ORIGINAL ARTICLE

Nocturnal Glucose Metabolism in Type 1 Diabetes: A Study Comparing Single Versus Dual Tracer Approaches

Ashwini Mallad, MD,¹ Ling Hinshaw, MD, PhD,¹ Chiara Dalla Man, PhD,² Claudio Cobelli, PhD,² Rita Basu, MD,¹ Ravi Lingineni, BSc,³ Rickey E. Carter, PhD,³ Yogish C. Kudva, MD,¹ and Ananda Basu, MD¹

Abstract

Background: Understanding the effect size, variability, and underlying physiology of the dawn phenomenon is important for next-generation closed-loop control algorithms for type 1 diabetes (T1D).

Subjects and Methods: We used an iterative protocol design to study 16 subjects with T1D on individualized insulin pump therapy for two successive nights. Endogenous glucose production (EGP) rates at 3 a.m. and 7 a.m. were measured with [6,6-²H₂]glucose as a single tracer, infused from midnight to 7 a.m. in all subjects. To explore possibility of tracer recycling due to prolonged [6,6-²H₂]glucose infusion, which was highly probable after preplanned interim data analyses, we infused a second tracer, [6-³H]glucose, from 4 a.m. to 7 a.m. in the last seven subjects to measure EGP at 7 a.m.

Results: Cortisol concentrations increased during both nights, but changes in glucagon and insulin concentration were inconsistent. Although the plasma glucose concentrations rose from midnight to 7 a.m. during both nights, EGP measured with [6,6-²H₂]glucose between 3 a.m. and 7 a.m. did not differ during Night 1 but fell in Night 2. However, EGP measured with [6-³H]glucose at 7 a.m. was higher than that measured with [6,6-²H₂]glucose during both nights, thereby suggesting tracer recycling probably underestimating EGP calculated at 7 a.m. with [6,6-²H₂]glucose. Likewise, EGP was higher at 7 a.m. with [6-³H]glucose than at 3 a.m. with [6,6-²H₂]glucose during both nights.

Conclusions: The data demonstrate a consistent overnight rise in glucose concentrations through increased EGP, mediated likely by rising cortisol concentrations. The observations with the dual tracer approach imply significant tracer recycling leading to underestimation of EGP measured by longer-duration tracer infusion.

Introduction

GLUCOSE VARIABILITY, BOTH NOCTURNAL and diurnal, is part of daily living in individuals with type 1 diabetes (T1D). Diurnal glucose variability is primarily related to meals and physical activity. Nocturnal glucose variability has been attributed in part to the putative “dawn” phenomenon, a term that was first coined more than 30 years ago to describe the rise in blood glucose concentrations in the early morning in patients with T1D treated with dinnertime dosages of ultralente, semilente, or NPH insulin.^{1,2} This phenomenon has been subsequently described in type 2 diabetes^{3–5} and, indirectly, in healthy controls.^{6,7} Its pathophysiological basis has been attributed variously to changes in plasma cortisol¹ or growth hormone⁸ concentrations, duration of action of din-

ner-time insulin injections,^{1,2,9,10} or, most likely, a combination of the two. Quantification of the effect size of the dawn phenomenon in T1D has demonstrated that the rise in glucose concentrations is approximately 15–25 mg/dL (0.8–1.4 mmol/mol), although recent reports have suggested mitigation of this effect with increasing use of long-acting insulin analogs.^{9,10}

Although increases in rates of endogenous glucose production (EGP) in the morning have been implicated in the cause of fasting hyperglycemia in type 2 diabetes and prediabetes,^{11–14} investigations related to nocturnal glucose turnover using state-of-the-art methods in T1D have, to the best of our knowledge, not been undertaken. Furthermore, in the era of modern diabetes technology, including in T1D patients using insulin pumps (continuous subcutaneous insulin infusion

Departments of ¹Endocrinology and ³Health Sciences Research, Mayo Clinic, Rochester, Minnesota.

²Department of Information Engineering, University of Padova, Padova, Italy.

[CSII]), further studies are necessary to determine the effect size, the pathophysiological basis, and the night-to-night variability of the “dawn” phenomenon not only to help manage the population of T1D patients on open-loop CSII therapy, but also to better inform closed-loop control algorithms that are currently being developed and refined to treat patients with T1D. This is especially critical because current best strategies for management confirm unacceptable overnight glucose control with nocturnal hypoglycemia rates ranging from 6% to 10%.¹⁵

Therefore, we designed an iterative series of experiments to determine nocturnal glucose metabolism during two successive nights using isotope dilution techniques. Each individual subject was permitted to maintain his or her customary overnight basal insulin pump infusion rate during both nights. The study design of two successive nights permitted assessment of the degree of consistency of nocturnal glucose metabolism in T1D. Furthermore, in order to assess differences, if any, in glucose turnover possibly due to glucose tracer recycling (i.e., tracer uptake into glycogen followed by re-appearance into glucose via glycogenolysis) that can result during prolonged tracer infusion in the postabsorptive state, we used a dual tracer approach to estimate overnight glucose fluxes in a subcohort of subjects.

Subjects and Methods

After approval from the Mayo Institutional Review Board, subjects with T1D signed informed consent to participate in the study.

Participants

Screen visit. Subjects reported in the morning after an overnight fast to the Clinical Research Unit (CRU) of the Mayo Center for Translational Science Activities for a history, physical examination, screening laboratory tests, standard urinalysis, and resting electrocardiogram. CSII data were downloaded and discussed with the subjects. All women of childbearing potential had a negative pregnancy test within 24 h of the study visit. A dietary history was taken to ensure adherence to a weight-maintaining diet consisting of at least 200 g of carbohydrates/day and that diet met American Diabetes Association guidelines for protein, fat, and carbohydrates. Body composition was measured using dual-energy X-ray absorptiometry.

Day 1–Night 1. Subjects meeting inclusion criteria were admitted to the CRU at approximately 4 p.m. A point-of-care urine pregnancy test was performed where appropriate to ensure that the test was negative before proceeding any further. Subjects were then provided a standard 10 kcal/kg meal (55% carbohydrate, 15% protein, and 30% fat) consumed between 5 and 5:30 p.m. Bolus insulin was administered at the start of the meal as per each individual subject’s insulin:carbohydrate ratio. No additional food was given until the next morning unless hypoglycemia occurred. Intravenous cannulas were inserted into both forearms at 8 p.m. for tracer infusion and periodic blood draws.

At 12 a.m., an adjusted primed continuous infusion of [6,6-²H₂]glucose was started. In the course of the experiment after completion of approximately half the total intended number of study subjects, glucose tracer recycling confounding glucose turnover calculations was strongly suspected based on

physiologically implausible EGP measurements at 7 a.m. using [6,6-²H₂]glucose as elucidated in the Results and Discussion below. Therefore, to indirectly prove the presence of tracer recycling, the protocol was modified by adding infusion of a second tracer, [6-³H]glucose, starting at 4 a.m. with an adjusted prime dose in the subsequent seven subjects. Blood was drawn periodically overnight to measure concentrations of glucose, [6,6-²H₂]glucose, [6-³H]glucose, and hormones.

Day 2. Study participants received three weighed meals prepared by the CRU metabolic kitchen, with each meal comprising 33% of total estimated calorie intake based on Harris Benedict calorie requirements (75 g of carbohydrate in each meal). The macronutrient contents that each participant consumed per meal were identical. No snacks or calorie-containing drinks were permitted between meals except to treat incident hypoglycemia. Prandial insulin boluses were administered through each subject’s insulin pump according to his or her customary insulin:carbohydrate ratios. Meals were served at the following times: breakfast at 7 a.m., lunch at 1 p.m., and dinner at 7 p.m.

Night 2. The tracer infusion protocols and periodic blood draws were identical to Night 1.

Day 3. After completion of the last blood draw, all cannulas were removed, and the subjects were provided breakfast along with their customary prandial insulin bolus and discharged from the CRU.

Analytical techniques

Hormone analyses. C-peptide assay was performed on the Cobas e411 (Roche Diagnostics, Indianapolis, IN). This is a two-site immunometric assay using electrochemiluminescence detection. Insulin was measured with a two-site immunoenzymatic assay performed on the DxI automated system (Beckman Instruments, Chaska, MN). Glucagon was measured by a direct, double-antibody radioimmunoassay (Linco Research, St. Charles, MO). Catecholamines were measured by reversed-phase high-performance liquid chromatography with electrochemical detection after extraction with activated alumina. Cortisol was measured with a competitive binding immunoenzymatic assay on the DxI automated immunoassay system. Human growth hormone was measured with a two-site immunoenzymatic assay performed on the DxI automated system.

Glucose tracers. Plasma samples were placed on ice, centrifuged at 4°C, separated, and stored at –80°C until assay. Plasma glucose concentration was measured by a glucose oxidase method (YSI, Inc., Yellow Springs, OH). Plasma [6-³H]glucose specific activity was measured by liquid scintillation counting. Plasma enrichment of [6,6-²H₂]glucose was measured using gas chromatography–mass spectrometry (Thermoquest, San Jose, CA).¹⁶

Calculations

Rates of EGP were estimated at 3 a.m. and at 7 a.m. with [6,6-²H₂]glucose and at 7 a.m. with [6-³H]glucose, all using the equation of Steele et al.¹⁷

FIG. 1. Mean (SD) concentrations of plasma (A) glucose (top left), insulin (top right), glucagon (bottom left), and cortisol (bottom right) and (B) growth hormone (top left), dopamine (top right), epinephrine (bottom left), and norepinephrine (bottom right) between 12 a.m. and 7 a.m. during Night 1 (solid line) and Night 2 (broken line) in the study cohort.

Statistical methods

Continuous variables are presented as mean (SD) values, and categorical variables are given as frequency (%). Longitudinal summary statistics¹⁸ were used to describe the changes in glucose, insulin, glucagon, and cortisol concentrations from 12 a.m. to 7 a.m. Linear trajectories were fit to each subject's serial observations in order to estimate the slope (or rate of change per hour). A one-sample *t* test was used to test if the slopes were equal to zero for each day, and a paired test was used to assess if the mean slopes were different between the two nights.

To further explore the changes in the measures, mixed models with random subject effect were used to assess the mean difference between the nights and between 3 a.m. and 7 a.m. for concentrations of hormones and glucose, as well as non-steady-state EGP using [6,6-²H₂]glucose and [6-³H]glucose. Because cortisol was obtained at 4 a.m. instead of 3 a.m., the change between 4 a.m. and 7 a.m. was calculated.

The effects of possible tracer recycling were also examined by comparing the estimated EGP using the first infused tracer ([6,6-²H₂]glucose) at 7 a.m. with the second infused tracer ([6-³H]glucose) at 7 a.m. for the subjects to whom both tracers were administered. Although some variation in estimates would be expected (random variation), bias was detected with the estimates from the first estimates. As such, comparisons of the rise in EGP from 3 a.m. to 7 a.m. are based on all available data obtained at 3 a.m. versus only those participants with the second tracer at 7 a.m. using a mixed model. This approach was essentially a generalized paired *t* test that allowed for subjects with missing 7 a.m. data to be used to estimate the 3 a.m. EGP measurements.

Statistical analyses were conducted using the SAS System (version 9.4; SAS Institute, Cary, NC). *P* values of <0.05 were taken as statistically significant. No correction for multiple testing has been applied to reported *P* values.

Results

Sixteen (eight males) subjects with T1D completed the study. The mean (SD) age and body mass index of the study population was 44.9 (12.5) years and 28.6 (5.5) kg/m², respectively. During the screen visit, the mean fasting blood glucose concentration was 9.8 (3.4) mM, hemoglobin A1c was 7.6% (0.7%) [60 (7.7) mmol/mol], percentage body fat was 34.9% (8.6%), and duration of diabetes was 27 (12.3) years. Subjects did not have any micro- or macrovascular complications apart from stable background retinopathy.

Overnight changes in glucose and hormone concentrations

The serial plasma concentrations of glucose, insulin, glucagon, and cortisol are presented in Figure 1A and summarized in Table 1. As illustrated, glucose values rose throughout the night on both nights; however, the mean rise on Night 1 (0.4 mM/h) reached statistical significance (*P*=0.02), and the rise on Night 2 did not (0.3 mM/h) (*P*=0.06). There were no statistical differences in these slopes between nights (*P*=0.4). Insulin levels decreased during Night 1 (−0.5 pM/h; *P*=0.02) and to a lesser extent during Night 2 (−0.3 pM/h; *P*=0.06). Although changes in glucagon concentrations within a given night were not found to be statistically significant, the dif-

TABLE 1. SUMMARY OF THE RATES OF CHANGE IN GLUCOSE AND HORMONE CONCENTRATIONS FROM 12 A.M. TO 7 A.M. (N=16)

Variable, night	Estimated hourly change in hormone ^a		
	Mean (SD)	95% CI	P
Glucose (mM/h)			
1	0.4 (0.7)	(0.1, 0.8)	0.0213
2	0.3 (0.5)	(−0.01, 0.5)	0.0576
Change	0.2 (0.8)	(−0.3, 0.6)	0.4008
Insulin (pM/h)			
1	−0.5 (0.8)	(−0.9, −0.1)	0.0226
2	−0.3 (0.6)	(−0.6, 0.01)	0.0564
Change	−0.2 (1.1)	(−0.8, 0.4)	0.4570
Glucagon (pg/mL×h)			
1	0.8 (1.8)	(−0.2, 1.7)	0.1130
2	−0.9 (1.8)	(−1.8, 0.1)	0.0786
Change	1.6 (2.8)	(0.1, 3.1)	0.0353
Cortisol (μg/dL×h)			
1	1.4 (0.7)	(1.1, 1.8)	<0.001
2	1.5 (0.5)	(1.3, 1.8)	<0.001
Change	−0.1 (0.6)	(−0.4, 0.2)	0.5279

P values are from one-sample *t* tests to test if the slopes were equal to zero (for Night 1 or 2) or if the slopes were statistically different between study days (change). The mean (SD) values are simple summary statistics of the data. The mean (95% confidence interval [CI]) represents model-based estimate of the change.

^aEstimated hourly changes are the summary of the patient-level rates of change (linear trajectory slope).

ference in slopes between nights was found to be statistically significant (*P*=0.04). Cortisol levels were observed to rise during both nights with no statistical differences in the slopes (*P*=0.5).

Growth hormone, dopamine, epinephrine, and norepinephrine concentrations

The serial plasma concentrations of growth hormone, dopamine, epinephrine, and norepinephrine are presented in Figure 1B. As illustrated, the nocturnal profiles or slopes of growth hormone, dopamine, epinephrine, and norepinephrine concentrations did not vary during both nights or between the two nights.

Tracer-tracee ratio

Despite a constant and identical infusion rate of [6,6-²H₂]glucose that was started at 12 a.m. at both nights, plasma enrichment of [6,6-²H₂]glucose tended to be higher at 7 a.m. than at 3 a.m. during both nights (Fig. 2, top panel), suggesting continuing recycling of [6,6-²H₂]glucose into glycogen and back to glucose as the duration of fast increased. In contrast, the specific activity of [6-³H]glucose in plasma was relatively unchanging after the infusion was started at 4 a.m.

EGP

The study design was modified to include a second tracer ([6-³H]glucose) to measure EGP at 7 a.m. to determine if EGP values obtained with [6-³H]glucose at 7 a.m. were concordant

FIG. 2. (Top panel) Tracer–tracee ratios and (bottom panel) rates of endogenous glucose production during Night 1 (solid line) and Night 2 (broken line): [6,6-²H₂]glucose (circles) and [6-³H]glucose (squares). FFM, fat free mass.

with those obtained at the same time with [6,6-²H₂]glucose. Therefore, there were reliable estimates of EGP from all 16 participants at 3 a.m. using [6,6-²H₂]glucose as the tracer, whereas only seven reliable estimates were available at 7 a.m. using [6-³H]glucose as the tracer (Fig. 2, bottom panel). Rates of EGP, measured using [6,6-²H₂]glucose at 3 a.m., were 18.6 (5.5) and 17.4 (5.4) $\mu\text{mol/kg/min}$ for Nights 1 and 2, respectively (Table 2). Furthermore, rates of EGP measured using [6,6-²H₂]glucose at 7 a.m. were 18.2 (5.0) and 15.4 (5.6) $\mu\text{mol/kg/min}$ for Nights 1 and 2, respectively. Although there was no difference in [6,6-²H₂]glucose-determined EGP between 3 a.m. and 7 a.m. in Night 1 ($P=0.8$), rates of EGP fell during Night 2 ($P<0.02$), despite increases in plasma glucose concentrations throughout both nights. The rate of EGP at 7 a.m. measured using [6-³H]glucose for Night 1 was 33.9 (11.2) $\mu\text{mol/kg/min}$, and that for Night 2 was 21.2 (8.0) $\mu\text{mol/kg/min}$. On both nights, we observed a rise in the rates of EGP from 3 a.m. to 7 a.m. ($P=0.011$ and $P<0.001$ for Nights 1 and 2, respectively); however, the rise observed on Night 1 was statistically greater than that observed on Night 2 ($P=0.029$).

Discussion

Applying state-of-the-art techniques, we have described the temporal profiles of nocturnal glucose kinetics and hormonal concentrations from midnight to 7 a.m. in a cohort of individuals with moderately controlled T1D on CSII therapy. Our observations include the following: (a) the rise in plasma glucose concentrations occurs continuously from 12 a.m. to 7 a.m.; (b) the pattern and extent of the rise in glucose from 12 a.m. to 7 a.m. were congruent on both consecutive nights; (c) there was a rise in plasma cortisol concentrations during both nights, whereas temporal patterns of insulin, glucagon, and growth hormone concentrations were inconsistent; and (d) the data suggest the presence of tracer recycling overnight, which confounded interpretation of EGP at 7 a.m. calculated by the longer-duration [6,6-²H₂]glucose infusion.

The impact of the duration of tracer infusion on isotope recycling with consequent errors in measurements of glucose turnover has been extensively described. The concept of glucose isotope cycling lies on the premise that glycogen

TABLE 2. TESTS FOR DIFFERENCES IN GLUCOSE, HORMONES, AND ENDOGENOUS GLUCOSE PRODUCTION BETWEEN 3 A.M. AND 7 A.M.

	Mean (SD)			
Night, variable	3 a.m.	7 a.m.	Difference [estimate (95% CI)] ^a	P
Night 1				
Glucose (mM)	8.1 (3.8)	10.1 (3.0)	1.9 (−0.3, 4.2)	0.0842
Insulin (pM)	12.8 (6.7)	10.2 (6.0)	−2.5 (−4.5, −0.4)	0.0220
Glucagon (pg/mL)	55.7 (13.9)	66.2 (16.9)	10.5 (3.8, 17.2)	0.0045
Cortisol (μg/dL) ^b	6.9 (2.9)	11.5 (2.8)	4.3 (2.5, 6.1)	<0.001
EGP (μM/kg of FFM/min) ^c	18.6 (5.5)	33.9 (11.2)	14.5 (4.6, 24.4)	0.0105
Night 2				
Glucose (mM)	7.9 (2.5)	9.3 (3.7)	1.4 (0.3, 2.5)	0.0138
Insulin (pM)	11.4 (6.2)	10.2 (5.9)	−1.2 (−4.4, 2.1)	0.4582
Glucagon (pg/mL)	59.8 (12.6)	60.8 (15.0)	1.1 (−7.8, 10.0)	0.8027
Cortisol (μg/dL) ^b	9.2 (4.5)	10.7 (2.8)	1.4 (−0.7, 3.5)	0.1748
EGP (μM/kg of FFM/min) ^c	17.4 (5.4)	21.2 (8.0)	2.9 (1.9, 3.9)	<0.001
Differences in the rise from 3 a.m. to 7 a.m. between study nights				
Glucose (mM)	—	—	0.5 (−1.9, 3.0)	0.6393
Insulin (pM)	—	—	−1.3 (−3.5, 1.0)	0.2368
Glucagon (pg/mL)	—	—	9.4 (−3.0, 21.8)	0.1254
Cortisol (μg/dL) ^b	—	—	2.9 (0.6, 5.2)	0.0163
EGP (μM/kg of FFM/min) ^c	—	—	11.6 (1.6, 21.5)	0.0289

The mean (SD) values are simple summary statistics of the data. The mean (95% confidence interval [CI]) represents model-based estimate of the change.

^aModel-based estimates for mean difference.

^bMean (SD) cortisol was at 4 a.m. instead of 3 a.m.

^cEndogenous glucose production (EGP) at 3 a.m. is based on [6,6-²H₂]glucose tracer (*n*=16) and at 7 a.m. using [6-³H]glucose tracer (*n*=7). For all other measurements, *n*=16 per day and time point except for one missing value for the Night 2 cortisol at 7 a.m. and Night 1 insulin at 7 a.m.

FFM, fat free mass.

synthesis from glucose and glycogen breakdown into glucose are simultaneously occurring phenomena in humans.^{19–21} Glucose cycling (i.e., cycling between glucose and glucose-6-phosphate [also termed futile cycling]) occurs in humans both in the fasted state and during hyperglycemia²² and is increased in type 2 diabetes^{23,24} but not in T1D either at euglycemia or at hyperglycemia.²⁵ However, the latter study did not measure nocturnal rates of EGP and infused several glucose tracers simultaneously for a 2-h infusion period only, before estimation of glucose turnover. The discrepancies in the rates of EGP between the tracers used were attributed to glucose–fructose cycling and to tracer impurities. Furthermore, the glucagon concentrations achieved in this report were severalfold higher than that achieved in the current study, although part of the differences could be because of changes in glucagon assay methodology over the years.

The relative contributions of glycogen synthesis and breakdown to glucose cycling depend on the prevailing insulin, glucagon, and glucose concentrations, the degree of hepatic insulin resistance,^{23,24} whether studies are performed in the fed²⁶ or fasted conditions,^{19–22} and the durations of postabsorptive or postprandial conditions. Both carbon- and hydrogen-labeled isotopes of glucose can be recycled by incorporation into glycogen through glycogen synthesis with subsequent release via glycogenolysis into glucose. Although glycogen synthesis could occur through both direct (from circulating glucose) and indirect (from circulating pyruvate through gluconeogenesis) pathways, it is noteworthy that only carbon-labeled glucose can potentially recycle via the indirect pathway.²⁷ In an elegant series of experiments in

overnight fasted healthy subjects when plasma glucose concentrations were unchanged at approximately 5.5 mM, Tigas et al.²⁸ concluded that in the postabsorptive stage, both carbon- and hydrogen-labeled glucose tracers resulted in comparable estimates of EGP, with longer duration of tracer infusions resulting in lower estimates of EGP, as is observed in our current study.

In our study both glucose isotopes used were hydrogen-labeled in the carbon-6 position. Hence it is unlikely that isotopic discrimination between these two isotopes could have confounded our results. Furthermore, plasma glucose concentrations rose gradually throughout both nights, and glucose concentrations were higher at 7 a.m. than at 3 a.m. Because EGP is the only source of glucose that could result in the observed rise in nocturnal plasma glucose concentrations, it seems logical that the unchanging rates of [6,6-²H₂]glucose-determined EGP calculated in Night 1 and the decreasing rates of EGP in Night 2 are erroneous by underestimating rates of EGP at 7 a.m. likely due to glucose recycling, with the contribution of recycling increasing as the duration of fasting progressively increased overnight. On the other hand, rates of EGP at 7 a.m. calculated by the shorter-duration (3-h) infusion of [6-³H]glucose were higher than the rates of EGP calculated at the same time with the longer-duration (7-h) infusion of [6,6-²H₂]glucose, thereby strengthening the biological plausibility of accuracy of EGP rates at 7 a.m. measured by [6-³H]glucose. Additionally, the rise in enrichment of [6,6-²H₂]glucose during both nights provides further proof of tracer recycling. Because [6,6-²H₂]glucose (tracer) infusion rates were identical

and constant throughout both nights, a rising tracer–tracee ratio in the presence of a steady increase in the glucose (tracee) concentration implies significant re-entry of tracer ($[6,6-^2\text{H}_2]\text{glucose}$) into the circulation through glycogen cycling. However, although our data imply tracer recycling occurring overnight, it does not prove it. To do so, ideally, would require monitoring of tracer incorporation into intermediary metabolites (e.g., glucose-1-phosphate or pyruvate).

Hepatic glycogen content is also a potential determinant of overnight EGP. None of the subjects developed hypoglycemia (plasma glucose concentrations <70 mg/dL) during the study protocol, hence minimizing possible confounding due to unequal glycogen stores related to accelerated glycogenolysis pertaining to hypoglycemia. Furthermore, the equivalent changes in plasma glucose concentrations together with a lack of difference in the slopes of plasma glucose profiles between 12 a.m. and 7 a.m. during both nights imply consistency of nocturnal glucose turnover, at least in this study cohort.

Changes in prevailing hormone concentrations could also potentially affect glucose cycling in humans.^{29–32} In an elegant series of experiments in healthy subjects,³³ glucose cycling increased with increasing glucagon concentrations, but this increase was inhibited when insulin concentrations were also permitted to rise. However, the plasma glucagon concentrations that augmented glucose cycling were approximately three- to ninefold higher than that observed in the current study, although part of the differences in glucagon concentrations could be related to changes in glucagon assay over time. During Night 1, there were slight but significant changes, in opposite directions, in insulin and glucagon concentrations between 3 a.m. and 7 a.m. that could have, at least in part, contributed to the rising glucose levels and rates of EGP. However, during Night 2, there was a similar rise in glucose concentrations between 3 a.m. and 7 a.m., without accompanying changes to insulin or glucagon levels during this time.

In contrast, cortisol concentrations rose during this period in both nights. It is interesting that in a study in healthy humans, high-dose, short-term dexamethasone administration increased glucose cycling without changing EGP.³⁴ Although plasma corticosteroid concentrations were not measured in that study, the high-dose dexamethasone used (3 mg twice a day) likely would have raised plasma corticosteroid concentrations much more than the physiological rise in nocturnal plasma cortisol concentrations observed in this study. Additionally, the temporal profiles (slope) of cortisol concentrations were similar during both nights, thus minimizing confounding in interpretation of glucose kinetics between nights. The relationship between the physiological nocturnal rises in cortisol concentrations and glucose kinetics in T1D is controversial. In an elegant series of experiments, Dinneen et al.³⁵ described that the nocturnal physiological rise in cortisol reduced postprandial glucose uptake and increased postprandial glucose appearance after breakfast in a cohort of T1D subjects. In other T1D studies,^{36,37} using the Biostator, overnight cortisol blockade with metyrapone or suppression of pituitary–adrenal axis with dexamethasone did not alter insulin requirements in the early morning. However, none of the above studies measured glucose turnover throughout the night, and they infused insulin intravenously to maintain euglycemia in the overnight period.

Like all studies, our study also has limitations. These data imply, but do not definitively prove, the presence of tracer recycling in the overnight fasted state because we did not monitor tracer enrichments in intermediary metabolites of glycogen and/or glucose as alluded to earlier. We suspected confounding due to tracer recycling on analyzing the EGP data obtained with $[6,6-^2\text{H}_2]\text{glucose}$. In order to accurately estimate EGP at regular 2–3-h intervals throughout the night, we would have ideally had to sequentially infuse at least one or two additional glucose tracers while monitoring for tracer enrichments in the intermediary metabolites. Such studies will need to be performed to elucidate this further. Additionally, we examined nocturnal glucose metabolism over only two consecutive nights. However, a recent longer-term study³⁸ demonstrated that the median occurrence of the dawn phenomenon was 56% of the nights over an 8-month observational period. Further investigations are therefore necessary to determine factors (e.g., antecedent daytime glucose control) that could better predict nocturnal rise in glucose concentrations in T1D.

To summarize, we have demonstrated that in reasonably well-controlled patients with uncomplicated T1D on CSII, there is a gradual, consistent, and continuous rise in glucose concentrations throughout the night that is related to increasing rates of EGP and appears to be correlated, at least in part, to rising cortisol concentrations. It is also noteworthy that the glucose rise was gradual and continuous without any abrupt changes in nocturnal glucose concentrations, thereby suggesting that the term "dawn phenomenon" may need to be rephrased. The effect size of the nocturnal rise in glucose concentrations is nontrivial and was comparable between the two consecutive nights examined. Hence, closed-loop control algorithms and open-loop therapeutic strategies would need to account for these changes from night to night to effectively manage patients with T1D. The Food and Drug Administration–approved T1D simulator may need to be refined to accommodate the effect size of the nocturnal rise in glucose concentrations.

Acknowledgments

We are deeply indebted to the research participants. We express our sincere thanks to the following: staff of the Mayo Clinic Center for Translational Science Activities CRU; GI Motility Core; the CRU Mass Spectroscopy Laboratory; CRU Immunochemical Core Laboratory; and Pamela Reich (research assistant), Chad Clark (laboratory technician), Brent McConahey (research assistant), Matthew Murphy (laboratory technician), and Shelly McCrady-Spitzer (research assistant). All persons mentioned above are with the Endocrine Research Unit, Mayo Clinic, Rochester, MN. This work was supported by grants DK R01 085561 and DK DP3 094331 from the National Institutes of Health and grant UL1 TR000135 from the National Center for Advancing Translational Science, a component of the National Institutes of Health. C.D.M. and C.C. are partially funded by the Italian Ministero dell'Istruzione, dell'Università e della Ricerca (Progetto FIRB 2009).

Author Disclosure Statement

No competing financial interests exist.

A.M., L.H., R.B., R.L., R.E.C., Y.C.K., and A.B. assisted in study conduct, data gathering and analyses, and manuscript writing and editing. C.D.M. and C.C. assisted in data analyses and manuscript editing. Y.C.K. and A.B. are the guarantors of this work, had full access to all the data, and take full responsibility for the integrity of data and the accuracy of data analysis.

References

- Schmidt MI, Hadji-Georgopoulos A, Rendell M, et al.: Fasting hyperglycemia and associated free insulin and cortisol changes in "Somogyi-like" patients. *Diabetes Care* 1979;2:457–464.
- Schmidt MI, Hadji-Georgopoulos A, Rendell M, et al.: The dawn phenomenon, an early morning glucose rise: implications for diabetic intraday blood glucose variation. *Diabetes Care* 1981;4:579–585.
- Bolli GB, Dimitriadis GD, Pehling GB, et al.: Abnormal glucose counterregulation after subcutaneous insulin in insulin-dependent diabetes mellitus. *N Engl J Med* 1984;310:1706–1711.
- Monnier L, Colette C, Sardinoux M, et al.: Frequency and severity of the dawn phenomenon in type 2 diabetes: relationship to age. *Diabetes Care* 2012;35:2597–2599.
- Monnier L, Dejager S, Colette C, et al.: Magnitude of the dawn phenomenon and its impact on the overall glucose exposure in type 2 diabetes. *Diabetes Care* 2013;36:4057–4062.
- Bolli GB, De Feo P, De Cosmo S, et al.: Demonstration of a dawn phenomenon in normal human volunteers. *Diabetes* 1984;33:1150–1153.
- Schmidt MI, Lin QX, Gwynne JT, et al.: Fasting early morning rise in peripheral insulin: evidence of the dawn phenomenon in nondiabetics. *Diabetes Care* 1984;7:32–35.
- Campbell PJ, Bolli GB, Cryer PE, et al.: Pathogenesis of the dawn phenomenon in patients with insulin-dependent diabetes mellitus. Accelerated glucose production and impaired glucose utilization due to nocturnal surges in growth hormone secretion. *N Engl J Med* 1985;312:1473–1479.
- Owens DR, Bolli GB: Beyond the era of NPH insulin—long-acting insulin analogs: chemistry, comparative pharmacology, and clinical application. *Diabetes Technol Ther* 2008;10:333–349.
- Porcellati F, Lucidi P, Bolli GB, et al.: Thirty years of research on the dawn phenomenon: lessons to optimize blood glucose control in diabetes. *Diabetes Care* 2013;36:3860–3862.
- Basu R, Barosa C, Jones J, et al.: Pathogenesis of prediabetes: role of the liver in isolated fasting hyperglycemia and combined fasting and postprandial hyperglycemia. *J Clin Endocrinol Metab* 2013;98:E409–E417.
- Bock G, Dalla Man C, Campioni M, et al.: Pathogenesis of pre-diabetes: mechanisms of fasting and postprandial hyperglycemia in people with impaired fasting glucose and/or impaired glucose tolerance. *Diabetes* 2006;55:3536–3549.
- Boden G, Chen X, Urbain JL: Evidence for a circadian rhythm of insulin sensitivity in patients with NIDDM caused by cyclic changes in hepatic glucose production. *Diabetes* 1996;45:1044–1050.
- Radziuk J, Pye S: Diurnal rhythm in endogenous glucose production is a major contributor to fasting hyperglycaemia in type 2 diabetes. Suprachiasmatic deficit or limit cycle behaviour? *Diabetologia* 2006;49:1619–1628.
- Bergental RM, Klonoff DC, Garg SK, et al.: Threshold-based insulin-pump interruption for reduction of hypoglycemia. *N Engl J Med* 2013;369:224–232.
- Hinshaw L, Dalla Man C, Nandy DK, et al.: Diurnal pattern of insulin action in type 1 diabetes: implications for a closed-loop system. *Diabetes* 2013;62:2223–2229.
- Steele R, Wall JS, De Bodo RC, et al.: Measurement of size and turnover rate of body glucose pool by the isotope dilution method. *Am J Physiol* 1956;187:15–24.
- Matthews JN, Altman DG, Campbell MJ, et al.: Analysis of serial measurements in medical research. *BMJ* 1990;300:230–235.
- Magnusson I, Rothman DL, Jucker B, et al.: Liver glycogen turnover in fed and fasted humans. *Am J Physiol* 1994;266:E796–E803.
- Wajngot A, Chandramouli V, Schumann WC, et al.: Testing of the assumptions made in estimating the extent of futile cycling. *Am J Physiol* 1989;256:E668–E675.
- Landau BR: Measuring glucose and fructose-6-phosphate cycling in liver in vivo. *Metabolism* 1993;42:457–462.
- Karlander S, Roovete A, Vranic M, et al.: Glucose and fructose 6-phosphate cycle in humans. *Am J Physiol* 1986;251:E530–E536.
- Efendic S, Karlander S, Vranic M: Mild type II diabetes markedly increases glucose cycling in the postabsorptive state and during glucose infusion irrespective of obesity. *J Clin Invest* 1988;81:1953–1961.
- Efendic S, Wajngot A, Vranic M: Increased activity of the glucose cycle in the liver: early characteristic of type 2 diabetes. *Proc Natl Acad Sci U S A* 1985;82:2965–2969.
- Bell PM, Firth RG, Rizza RA: The effects of hyperglycaemia on isotopic measurement of glucose utilisation using [^2H], [^3H] and [^{14}C] glucose in patients with type 1 (insulin-dependent) diabetes mellitus. *Diabetologia* 1987;30:365–371.
- Stingl H, Chandramouli V, Schumann WC, et al.: Changes in hepatic glycogen cycling during a glucose load in healthy humans. *Diabetologia* 2006;49:360–368.
- Kalhan SC, Bier DM, Savin SM, et al.: Estimation of glucose turnover and ^{13}C recycling in the human newborn by simultaneous [$1\text{-}^{13}\text{C}$]glucose and [$6,6\text{-}^1\text{H}_2$]glucose tracers. *J Clin Endocrinol Metab* 1980;50:456–460.
- Tigas SK, Snehag AL, Haymond MW: Impact of duration of infusion and choice of isotope label on isotope recycling in glucose homeostasis. *Diabetes* 2002;51:3170–3175.
- Shulman GI, Ladenson PW, Wolfe MH, et al.: Substrate cycling between gluconeogenesis and glycolysis in euthyroid, hypothyroid, and hyperthyroid man. *J Clin Invest* 1985;76:757–764.
- Wolfe RR, Herndon DN, Jahoor F, et al.: Effect of severe burn injury on substrate cycling by glucose and fatty acids. *N Engl J Med* 1987;317:403–408.
- Bell PM, Firth RG, Rizza RA: Effects of hyperglycemia on glucose production and utilization in human. Measurement with [$2\text{-}^3\text{H}$]-, [$3\text{-}^3\text{H}$]-, and [$6\text{-}^{14}\text{C}$] glucose. *Diabetes* 1986;35:642–648.
- Karlander S, Vranic M, Efendic S: Increased glucose turnover and glucose cycling in acromegalic patients with normal glucose tolerance. *Diabetologia* 1986;29:778–783.
- Miyoshi H, Shulman GI, Peters EJ, et al.: Hormonal control of substrate cycling in humans. *J Clin Invest* 1988;81:1545–1555.

34. Wajngot A, Khan A, Giacca A, et al.: Dexamethasone increases glucose cycling, but not glucose production, in healthy subjects. *Am J Physiol* 1990;259:E626–E632.
35. Dinneen S, Alzaid A, Miles J, et al.: Effects of the normal nocturnal rise in cortisol on carbohydrate and fat metabolism in IDDM. *Am J Physiol* 1995;268:E595–E603.
36. Bright GM, Melton TW, Rogol AD, et al.: Failure of cortisol blockade to inhibit early morning increases in basal insulin requirements in fasting insulin-dependent diabetics. *Diabetes* 1980;29:662–664.
37. Skor DA, White NH, Thomas L, et al.: Examination of the role of the pituitary-adrenocortical axis, counterregulatory hormones, and insulin clearance in variable nocturnal insulin requirements in insulin-dependent diabetes. *Diabetes* 1983;32:403–407.
38. Bouchonville MF, Jaghab JJ, Duran-Valdez E, et al.: The effectiveness and risks of programming an insulin pump to counteract the dawn phenomenon in type 1 diabetes. *Endocr Pract* 2014;20:1290–1296.

Address correspondence to:
Ananda Basu, MD

5-194 Joseph, Endocrine Research Unit
Division of Endocrinology and Metabolism
Mayo College of Medicine
Rochester, MN 55905

E-mail: basu.ananda@mayo.edu