Journal of Diabetes and Its Complications xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Journal of Diabetes and Its Complications

journal homepage: WWW.JDCJOURNAL.COM

Original Article

Glycemic control is impaired in the evening in prediabetes through multiple diurnal rhythms

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ARTICLE INFO

Article history:
Received 4 December 2013
Received in revised form 11 March 2014
Accepted 1 April 2014
Available online xxxx

Keywords:
Circadian rhythms
Diurnal rhythm
Glucose tolerance
Cortisol
Prediabetes
Oral Glucose Tolerance Test (OGTT)

ABSTRACT

Aims: Recent studies suggest that circadian rhythms regulate glucose metabolism, weight loss, and even drug efficacy. Moreover, molecules targeted at the circadian clock show promise in treating metabolic disease. Therefore, this study set out to better characterize interactions among diurnal rhythms in prediabetes.
Methods: Ten subjects with prediabetes completed oral glucose tolerance tests at 0700 h and 1900 h on the same day. Lipids and hormones were also measured.
Results: Two-hour and three-hour glucose tolerances were worse in the evening by 40 ± 52 mg/dl ($p = 0.02$) and 62 ± 46 mg/dl ($p = 0.001$), respectively. These impairments were explained by lower insulin sensitivity (OGIS; 5.14 ± 1.02 vs. 4.74 ± 0.77 mg/kg/min; $p = 0.03$) and 2-hour AUC insulin levels (87.4 ±37.6 vs. 69.8 ± 40.2 mU·hr/l; $p = 0.02$) in the evening. Intriguingly, more insulin resistant subjects had weaker rhythms in insulin sensitivity ($r = -0.66$; $p = 0.04$) but enhanced rhythms in insulin ($r = -0.67$; $p = 0.03$) and cortisol ($r = -0.78$; $p = 0.008$) levels. Importantly, the rhythms in cortisol primarily but also insulin sensitivity drove the declines in evening glucose tolerance ($r = 0.86$; $p = 0.002$).
Conclusions: Glycemic control is dramatically impaired in the evening in people with prediabetes, particularly when the cortisol rhythm is weak, but is unrelated to the rhythm in insulin levels. Therefore, food intake at dinnertime may need to be curbed in prediabetes.

1. Introduction

Within the past decade, there has been renewed interest in the role of circadian rhythms as contributing regulators of energy metabolism and disease risk factors (Gimble, Bray, & Young, 2009; Green, Takahashi, & Bass, 2008). The body's circadian clock operates within the suprachiasmatic nucleus of the brain where the molecular machinery oscillates at the protein and RNA levels in accordance with a 24-hour rhythm (Welsh, Takahashi, & Kay, 2010). The circadian clock's molecular apparatus has been detected throughout the body and is expressed within metabolically active organs such as adipose tissue and liver (Ando et al., 2005; Loboda et al., 2009; Zvonic et al., 2006), affecting an estimated 8–10% of all genes. Moreover, it has physiologically important effects. For instance, intravenous glucose tolerance testing in rodents shows that glucose uptake is higher at the start of their daily activity period (equivalent to breakfast) compared to the end of their activity period, but lesioning of the suprachiasmatic nucleus eliminates this difference (la Fleur, Kalsbeek, Wortel, Fekkes, & Buijs, 2001). Similarly, hamsters increase their lipogenic response to insulin five-fold during their active period compared to their inactive sleeping period (Cincotta & Meier, 1984).

There is also evidence that glucose metabolism displays a circadian rhythm in human subjects (Bowen & Reeves, 1967; Jarrett & Keen, 1969; Lee, Ader, Bray, & Bergman, 1992; Roberts, 1964; Saad et al., 2012; Schulz, Ratzmann, Albrecht, & Bibergeil, 1983; Shapiro et al., 1988). Clinical studies have used both oral glucose tolerance and glucose infusion tests to evaluate the influence of time of day on glucose metabolism in healthy control, prediabetic, and diabetic subjects. In most cases, declines in glucose tolerance and/or other facets of glycemic control have been reported later in the day. These metabolic mechanisms have in turn been linked to the diurnal oscillations of cortisol, catecholamines, and growth hormone (Bolli &

Grant Support: Jenny Craig and the Pennington Biomedical Research Foundation.
Disclosure Statement: T.S., J.R., and C.M.P. have no conflicts of interest to declare. J.M.G. is the co-founder, co-owner, and Chief Scientific Officer of LaCell LLC, a Louisiana-based biotechnology company focusing on the use of adult stem cells isolated from adipose tissue.
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http://dx.doi.org/10.1016/j.jdiacomp.2014.04.001
1056-8727/

Gerich, 1984; Bolli et al., 1984; Plat et al., 1996), but the reasons and interrelationships among these factors have remained unclear.

Moreover, the timing of food intake may impact obesity and other metabolic endpoints. In a three-week study in 12 human subjects, when subjects consumed all their food in the morning within one hour after awakening, they lost weight; however, when study participants shifted back the timing of the meal to 12 hours later, eating all calories in the evening, they lost no weight (Halberg, 1989). Also, mice fed a high-fat meal at the beginning of their active period and a high carbohydrate meal at the end of the active period increased fat oxidation throughout the active period and maintained a lower body weight, in comparison to animals who ate the same meals and same calories but in the reverse order (Bray et al., 2010).

In addition, there is increasing evidence that small molecules and proteins can be used to modify circadian clocks to treat metabolic disease (Chen, Yoo, & Takahashi, 2013; Kumar et al., 2010). For example, treatment with bromocriptine reduces prolactin, a hormone involved in circadian rhythms, and consequently increases insulin sensitivity in humans (Barnett, Chapman, Gailer, & Hayter, 1980). When bromocriptine was given in the morning, glycemic control as measured by hemoglobin A1c improved (Aminorroaya, Janghorbani, Ramezani, Haghighi, & Amini, 2004), but when bromocriptine was given twice a day, there was no improvement, suggesting that diurnal variation in prolactin was needed to improve glycemic control (Wasada, Kawahara, & Iwamoto, 2000). Consequently, bromocriptine administration in the morning is now approved for the treatment of type 2 diabetes and has been shown to improve insulin sensitivity and hard cardiovascular endpoints like myocardial infarction and stroke (Gaziano et al., 2010). Interestingly, Olanzapine, an anti-psychotic medication known to induce insulin resistance and increase body weight, also increases prolactin secretion (Vidarsdottir, Roelfsema, Frolich, & Pijl, 2009).

Based on these findings in animal and human models, the current study set out to evaluate the diurnal rhythms in glucose and insulin dynamics in prediabetic human subjects. The primary goal was to provide a more sophisticated characterization of the interrelationships among various diurnal patterns, in order to determine which factor(s) are the largest contributors to the evening decline in glucose tolerance; to our knowledge, this is the first such analytic approach of its kind. A secondary aim of this study was to determine whether sequential fasting oral glucose tolerance tests administered within a single day can be used as a simple but robust measure of diurnal patterns in glucose homeostasis.

2. Subjects, materials, and methods

The study protocol was reviewed and approved by the Institutional Review Board at Pennington Biomedical Research Center prior to implementation, and the trial was registered under number NCT01546545 on ClinicalTrials.gov. The study was performed in accordance with the Declaration of Helsinki, and all study subjects gave written informed consent prior to participating.

2.1. Participants

Study subjects were required to have impaired fasting glucose (100–125 mg/dl), indicative of prediabetes, at screening and to be between 18 and 70 years of age. Potential participants were excluded if they were pregnant or nursing; taking a medication for diabetes or a medication like systemic glucocorticoids that affects glucose or insulin; on a chronic medication without a stable dose for at least 1 month; or taking any anti-psychotic medication with known circadian effects. None of the study participants were shift workers. Also, prior to testing, sleep and feeding schedules were not regularized or standardized in study participants; instead, the goal of this study was to investigate the natural variations in diurnal patterns in glucose homeostasis without any intervention.

2.2. Study design

This study consisted of one screening visit and one clinic visit. The screening visit involved the completion of a health questionnaire and anthropometric measurements with the study coordinator (TS). Eleven eligible subjects were recruited between May 2012 and April 2013, and ten completed the protocol, undergoing two oral glucose tolerance tests (OGTTs) separated by 12 hours: one at 0700 h (morning or AM test) and a second at 1900 h (evening or PM test). On the night prior to testing, subjects fasted from 2100 h until 0700 h the following morning. At 0700 h, the morning OGTT was performed by administering a 75 gram oral glucose load. Blood was drawn at −15, 0, 30, 60, 120 and 180 minutes for measurement of glucose and insulin. Following the 180-minute blood draw (1000 h), subjects were served a brunch and allowed to eat to their satisfaction. After 1100 h, subjects were not allowed food or liquids other than water. After 8 hours of fasting (at 1900 h), the evening OGTT was performed, and the subjects repeated the same three-hour OGTT procedure as in the morning.

2.3. Measurement of serum markers

Immediately following collection, blood samples were centrifuged at room temperature, and the serum was dispensed into aliquots and stored at −20°C. All serum samples were later thawed and assayed for glucose and insulin levels. The fasting serum samples (both AM and PM) were also assayed for the following hormones and lipids: cortisol, leptin, growth hormone, free fatty acids, cholesterol, HDL, LDL, and triglycerides. All assays were conducted in the Clinical Chemistry Core Facility at Pennington Biomedical. The between-assay (within-assay) coefficients of variation (CVs) for glucose, insulin, cortisol, leptin, and growth hormone are 1.87% (1.65%), 7.3% (5.5%), 8.2% (6.1%), 8.3% (6.2%), and 6.6% (4.6%). Similarly, the CVs for cholesterol and free fatty acids are 4.5% (3.0%) and 5.65%.

2.4. Calculations

Areas under the curve (AUCs) for glucose and insulin during the OGTTs were determined using the trapezoidal rule. Incremental AUCs were calculated by subtracting the values at 0 minutes from each time point and then calculating the AUCs with the modified values. HOMA-IR values were calculated according to the formula: fasting glucose (mg/dl) x fasting insulin (mU/l)/405 (Matthews et al., 1985). To assess β-cell function, two OGTT-derived indices of insulin release were used: the insulinogenic index and the AUC ratio. The insulinogenic index, an indicator of early-phase β-cell function, is the ratio of the change in insulin levels to the change in glucose levels during the first 30 minutes of the OGTT ($\Delta Ins_{30}/\Delta Glu_{30}$) (Seltzer, Allen, Herron, & Brennan, 1967). The AUC ratio is the ratio of AUC insulin to AUC glucose and has been validated as a robust measure of β-cell function (Retnakaran et al., 2008). As estimates of insulin sensitivity, the glucose clearance rates for an OGTT (Cl_{OGTT}) and a hyperinsulinemic euglycemic clamp (OGIS) were calculated as formulated by Mari, Pacini, Murphy, Ludvik, and Nolan (2001) (Mari et al., 2001); these dynamically-based estimates represent the clearance of glucose at hyperglycemia and euglycemia, respectively. Finally, the AM and PM values for fasting glucose, glucose tolerance, insulin levels, and their derivative indices, along with cortisol and leptin, were compared using one-tailed paired t-tests because of prior evidence indicating one-sided behavior. All other variables were compared with two-tailed paired t-tests. Statistical significance was set at $p \leq 0.05$. Values are reported as mean ± SD, with AM values listed first, except where noted otherwise. However, the data in figures are shown as mean ± SEM for visual clarity.

Please cite this article as: Sonnier, T., et al., Glycemic control is impaired in the evening in prediabetes through multiple diurnal rhythms, *Journal of Diabetes and Its Complications* (2014), http://dx.doi.org/10.1016/j.jdiacomp.2014.04.001