SUPPLEMENTARY DATA

Rat in-vivo study protocols

Rat protocol 1. Rats (n=14) were studied 5 days after surgical implantation of catheters as previously described (6). Endogenous insulin secretion was suppressed by somatostatin (10 $\mu g \cdot kg^{-1} \cdot min^{-1}$) with basal glucagon replacement (0.65 $ng \cdot kg^{-1} \cdot min^{-1}$) via a jugular vein (0-240 minutes). In 5 rats (pulsatile insulin delivery), insulin was infused intraportally to reproduce fasting pulsatile insulin secretion in rats (5 pmol/kg/min) delivered as 70% pulses at 5 minute intervals and 30% constant basal insulin infusion. In 4 rats (constant insulin delivery), the same total insulin infusion rate was administered as 100% constant infusion. In 5 rats (T2DM insulin delivery), insulin was delivered with the pulsatile component decreased by 50% (rate=3.25 pmol/kg/min) at the same frequency and with the same basal constant insulin delivery as in pulsatile group, reproducing the pattern in T2DM. Blood was sampled at 15-minute intervals (0-240 min) from the carotid artery catheter for measurement of plasma glucose concentrations.

Rat protocol 2. Rats (n=14) were evaluated by modified hyperinsulinemic-euglycemic clamp to examine insulin sensitivity following portal vein delivery of insulin either in pulsatile, constant or in T2DM pattern as already described. In short, during somatostatin inhibition of endogenous insulin secretion, one cohort of rats (pulsatile; n=6) received insulin infused into the portal vein at high physiological rate (70 pmol/kg/min) in 5 minute pulses to recapitulate postprandial insulin secretion. 5 rats received the same total insulin infusion rate but as a constant infusion. 3 rats (T2DM) the pulsatile component decreased by 50% (rate=46 pmol/kg/min) at the same frequency and with the same basal insulin delivery as in pulsatile group. Blood samples to check plasma glucose were drawn from the carotid catheter every 10 minutes and any decrement in arterial glucose was prevented via variable jugular vein 50% dextrose infusion. Rates of exogenous glucose infusion were recorded to assess insulin sensitivity.

Rat protocol 3. To examine acute changes in hepatic insulin signaling anesthetized rats (n=18) were exposed to one of three patterns of insulin delivery (vida supra) and sequential liver biopsies were obtained. Immediately following catheter implantation under anesthesia, a 10 $\mu g \cdot kg^{-1} \cdot min^{-1}$ somatostatin infusion was started via jugular vein catheter (-30 to 0 min) to suppress endogenous insulin secretion. For pulsatile, (n=6) insulin was infused intraportally at 70 $pmol \cdot kg^{-1} \cdot min^{-1}$ with 70% of insulin given in min insulin pulses at 5 minute intervals and with 30% as a constant infusion. For constant (n=6) insulin was infused intraportally at a constant rate at 70 $pmol \cdot kg^{-1} \cdot min^{-1}$. For T2DM (n=6) the insulin infusion was delivered as in the first group but with the insulin pulses decreased by 50% at the same frequency and with the same basal insulin delivery (rate=46 pmol/kg/min). Sequential liver biopsies were taken using a non-traumatic tissue clamp and prefrozen dressing forceps (24) immediately before (t = 0 min) and following one (2 minutes), two (6 minutes) or three (10 minutes) pulses of insulin. Biopsies were immediately snapped frozen in liquid nitrogen and stored in -80^{o}C for subsequent analysis.

Rat protocol 4. In order to further investigate the actions of pulsatile insulin on hepatic insulin signaling 30 minutes after exposure to these three portal vein insulin protocols, the studies above were repeated in a second group of rats but continued for 30 minutes of pulsatile (n=5), constant (n=6) or T2DM (n=6) insulin infusions. Glucose was infused to clamp glucose concentrations. Liver biopsies were taken at 0 and 30 minutes and immediately 1) snapped frozen in liquid nitrogen and stored in -80^{o}C, 2) fixed in 4% cold paraformaldehyde for paraffin embedding, 3) preserved for subsequent real-time PCR analysis (RNA*later*, Sigma-Aldrich, St. Louis, MO, USA)

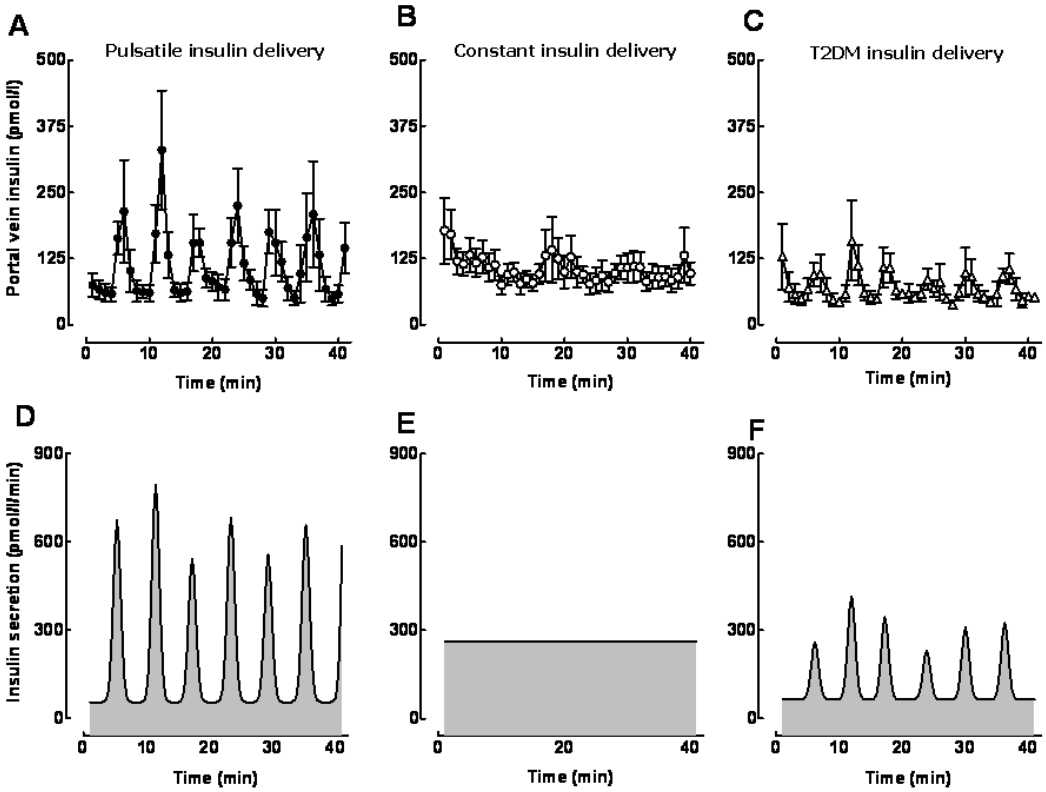
SUPPLEMENTARY DATA

Rat protocol 5. In, rat protocol 1-4, we examined the effects of different patterns of portal vein insulin delivery on concurrent hepatic insulin signaling. In protocol 5 we examined the actions of antecedent portal vein pulsatile (n=4) or constant (n=4) insulin delivery (120 minutes) on a subsequent identical 10 fold increment of portal vein insulin delivery for a further 30 minutes on hepatic insulin signaling and gene expression, the glucose being clamped by a glucose infusion. During somatostatin inhibition of endogenous insulin secretion, one cohort of rats (pulsatile) received 120-minute insulin infusion into the mesenteric vein at a basal physiological rate (7 pmol/kg/min) in 5-minute pulses to recapitulate fasting insulin secretion. Another cohort of rats received the same insulin infusion rate as a constant infusion for 120-minute insulin infusion into the mesenteric vein. Following the 120-min portal vein insulin infusion all rats received, an identical 30-min bolus of insulin (70 pmol/kg/min) designed to recapitulate the postprandial rise in insulin secretion. After the 30-min bolus insulin infusion (minute 150), all rats were euthanized and livers quickly removed for subsequent analysis of protein and gene expression and processed as described above.

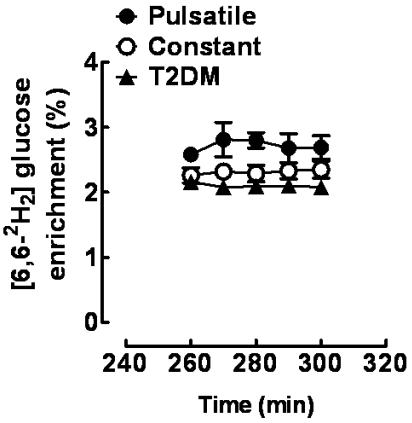
HIP rat study. In addition, in rat protocol-6 we used 39 Sprague Dawley male rats expressing h-IAPP (HIP rats) and 36 Sprague Dawley wild type littermate controls (WT) at ages 2, 7 and 12 months bred and housed individually throughout the study at the University of California Los Angeles animal housing facility. The generation and metabolic characteristics of diabetes-prone HIP rats was previously described in detail (6). A subset of HIP (n=19) and WT (n=20) rats at ages 2, 7 and 12 months were anesthetized and outfitted with portal vein sampling catheters for subsequent determination of pulsatile insulin secretion as previously described (23). A different subset of HIP (n=19) and WT (n=17) rats at ages 2, 7 and 12 months was used for determination of hepatic *glucokinase* gene expression. Insulin secretion, fasting glucose and beta cell mass in the WT rats was reported previously in a study describing the effects of aging on pulsatile insulin secretion (22).

SUPPLEMENTARY DATA

Supplementary Figure 1. Confirmation of portal vein insulin concentration and secretion profiles in dogs during the fasting insulin replacement protocol. (A-C) Portal vein insulin concentration and resultant portal vein insulin secretion (D-F) profiles measured at 1-minute intervals during the fasting insulin replacement period (140-180 min) following normal pulsatile (A and D), constant (B and E) or T2DM (C and F) insulin delivery into the portal vein of dogs.

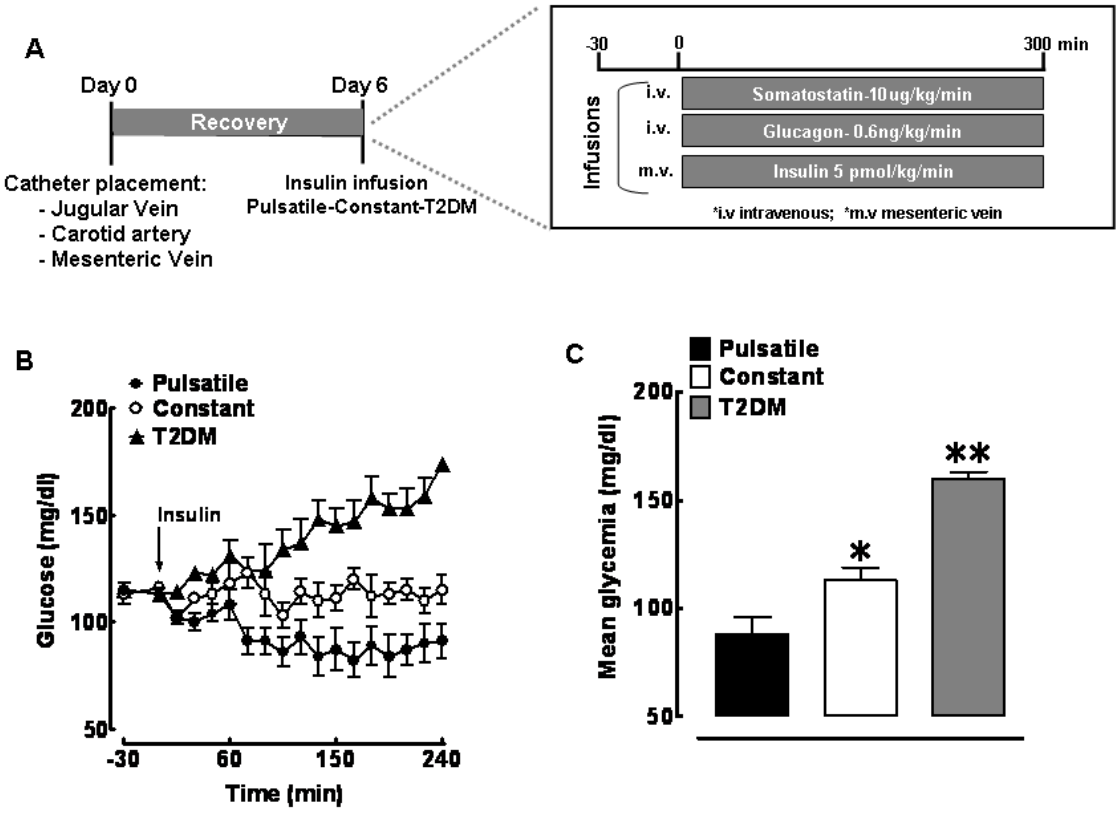


Supplementary Figure 2. Confirmation of the steady state [6, 6-2H2] Glucose isotope enrichment in dogs during the fasting insulin replacement protocol. Percent [6, 6-2H2] Glucose isotope enrichment in plasma during the steady state fasting insulin replacement period (260-300 min) following normal pulsatile, constant or T2DM insulin delivery into the portal vein of dogs.



SUPPLEMENTARY DATA

Supplementary Figure 3. Intraportal fasting insulin replacement protocol in rats (rat protocol 1). (A) All rats had a mesenteric and jugular vein infusion catheter and carotid artery sampling catheter placed surgically under anesthesia. Following 6 day recovery 3 separate cohorts of rats (n=14) were exposed in random order to (1) pulsatile portal vein insulin infusion, 2) constant portal vein insulin infusion, 3) portal vein insulin infusion designed to reproduce that observed in patients with T2DM which was accomplished by halving the insulin pulse mass. For each of these protocols, somatostatin (10ug/kg/min) and glucagon (0.65ng/kg/min) was infused jugular vein catheter throughout the study (0-240 min). For the pulsatile protocol, insulin was infused into the mesenteric vein catheter with 70% of insulin delivered in pulses and 30% as a basal constant insulin infusion. For constant insulin infusion, the same total amount of insulin was delivered at a constant rate. For the T2DM protocol, insulin was delivered with 50% diminished pulses at the same frequency and wit same basal insulin delivery. (B) Plasma glucose levels sampled every 15 minutes at baseline (-30 to 0 min) and during insulin replacement study period (0-240 min). (C) Mean plasma glucose levels during the final 60 minutes of insulin replacement period (180-240 min) following normal pulsatile (dark bars), constant (open bars) or T2DM (grey bars) pulsatile insulin delivery into the portal vein of rats.



SUPPLEMENTARY DATA

Supplementary Figure 4. Intraportal insulin replacement hyperinsulinemic-euglycemic clamp protocol in rats (rat protocol 2). (A) All rats had a mesenteric and jugular vein infusion catheter and carotid artery sampling catheter placed surgically under anesthesia. Following 6-day recovery 3 separate cohorts of rats (n=14) were exposed to portal vein insulin replacement hyperinsulinemic-euglycemic clamp. During somatostatin inhibition of endogenous insulin secretion, one cohort of rats (pulsatile) received insulin infused into the mesenteric vein at high physiological rate (70 pmol/kg/min) in 5-minute pulses to recapitulate insulin secretion after the meal. Another cohort of rats (constant), received the same total insulin infusion rate but as a constant infusion. In the third cohort of rats (T2DM), insulin was delivered with the pulsatile component decreased by 50% (rate=46pmol/kg/min) at the same frequency and with the same basal insulin delivery as in pulsatile group. Blood samples were drawn from the carotid catheter every 10 minutes and any decrement in arterial glucose was prevented via variable jugular vein 50% dextrose infusion. Rates of exogenous glucose infusion were recorded to assess insulin sensitivity. (B) Plasma glucose levels sampled every 10 minutes at baseline (-30 to 0 min) and during insulin replacement study period (0-120 min). (C) Mean glucose infusion rates during the final 30 minutes of insulin replacement period (90-120 min) following normal pulsatile (dark bars), constant (open bars) or T2DM (grey bars) pulsatile insulin delivery into the portal vein.