The Islet Circadian Clock: Entrainment Mechanisms, Function and Role in Glucose Homeostasis

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Abstract

Circadian regulation of glucose homeostasis and insulin secretion has long been appreciated as an important feature of metabolic control in humans. Circadian disruption is becoming increasingly prevalent in today's society and is likely responsible in part for the considerable rise in Type 2 diabetes (T2DM) and metabolic syndrome worldwide. Thus, understanding molecular mechanisms driving the inter-relationship between circadian disruption and T2DM is important in context of disease prevention and therapeutics. In this regard, the goal of this manuscript is to highlight the role of the circadian system, and islet circadian clocks in particular, as potential regulators of β-cell function and survival. To date, studies have shown that islet clocks respond to changes in feeding patterns, and regulate a multitude of critical cellular processes in insulin secreting β-cells (e.g. insulin exocytosis, mitochondrial function and response to oxidative stress). Subsequently, either genetic or environmental disruption of normal islet clock performance compromises β-cell function and leads to loss of glycemic control. Future work is warranted to further unravel the role of circadian clocks in human islet function in health and contributions to pathogenesis of T2DM.

Keywords

Introduction

The incidence of type 2 diabetes (T2DM) has demonstrated a consistent rise, reaching epidemic proportions worldwide [1]. This debilitating disease greatly increases population morbidity and mortality, and places substantial burden on the health care systems globally [1]. For this reason, understanding the molecular and physiological mechanisms underlying

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induction of T2DM is essential for development of novel therapeutic and preventative approaches. Initiation of fasting and postprandial hyperglycaemia in T2DM is due to the complex interplay between diminished insulin action (primarily in the liver and skeletal muscle), and pancreatic islet failure characterized by inappropriate stimulation of insulin secretory response and impaired suppression of glucagon release [2]. Islet failure is a hallmark abnormality in T2DM that often precedes diabetes diagnosis and is already present in non-diabetic individuals who are at increased risk for development of the disease [3, 4]. Impaired insulin secretion is a key feature of islet failure in T2DM and is attributed to β-cell secretory dysfunction and loss of β-cell mass [5, 6]. β-cell secretory dysfunction in T2DM is multifaceted and manifests as defects in 1) glucose–stimulated insulin secretion [5], 2) meal-stimulated insulin secretion [7], 3) pulsatile mode of insulin release [8] and 4) incretin-stimulated amplification of insulin response [9]. β-cell loss in T2DM patients has been attributed to increased β-cell apoptosis [6], although recent studies suggest that β-cell dedifferentiation may also play a contributory role [10].

T2DM is a complex polygenic disease, pathophysiology of which involves interactions between an array of genetic predispositions and environmental triggers. Although genetic susceptibilities clearly play a role in predisposition to T2DM, environmental factors appear to be significantly greater predictors of diabetes onset and progression in human populations [11]. Environmental triggers such as increased caloric intake and physical inactivity has long been known to increase susceptibility to T2DM [12]. More recently, circadian disruption has gained greater appreciation as a novel environmental factor contributing to T2DM development with multiple strands of evidence supporting the correlative relationship between circadian disruption and T2DM susceptibility [13–24].

Firstly, individuals performing rotational shift work or people engaged in work conditions characterized by circadian misalignment exhibit significantly higher prevalence of diabetes, impaired glucose tolerance, and metabolic syndrome compared to their counterparts exposed to standard work schedules [13–15]. Also, the quantity and quality of sleep serves as an independent risk factor for development of T2DM [16–18]; however it is important to note that sleep loss may have circadian-independent effects on glucose metabolism. In addition, clinical studies performed in controlled laboratory settings also show that acute exposure to circadian misalignment and/or sleep loss results in dysregulation of glucose metabolism characterized by impaired insulin action and insulin secretory response [19–25]. Moreover, circadian disruption appears to worsen glycemic control in those patients already diagnosed with T2DM [26]. Taken together, accrual of evidence suggests that circadian disruption/ misalignment is a risk factor for development of T2DM. This highlights the necessity to understand molecular and physiological underpinnings responsible for this phenomenon. Subsequently, the goal of this manuscript is to overview the role of the circadian system in regulating pancreatic islet function, placing a particular emphasis on the regulation of insulin secretion and β-cell survival in context of diabetes development.

The circadian system and regulation of insulin secretion

The circadian system is a distinctive feature of nearly all living organisms. It permits an adaptive advantage to organisms by allowing them to anticipate and adjust their intracellular

biological processes in response to environmental changes created by the earth's rotation (i.e. changes in light/dark (LD) cycle). In mammals, numerous behavioral (i.e. sleep/wake cycles), physiological (i.e. glucose homeostasis) and cellular (i.e. cell cycle progression) processes are under direct circadian control [27]. Regulation of glucose homeostasis provides an excellent example of how the circadian system exerts control over essential physiological functions and coordinates metabolic control in response to changing sleep/wake and feeding/fasting circadian cycles [28]. Thus, plasma glucose levels are tightly controlled throughout the 24-h period in healthy individuals, since hypoglycaemia compromises neuronal function whereas prolonged hyperglycaemia episodes can impart oxidative tissue damage. Subsequently, circadian regulation of β-cell insulin production, secretion, and growth provides an important balancing mechanism between allowing sufficient cellular metabolic fuel and control of diurnal glycaemia.

Circadian rhythms in glucose tolerance have been consistently observed in human studies, demonstrating enhanced glucose/meal tolerance during the morning over the night-time hours [29–31]. Although regulation of glucose/meal tolerance is complex and requires coordinated interaction between insulin/glucagon secretion and insulin sensitivity, insulin secretory response demonstrates robust circadian variation even when changes in insulin sensitivity and other hormonal parameters are taken into the account [29–31]. Furthermore, robust circadian rhythms in human insulin secretion are also present following administration of glucose-independent insulin secretagogues such as tolbutamide (K_{ATP} channel blocker), emphasizing circadian regulation of β-cell secretory capacity [29]. Classic studies by Boden and colleagues [32] provided comprehensive assessment of circadian insulin response in humans studied under glucose clamp conditions over a 3 day period. These studies confirmed robust 24-h circadian rhythms in insulin secretory response which interestingly became more pronounced under hyperglycemic clamp conditions [32]. Finally, it is important to note that circadian rhythms in insulin secretion are disrupted in states associated with metabolic disease such as obesity and T2DM [30, 33], implying an intriguing possibility that loss of circadian rhythmicity in insulin secretion may serve as an early biomarker of β-cell dysfunction in T2DM [34].

Pre-clinical studies in rodents provide further support for circadian control of insulin release. Rodent islets exhibit circadian rhythms in insulin secretion studied in *in vitro* islet perifusion setting [35]. Interestingly, the phase of circadian insulin secretion *in vitro* can be modulated by administration of melatonin, and regulated by changes in β-cell K_{ATP} channel activity [35, 36]. In addition, disrupting the central or pancreas-specific circadian clock function leads to loss of circadian rhythms in insulin secretion and glucose tolerance [37–39]. Thus, accumulating evidence from human as well as preclinical animal studies indicates that insulin secretory capacity and function is under circadian control and appears to be disrupted in T2DM. In this context, understanding the molecular and physiological underpinnings of circadian regulation of insulin secretion becomes increasingly important.

Islet circadian clock function and entrainment mechanisms

The "master clock" of the circadian system in mammals is contained in the suprachiasmatic nucleus (SCN) of the hypothalamus. The SCN is comprised of molecular oscillators

(clocks), set by distinct transcriptional-translational molecular feedback loops [40]. However, more recently autonomous molecular clocks have been shown to be present in numerous tissues outside of the SCN [41], including pancreatic islets [39, 42, 43]. Thus the SCN integrates and synchronizes peripheral oscillators by employing a combination of neuronal, behavioral, and endocrine outputs (44). The molecular clock mechanism is highly conserved among various cell types and is driven by a set of core "clock genes" that form inter-related transcriptional-translational complex (for detailed reviewed see [27]). In short, the positive limb of the clock gene feedback circuit includes genes *Circadian locomotor output cycles kaput (Clock)* and *Brain and muscle ARNT-like1* (*Bmal1*), which encode basic helix-loop-helix Per-Arnt-Single-minded (bHLH-PAS) proteins that initiate transcription by binding to promoter regions of target genes [45]. *Period* (*Per1/2/3*) and *Cryptochrome* (*Cry1/2*) genes comprise the negative limb of the clock gene feedback loop where PER and CRY translocate to the nucleus upon translation and where they function as negative regulators of their own transcription by interacting with CLOCK/BMAL1 complexes. [46]. This complex oscillatory network sustains 24-h transcriptional oscillations and synchronizes the transcription of clock effector genes to changes in LD cycles. Importantly, accumulating evidence suggests that molecular underpinnings of the islet circadian clock are essential for proper regulation of β-cell function, survival and glucose control [47].

Presence of robust circadian clock gene expression in pancreatic islets has been previously shown by rtPCR analysis in islets isolated by either the collagenase method or laser microdissection from pancreatic tissues collected at multiple time points throughout the 24-h cycle [39, 42, 43]. In addition, tracking of real-time islet cell bioluminescence with a clock gene luciferase fusion construct has been used for longitudinal monitoring and assessment of the islet clock function *in vitro* [39, 42, 43]. Seminal studies by Marcheva and colleagues used bioluminescent imaging of islets isolated from *Per2:Luc* mice to first demonstrate autonomous clock expression in pancreatic islets [42]. Consistent with these observations, our studies utilizing *Per1:Luc* transgenic rats (in which mouse *Per1* promoter is linked to a luciferase reporter) reported well defined in phase high amplitude circadian cycles of *Per1*-driven luciferase expression in individual rat islets with ~24-h period and the phase reflecting temporal *in vivo* profile of *Per1* mRNA expression [39] (Fig. 1A,B). Interestingly, islet clocks demonstrated robust glucose sensitivity particularly with islets cultured at low glucose concentrations (<5 mM), displaying substantial reduction in the amplitude, lengthening of the oscillatory period, and altered phase of *Per1* bioluminescence oscillations [39] suggesting potentially important inter-relationships between cellular, metabolic, and circadian cycles in pancreatic islets [48].

While the primary environmental cue (i.e. zeitgeber) modulating SCN clock is light, peripheral clocks are believed to be responsive to a variety of signals including autonomic nervous system control by the SCN, neuroendocrine hormonal signals, as well as indirect signals such alterations in body temperature and timing of food intake [49–51]. To investigate potential mechanisms mediating entrainment of islet circadian clocks, we first examined whether sudden changes in LD cycle can modulate and reset the phase of transcriptional oscillations in islet clocks. Subsequently, inversion of the phase of the LD cycle in *Per1:Luc* transgenic rats by 12 h from LD (lights on at 6:00 h, lights off 18:00 h) to

DL (lights on at 18:00 h, lights off 6:00 h) resulted in near 12-h phase reversal of the islet *Per1:Luc* bioluminescence signal with no change in oscillatory period or the amplitude [39] (Fig. 1C, D). Feeding has been previously shown to entrain peripheral clocks and uncouple peripheral oscillators from the SCN pacemaker, an observation particularly evident in organs responsive to nutritional load, such as the liver [52, 53]. Thus *Per1:Luc* transgenic rats were used to assess the ability of feeding to entrain islet clocks. Restricting food availability for 6 h during the light phase of standard LD cycle entrained the islet clocks as evident by a rapid 6-h shift in phase of islet *Per1:Luc* bioluminescence rhythm (Fig. 1C, D). Similar to sudden changes in LD cycle, restricting feeding time appears to reset the phase of islet clocks, suggesting that peripheral oscillators in the islet may be coupled to the SCN via modulation of feeding rhythmicity. In fact, feeding patterns have been previously shown to directly entrain peripheral clocks in a variety of organs independent of the SCN, although the degree and timing of feeding-induced clock phase shifts vary among different organs [52].

Mechanisms driving feeding-induced entrainment of islet clocks remain unclear; however, some clues can be gleaned from previous studies that examined feeding entrainment of various other peripheral oscillators. A number of potential candidates mediating feeding-induced entrainment have been considered which include, but are not limited to 1) feeding-generated metabolites, 2) feeding-induced hormonal factors, and 3) feeding-induced regulation of body temperature. Changes in glucose availability and subsequent changes in intracellular metabolic milieu may constitute a logical candidate given that the former has been shown to alter clock gene expression in cultured cells, including pancreatic islets [39, 54]. Also, the oscillation of the intracellular redox state due to metabolic changes, can affect CLOCK and BMAL1 dimerization and their binding to DNA elements *in vitro*, thus contributing to another entrainment mechanism [55]. Furthermore, AMP-dependent kinase (AMPK)-mediated phosphorylation and degradation of clock proteins in peripheral tissues may be yet another mechanism that could potentially play a role in feeding-induced entrainment of islet clocks [56]. Finally, the availability of other food-generated metabolites (e.g. lipids, amino acids, ketones, etc.) which are capable of altering intracellular metabolic environment may also potentially contribute to resetting the islet circadian oscillators.

Circadian release of feeding-induced hormonal factors may also play a role in entrainment of islet oscillators. Gut incretin peptides such as Glucagon-like Peptide (GLP-1) and Gastric inhibitory polypeptide (GIP) constitute plausible candidates given the recent demonstration of their secretion under clock control [57], high expression of incretin receptors in islets, and the fact that incretins activate cAMP-pCREB intracellular signaling cascade known to be important for clock entrainment [58]. In addition, autocrine effects of insulin release may also contribute to islet clock entrainment, since insulin has been recently shown to entrain circadian oscillators in the liver [59]. Finally, feeding cycles have been previously shown to impact body temperature regulation, thus providing yet another potential mechanism responsible for feeding-induced islet clock entrainment [52]. Indeed, temperature can sufficiently entrain clocks both *in vitro* and *in vivo* [60]. Clearly, additional work is needed to dissect and unravel exact mechanisms driving the entrainment of islet clocks and how

disruption of islet clock entrainment contributes to increased susceptibility for metabolic disease and T2DM.

Circadian disruption, islet clock function and contribution to islet failure in T2DM

A functional circadian system and resultant synchronous inter-relationships between peripheral and central oscillators are essential for maintaining appropriate homeostatic function. However, in instances when environmental factors (e.g. rotational shift work) induce misalignment between internal circadian oscillators and the external environment, functional regulation of multiple physiological systems is compromised, including glucose homeostasis and β-cell function [28, 61, 62]. One plausible mechanism underpinning this phenomenon is the impairment in autonomous islet clock function. Indeed, circadian disruption due to continuous exposure to constant light (LL) and resultant loss of circadian rhythms in locomotor activity, feeding and hormonal secretion appears to significantly compromise islet clock integrity [63] (Fig. 2). Specifically, circadian misalignment induced by LL results in impaired amplitude, phase and inter-islet synchrony of clock gene oscillations [63] (Fig. 2). The functional significance of uncoupling and dampening of autonomous circadian oscillators in islets remains to be further explored; however, islets isolated from LL-exposed animals display significant loss of β-cell function (Fig. 2) [63]. Furthermore, continuous exposure to LL (as well as chronic jetlag-like conditions) significantly accelerates the development of fasting hyperglycaemia and islet failure in diabetes-prone human islet amyloid transgenic (HIP) rats [64] (Fig. 3). Interestingly, the timing and extent of diabetes phenotype acceleration induced by circadian disruption in diabetes-prone HIP rats is comparable to that achieved by exposure to 60% high fat diet [65]. Importantly, induction of islet failure in circadian-disrupted HIP rats was due to loss of insulin secretory function and β-cell mass, the latter attributed to increased β-cell apoptosis [64] (Fig. 3). Similar results were also observed in mice where continuous exposure to shift-work conditions resulted in development of fasting hyperglycaemia and loss of glucose-stimulated insulin secretion [66].

Over the past few years, a number of pancreas and β-cell specific clock gene mutants have been established to investigate molecular mechanisms underlying the inter-relationship between autonomous β-cell clocks and regulation of β-cell health [42, 66–68]. These studies have clearly shown that embryonic deletion of key components of the β-cell circadian clock compromise the regulation of glucose homeostasis via impaired β-cell function, associated with defective insulin vesicular exocytosis and/or diminished mitochondrial function [42, 66–68]. Clock-controlled regulation of cellular mitochondrial function is particularly noteworthy given the critical importance of mitochondrial respiration for stimulation of glucose-mediated insulin secretion and well characterized mitochondrial dysfunction in diabetic β-cells [69, 70]. In addition, β-cell circadian clock appears to be also involved in regulation of key transcription factors regulating β-cell growth, proliferation as well as maturation [42]. Finally, recent work in β-cell specific *Bmal1* knockout mice shows that β-cell clock is also involved in orchestrating cellular response to oxidative stress via regulation of *nuclear factor erythroid 2-related factor 2* (*Nrf2*) [66]. This would suggest that an

autonomous islet clock regulates β-cell homeostasis by coordinating its response to oxidative stress. This is particularly relevant in context of T2DM metabolic environment where β-cells are exposed to an array of pro-oxidative stressors such as glucotoxicity, lipotoxicity and proteotoxicity [71, 72]. This postulate is supported by the recent observation of improved response to oxidative stress and consequent restoration of β-cell function and survival in diabetic β-cells subsequent to activation of melatonin receptor signaling [73], a signaling pathway recently shown to enhance the circadian clock function in β-cells [74].

Conclusion

Circadian regulation of β-cell insulin secretory capacity and secretion in humans are well characterized. Furthermore, loss of circadian rhythmicity of insulin secretion appears to serve as an early biomarker of β-cell dysfunction in obesity and T2DM. Circadian misalignment *per se* is associated with increased susceptibility for development of T2DM, mediated partly through decline in β-cell function, and plausibly, compromised β-cell survival. It is currently unknown whether islet clock function in humans is compromised as consequences of circadian disruption and/or T2DM. However, preclinical rodent work suggests that islet clocks regulate a multitude of critical cellular processes including insulin exocytosis, mitochondrial function, and response to oxidative stress. Consequently, the loss of islet clock function in rodents leads to loss of glucose control mediated by a decline in β-cell function and survival. Clearly, the interworking of the circadian control of β-cell function is highly complex and likely entails a coordinate regulation of multiple cellular and physiological systems. That being said, understanding the molecular targets of islet clocks and translating this work to human disease will be an important step toward development of novel chronotheraputic approaches to treat metabolic diseases.

Acknowledgments

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Callout 1 (relates to the text described in pages 4–6)

Insulin secretory capacity and function is under circadian control and disrupted in obesity and T2DM, implying an intriguing possibility that loss of circadian rhythmicity in insulin secretion may serve as an early biomarker of β-cell dysfunction in T2DM.

Callout 2 (relates to the text described in pages 7–10)

Restricting feeding time resets the phase of islet clocks suggesting that peripheral oscillators in the islet may be coupled to the SCN via modulation of daily feeding cycles.

Callout 3 (relates to the text described in pages 10–12)

Temporal profile and the extent of diabetes phenotype acceleration induced by circadian disruption in diabetes-prone HIP rats are comparable to that achieved by exposure to 60% high fat diet.
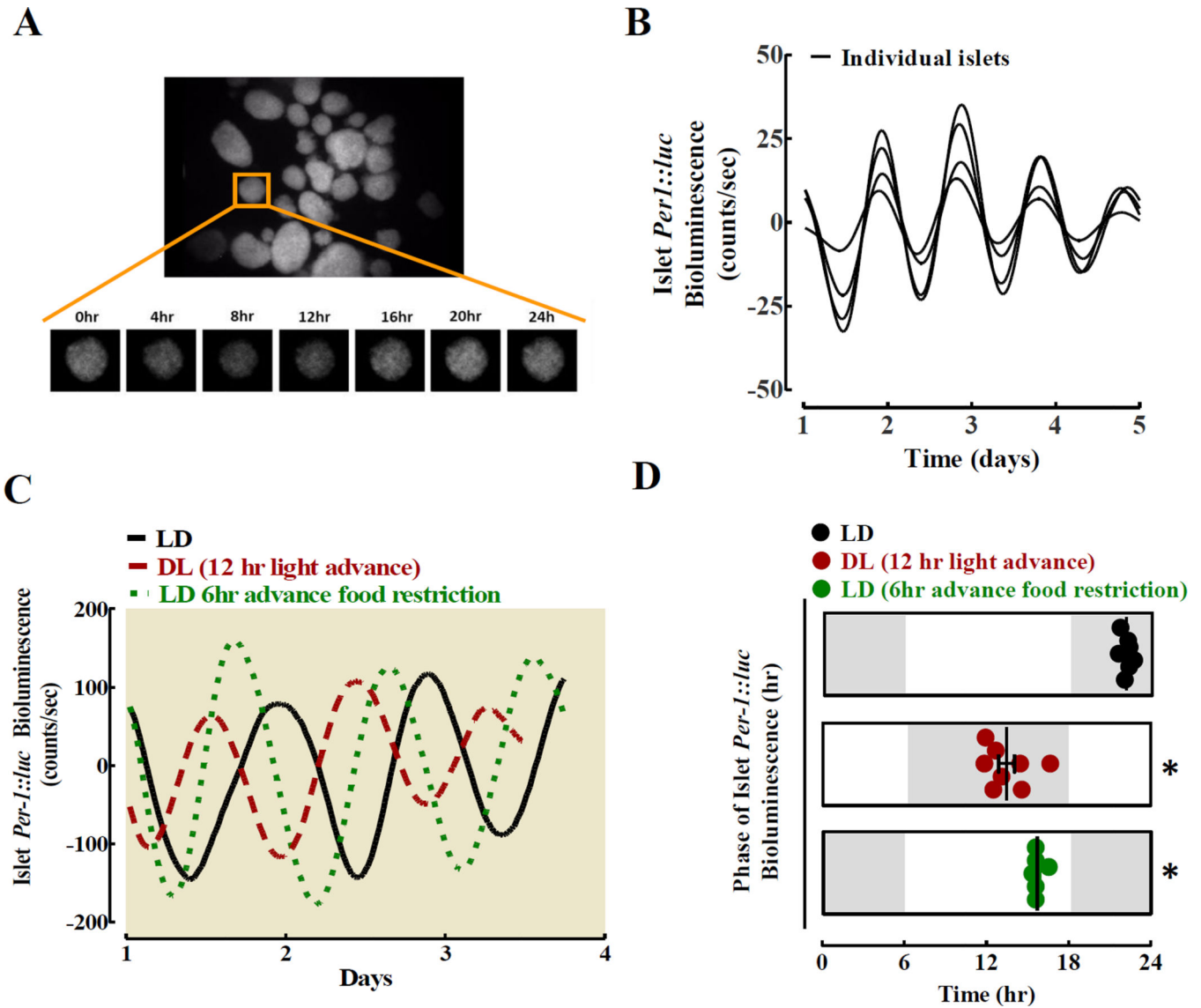
A

B

C

D

Figure 1. Examination of the islet clock entrainment by longitudinal monitoring of islets isolated from *Per-1:Luc* transgenic rats

(A) Whole-field image (10×) of islets isolated from *Per-1:Luc* transgenic rats under standard LD condition obtained by intensified charge-coupled device (ICCD) camera. Individual islet images collected over 24 h from the islet shown in orange square insert in the figure. (B) Representative examples of corresponding *Per*-driven bioluminescence rhythms obtained from 4 individual islets isolated from *Per1:Luc* rats housed under standard LD cycle conditions. Note the robust in phase oscillations among individual islets. (C) Representative examples of *Per*-driven diurnal bioluminescence rhythms in batches of 50 islets isolated from *Per1:Luc* rats housed under 1) standard LD (lights on at 6:00 h, lights off 18:00 h) cycle and given ad-libitum food intake (black lines), 2) 12 h advance in the light cycle DL (lights on at 18:00 h, lights off 6:00 h)) and given ad-libitum food intake (red lines), and 3) standard LD (lights on at 6:00 h, lights off 18:00 h) cycle with food intake restricted to 6 h period (12:00 h to 18:00 h) during the light phase of the LD cycle for 7 days (green lines).

(D) Peak phase (mean ± S.E.M) of *Per*-driven bioluminescence rhythms in (n=6–8) independent batches of 50 islets isolated from *Per1:Luc* rats housed under 1) standard LD cycle and given ad-libitum food intake (black circles), 2) 12 h advance in the light cycle DL and given ad-libitum food intake (red circles), and 3) standard LD cycle with food intake restricted to 6 h period (12:00 h to 18:00 h) during the light phase of the LD cycle (green circles). For clarity, data are plotted against the x-axis showing circadian time (0–24 h) with dark and light phases of LD cycle indicated by white vs. grey area shading. *P<0.05 denotes statistical significance vs. LD. *Reprinted with permission from American Diabetes Association from Ref [63]*
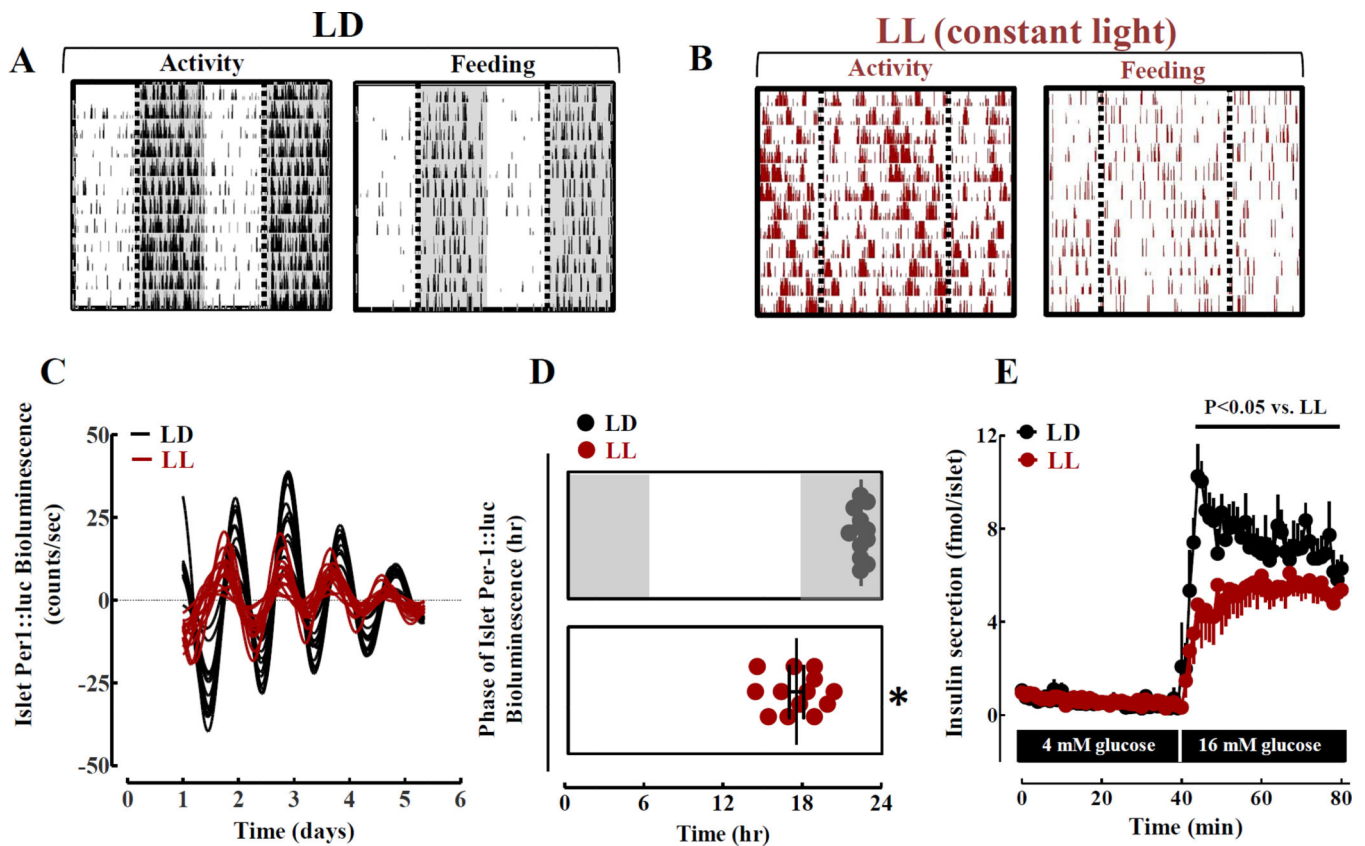
Figure 2. Continuous exposure to constant light disrupts islet clock and β-cell function

Actogram recordings of gross motor (left) and feeding (right) activity in representative rats exposed to 10 weeks (last 14 days is shown) of either (A) standard LD cycle (shown in black) or (B) constant light (LL) (shown in red). Note the complete behavioral arhythmicity in LL rats. (C) Representative examples of *Per*-driven diurnal bioluminescence rhythms imaged by ICCD camera at the level of individual islets (n=13) from *Per1:Luc* rats exposed to either 10 weeks of LD (black lines) or aberrant LL (red lines) cycles. Each black or red line represents *Per*-driven bioluminescence signal from an individual islet. Note the apparent loss of amplitude and synchrony in *Per1:Luc* expression among individual islets in LL rats. (D) Peak phase (mean ± S.E.M) of *Per*-driven bioluminescence rhythms in individual islets isolated from *Per1:Luc* rats exposed to either 10 weeks of LD (black circles) or LL (red circles) cycles. For clarity, data are plotted against the x-axis showing circadian time (0–24 h) with dark and light phases of LD cycle indicated by white vs. grey area shading. *P<0.05 denotes statistical significance vs. LD. (E) Mean insulin concentration profiles obtained during isolated islet perifusion at low 4 mM (0–40 min) and high 16 mM glucose (40–80 min) concentrations in isolated islets from rats exposed to 10 weeks of either standard LD (black lines) or LL (red lines) cycle (n=5 per condition). Note the apparent loss of glucose-stimulated insulin response in LL islets. *Reprinted with permission from American Diabetes Association from Ref [63]*
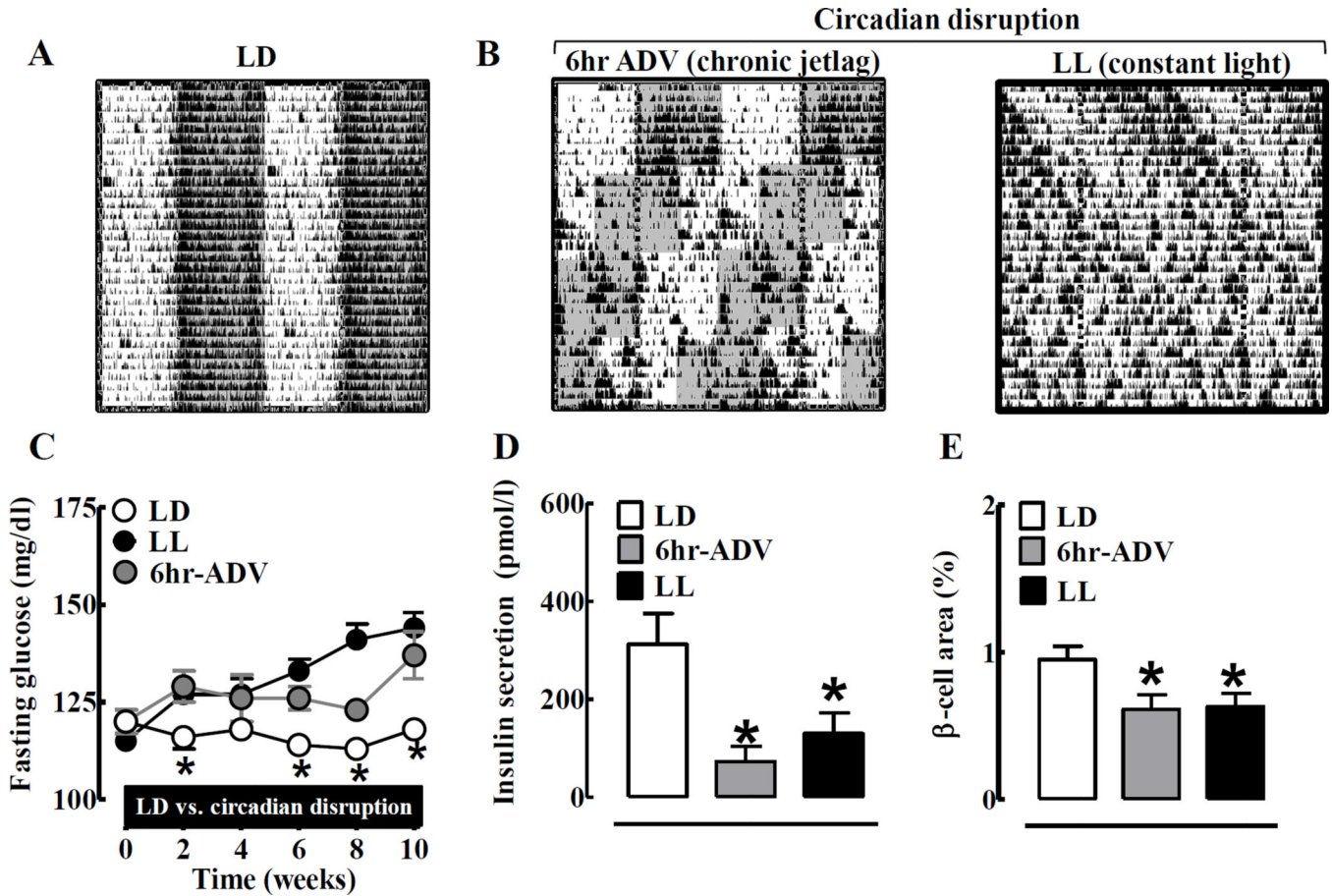
Figure 3. Circadian disruption accelerates development of diabetes in diabetes-prone HIP rats due to loss of β-cell function and mass

Representative double-plotted actograms of rats exposed to either standard LD cycle (A) or experimental changes in the LD cycle (B) produced by either 6 h advance of the light cycle every 3 days (chronic jetlag) or by constant light (LL). (C) Changes in plasma fasting glucose concentrations in diabetes-prone HIP rats exposed to 10 weeks of either standard LD (white circles), LL (black circles) or 6 h advances (grey circles) light regiments. Data are expressed as mean ± SEM, *P<0.05 statistical significance for changes in plasma glucose in LD vs. 6 h advance and LL. (D) Mean insulin response to glucose challenge during the hyperglycemic clamp in diabetes-prone HIP rats following 10 week exposure to LD (open bars), LL (black bars) or 6 h advance (grey bars). Data are expressed as mean ± SEM, *P<0.05 statistical significance vs. LD. (E) Mean β-cell fractional area in diabetes-prone HIP rats following 10 week exposure to LD (open bars), LL (black bars) and 6 h advance (gray bars). Data are expressed as mean ± SEM, *P<0.05 statistical significance vs. LD. *Reprinted with permission from Society for Research on Biological Rhythms from Ref [64*