Adaptations in Pulsatile Insulin Secretion, Hepatic Insulin Clearance and Beta-Cell Mass to Age-Related Insulin Resistance in the Rat.

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Abstract

In health insulin is secreted in discrete insulin secretory bursts from pancreatic beta cells, collectively referred to as beta-cell mass. We sought to establish the relationship between beta-cell mass, insulin secretory-burst mass and hepatic insulin clearance over a range of age-related insulin sensitivity in adult rats. To address this we used a novel rat model with chronically implanted portal vein catheters in which we recently established the parameters to permit deconvolution of portal vein insulin concentrations profiles to measure insulin secretion and resolve its pulsatile components. In the present study we examined total and pulsatile insulin secretion, insulin sensitivity, hepatic insulin clearance and beta-cell mass in 35 rats aged 2-12 months. With aging, insulin sensitivity declined but euglycemia was sustained by an adaptive increase in fasting and glucose-stimulated insulin secretion through the mechanism of a selective augmentation of insulin pulse mass. The latter was attributable to a closely related increase in beta-cell mass (r=0.8, p<0.001). Hepatic insulin clearance increased with increasing portal vein insulin pulse amplitude, damping the delivery of insulin into the systemic circulation. In consequence the curvilinear relationship previously reported between insulin secretion and insulin sensitivity was extended to both insulin pulse mass and beta-cell mass versus insulin sensitivity. These data support a central role of adaptive changes in beta-cell mass to permit appropriate insulin secretion in the setting of decreasing insulin sensitivity in the aging animal. They emphasize the cooperative role of pancreatic beta cells and the liver in regulating the secretion and delivery of insulin to the systemic circulation.
In health the blood glucose concentration is closely regulated. Pancreatic beta cells play a central role by secreting insulin in a glucose concentration-dependent manner. Both regulation of insulin secretion and the delivery of insulin to target tissues are complex. Insulin is secreted in discrete secretory bursts at approximately 4 minute intervals with regulation accomplished through modulation of burst size (56). As a result hepatocytes (directly exposed to portal venous blood in hepatic sinusoids) are exposed to an insulin concentration wavefront with oscillations of approximately 200-500 pmol/l in the fasting state and increasing to 1000-5000 pmol/l after meal ingestion (50, 56). Regulation of insulin delivery to extrahepatic insulin tissues depends on both the rate of insulin secretion and the extent of hepatic insulin clearance (40). In addition to minute-by-minute regulation of insulin secretion, pancreatic beta cells also adapt over a longer period to overall demand. For example in response to an increased insulin demand in pregnancy, there is an adaptive increase in beta cell number per given pancreatic weight (often collectively termed beta cell mass) (28).

In type 2 diabetes there is an approximately 65% deficit in beta-cell mass (8) and impaired insulin secretion (49, 55, 58) in the setting of insulin resistance (15). The mechanism subserving impaired insulin secretion is reduced amplitude of insulin secretory bursts, particularly in response to hyperglycemia (32). However, impaired insulin secretion is partially offset by decreased hepatic clearance (7, 38, 54). These findings highlight the important relationships between insulin secretion, insulin clearance and beta cell mass. While elegant studies have examined the relationship between insulin secretion and insulin sensitivity (6, 27), none has established a relationship between beta-cell mass and insulin sensitivity. Moreover, most clinical analysis have utilized systemic measurements an inadequate sampling intensity to permit evaluation of
pulsatile insulin (27). Evaluation of insulin secretion by use of insulin concentrations measured in the systemic circulation, while technically feasible in humans, presents significant limitations. Since hepatic insulin clearance is related to the magnitude of insulin pulses in the portal vein (40), and approximately eighty percent of insulin is cleared in the first pass through the liver (40), systemic insulin measurements predictably underestimate decreased insulin secretion. Estimates of beta-cell mass in humans have been confined to autopsy studies precluding analysis of the relationships among beta-cell mass, insulin secretion and insulin action (8, 30).

Our goal was to examine the relationship among beta-cell mass, insulin secretion (to include direct sampling of the portal vein for pulsatile insulin secretion), hepatic insulin clearance and insulin sensitivity in the same animal. Moreover we sought to establish how these relationships adapt to increasing insulin demand (decreasing insulin sensitivity) during normal aging. We hypothesized that the primary adaptive response to an age-related reduction in insulin sensitivity is an adaptive increase in beta cell mass. We further postulated that an increase in beta-cell mass could permit appropriate glucose-stimulated insulin secretion in response to hyperglycemia through the mechanism of increased insulin burst mass presented to the liver. Finally we hypothesized that the extent of increased insulin secretion in the portal vein is attenuated by a related increase in hepatic insulin clearance.
Research Design and Methods

Study design We applied newly developed methods of direct measurement of pulsatile insulin secretion in the rat portal vein (37) along with those previously available to quantify the fasting and glucose-stimulated insulin secretion, hepatic insulin clearance and insulin sensitivity as well as beta cell mass in Sprague Dawley rats at age 2, 7 and 12 months. With this ensemble of data we were thus able to examine the adaptive changes of beta cell mass, insulin secretion and hepatic insulin clearance in health to age-related declining insulin sensitivity.

Animal studies were approved by The University of California Los Angeles Institutional Animal Care and Use Committee. Rats were bred and housed individually at University of California Los Angeles animal housing facility. Rats were maintained in a standard 12-hour light-dark cycle and fed ad libitum regular rodent chow.

Surgical Implantation of Catheters The surgical implantation of the portal vein and arterial sampling catheters as well as jugular vein infusion catheters has been described in detail (37). In brief, rats were anesthetized by isoflurane (2.5%) inhalation until effect. Under aseptic conditions portal vein was dissected free of surrounding connective tissue and the portal vein sampling catheter was placed into the hepatic portal vein at the bifurcation of the portal vein in the liver. Additionally, indwelling catheters were inserted into the right internal jugular vein and left carotid artery. All catheters were exteriorized to the back of the neck and encased in the infusion harness (Instech Inc, PA). Immediately postoperatively, and for the next 3 days all animals received preventative antibiotic treatment (Sulfamethoxazole, Hi-Tech Pharmaceutical, Amityville, NY; 200mg in drinking water). The rats maintained their preoperative body weight, had normal food intake and a normal hematocrit (42±2%) at the time of subsequent studies.
Fasting Insulin secretion and clearance

To assess the impact of aging on fasting insulin secretion male Sprague Dawley rats ages 2 (n=7), 7 (n=5) and 12 (n=5) month old rats were studied in the conscious unrestrained state 12 hours after food withdrawal the preceding day and 5-7 days after surgery as described in detail (37). In short, rats were placed in a cage outfitted with a counter-weighted-swivel mount (Instech Inc, PA), and following a 30 min equilibration period (-30 to 0 min), portal vein blood (~80 μl) was sampled every minute for 35 minutes and arterial samples (~100 μl) were sampled every 10 minutes. Blood samples were collected in pre-chilled microcentrifuge tubes containing protease inhibitor cocktail solution and EDTA and immediately centrifuged. At the end of the sampling period animals were euthanised and pancreas was removed for subsequent morphological analysis.

Glucose-stimulated insulin secretion and clearance

To assess impact of aging on glucose-stimulated insulin secretion Sprague Dawley rats age 2 (n=7), 7 (n=5) and 12 (n=6) months underwent a hyperglycemic clamp. On the morning of the study animals were weighed and the jugular vein venous line was connected to an adjustable infusion pump (Harvard Apparatus, Holliston, MA) for glucose infusion. The portal vein and carotid artery line were used for simultaneous blood sampling. Following an equilibration period, 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. Arterial blood samples (50ul) were taken at baseline and every 15 minutes during the clamp for immediate determination of plasma glucose and arterial insulin concentrations. Portal vein blood (~80 μl) was sampled every minute for 35 minutes (5-40 min) during the steady state
period of the hyperglycemic clamp. At the end of the sampling period animals were euthanised and pancreas was removed for subsequent morphological analysis.

**Islet morphology and immunohistochemistry** Pancreas was rapidly removed from euthanised rats and all non-pancreatic tissue was removed, pancreas was weighed and immediately fixed in 4% paraformaldehyde overnight at 4°C. The pancreas was embedded in paraffin and subsequently complete longitudinal sections (4-μm) of pancreas (head, body and tail) were obtained through its maximal width. These sections were stained for hematoxylin/eosin and insulin (guinea-pig anti-insulin, 1:100; Zymed, Carlsbad, CA). The β-cell mass was measured by first quantifying pancreatic cross-sectional area positive for insulin and multiplying this by pancreatic weight.

**Analytical procedures** Insulin concentrations were measured in duplicate by an insulin ELISA (ALPCO Diagnostics, Salem, NH). C-peptide levels were measured using a competitive colorimetric enzyme-linked immunosorbent assay (ALPCO Diagnostics, Salem, NH). Plasma free fatty acid levels were measured using in-vitro standard enzymatic colorimetric method (WAKO Chemicals, Richmond, VA). Glucose concentrations were measured by the glucose oxidase method (Beckman Glucose Analyzer 2, Fullerton, CA).

**Calculations** The portal vein plasma insulin concentrations were subjected to multiparameter deconvolution analysis as previously described in detail and recently validated for use in rats (37). Briefly, multiparameter deconvolution technique used in this study assumes that time varying insulin concentrations can be decomposed mathematically into: 1) a finite number of discrete insulin secretory bursts occurring at specific times and having 2) individual amplitudes and 3) a common half-duration, wherein bursts are superimposed upon 4) a basal time-invariant insulin secretory rate and
5) insulin disappearance in the portal vein is modeled via rapid and slow half-lives of 0.18 and 1.67 min and fractional slow-component amplitude of 0.065. Mean rates of insulin clearance were calculated during the fasting sampling period and during the hyperglycemic clamp studies in each rat using the following equation: \( C \text{ (l/min)} = \frac{S \text{ (pmol/min)}}{I \text{ (pmol/l)}} \) where \( C \) = insulin clearance rate, \( S \) = insulin secretion rate (by deconvolution), and \( I \) = corresponding arterial plasma insulin concentration. Insulin sensitivity index was calculated as a ratio of a mean glucose infusion rate during the hyperglycemic clamp in (mg/kg/min) to prevailing insulin concentrations in (pmol/l) and has been previously validated for the use in rats (45).

**Statistical analysis**

Statistical analysis was performed using standard one-way ANOVA analysis and regression analysis as stated (Statsoft, Tulsa, OK). Data in graphs and tables are presented as means ± SEM. Findings were taken to be statistically significant at the P<0.05.

**Results**

**Fasting metabolic characteristics**

As expected, aging resulted in a progressive increase in body weight from 2 to 12 months of age (p<0.05 between the groups, Table 1). Fasting plasma glucose levels remained within the non-diabetic range in all groups. However the mean in 12 month-old rats was slightly but significantly elevated compared with that in 2 and 7 month-old rats (108±1 vs. 100±3 and 93±3 mg/dl, P<0.05; Table 1). Aging led to a progressive increase in arterial insulin and C-peptide concentrations from 2 to 12 months age (P<0.05, Table 1), which
was associated with a progressive decrement in insulin sensitivity (P<0.05 between all groups; Table 1).

**Fasting pulsatile insulin secretion**

Visual examination of portal vein insulin concentration profiles in the fasting state (representative examples shown in Fig. 2) revealed distinct insulin oscillations with a periodicity of ~5 minutes and an amplitude of ~400 pmol/l at 2 months of age increasing to ~2000 pmol/l by 12 months of age. Deconvolution of fasting insulin concentration time series revealed that the fasting insulin secretion rate increased by 12-fold between ages 2 and 12 months (4±1 vs. 21±5 vs. 49±7 pmol/kg/min for 2, 7 and 12 month-old rats respectively, P<0.05 between all groups; Table 2 and Figure 2,4). Increased insulin secretion was achieved through the mechanism of increased insulin secretory-burst mass (5±1 vs. 34±5 vs. 77±22 pmol/pulse for 2, 7 and 12 month-old rats respectively, P<0.05 between all groups; Table 2 and Figure 4). There was no change in the frequency of insulin pulses (P>0.05). Deconvolution analysis indicated that the majority of insulin secreted (62%) was pulsatile.

**Glucose-stimulated pulsatile insulin secretion**

Inspection of individual 1-min sampled portal vein insulin concentration profiles during the hyperglycemic clamp (Figure 3) revealed that the amplitude of insulin oscillations increased in response to hyperglycemia at all ages. The glucose-stimulated increase in insulin secretion rate compared with baseline was achieved by a selective increase in insulin pulse mass (P<0.05 for all age groups) with no change in pulse interval (P>0.05). Despite comparable arterial glucose concentrations during the hyperglycemic clamps
(Fig. 1), glucose-stimulated insulin secretion was approximately three-fold greater in 12 month old than 2 months old rats (P<0.01). The age-related increase in glucose-stimulated insulin secretion was also attributed to a selective 3-fold increase in insulin pulse mass (59±7 vs. 117±19 vs. 182±25 pmol/pulse for 2, 7 and 12 months old rats respectively, P<0.05 between all groups; Table 2 and Figure 4) with no change in the frequency of insulin release (P>0.05).

**Insulin clearance**

The calculated clearance rate of endogenously secreted insulin increased with age both at fasting (19±3 vs. 54±9 vs. 82±8 ml/min for 2, 7 and 12 months old rats respectively (P<0.05 between all groups; Table 2) and glucose-stimulated states (35±5 vs. 48±4 vs. 62±7 ml/min for 2, 7 and 12 months old rats respectively, P<0.05; Table 2). Thus the systemic accessibility of increased insulin secretion in response to the age-related decline in insulin sensitivity is attenuated by greater hepatic insulin clearance. Consequently exposure of the liver to age-related elevation of insulin concentrations was greater than that of extrahepatic tissues. Moreover had we relied on sampling insulin at an extrahepatic sampling site, we would have markedly underestimated the extent of increased insulin secretion with aging. In extension of prior studies in which we have shown that hepatic clearance of endogenously secreted insulin is proportionate to the amplitude of portal vein insulin concentrations (29, 38, 40, 52), insulin clearance in this study was correlated with the magnitude of insulin pulses under fasting (r=0.7, P<0.001; Fig. 5) and hyperglycemic clamp conditions (r=0.5, P<0.05; Fig.5).
Relationship of insulin pulse mass and total insulin secretion to beta-cell mass

To putatively compensate for increased insulin demand in response to the age-associated decline in insulin sensitivity, beta-cell mass increased almost 3-fold from 2 to 12 months of age (8.6±0.8 vs. 14.4±1.6 vs. 23.4±1.8 mg for 2, 7 and 12 months old rats respectively, P<0.05 between all groups). To test the postulate that beta-cell mass adaptively increases to permit the appropriate rate of insulin secretion, we examined the relationship between insulin secretion and beta-cell mass in the same animal. There was a strong linear relationship between the rate of glucose-stimulated insulin secretion and beta-cell mass when all age groups were included (r=0.8, P<0.001, Fig 6B). Since insulin secretion is derived from insulin secretory bursts, not surprisingly there was a comparable close relationship between insulin pulse mass and beta-cell mass (r=0.8, P<0.001). These data imply that in the aging rat beta-cell mass adaptively increases in response to the demand for insulin secretion under glucose drive. The relationship between beta-cell mass and insulin secretion in the fasting state was also linear (r=0.7, P<0.01, Fig. 6A), however the rate of insulin secretion initially increased more rapidly than beta-cell mass. As a consequence of these relationships the beta-cell workload (insulin secretion per mg/beta-cells) increased ~10 fold in the fasting state (Fig. 6C), and ~2 fold in the glucose-stimulated state from age 2 to 12 (Fig. 6D).

Relationship between beta-cell mass, insulin secretion, hepatic insulin clearance and insulin sensitivity

Uniquely in this study we were able to examine the interrelationship among adaptive changes in beta cell mass, insulin secretion (including direct measurement of prehepatic
insulin pulses), hepatic insulin clearance and age-associated increased insulin demand (decline in insulin sensitivity). We reaffirm the hyperbolic relationship between insulin sensitivity and insulin secretion ($r=0.8$, Fig.7) previously noted in humans (6, 23). We demonstrate for the first time a similar curvilinear relationship between both beta-cell mass ($r=0.8$, Fig. 7) and pulse mass ($r=0.9$, Fig. 7) versus insulin sensitivity.

**Discussion**

In the present study we report that consistent with canine and human studies (51, 56) 1) most insulin in rats is secreted in a high-frequency pulsatile manner (pulse interval ~ 4-5 min); 2) glucose concentrations determine insulin secretion by amplification of insulin burst mass with no change in pulse frequency; 3) as a consequence of this mode of insulin secretion the liver is exposed to insulin oscillations of ~1000 pmol/l in the fasting state increasing to 6-10,000 pmol/l in the glucose-stimulated state and 4) hepatic clearance of endogenous insulin secretion is proportionate to the amplitude of portal vein insulin pulses.

Having recently established this methodology in rats, we were able to examine the adaptive changes in pulsatile insulin secretion and beta-cell mass over a range of insulin sensitivity and age. As previously reported beta-cell mass in rats increases from 2 to 12 months of age (36, 44). While there is a rapid period of post-natal expansion of beta-cell mass in the first 2 months after birth in rodents, growth of beta cell mass thereafter appears to be determined by insulin sensitivity (18, 44). Consistent with this impression, beta-cell mass increased as insulin sensitivity declined in the present studies (Figure 7). As insulin sensitivity declined, normal blood glucose values were maintained by a
progressive increase in insulin secretion accomplished by an increased pulse mass exposing the liver to markedly greater oscillations in insulin concentrations (Figure 2,3).

The deconvolution approach used here assumes that insulin clearance is constant at the sampling site. To the extent that the insulin clearance varies from minute to minute at the sampling site, the deconvolved insulin secretory rate may be in error. We previously reported that first pass hepatic insulin extraction of endogenous insulin secretion increases in phase with each secretory burst (40) and is proportionate to amplitude of the insulin pulse in the portal vein (38, 40, 50). As a result the deconvolved insulin secretion rate during insulin pulses may be underestimated when sampling from the systemic circulation. Fortunately, this error appears to have little impact on measured insulin secretion when sampling at the portal vein sampling site since the major determinants of insulin concentration at this sampling site are insulin secretion and blood flow through the portal vein as previously discussed (37, 51). Of note, the deconvolution approach used here was directly validated in the portal vein previously in the dog (51) over a threefold range of insulin secretion implying that variance in first pass hepatic insulin extraction (and therefore insulin clearance) at the portal vein sampling site has a negligible effect on the dynamics of insulin concentration in comparison to the rate of insulin secretion.

Hepatic clearance of endogenously secreted insulin was directly related to the amplitude of portal-vein insulin oscillations (Fig 5), consistent with prior studies in the dog and human (38, 40, 52). We have previously reported that hepatic insulin clearance is pulsatile, the pulses being synchronous with pulsatile insulin secretion (40). In a variety of other studies in which blood was sampled directly from the portal vein we were able to report that hepatic insulin clearance of endogenously secreted insulin increases with the amplitude of portal vein insulin pulses (38,50). Collectively these observations imply that
hepatocytes appear to respond minute-by-minute to changes in the delivered insulin concentration by adaptively changing insulin clearance. Hepatic insulin clearance and subsequent degradation is a receptor-mediated endocytosis process (16). Receptor-bound insulin is internalized for degradation within endosomes and this process requires insulin receptor (2), but not necessarily IRS-1 or IRS-2, tyrosine phosphorylation (11). More recently, evidence has emerged to reveal an important role of the transmembrane glycoprotein CEACAM1 in regulation of hepatic insulin clearance (53). CEACAM1 is phosphorylated by the insulin receptor kinase after insulin binding and can subsequently bind to insulin receptor and is thus internalized (46). Since CAECAM1 null mice or mice that express a dominant-negative CAECAM1 mutant have impaired hepatic insulin clearance, this close relationship between CAECAM1 and the insulin receptor clearly has mechanistic importance (14, 53). Based on this data it is plausible that the explanation for the relationship between hepatic insulin clearance and the amplitude of insulin presented to the liver in a pulse is mediated at least in part through the property of insulin stimulated CAECAM1 facilitation of hepatic insulin clearance. Whether this mechanism is responsible for the increase in insulin clearance observed in aging-associated insulin resistance in the present study remains to be elucidated.

Our data again emphasize that under conditions of daily living the liver is intermittently exposed to much higher insulin concentrations than extrahepatic tissues. Moreover, the insulin concentration profile to which the liver is exposed in health far exceeds the range of insulin concentrations represented during hyperinsulinemic-euglycemic clamp studies in which suppression of hepatic glucose production is conventionally measured (31). It remains to be established what role the large insulin concentration oscillations in the portal vein have, if any, on hepatic insulin signaling. They
do not appear to be important in insulin mediated hepatic glucose uptake (21) but their role in suppression of hepatic glucose release remains unresolved.

Another consequence of increased hepatic insulin clearance of increasing portal vein insulin concentration oscillations is that changes in insulin secretion across the range present in healthy individuals will be markedly damped when evaluated by measurement of insulin concentrations in the systemic circulation. Defective insulin secretion in type 2 and early type 1 diabetes is characterized by loss of insulin pulse mass (32, 47). It is therefore not surprising that the systemic insulin concentrations are often comparable in individuals with T2DM and non-diabetic controls in the basal state despite decreased C-peptide concentrations, contributing to an under appreciation of the importance of reduced insulin secretion in the pathophysiology of type 2 diabetes (7, 54).

When evaluating the relationship between the rate of insulin secretion and beta-cell mass (Fig. 6) we noted distinct relationships in the fasting and glucose-stimulated states. There was a close linear relationship between the rate of glucose-stimulated insulin secretion and the beta-cell mass (r=0.8). Not surprisingly, since most insulin is derived in secretory bursts the relationships were comparable between total rate or insulin pulse mass and beta cell mass during glucose stimulation (r=0.8). The data serve to underscore the importance of beta cell mass in defining glucose-stimulated insulin secretion. The relationship between beta-cell mass and insulin secretion in the fasting state was also linear (r=0.7), however the rate of insulin secretion initially increased more rapidly than beta-cell mass. Although it remains controversial precisely how beta-cell mass adapts to increased insulin demand (insulin resistance) (24), one hypothesis is that beta cell formation is prompted by the unfolded-protein response of the endoplasmic reticulum under an increased load of protein folding and processing. The present data
would suggest that it is the glucose-stimulated rate of insulin secretion that drives expansion of beta cell mass rather than the rate of insulin secretion in the fasting state. The data also serve to emphasize the importance of beta cell mass in the adaptive response to increased demand for insulin secretion.

However, it also important to point out that the close relationship between the adaptive change in beta cell mass and pulsatile insulin secretion in response insulin resistance observed in the current study doesn’t necessarily imply that the same relationship is true in humans. Although the beta-cell mass can adaptively increase in humans in response to obesity (8, 30) the extent of this increase is less than that reported in rodent studies (9). It is also important to emphasize that the current study was designed to establish the adaptive changes in beta-cell mass and insulin secretion (specifically the pulsatile component) to age related increasing insulin demand (decreasing insulin sensitivity) with normal aging in rats. The same relationships may not necessarily occur in response to insulin resistance induced through other mechanisms (for example obesity). Also adaptive changes in beta-cell mass and insulin secretion to insulin resistance may also be different with onset of insulin resistance at different ages, since the ability to expand beta-cell mass and function may be altered in animals and humans with aging (39, 59).

The relationship between insulin secretion and insulin sensitivity in-vivo is often portrayed as a hyperbolic relationship as first described by Bergman and colleagues (6). It has been postulated that a feedback mechanism operates between insulin sensitivity and beta-cell function such that as insulin sensitivity declines (e.g. in response to obesity), insulin secretion increases thereby maintaining a constant product of insulin secretion and insulin sensitivity (27). Here we report that there is a comparable curvilinear relationship
between the adaptive increase in insulin secretion and beta cell mass to declining insulin sensitivity. As expected since most insulin secretion is derived from insulin secretory bursts, a relationship can be delineated between insulin pulse mass and insulin sensitivity (Fig. 7).

In humans, evaluation of the effects of aging on beta-cell function has yielded conflicting results, with some investigators reporting increased in both fasting (1, 5, 12, 19, 22) and glucose-stimulated (13, 19, 22, 26) insulin and/or C-peptide levels and others a decrease or no change in insulin secretion (1, 12, 20, 41, 48). These differences are likely attributed to many factors such as differences in methods used to measure insulin secretion (12, 22), varying degrees of insulin sensitivity (27), differences in body composition and physical fitness (13), and varying levels of impaired fasting glucose and glucose tolerance often associated with aging (1, 12, 20). Most of these studies used systemic insulin concentrations to assess insulin secretion (12, 13, 20, 41). Studies that assessed the rates of pre-hepatic insulin secretion via deconvolution analysis of C-peptide concentrations reported both increased fasting and glucose-stimulated insulin secretion with aging in humans (22) and increased hepatic insulin clearance (5).

In the present study, the aging-associated increase in insulin pulse mass correlated with increased hepatic clearance of endogenously secreted insulin. We have previously reported that the amplitude of insulin pulses directed to the liver is related to hepatic insulin clearance (40), implying that the islet dictates the rate of systemic insulin delivery through the dual mechanisms of pulsatile insulin secretion and the influence of the latter on hepatic insulin clearance. The present data support this hypothesis. Additionally, consistent with the present model others have shown an increase in hepatic insulin extraction with aging in humans (1, 5). Thus as pulse amplitude presented to the
liver increases in response to insulin resistance, the liver preferentially extracts large
insulin pulses thus regulating systemic insulin delivery. Subsequently the marked
increase in fasting pre-hepatic pulsatile insulin secretion (~12 fold) is largely offset by a
compensatory increase in hepatic insulin clearance resulting in lesser (~4 fold) increase
in fasting systemic insulin concentrations. This observation suggests that an adaptive
increase in pulsatile insulin secretion in response to aging-associated insulin resistance
primarily modulates hepatic glucose production and not necessarily extra-hepatic glucose
disposal, an observation supported by others (3, 4, 23).

Our observation that the clearance of endogenously secreted insulin increased in
response to age-related insulin resistance in the rat contradicts previous data that reports
a decline in insulin clearance in insulin resistant states such as obesity (17) and high fat
feeding (43). These differences are likely attributed to many factors one of which is the
methods used to measure insulin clearance. It is important to emphasize that the insulin
clearance reported here is that of endogenously secreted insulin which is delivered into
the portal vein and thus directly to the liver in large discrete pulses. Insulin clearance of
endogenously secreted insulin was obtained by dividing the insulin secretion rate
(obtained by direct sampling from the portal vein) by the insulin concentration in the
systemic circulation.

In contrast, most prior studies either measure the clearance of exogenously
delivered insulin by a constant systemic or intraportal insulin infusion (43, 60) or estimate
the clearance of endogenously secreted insulin by comparing systemic insulin and C-
peptide concentrations (17). Moreover, differences in plasma free fatty acid levels in the
current study (no change between 2-12 months old rats) and others (elevated FFA levels
observed in obesity) may account for the discrepancy in the results. Elevation in
systemic free fatty acid concentrations have been shown in vivo and in-vitro to impair hepatic insulin clearance (57, 60) presumably by direct inhibition of insulin binding and subsequent degradation (60).

In summary, we report that the beta-cell adaptation to age-associated insulin resistance is accomplished via a selective increase in both fasting and glucose-stimulated insulin secretory burst mass. Moreover the increase in insulin secretory burst mass in the rat can be attributed to a parallel increase in beta-cell mass. Concurrently hepatic insulin clearance increases due to higher amplitude of portal vein insulin oscillations, so that the age-related increase in systemic insulin concentrations is substantially damped. These data serve to emphasize the complex but highly coordinated relationship between pancreatic islets (through pulsatile insulin secretion) and the liver (through both metabolic responses and regulation of systemic insulin delivery). The data also permit an appreciation of the primacy of beta-cell mass in determining insulin secretion over a range of insulin sensitivity.

Acknowledgments.

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**References**

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Figure Legends

Figure 1. The mean arterial glucose (top), arterial insulin (middle) and portal vein insulin concentrations (bottom) at fasting (-30-0 min) and during a hyperglycemic clamp (0-75 min) in rats at 2, 7 and 12 months of age.

Figure 2. Fasting state. Insulin concentration (top panel) and deconvolved corresponding insulin secretion rates (bottom panel) (measured at 1-min intervals) at the portal vein in representative rats at 2, 7 and 12 months of age in the fasting state.

Figure 3. Glucose-stimulated state. Insulin concentration (top panel) and deconvolved corresponding insulin secretion rates (bottom panel) (measured at 1-min intervals) at the portal vein in representative rats at 2, 7 and 12 months of age during the hyperglycemic clamp.

Figure 4. The mean insulin secretion rate (left), insulin pulse mass (middle) and interpulse interval (right) at fasting and during the hyperglycemic clamp in rats at 2, 7 and 12 months of age. Data are expressed as mean ± SE. Statistical analysis was restricted to effects of aging. *P<0.05 for 7 vs. 2 months, †P<0.05 for 12 vs. 2 months, ‡P<0.05 for 12 vs. 7 months.

Figure 5. The relationship between the clearance of endogenously secreted insulin and the mean insulin pulse amplitude in each rat at fasting (left) and during the hyperglycemic clamp at 2, 7 and 12 months of age.

Figure 6. The relationship between the individual beta-cell mass (mg) and deconvolved total insulin secretion rate in each rat at 2, 7 and 12 months of age at fasting (A) and during the hyperglycemic clamp (B). The mean fasting (C) and glucose-stimulated (D) beta-cell workload calculated as the ratio of insulin secretion to mg of beta-cell mass. Data are expressed as mean ± SE. *P<0.05 for 7 vs. 2 months, †P<0.05 for 12 vs. 2 months.

Figure 7. The hyperbolic relationship between insulin sensitivity vs. the beta-cell mass (left), insulin pulse mass (middle), insulin and deconvolved total insulin secretion (right) in each rat at 2, 7 and 12 months of age.
Table 1 (Metabolic and demographic characteristics)

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Data are expressed as mean ± SE.

*P<0.05 for 7 vs. 2 months, †P<0.05 for 12 vs. 2 months, ‡ P<0.05 for 12 vs. 7 months.

§ Insulin sensitivity index was calculated as a ratio of a mean glucose infusion rate during the Hyperglycemic clamp in (mg/kg/min) to prevailing insulin concentrations in (pmol/l).
### Table 2 (Pulsatile Insulin Secretion)

<table>
<thead>
<tr>
<th></th>
<th>Pulse mass (pmol)</th>
<th>Pulse Amplitude (pmol/l)</th>
<th>Pulse Interval (min)</th>
<th>Insulin Secretion (pmol/kg/min)</th>
<th>Insulin Clearance (ml/min)</th>
<th>%Pulsatile Secretion</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>2 Months</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting</td>
<td>5 ± 1</td>
<td>666 ± 108</td>
<td>4.7 ± 0.3</td>
<td>4 ± 1</td>
<td>19 ± 3</td>
<td>72 ± 6</td>
</tr>
<tr>
<td>Glucose-Stimulated</td>
<td>59 ± 7</td>
<td>7066 ± 594</td>
<td>4.7 ± 0.3</td>
<td>56 ± 4</td>
<td>35 ± 5</td>
<td>61 ± 6</td>
</tr>
<tr>
<td><strong>7 Months</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting</td>
<td>34 ± 9*</td>
<td>2407 ± 723*</td>
<td>4.7 ± 0.4</td>
<td>21 ± 5*</td>
<td>54 ± 9*</td>
<td>59 ± 8</td>
</tr>
<tr>
<td>Glucose-Stimulated</td>
<td>117 ± 19*</td>
<td>9437 ± 1427*</td>
<td>4.6 ± 0.4</td>
<td>79 ± 6</td>
<td>48 ± 4</td>
<td>58 ± 9</td>
</tr>
<tr>
<td><strong>12 months</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting</td>
<td>77 ± 22†</td>
<td>5206 ± 1627†</td>
<td>4.5 ± 0.7</td>
<td>49 ± 7†</td>
<td>82 ± 8†</td>
<td>57 ± 5</td>
</tr>
<tr>
<td>Glucose-Stimulated</td>
<td>182 ± 25‡</td>
<td>13356 ± 1882‡</td>
<td>4.6 ± 0.3</td>
<td>134 ± 12‡</td>
<td>62 ± 7†</td>
<td>46 ± 3</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SE and statistical analysis was restricted to effects of aging. *P<0.05 for 7 vs. 2 months, †P<0.05 for 12 vs. 2 months, ‡P<0.05 for 12 vs. 7 month