Successful versus failed Adaptation to High Fat Diet induced Insulin Resistance; the role of IAPP induced Beta Cell Endoplasmic Reticulum Stress.

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ABSTRACT

Objective. Obesity is a known risk factor for Type 2 diabetes (T2DM). However, most obese individuals do not develop diabetes because they adapt to insulin resistance by increasing beta-cell mass and insulin secretion. Islet pathology in T2DM is characterized by beta-cell loss, islet amyloid derived from islet amyloid polypeptide (IAPP) and increased beta cell apoptosis characterized by endoplasmic reticulum (ER) stress. We hypothesized that IAPP-induced ER stress distinguishes successful versus unsuccessful islet adaptation to insulin resistance.

Research Design and Methods. To address this we fed wild type (WT) and human IAPP transgenic rats (HIP rats) either 10 weeks of regular chow or high fat diet and prospectively examined the relationships between: 1) beta cell mass and turnover, 2) beta cell ER stress 3) insulin secretion and 4) insulin sensitivity.

Results. A high fat diet led to comparable insulin resistance in WT and HIP rats. WT rats compensated with increased insulin secretion and beta cell mass. In contrast, in HIP rats neither beta cell function or mass compensated for the increased insulin demand, leading to diabetes. The failure to increase beta cell mass in HIP rats was due to ER stress induced beta cell apoptosis that increased in proportion to diet induced insulin resistance.

Conclusions. IAPP-induced ER stress distinguishes the successful versus unsuccessful islet adaptation to a high fat diet in rats. These studies are consistent with the hypothesis that IAPP oligomers contribute to increased beta-cell apoptosis and beta cell failure in humans with Type 2 diabetes.
Insulin resistance, most often attributed to high-caloric food intake and consequent obesity (1), is a well characterized risk factor for Type 2 diabetes (T2DM) (2,3). However, most obese individuals (>80%) do not develop diabetes (4) because they are able to compensate for insulin resistance by an adaptive increase in insulin secretion (5, 6). T2DM develops as a result of a failure to adequately increase insulin secretion to meet demands of insulin resistance (5,7,8). Consistent with this, genome wide studies imply that the genetic variance that underlies predisposition to T2DM is manifest in pancreatic beta cells (9-11). By implication there is a subset of individuals in the general population at risk of developing T2DM if subject to insulin resistance. These observations raise the question, what underlies the failure of beta cell adaptation to insulin resistance in those predisposed to T2DM?

Some insights arise from the pathology of the islet in T2DM. There is a 60% deficit in beta cells in individuals who are obese with T2DM compared to those comparably obese but non diabetic (12,13). The islet in T2DM is also characterized by islet amyloid derived from islet amyloid polypeptide (IAPP) (12). High expression rates of human-IAPP in some rodent models recapitulate this islet pathology and lead to diabetes (14-17). For example, in the HIP rat (Sprague Dawley rats transgenic for human IAPP) there is a gradual decline in beta cell mass leading to impaired fasting glucose at 5 months of age and diabetes by 10 months of age (16,18). In both humans with T2DM (12) and rodents with high expression rates of human IAPP, there is increased beta-cell apoptosis characterized by endoplasmic reticulum (ER) stress (19,20). Therefore beta cell loss in T2DM has many parallels with that in neurodegenerative diseases also characterized by protein misfolding (21). For example, in Alzheimer’s and Parkinson’s disease, neuronal cell loss is characterized by increased ER stress induced apoptosis accompanied by formation of toxic oligomers of locally expressed amyloidogenic proteins (22). Collectively these diseases have been referred to as protein misfolding diseases.

In occasional families, protein misfolding diseases can be attributed to a mutation that increases the propensity of the respective amyloidogenic protein to aggregate (23). However in most cases, the basis for protein misfolding is not due to a mutant amyloidogenic protein but rather a consequence of an imbalance between the delivery of nascent protein to the ER and the capacity of the ER to fold, traffic and process the protein (22). Support for the concept of a threshold of expression rate above which ER fails to adequately process IAPP is provided by human IAPP transgenic rodent models recently reviewed elsewhere (24). Given the complexity of ER function (including calcium homeostasis, chaperone proteins, glycosylation, clearance of misfolded proteins) it is likely that this threshold varies widely between individuals within the population, perhaps contributing to the genetic variance in predisposition to T2DM. In this regard it is of note that IAPP expression increases with insulin resistance (25).

It is therefore plausible that the link between the increased risk for T2DM in obesity is mediated through an imbalance between the delivery of nascent IAPP to the ER and the capacity of the ER to fold, traffic and process this amyloidogenic protein in vulnerable individuals. It is not possible to address this hypothesis experimentally in humans. To gain insight...
into this postulate, we undertook studies in wild type (WT) Sprague Dawley rats and Sprague Dawley rats transgenic for human IAPP (HIP rats) on a high fat diet. We hypothesized that WT rats would adapt to a high fat diet to remain non diabetic where as HIP rats would fail to adapt and develop diabetes. This hypothesis was affirmed, permitting us to further establish the molecular, pathological and physiological basis of successful versus failed adaptation to a high fat diet in vivo as well as at the level of the pancreas.

**EXPERIMENTAL PROCEDURES**

**Animal housing, diet administration and surgical procedures.** A total of 54 Sprague Dawley rats (WT; n=25) and rats expressing h-IAPP (HIP rats; n=29) were used. The generation of the h-IAPP transgenic rats has been described in detail previously (16). Rats were bred and housed individually throughout the study at the University of California Los Angeles animal housing facility and subjected to standard 12-hour light-dark cycle. The University of California Los Angeles Institutional Animal Care and Use Committee approved all surgical and experimental procedures. At 2 months of age WT and HIP rats were randomly assigned to either high fat diet (HFD) (60% Fat, 20% protein and 20% carbohydrates, Research Diets Inc, New Brunswick, NJ) or control regular chow diet (10% Fat, 20% protein and 70% carbohydrates, Research Diets Inc, New Brunswick, NJ) and fed ad libitum for 10 weeks. Following 10 weeks of either diet treatment animals were anesthetized with isoflurane (2.5%) by inhalation until effect and indwelling catheters were then inserted into the right internal jugular vein and left carotid artery for subsequent in-vivo metabolic studies as previously described (26). All catheters were filled with 100 U/ml Heparin/Saline solution, exteriorized to the back of the neck and encased in the infusion harness (Instech Inc, PA). After surgery all rats maintained preoperative body weight, had normal food intake and mean hematocrit (>40%).

**Hyperglycemic Clamp and arginine bolus injection.** To assess glucose and arginine-stimulated insulin secretion HIP (n=10), HIP+HFD (n=9), WT (n=6) and WT+HFD (n=12) rats underwent a hyperglycemic clamp followed by an arginine bolus injection as previously described (18). In brief, following a 30 min equilibration period, plasma samples were taken for measurements of fasting glucose, insulin, c-peptide and free fatty acids. Thereafter animals received an intravenous glucose bolus (375 mg/kg) followed by a variable 50% (wt/vol) glucose infusion to clamp arterial glucose at ~250 mg/dl (0-70 min). At t=60 min rats received a bolus injection of L-Arginine solution (1 mmol/kg: Sigma, St. Louis, MI). Arterial blood samples (50ul) were taken at baseline (-30 and 0 min), at 1, 5 min and every 15 minutes thereafter during the clamp for immediate determination of plasma glucose and subsequent analysis for insulin and c-peptide.

**Hyperinsulinemic-Euglycemic Clamp + ^3^H-glucose infusion.** To assess insulin sensitivity and glucose turnover HIP (n=7), HIP+HFD (n=7), WT (n=6), and WT+HFD (n=7) underwent a hyperinsulinemic-euglycemic clamp with concomitant infusion of [3-^3^H] glucose to assess glucose turnover as previously described (18). Briefly, rats received primed (3μCi) continuous (0.05μCi/min) infusion of [3-^3^H] glucose; Perkin Elmer, Boston, MA) for a 90 minutes basal period increased to 0.2μCi/min for 120 minutes throughout the hyperinsulinemic-
euglycemic clamp. Plasma glucose was determined every 10 minutes and additional blood samples (~150 µl) for determination of tracer specific activity at fasting were drawn from (~40 to 0 min) and during insulin infusion from (120 to 150 min).

Pancreas morphology. At the end of the hyperinsulinemic-euglycemic clamp, rats were euthanised and the pancreas was then rapidly removed and fixed in 4% paraformaldehyde overnight at 4°C. Paraffin embedded pancreatic sections were stained first for hematoxylin/eosin, insulin (guinea-pig anti-insulin, 1:100; Zymed, Carlsbad, CA). The β-cell mass was measured by first quantifying the pancreatic cross-sectional area positive for insulin and multiplying this by the pancreatic weight. β-cell size was determined as a mean distance between neighboring insulin positive nuclei in 10 representative islets per animal. β-cell nuclear diameter was determined as a mean of (of 180 measurements per nucleus) nuclear diameter in insulin positive nuclei in 10 representative islets per animal. In addition, sections were co-stained by immunofluorescence for insulin (guinea-pig anti-insulin, 1:100; Zymed, Carlsbad, CA) and terminal deoxynucleotidyl transferase biotin-dUTP nick end-labelling (TUNEL method, Roche Diagnostics, Mannheim, Germany) for quantification of beta-cell apoptosis, and insulin (guinea-pig anti-insulin, 1:100; Zymed, Carlsbad, CA) and Ki-67 (mouse anti Ki-67, 1:50; Dako, Carpinteria, CA) for determination of beta-cell replication. Additional sections were co-stained by immunofluorescence for insulin (guinea-pig anti-insulin, 1:100; Zymed, Carlsbad, CA) and CHOP (rabbit anti-CHOP, Santa Cruz Biotechnology) as previously described in detail (19; 20). All islets per pancreatic section (~100 islets) were examined in detail at ×200 magnification (×20 objective, ×10 ocular) for the total number of TUNEL, Ki-67 and CHOP positive beta cells. The frequency of TUNEL, Ki-67 and CHOP expression in each animal was presented as total number of stained beta-cells per total number of islets. Fluorescent slides were analyzed and imaged using a Leica DM600 microscope (Leica Microsystems, Wetzlar, Germany) and images acquired using OpenLab software (Improvision) and analyzed using ImagePro Plus software.

Islet Isolation and western blotting. To obtain rat islets, HIP (n=4), HIP+HFD (n=4), WT (n=3) and WT+HFD (n=3) rats were sacrificed and islets were isolated as previously described (19). For Western blotting experiments, islets were washed with ice cold PBS and lysed in 2x Laemmli sample buffer immediately following the isolation procedure. Proteins (50 µg/lane) were separated on a 4–12% Bis-Tris NuPAGE gel and transferred to polyvinylidine fluoride membranes (Bio-Rad). Membranes were blocked with 5% nonfat dry milk in Tris-buffered solution (TBS)/0.1% Tween-20 and incubated overnight at 4°C with, anti–C/EBP homologous protein (CHOP) (1:500; Santa Cruz Biotechnology, Santa Cruz, CA), anti–P-PE RK (1:2,000; Cell Signaling Technology, Beverly, MA), anti–GRP78 (1:10000, Stressgen, Victoria, BC, Canada), anti–hiAPP (1:6000, 25–37 aa; Peninsula Laboratories, San Carlos, CA), anti–insulin (1:100, Zymed, Carlsbad, CA) and anti–GAPDH (1:8000, Cell Signaling Technologies, Beverly, MA). Membranes were washed with TBS/0.1% Tween-20 and incubated with horseradish peroxidase–conjugated secondary antibodies (1:3,000; Jackson Laboratories, Bar Harbor, ME) for 1 h. After washes, proteins were visualized...
using enhanced chemiluminescence (Bio-Rad) and protein expression levels were quantified using UN-SCAN-IT software (Silk Scientific Inc., Orem, UT).

**Analytical procedures.** Plasma glucose concentrations were measured by the glucose oxidase method (Beckman Glucose Analyzer 2, Fullerton, CA). Plasma insulin and c-peptide levels were measured using ELIZA assay (Alpco Diagnostics, Salem NH). Plasma free fatty acid levels were measured using in-vitro standard enzymatic colorimetric method (WAKO Chemicals, Richmond, VA). Plasma glucose specific activity, hepatic glucose production and glucose disposal was calculated as previously described in detail (18).

**Statistical analysis.** Statistical analysis was performed using ANOVA analysis with Fisher’s post-hoc where appropriate. Regression analysis was performed using Statistica, version 6 (Statsoft, Tulsa, OK). Date in graphs and tables are presented as means ± SEM. Findings were assumed statistically significant at the P<0.05.

**Results**

**Development of diet-induced diabetes in HIP rats on high fat diet.** Food intake, body weight and plasma free fatty acid levels increased comparably in WT and HIP rats on a high fat diet. (Fig. 1B,C and Table 1). The fasting plasma glucose concentrations in HIP and WT rats were comparable at the beginning of the study (p=0.85 between groups). On a regular chow diet fasting plasma glucose values remained unchanged in the WT rats but increased to the impaired fasting glucose concentration range in the HIP rats consistent with prior studies over the same age range (16,18). On the high fat diet there was a modest increase in blood glucose in WT rats (110 ± 3 vs 98 ± 2 mg/dl; p=NS for WT+HFD vs. WT, Fig. 1A) but in contrast HIP+HFD rats developed diabetes (178 ± 20 mg/dl; p<0.0001 vs. all other groups, Fig. 1A). While there was no relationship between change in either body weight or food intake and the fasting blood glucose concentration in WT rats, both food intake (r=0.9, P<0.01) and increase in body weight (r=0.9, P<0.01) during the 10 week study predicted the subsequent fasting blood glucose concentrations in HIP rats (Fig. 1D and E).

**Failure of the compensatory increase in beta-cell mass in HIP rats.** The HFD provoked a 46% increase in β-cell mass in WT rats (13 ± 2 vs. 19 ± 2 mg, WT vs. WT+HFD; p<0.05), but no change of beta cell mass in HIP rats (12 ± 1 vs. 13 ± 2 mg, HIP vs. HIP+HFD; P=NS) (Fig. 2A and B). The increase in β-cell mass in WT rats on a HFD was due to β-cell hyperplasia rather than hypertrophy as β-cell size and nuclear diameter was unchanged (Fig. 2D and E). Beta cell apoptosis was increased almost 13 fold (0.04±0.006 vs. 0.003±0.001 cells/islet, Fig 3A and C, p<0.001) in HIP versus WT rats on a regular diet but this was partially offset by a 6 fold increase in beta cell replication (0.018±0.003 vs. 0.003±0.003 cells/islet, Fig 3B and D). However, while there was no increase (p=0.22) in beta cell apoptosis in WT rats on a HFD, beta cell apoptosis increased still further in HIP rats on a HFD (Fig. 3A and C, p<0.0001, 34-fold versus WT rats). In contrast, there was no further increase in beta cell replication in HIP rats on HFD (Fig. 3B and D).

**Unfolded Protein Response and ER stress and high fat feeding in WT versus HIP rats.** To further elucidate the underlying mechanism by which a HFD led to increased beta cell apoptosis in the HIP rat but not the WT rats, we next
investigated the unfolded protein response (p-PERK and GRP-78) and ER stress by examining the protein expression and nuclear co-localization of the ER stress marker CHOP. In WT rats, the HFD increased both PERK phosphorylation and GRP78 expression in relation to the primary ER client protein insulin (Fig. 4A-C). In HIP rats, P-PERK and GRP78 expression were already increased on a regular chow diet in comparison to WT rats in relation to insulin expression. However in HIP rats neither P-PERK or GRP78 expression were further increased in response to the high fat diet (Fig. 4B and D) despite the further increased expression of both insulin and IAPP, so the protective unfolded protein response was not further increased in the high fat fed despite this marked increased ER load. Nuclear CHOP expression was 13-fold increased in HIP vs. WT rats on a regular diet (0.049±0.011 vs. 0.0037±0.002 β-cells/islet, P<0.01; Fig. 4D and E) and was increased further in HIP, but not WT rats with a HFD (0.049 ± 0.011 vs. 0.09 ± 0.01 β-cells/islet, P<0.01; Fig. 4D and E). Furthermore, there was a positive correlation between the frequency of β-cell nuclear CHOP and β-cell apoptosis (r=0.6, P<0.001; Fig. 4F). Western blot analysis of protein lysates from isolated islets confirmed the immunohistochemically detected increased CHOP protein expression in HIP rats on a regular diet increased further on the HFD (Fig. 4A). These data reveal that a high fat diet exacerbated ER stress induced apoptosis in the HIP rat, but did not induce ER stress in WT rats, implying that the propensity of h-IAPP to form toxic oligomers is an important link between a high fat diet and ER stress induced beta cell apoptosis.

HIP rats fail to adaptively increase fasting, glucose-stimulated and arginine-stimulated insulin secretion in response to high fat diet, and have impaired hepatic insulin clearance. In the fasting state insulin secretion was impaired in HIP rats on a high fat diet as illustrated by failure to increase C-peptide concentrations despite hyperglycemia (Fig 5A, D and G). In contrast, C-peptide concentrations increased by 1.6 fold in WT rats on a HFD (1.9±0.2 vs. 3.1±0.5 ng/ml, P<0.05, Fig. 5A, D and G) in response to a HFD. In contrast, while insulin secretion was impaired in HIP rats in the fasting state (evaluated by C-peptide concentrations), systemic insulin concentrations were comparable to those in WT rats (Table 1), indicating that hepatic insulin clearance of endogenously secreted insulin was decreased in HIP rats on a chow or HF diet. In order to evaluate glucose mediated insulin secretion under comparable conditions we performed hyperglycemic clamp studies in which by design plasma glucose levels were maintained at 265±10 mg/dl (Supplemental Fig 1). In response to HFD, in WT rats the first phase insulin response to glucose was increased by 1.7 fold (966±90 vs. 1,677±108 pmol/l, p<0.01, WT vs. WT+HFD; Fig 5B, E and H) and the second phase by 2 fold (705±33 vs 1,462±116 pmol/l, p<0.01, WT vs. WT+HFD, Supplemental Fig. 1). In contrast, in HIP rats there was a markedly blunted first and second phase response to hyperglycemia and no increment with a HFD (Fig. 5B, E and H). Likewise, while there was an 1.5 fold increase in arginine stimulated insulin release in WT rats in response to a HFD, there was no increase in arginine induced insulin secretion at the same glucose concentration in the HIP rat on a regular
or HFD (Fig 5C, F and I). Consistent with these findings we observed a positive correlation between beta-cell mass and fasting C-peptide (r=0.5, p<0.01; Fig. 5D and G), first phase insulin response to glucose (r=0.4, p<0.01; 5E and H) and first phase insulin response to arginine (r=0.5, p<0.01; 5F and I).

**HIP rats on high fat diet exhibit increased fasting and insulin-stimulated hepatic glucose production.** To further elucidate the relationship between hepatic and extrahepatic insulin sensitivity and beta cell failure in HIP rats exposed to a HFD we undertook hyperinsulinemic-euglycemic clamps while measuring glucose turnover. By design, plasma glucose and insulin concentrations were comparable during the hyperinsulinemic-euglycemic clamps in HIP and WT rats on regular chow or HFD (Fig. 6A). Whole-body insulin sensitivity, assessed by the mean glucose infusion rates during the hyperinsulinemic clamp was comparably decreased by HFD in HIP and WT rats (Fig. 6B). The isotope dilution technique revealed that fasting hyperglycemia in the HIP+HFD group was attributed to a 2 fold increase in fasting hepatic glucose production (Fig. 6C, p<0.05 vs. all other groups). Furthermore, in HIP rats there was hepatic insulin resistance (impaired suppression of hepatic glucose release during the clamp). (Fig. 6C). Isotopically measured insulin–stimulated glucose uptake was comparably decreased by HFD in both HIP and WT rats (Fig. 6D). In conclusion a HFD had its primary effect on insulin sensitivity by inducing extrahepatic insulin resistance, while the HIP rat has a selective defect in hepatic insulin sensitivity independent of the HFD.

**The interaction between insulin sensitivity and successful versus failed beta cell adaptation.** In summary, WT and HIP rats provided either a regular chow or HFD for ten weeks had an average daily food intake that varied from 100-200 Kcal/day. Weight gain and subsequent insulin resistance was proportionate to average food intake which was comparable in WT and HIP rats. In WT rats beta cell mass and function successfully adapted to insulin resistance. The adaptive increase in beta cell mass was accomplished by increased beta cell replication in the absence of any increase in beta cell apoptosis (Fig 7B, D, F). In contrast, in HIP rats neither beta cell function or mass adaptively increased to the increased demand imposed by a high fat diet (Fig 7E). In HIP rats, increasing insulin resistance provoked increased beta cell apoptosis (Fig 7A) but with no effective increase in beta cell replication (Fig 7B).

**DISCUSSION**

A high fat diet leads to obesity and insulin resistance (1) that in most individuals is compensated by increased beta cell mass and insulin secretion (6,12). In those vulnerable to T2DM, hyperglycemia occurs as a consequence of impaired insulin secretion in response to insulin resistance (27,28) with characteristic islet pathology including a deficit in beta cell mass (12,13), islet amyloid derived from IAPP and ER stress induced beta cell apoptosis (20,29). In the present study we sought to gain insights into the mechanisms underlying the appropriate adaptive versus maladaptive beta cell response to high fat diet induced insulin resistance.

In humans, prospective measurements of the adaptive changes in β-cell mass and corresponding changes in insulin secretion and insulin resistance in response to obesity are unavailable,
because longitudinal studies of pancreatic morphology cannot be performed. Cross-sectional studies of islet morphology in humans report a 50% increase in beta-cell mass in obese individuals compared to their lean counterparts (12). Similarly, cross-sectional studies that examined changes in insulin secretion and insulin sensitivity in obesity, report an ~1.5–3 fold increase in fasting and meal-stimulated insulin secretion (6,7,28) and ~ 50% decrease in insulin sensitivity (28, 30). Here we report that 10 weeks of high fat feeding initiated at two months of age in the Sprague Dawley rat induced a 46 percent increase in beta-cell mass, 1.5 to 2 fold increase in fasting and glucose-stimulated insulin secretion, in response to a 50% decline in whole body insulin sensitivity. Therefore the adaptive changes in the WT rat to a high fat diet recapitulate those observed in non-diabetic obese humans. Here, we report for the first time in the same animals, that the adaptive changes in insulin secretion in WT high fat fed animals is closely related to the adaptive increase in beta-cell mass.

In contrast to WT rats, HIP rats failed to adaptively increase beta-cell mass and insulin secretion in response to high fat diet-induced insulin resistance. Beta cell replication was increased in HIP rats compared to WT rats at baseline, but this increased beta cell replication was offset by increased beta cell apoptosis so that beta cell mass was comparable in WT and HIP rats. However beta cell replication failed to increase further in HIP rats on a high fat diet, despite a marked further increase in beta cell apoptosis, consistent with prior studies showing that newly forming beta cells have increased vulnerability to IAPP induced apoptosis (31). A primary goal of the present studies was to gain insights into the successful versus failed adaptation to high fat induced obesity. Having noted that failure to expand beta cell mass in HIP rats fed a high fat diet was due to ER stress induced beta cell apoptosis, we turned our attention to the role of the ER in the successful versus failed adaptation to a high fat diet.

The ER in the beta-cell is well developed to permit synthesis, folding, processing and export of the primary client secretory proteins, insulin and IAPP. The unfolded protein response (UPR) is an important adaptive regulatory mechanism that seeks to balance the rate of delivery of nascent proteins into the ER with the capacity of the ER to fold and export these proteins (32). The UPR accomplishes this both by constraining the rate of protein synthesis as well as enhancing the capacity of this system, for example by increasing the ER chaperone protein BIP that binds to all nascent ER proteins as they are inserted into the ER (33). Evidence that the UPR was intact in WT rats is provided by increased PERK phosphorylation and GRP78 expression in relation to the primary ER client proteins insulin and IAPP. In contrast, while PERK phosphorylation and GRP78 expression were increased in HIP rats on a regular chow diet in comparison to WT rats, in relation to insulin expression, the UPR was not further increased in response to the high fat diet. It is therefore plausible that high expression rates of oligomeric proteins (such as human IAPP) may lead to ER stress in part due to the failure of an adequate unfolded protein response. Consistent with action of CHOP to mediate ER-stress–induced apoptosis we observed a similar 2 fold increase in beta-cell apoptosis in response to high fat diet in HIP, but not in WT rats. Moreover, there was a positive correlation between
nuclear CHOP and beta-cell apoptosis \( (r=0.6, P=0.0001; \text{Fig. 4F}) \).

To underscore the postulate that beta cell ER stress induced apoptosis is related to the extent of insulin resistance in those genetically vulnerable, the frequency of beta-cell apoptosis was positively related to the degree of insulin resistance in HIP rats \((P<0.05)\), but not WT rats \((P=0.6)\). The close parallels in the islet pathology between the high fat fed HIP rat and obese humans with T2DM (beta cell deficit due to ER stress induced beta cell apoptosis) are consistent with the postulate that humans genetically at risk of T2DM, like the HIP rat, have a marginal capacity for ER to traffic IAPP that is readily overcome by induction of insulin resistance. In contrast humans who are not genetically vulnerable to T2DM are capable of adapting to insulin resistance and so presumably have a much higher threshold for expression and trafficking of IAPP.

Here we note that both WT and HIP rats developed comparable insulin resistance in extrahepatic tissues on a high fat diet. However hepatic insulin resistance in response to high fat feeding was more prominent in the HIP rats. We previously reported that the loss of beta cell mass in the HIP rat leads to hepatic insulin resistance \((\text{18})\). One possible explanation for this finding is that the impaired pulsatile delivery of insulin to the liver leads to hepatic insulin resistance. In health, insulin is secreted in discrete insulin secretory bursts that present the liver with an insulin concentration profile that oscillates with an amplitude of ~1000 pmol/l in the fasting state and up to 5000 pmol/l after meal ingestion \((\text{34, 35})\), an observation recently confirmed in-vivo in rats \((\text{26})\). The magnitude of these bursts is substantially decreased in patients with Type 2 diabetes \((\text{36})\) and in animal models of reduced beta-cell mass \((\text{37, 38})\), including the HIP rat \((\text{Unpublished observations})\). It has been proposed that pulsatile mode of insulin delivery to liver increases hepatic insulin sensitivity \((\text{39, 40})\), possibly by avoiding insulin receptor down regulation and/or sustained activation of AKT with attendant feedback inhibition of IRS-2 \((\text{41})\). We postulate that the more prominent hepatic insulin resistance in the HIP rat on high fat diet (and in obese patients with Type 2 diabetes) may relate at least in part to the decline in \(\beta\)-cell mass leading to an abnormal pattern of insulin delivery to the liver.

In the current study we also document a decrease in hepatic insulin clearance in HIP rats fed high fat diet. While fasting insulin levels in HIP rats increased almost 3 fold in response to high fat diet, C-peptide levels remained relatively unchanged (Table 1). Thus in the HIP rat the C-peptide to insulin molar ratio, a measure of insulin clearance, was reduced by 40% in high fat fed HIP rats. We have previously reported that the amplitude of insulin pulses directed to the liver is directly related to hepatic insulin clearance in humans \((\text{42})\). Consistent with this hypothesis hepatic insulin clearance is decreased in animal models of reduced beta-cell mass/insulin pulse mass as well as in humans with diabetes \((\text{37,38,43,44})\). Since hepatic insulin extraction depends on the amplitude of insulin pulses presented to the liver \((\text{42})\), deficits in insulin secretion, at least in the fasting state, would be largely offset in the systemic circulation by decreased hepatic insulin clearance.

In summary, a high fat diet induced a comparable increase in expression of IAPP and induction of UPR in beta cells irrespective of amyloidogenic potential of
expressed IAPP. However, despite the induction of the UPR, a high fat diet still led to beta-cell ER stress mediated apoptosis in the HIP but not WT rats, with islet pathology that recapitulates that in humans with T2DM. Specifically the beta-cell mass was deficient in the high fat fed HIP rats, because the increased beta cell apoptosis prevented an appropriate adaptive increase in beta cell mass. The consequence of beta cell ER stress and a deficit in beta cell mass in high fat fed HIP rats was impaired insulin secretion and hyperglycemia. Of interest, this beta cell failure led to a further exacerbation of the high fat diet induced insulin resistance in the HIP rat.

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Table 1.
(Metabolic characteristics of HIP and WT rats following 10 weeks on high fat (HFD) or regular chow diet)

|                  | WT      | WT+HFD  | HIP     | HIP+HFD |
|------------------|---------|---------|---------|---------|
| n                | 6       | 13      | 10      | 11      |
| Weight (g)       | 571±27  | 725±30* | 541±15  | 705±30* |
| Fasting Glucose (mg/dl) | 98±2    | 110±3   | 121±3   | 178±20‡ |
| Fasting Insulin (pmol/l) | 299±57  | 610±80* | 179±28  | 518±113*|
| Fasting C-Peptide (ng/ml) | 1.9±0.2 | 3.1±0.5*§ | 1.3±0.2 | 1.8±0.2 |
| Fasting FFA (mmol/l) | 0.4±0.1 | 1.1±0.4 | 0.5±0.1 | 0.8±0.2 |

Data are expressed as mean ± SEM.
*P<0.05 for HFD vs. regular chow, ‡P<0.05 for HIP+HFD vs. all other groups, §P<0.05 for WT+HFD vs. all groups
Figure 1. Development of diet-induced diabetes in HIP rats on high fat diet. Fasting plasma glucose (A), body weight (B) and mean daily food intake (C) during 10 week treatment with 60% high fat (HFD) or regular chow diet in wild type (WT: n=19) and HIP rats (HIP: n=21). The relationship between the change in body weight (D) and daily caloric food intake (E) vs. fasting glucose levels in HIP rats (solid line; —) and Wild Type rats (broken line; ---) during 10 week treatment with 60% high fat (HFD) or regular chow diet. (F) The relationship between the mean daily caloric food intake and change in body weight in individual Wild Type rats (broken line; ---) and HIP rats (solid line; —). Data are expressed as mean ± SE. *P<0.001 for HIP+HFD vs. all groups, ‡ P<0.001 for regular chow vs. high fat diet.
Figure 2. Compensatory increase in beta-cell mass in response to high fat diet is absent in HIP rats. (A) Typical islets from wild type (WT) and HIP rats stained for insulin and hematoxylin after 10 weeks of 60% high fat (HFD) or regular chow diet. Quantification of beta-cell mass (B), mean islet number per section (C), mean β-cell size (D) and β-cell nuclear diameter (E) after 10 weeks of 60% high fat (HFD) or regular chow diet in wild type (WT: n=19) and HIP rats (HIP: n=21). Data are expressed as mean ± SE, *P<0.05, **P<0.01.
Figure 3. High fat diet leads to increased beta-cell apoptosis in HIP rats and increased beta-cell replication in wild type rats. (A) Examples of islets stained for insulin (green) and apoptosis marker TUNEL (red) and (B) islets stained for insulin and replication marker Ki-67 (red) and nuclear stain Dapi (blue) imaged at 200X. (C and D) Quantified frequency of beta-cell apoptosis and replication following 10 weeks of 60% high fat (HFD) or regular chow diet in wild type (WT: n=19) and HIP rats (HIP: n=21). Data are expressed as mean ± SE, *P<0.05, **P<0.01. Please note the frequency of TUNEL and Ki67 positive cells in islets shown in A and B are higher than the mean to reveal the fidelity of the immunostaining rather than to be representative of the mean.
**Figure 4.** High fat diet leads to increased ER stress in HIP rats. (A) Immunoblot analysis of P-PERK, GRP-78, Insulin, IAPP and CHOP in protein lysates from islets isolated following 10 weeks of 60% high fat (HFD) or regular chow diet in wild type (WT: n=6) and HIP rats (HIP: n=8). (B-E) Quantification of P-PERK and GRP-78 expression normalized to insulin expression. (F) Representative examples of islets stained by immunofluorescence for insulin (green), endoplasmic reticulum stress marker CHOP (red) and nuclear stain Dapi (blue) imaged at 200X. (G) Quantified frequency of nuclear CHOP expression per islet and (H) a relationship between the frequency of beta-cell CHOP vs. TUNEL expression following 10 weeks of 60% high fat (HFD) or regular chow diet in wild type (WT: n=19) and HIP rats (HIP: n=21) rats. Data are expressed as mean ± SE, *P<0.05, **P<0.05. Please note the frequency of CHOP positive cells in islets shown in D are higher than the mean to reveal the fidelity of the immunostaining rather than to be representative of the mean.
Figure 5. HIP rats fail to adaptively increase fasting, glucose-stimulated and arginine-stimulated insulin secretion in response to high fat diet. Mean fasting C-Peptide (A) levels, first phase glucose (B) and arginine-stimulated (C) insulin secretion. (D-I) regression analysis of relationships between changes in beta-cell mass and fasting, glucose-stimulated and arginine-stimulated insulin secretion in wild type (WT: n=18) and HIP rats (HIP: n=19) after 10 week treatment with 60% high fat (HFD) or regular chow diet. Data are expressed as mean ± SE, *P<0.05, **P<0.01.
Figure 6. HIP rats on high fat diet exhibit increased fasting and insulin-stimulated hepatic glucose production. (A) Fasting (-30 and 0 min) and hyperinsulinemic-euglycemic clamp glucose concentrations. (B) Mean glucose infusion rates required to maintain euglycemia during the hyperinsulinemic clamp. (C) Mean rates of hepatic glucose production and glucose disposal (D) at fasting and during final 30 minutes of the hyperinsulinemic-euglycemic clamp in wild type (WT: n=13) and HIP rats (HIP: n=14) after 10 week treatment with 60% high fat (HFD) or regular chow. Data are expressed as means ± SE, **P<0.05 for HIP+HFD vs. all groups, *P<0.05 for HIP vs WT, ‡P<0.05 for HFD vs. chow.
Figure 7. High-fat diet-induced insulin resistance correlates with the increase in beta-cell apoptosis in HIP rats and increase in beta-cell replication in WT rats. Regression analysis of relationships between the beta-cell apoptosis, beta-cell replication and beta-cell mass versus hyperinsulinemic-euglycemic clamp determined insulin sensitivity in wild type rats (WT: n=13) (B, D, F) and HIP rats (HIP: n=14) (A, C, E) after 10 week treatment with 60% high fat (HFD) or regular chow.