circulating insulin in response to glucose. Although there was a significant increase in plasma insulin in response to the glucose challenge in animals fed HFD for 1 wk, this response was reduced by 50% compared with the response of the CD-fed group (Fig. 4A). The CD-fed plasma insulin response remained consistent through week 11 (Fig. 4, B and C). Plasma insulin values for HFD-fed mice were virtually unchanged for weeks 1–7 (Fig. 4, A and B, and data not shown). As predicted, hyperinsulinemia was apparent in long-term HFD-fed mice (Fig. 4C). Fasting plasma insulin was elevated in HFD-fed mice by week 9 (data not shown) and

Fig. 4. Hyperinsulinemia became evident after 11 wk of HFD feeding. Fasting and glucose-induced plasma insulin secretion were assayed at weeks 1 (A), 5 (B), and 11 (C). Open bars, CD; black bars, HFD. Data are shown as means ± SE (n = 5–6); significance was calculated with the paired t-test and 2-way ANOVA with Sidek correction for calculation of multiple comparisons. *P ≤ 0.05; **P ≤ 0.01.

Fig. 5. Insulin sensitivity is not modified after 1 wk of HFD. A: blood glucose during hyperinsulinemic euglycemic clamp. B: the average glucose infusion rate is calculated during the 90- to 120-min period where blood glucose levels are identical between CD- and HFD-fed mice. C: plasma insulin at 120 min trends higher for HFD mice but is not significantly different from CD mice. D: insulin sensitivity between CD and HFD is not changed, as indicated by similar insulin sensitivity index (M/I) values. Data are shown as means ± SE. Open bars, CD (n = 6); black bars, HFD (n = 7).
exacerbated further in week 11 (Fig. 4C), at which point it was nearly fourfold higher in the HFD-fed group compared with the CD-fed group \((P < 0.02)\). Despite elevated fasting plasma insulin in HFD-fed mice at week 11, they still presented with a moderate but significant response to the initial glucose challenge at 15 min \((P < 0.03)\).

Our data at later time points agree with a wealth of previously published studies demonstrating insulin resistance after prolonged HFD feeding. The more provocative finding of the present study is a lack of apparent insulin resistance 1 wk after initiation of HFD. To more rigorously investigate whether insulin resistance is present after 1 wk of HFD feeding, we performed hyperinsulinemic euglycemic clamps (Fig. 5). The GIR required to maintain blood glucose levels (Fig. 5A) at the target level was not different between the two groups (Fig. 5B). Although circulating insulin levels at the end of the clamp were slightly higher in the HFD-fed group (Fig. 5C), this difference was not statistically significant, and the M/I index was not different between both groups (Fig. 5D). These results suggest that insulin sensitivity was unaltered after 1 wk of HFD.

In humans, elevations of the metabolite \( \alpha \)-hydroxybutyrate (\( \alpha \)-HB) in plasma are indicative of insulin resistance \((11, 13)\). Given the results from the IPITT and hyperinsulinemic euglycemic clamps suggesting lack of insulin resistance 1 wk after initiation of HFD, we queried results from metabolomic analyses of metabolically relevant tissues (including plasma) to determine whether levels of \( \alpha \)-HB were increased in response to HFD at this time point. Metabolomic analysis of liver, skeletal muscle, epididymal fat, bone, and plasma collected from 1-wk-HFD- and -CD-fed mice revealed decreased \( \alpha \)-HB in the liver and adipose tissue of HFD-fed mice; \( \alpha \)-HB was unchanged in other tissues, including plasma, at this time point (Fig. 6).

\( \beta \)-Cell mass expansion begins after 3 wk of HFD consumption. \( \beta \)-Cell mass expansion is well established in long-term HFD feeding studies; however, the timing of this expansion is less clear. To address this, \( \beta \)-cell mass was determined every other week between 1 and 11 wk (Fig. 7). A significant increase in \( \beta \)-cell mass was observed after 3 wk of HFD feeding; however, this increase was not sustained throughout the treatment period. The \( \beta \)-cell mass within each diet group remained relatively constant until week 9, when a significant increase was observed in both groups. Compared with the chow-fed animals, a further increase in \( \beta \)-cell mass was observed between weeks 9 and 11 in the HFD-fed group. Comparison between the two

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**Fig. 6.** Assessment of \( \alpha \)-hydroxybutyrate (\( \alpha \)-HB) in plasma, bone, muscle, liver, and adipose tissue collected from 1-wk-HFD- (gray boxes) and CD-fed mice (white boxes) revealed significantly decreased \( \alpha \)-HB in the liver and adipose tissue of HFD-fed mice. Data are shown as means ± SE; \( P \) values were calculated using the unpaired \( t \)-test \((n = 8)\). *\( P \leq 0.05\); **\( P \leq 0.01\).

**Fig. 7.** \( \beta \)-Cell mass expansion in response to HFD first becomes evident in week 3. \( \beta \)-Cell mass in HFD-fed mice compared with CD-fed mice was slightly elevated in week 3, becoming significantly elevated during weeks 9–11. Open bars, CD; black bars, HFD. Data are shown as means ± SE \((n = 3–6)\); \( P \) values were calculated using the unpaired \( t \)-test. *\( P \leq 0.05\); **\( P \leq 0.01\); ***\( P \leq 0.001\).
Diets at the 11-wk time point revealed a 1.3-fold increase in β-cell mass for HFD-fed mice over CD-fed mice (P = 0.001).

β-Cell proliferation was induced with short-term HFD feeding. To ascertain the kinetics of the β-cell-proliferative response to HFD feeding, pancreata were assayed at different durations of HFD for β-cell proliferation using Ki-67 and pH3 immunolabeling. Whereas CD-fed mice exhibited a constant low percentage of Ki-67/insulin double-positive cells throughout the study, β-cell proliferation in the HFD-fed group was dynamic and enhanced after just 3 days (Fig. 8A). Compared with CD, β-cell proliferation was enhanced 2.6-, 2.2-, 2.9-, and 2.3-fold for the HFD-fed mice at 3 days (P = 0.05), 1 wk (P = 0.0005), 5 wk (P = 0.0001), and 7 wk (P = 0.003), respectively. Similar results were obtained using pH3 as a proliferative marker; the greatest enhancement in β-cell proliferation was observed after only 3 days of HFD, and an increase in β-cell proliferation was maintained at 1 wk of HFD but was no longer apparent at 11 wk of HFD (Fig. 8B).

Since a significant increase in β-cell proliferation was detected within 1 wk of HFD treatment, qRT-PCR was conducted on RNA from isolated islets at 1 wk to analyze changes in gene expression for key proliferative genes. Expression of Ki67, Foxm1, cyclin A2, and cyclin B1 was upregulated 1.6-, 1.6-, 1.7-, and twofold (P = 0.003, 0.0004, 0.03, and 0.01), respectively (Fig. 8C).

Fig. 8. β-Cell proliferation was elevated substantially after 3 days (0.4 wk) of HFD feeding. β-Cell proliferation in CD-fed mice did not significantly change throughout the study, as assessed by immunolabeling for either Ki-67 (A) or phosphorylated histone H3 (B); β-cell proliferation was acutely affected by HFD feeding after only 3 days. C: quantitative RT-PCR for genes positively and negatively associated with β-cell proliferation was performed after 1 wk of diet. Open bars, CD; black bars, HFD. Data are shown as means ± SE (n = 3–6); significance was calculated with the paired t-test and 2-way ANOVA with Sidak correction for calculation of multiple comparisons. *P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001; #P < 0.0597.
respectively, in 1-wk-HFD-fed mouse islets compared with CD islets. Expression of cyclins D1 and D2 was unchanged. Expression of the cell cycle inhibitors cdkn2A (p16), cdkn1A (p21), and cdkn1B (p27) was not changed significantly in HFD-fed mice (2, 24, 28, 29, 39, 42). Our study agrees with a previous report that impaired glucose tolerance occurs after 3 days of HFD and is maintained through 12 wk of HFD feeding (44). However, this previous study utilized only AUC to report the IPGTT results. The present study clearly indicates that the impaired glucose tolerance observed 1 wk after HFD feeding is confined to the 30-min time point, suggesting an intact early insulin response after short-term HFD feeding and a diminished second-phase response. Whereas IPGTTs for CD-fed animals remained relatively steady from week to week throughout the study, the HFD-fed group showed worsening glucose tolerance with increased diet duration, particularly during the first 30 min. This effect may be attributable to a number of factors, including adiposity-related changes in the rate of glucose absorption and impaired first-phase insulin secretion. Interestingly, the latter 90 min of the HFD-fed IPGTTs did not deviate until week 11. A significant increase in circulating insulin in response to glucose was observed for both diets at week 1; however, the insulin levels in HFD-fed mice were not sufficient to maintain proper glucose homeostasis. Although the elevated fasting plasma insulin in week 11 HFD-fed mice suggested insulin resistance, there was still an intact response to glucose injection.

Mice fed HFD for 1 wk showed a more significant decline in blood glucose in response to exogenous insulin than CD-fed mice. These data are supported by a previous report where nonfasted 1-wk-HFD-fed mice exhibited increased insulin sensitivity, which becomes apparent when results in that study are normalized for fasting blood glucose (35). On the surface, it appears that HFD-fed mice are more insulin sensitive; however, further scrutiny suggests otherwise. Insulin has an estimated circulating half-life of 10 min in mice (8); therefore, after the first 30 min of the IPITT, all exogenous insulin should be cleared. The blood glucose measurements between 30 and 60 min are more indicative of secondary effects (e.g., counter-regulatory hormone responses). Interestingly, a study done in humans more than four decades ago reported a similar phenomenon where “mild” diabetic test subjects were more insulin tolerant during the latter time points of the test (40). These authors suggested that this may be due to increasing endogenous insulin levels in the “mild” diabetic patients; contrary to this, our plasma insulin data imply that the lowering of blood glucose during the latter part of the IPITT is not due to an increase in endogenous insulin. Thus, at present it is unclear why 1 wk HFD feeding appears to improve insulin tolerance, but these results warrant further investigation.

### Table 2. Summary of results

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</table>

HFD, high-fat diet; ND, not determined. All caparisons are with animals on control diet for the same duration.
β-Cell proliferation was increased as early as 3 days after HFD feeding was initiated, a finding supported by the upregulation of key β-cell-proliferative genes at 1 wk. FoxM1, a transcription factor required for postnatal β-cell proliferation and elevated in islets from HFD-fed mice (14, 47), is activated by the G2 cyclin, cyclin A2 (21). Furthermore, cyclin B1 is a downstream target of FoxM1 during the G2/M transition, whereas cyclins D1 and D2, predominant during G1, are unaffected by Foxm1 overexpression (23). These data are further supported by a recent study that found similar changes in proliferative gene expression in islets from mice fed HFD for 1 wk (35). The impaired glucose tolerance and diminished plasma insulin concentration after 1 wk of HFD feeding may be due in part to this observed increase in β-cell proliferation. Oncology studies have shown that proliferating cells effectively switch to a less efficient metabolism (45), and although controversial, some studies suggest that proliferating β-cells are partially dedifferentiated (reviewed in Ref. 17). Therefore, actively proliferating β-cells may temporarily experience decreased functionality. Although maximal β-cell proliferation is observed after 3 days or 1 wk of HFD, a detectable increase in β-cell mass is not observed until 3 wk. A delay between increased β-cell proliferation and a detectable increase in β-cell mass was not unexpected, as this also occurs in maternal islets during pregnancy, where productive cell divisions must accumulate to translate into measurable increases in β-cell mass. We also assessed whether there was an increase in β-cell death in response to HFD feeding that might explain the delay in β-cell mass increase but were unable to detect any increase in TUNEL-labeled insulin+ cells in the HFD-fed animals compared with controls at 3 days, 1 wk, or 11 wk of HFD (data not shown).

Based on previous studies that reported HFD-induced insulin resistance in as few as 3–7 days after initiation of HFD feeding (2, 22, 32, 44), we expected to detect symptoms of insulin resistance in 1-wk-HFD-fed mice. Instead, the present studies suggest that insulin resistance occurs much later, after at least 5 wk of HFD-feeding. The later time points are concurrent with an increase in insulin resistance, as evidenced by the growing impairment in glucose tolerance, hyperinsulinemia, and the worsening insulin tolerance in weeks 9 and 11. The absence of insulin resistance after 1 wk of HFD was supported by similar M/I indices in hyperinsulinemic euglycemic clamps. Metabolomics assessment of α-HB, a plasma biomarker of insulin resistance in humans (11), confirmed the absence of insulin resistance in multiple tissues of 1-wk-CD- and -HFD-fed mice, thus validating this biomarker in mouse models. Our results are supported by two studies; one study failed to detect insulin resistance in 1-wk-HFD-fed mice, and another measured insulin resistance in skeletal muscle, adipose tissue, and liver and found that insulin resistance was detectable after 3 wk of HFD feeding (28, 35). Together, these results suggest that β-cells initially proliferate in response to a change in diet or an unknown HFD-induced factor before they respond to insulin resistance. As HFD consumption continues, insulin resistance increases, and hyperinsulinemia and enhanced β-cell mass ensue. However, at later stages of HFD feeding, β-cell proliferation declines and glucose tolerance becomes more impaired.

In conclusion, our data suggest that β-cell proliferation in response to HFD feeding is highly dynamic, occurring within 3 days of starting a diet high in fat. This is exciting evidence showing that adult pancreatic β-cells can indeed respond robustly to proliferative cues without the complications of insulin resistance. To our knowledge, this is the first study to definitively indicate that HFD-induced β-cell proliferation precedes overt insulin resistance, although this was suggested by the study from Stamateris et al (35). These studies lay the groundwork for defining the proliferative signals that are present in response to HFD.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

R.E.M., V.P., and M.G. conception and design of research; R.E.M., M.F.M., R.S.M., J.C.D., B.A.C., K.A., and K.P. performed experiments; R.E.M., V.S.M., J.C.D., B.A.C., K.P., and M.G. analyzed data; R.E.M., K.P., and M.G. interpreted results of experiments; R.E.M., B.A.C., K.P., and M.G. prepared figures; R.E.M. drafted manuscript; R.E.M., K.A., K.P., V.P., and M.G. edited and revised manuscript; B.A.C. and M.G. approved final version of manuscript.

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