Impaired Diurnal Pattern of Meal Tolerance and Insulin Sensitivity in Type 2 Diabetes: Implications for Therapy

Yogesh Yadav,[1] Davide Romeres,[1] Claudio Cobelli,[2] Chiara Dalla Man,[3] Rickey Carter,[4] Ananda Basu,[1] and Rita Basu[1]

Diabetes 2023;72:223–232 | https://doi.org/10.2337/db22-0238

To assess the diurnal patterns of postprandial glucose tolerance and insulin sensitivity, 19 subjects with type 2 diabetes (8 women; 60 ± 11 years; BMI 32 ± 5 kg/m^2) and 19 anthropometrically matched subjects with no diabetes (ND; 11 women; 53 ± 12 years; BMI 29 ± 5 kg/m^2) were studied during breakfast (B), lunch (L), and dinner (D) with identical mixed meals (75 g carbohydrates) on 3 consecutive days in a randomized Latin square design. Three stable isotopes of glucose were ustilized to estimate meal fluxes, and mathematical models were used in estimating indices of insulin action and β-cell function. Postmeal glucose excursions were higher at D versus B and at D versus L in type 2 diabetes ($P < 0.05$), while in ND they were higher at D versus B ($P = 0.025$) and at L versus B ($P = 0.04$). The insulin area under the curve was highest at B compared with L and D in type 2 diabetes, while no differences were observed in ND. Disposition index (DI) was higher at B than at L ($P < 0.01$) and at D ($P < 0.001$) in ND subjects, whereas DI was low with unchanging pattern across B-L-D in individuals with type 2 diabetes. Furthermore, between-meal differences in β-cell responsivity to glucose (F) and insulin sensitivity (SI) were concurrent with changes in the DI within groups. Fasting and postmeal glucose, insulin, and C-peptide concentrations, along with estimates of endogenous glucose production (EGP), R_d, SI, F, hepatic extraction of insulin, insulin secretion rate, extracted insulin, and DI, were altered in type 2 diabetes compared with ND ($P < 0.011$ for all). The data show a diurnal pattern of postprandial glucose tolerance in overweight otherwise glucose-tolerant ND individuals that differs from overweight individuals with type 2 diabetes. The results not only provide valuable insight into management strategies for better glycemic control in people with type 2 diabetes, but also improved understanding of daytime glucose metabolism in overweight individuals without impaired glucose tolerance or overt diabetes.

It is well established that people with type 2 diabetes (T2D) have fasting and postprandial hyperglycemia due to increased endogenous glucose production (EGP) and decreased glucose uptake (1,2). In both cases, the degree of hyperglycemia is determined by the net balance of R_a and R_d in the circulation. While 80% of R_a comes from meal-ingested glucose (3) in the postprandial state, other contributors to R_a include gluconeogenesis and glycogenolysis, both of which are increased in T2D (4,5) in the fasted state. While R_a increases in T2D, R_d decreases (1) because of decreased rates of peripheral and splanchnic glucose uptake (6). Using the triple-tracer technique, we previously demonstrated that postprandial hyperglycemia after a mixed meal at breakfast (B) in T2D is due to higher EGP and lower R_d coupled with abnormalities in indices of insulin sensitivity (SI) and β-cell responsivity (Φ) (1). Whether these abnormalities in glucose turnover persist throughout the day and/or whether the diurnal pattern of insulin action and secretion is abnormal in T2D is currently unknown.

We previously examined the diurnal pattern of postprandial SI, Φ, and disposition index (DI) in lean, healthy subjects (7). Likewise, we characterized a diurnal pattern of SI in lean subjects with type 1 diabetes (8) that has now been incorporated into the type 1 diabetes simulator for the next generation of closed-loop control algorithms. A better understanding of diurnal changes to insulin secretion and action in overweight or obese people with/without T2D could help design rational therapeutic and nutritional strategies in these individuals.

[1]Division of Endocrinology, Department of Medicine, University of Virginia School of Medicine, Charlottesville, VA
[2]Department of Woman and Child's Health, University of Padova, Padova, Italy
[3]Department of Information Engineering, University of Padova, Padova, Italy
[4]Department of Health Sciences Research, Mayo Clinic, Jacksonville, FL

Corresponding author: Rita Basu, basu.rita@virginia.edu

Received 7 March 2022 and accepted 3 November 2022

Y.Y. and D.R. contributed equally.

RESEARCH DESIGN AND METHODS

The University of Virginia's Institutional Review Board approved the study. Eligible subjects reported the morning after an overnight fast to the University of Virginia Clinical Research Unit for a screening visit. After written informed consent, a history, and a physical examination, blood and urine samples were tested to ensure that subjects met enrollment criteria. A pregnancy test was performed when appropriate, and a negative result was confirmed before proceeding. Body composition was measured by Lunar iDXA version 15.0 software (GE Healthcare Technologies, Chicago, IL). Inclusion criteria for all subjects was 30–75 years of age and a BMI between 25 and 45 kg/m^2. Subjects with no diabetes (ND) underwent a 2-h standard oral glucose tolerance test to exclude prediabetes and T2D. Subjects with type 2 diabetes had HbA_{1c} $\leq$10% either on lifestyle treatment or mono/combination therapy with oral hypoglycemic agents (metformin, sulfonylurea). Patients with type 2 diabetes on thiazolidinediones, sodium–glucose cotransporter 2 inhibitors, dipeptidyl peptidase 4 inhibitors, glucagon-like peptide 1 receptor agonists, or insulin, as well as those with microvascular complications (except stable background retinopathy) were excluded from participation. People with chronic debilitating illnesses, anemia, cardiac, hepatic, or renal disease, or on medications such as corticosteroids, barbiturates, opiates, and anticoagulant therapy, were excluded.

After meeting enrollment criteria, subjects with T2D were instructed to withdraw oral hypoglycemic agents 7–10 days prior to the study visit and regularly monitor their fasting capillary glucose. If three successive fasting glucose values >300 mg/dL, they resumed their medication(s) and were withdrawn from the study. Subjects were asked to refrain from unaccustomed physical exercise and maintain their weight within 2% between screen and study visits. Subjects wore Fitbits to keep track of their daily activity. Subjects meeting the enrollment criteria were scheduled for study visits. They consumed a standard 10 kcal/kg meal (50% carbohydrate, 20% protein, and 30% fat) provided to them at 5 P.M. the day before the study admission (4). No additional food was eaten until the next morning. Subjects were provided 3 days of identical caffeine-free meals (8 kcal/kg/meal;75 gm carbohydrate) for B (7 A.M.), lunch (L) (1 P.M.), and dinner (D) (7 P.M.), each of which consisted of 33% of the subject's total estimated calorie intake based on Harris Benedict + 20% calorie requirements. Each day, one of the meals provided was labeled for measurement of glucose turnover. The order was randomly selected per the Latin square design (7,8).

Triple-Tracer Mixed Meal

The triple-tracer method previously published (9) was modified to use all stable isotopes (i.e., [2-^{13}C]glucose in lieu of [6-^{3}H]glucose with no other changes in isotopes) (Experimental design, Fig. 1*A*). At 2 h (−120 min) prior to the labeled meal of the day, subjects received a primed (11.8 mg/kg) and continuous infusion (0.1184 mg/kg/min) of [6,6-$^2H_2$]glucose until the end of the study. The mixed meal, which consisted of flavored Jell-O containing 75 g of dextrose labeled with [1-^{13}C]glucose, along with scrambled eggs and Canadian bacon, was fed at time zero (0 min) and consumed within 15 min. Sugar-free gelatin was used for the Jell-O preparation. An infusion of [2-^{13}C]glucose was started at time zero minutes concurrently with the first bite of the meal, and the rate of the infusion varied to mimic the anticipated R_a of the [1-^{13}C]glucose for the remainder of the study (9). Simultaneously, at time zero minutes, the rate of infusion of [6,6-$^2H_2$]glucose was altered to approximate the anticipated pattern of change in EGP (9). Blood was sampled (1,9) periodically for measurement of glucose, hormones, and other analytes.

Analytical Techniques

Plasma samples were placed on ice, centrifuged at 4°C, separated, and stored at –80°C until assayed. Plasma glucose concentrations were measured using a glucose oxidase method (YSI, Yellow Springs, OH). Plasma insulin and C-peptide were measured as previously described (1). We differentiated [1-^{13}C]glucose and [2-^{13}C]glucose enrichments using gas chromatography-mass spectrometry by quantifying C-1 and C-2 to C-3 fragments of a permethyl derivative of glucose, as previously described (10). Plasma enrichments of [1-^{13}C]glucose and [6,6-$^2H_2$]glucose were determined using gas chromatography-mass spectrometry via simultaneous quantitation of the C-1 to C-2 and C-3 to C-6 fragments of a methyloxime derivative of glucose, as previously described (11,12).

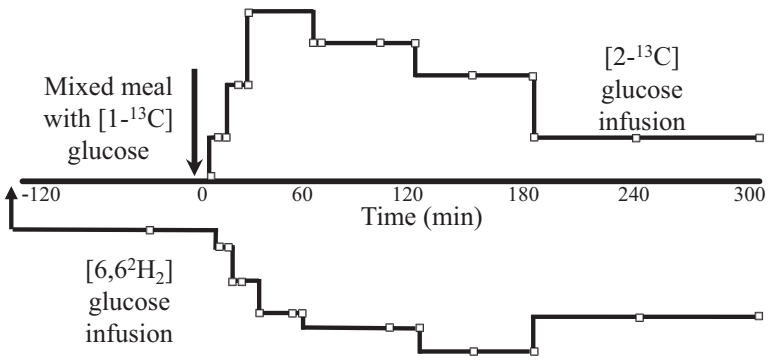
Calculations

Fasting and postprandial rates of glucose turnover were calculated according to prior publications (9). Briefly, the systemically infused [2-^{13}C]glucose was used to trace the systemic R_a of [1-^{13}C]glucose contained in the meal, whereas [6,6-$^2H_2$]glucose was used to trace the R_a of endogenously produced glucose. The plasma concentration ratio of [2-^{13}C]glucose to [1-^{13}C]glucose was used to calculate the R_a of ingested [1-^{13}C]glucose, and the plasma concentration ratio of [6,6-$^2H_2$]glucose to endogenous glucose concentration was used to calculate EGP. The endogenous glucose concentration was calculated by subtracting the concentration of exogenously derived (ingested + infused) glucose (i.e., plasma [1-^{13}C]glucose concentration divided by meal [1-^{13}C]glucose enrichment + plasma [2-^{13}C]glucose concentration + plasma [6,6-$^2H_2$]glucose concentrations) from the total plasma glucose concentration (3,9).

Indices of Insulin Action and Secretion

Insulin action or SI is the composite effect of insulin to stimulate R_d and inhibit EGP, and it was estimated in this study using the oral glucose minimal model (7). The oral C-peptide and oral insulin minimal models (3,9,13–16) were used to measure the interactions of plasma glucose, C-peptide, and insulin to derive measures of Φ and

A



B

	ND (n=19)	Type 2 Diabetes (n=19)	*P* value
Sex (M/F)	8/11	11/8	0.330
Age (years)	53.7 ± 11.9	60.8 ± 11.0	0.063
Body weight (kg)	85.9 ± 12.3	91.4 ± 18.8	0.292
Height (m)	1.7 ± 0.1	1.7 ± 0.1	0.220
Body Mass Index (kg/m^2)	29.3 ± 5.0	32.1 ± 4.9	0.087
Lean body mass (kg)	53.7 ± 7.7	58.6 ± 12.2	0.147
Body fat (%)	37.9 ± 8.4	35.2 ± 7.5	0.399
Fasting plasma glucose (mmol/L)	4.9 ± 0.6	8.4 ± 3.9	< 0.001
HbA_{1C} (%)	5.3 ± 0.4	7.2 ± 1.2	< 0.001
HbA_{1C} (mmol/mol)	35.2 ± 4.0	55.0 ± 12.8	< 0.001
Duration of diabetes (years)	NA	7.3 ± 5.3	
eGFR (mL/min/1.73m^2)	89.1 ± 16.9	88.5 ± 13.2	0.928
Creatinine (mg/dL)	0.9 ± 0.2	0.8 ± 0.2	0.980

Subject Characteristics

Figure 1—*A*: The triple-tracer technique modified using all three stable isotopes is shown. *B*: Baseline subject characteristics (mean ± SD) are shown in the ND and T2D group. eGFR, estimated glomerular filtration rate.

hepatic insulin extraction (HE). Additionally, a measure of insulin secretion appropriate for the level of insulin resistance or the DI was derived from the products of SI and corresponding Φ (16).

Statistical Analyses

The initial sample size calculations considering the preliminary data indicated that subjects with T2D, on average, had 3.0 μmol/kg of fat-free mass (FFM)/min higher EGP overnight within group and within assessment time, with a SD of ∼6.0 μmol/kgFFM/min (17). This resulted in an effective sample size of 64 per group, assuming independence, 80% power, and a 0.05 (two-sided) level of significance. Given subjects were to be repeatedly assessed over three meals, the total number of participants was estimated to be less when accounting for the design effect (i.e., $1 + [m-1]^*\rho$) where m is the number of meals and ρ is the intraclass correlation coefficient. With three meals per participant and an intraclass correlation coefficient ranging from 0 to 0.1, an initial total of 21 to 25 participants per group was estimated to be required to produce the effective sample size of 64 per group at the $\alpha = 0.05$ level of significance.

The Latin squares design (7,8) was used to randomize the labeling of mixed meals (treatment) to one of three meal conditions (B, L, and D) over 3 experimental days. Prior to the primary analyses, data were visualized in both their native time series format as well as summarized data that provided a meal-level response for each participant. For the latter summary, the incremental area under the curve (iAUC) was used to better isolate the effect of the labeled meal intervention. Prior to the calculation of the AUC, intermittent missing data over the 300-min sampling period were imputed using random forest imputations. When the iAUC was used as the dependent variable, the study design became statistically equivalent to a 3-period, 3-treatment crossover study with no period or carryover effects. As was considered in prior studies with this design (7,8), model-based means were estimated in order to provide a means to construct statistical contrasts that would test for differences among meals and between combinations of meals and the study cohort.

The primary end point was the iAUC for EGP. These EGP profile summaries were modeled using a mixed-model

formulation that included the main effects of study cohort, meal, and cohort by meal interaction with a random effect for the individual participant. The primary hypothesis was the test of the model-based mean for the study cohort effect, which was a weighted average of the mean iAUC over the three meal periods between groups. As a secondary analysis, we examined the significances of the cohort by meal interaction that described whether the difference in EGP between the study cohorts is modulated by the meal timing. Model contrasts were configured to compare changes in EGP over each of the experimental conditions (meal timing within study cohort and the meal by study cohort interaction). Additional outcome measures and derived indices obtained from the study protocol were summarized similarly. For a simplified comparison of the data by study cohort and meal, Wilcoxon rank sum tests were performed within each stratum.

Statistical analysis was conducted using R version 3.6.2, primarily using the lme4 (version 1.1-25) and emmeans (version 1.5.2-1) packages.

Data and Resource Availability

Data and resources are available upon request.

RESULTS

Subject Characteristics

Of 54 subjects (24 with T2D, 30 with ND) who were screened for the study (Fig. 1*B*), there were 9 subjects that did not meet enrollment criteria and 7 additional subjects withdrew after successful screening due to non–study-related reasons. A total of 38 subjects (19 with T2D, 19 with ND) completed all study procedures. Due to pandemic conditions, additional subjects were not enrolled in the study. Age, sex, BMI, weight, lean body mass, and percentage of body fat did not differ statistically between groups, but fasting glucose concentrations and HbA_{1c} levels were higher by design in subjects with T2D ($P < 0.001$).

Glucose, Insulin, and C-Peptide Concentrations

Serial hormone data over the course of the study are presented in Fig. 2 (left panel). The profiles of the trajectories matched the expected physiology of T2D and were all statistically significant between cohorts at baseline and over the postprandial period (Table 1 and Fig. 2). Glucose iAUC was higher in subjects T2D than in ND subjects for all three meals (each $P < 0.0012$) (Table 1 and Fig. 2, right panel).

Figure 2 (right panel) examines these data in more detail. Within the cohorts, the glucose iAUC for D was higher than B for both T2D ($P = 0.039$) and ND ($P = 0.025$). Within the cohort with T2D, the insulin iAUC was significantly higher for B than for both L and D (each $P < 0.04$). Within the ND group, there was no significant difference between insulin iAUC for B, L, and D meals. However, C-peptide iAUC for all three meals was significantly lower in subjects with T2D than in ND subjects (each $P < 0.020$). Within the T2D group, C-peptide iAUC for B was significantly higher than L ($P = 0.013$), but there was no significant difference in iAUC C-peptide for the three meals within the ND group.

Meal R_a, EGP, and R_d

Figure 3 (left panel) presents the serial trends in the meal fluxes over the study period by meal and cohort. While meal R_a (MR_a) did not differ between subjects with T2D and those with ND for any meals ($P > 0.13$) (Table 1), there were differences in EGP and the R_d. Preprandial EGP was significantly higher in T2D than in ND for B (18.2 ± 4.64 vs. 14.4 ± 1.91 μmol/kgFFM/min, $P = 0.0071$), L (20.2 ± 6.87 vs. 15.4 ± 1.84 μmol/kgFFM/min, $P = 0.0008$), and D (18.7 ± 5.19 vs. 15.8 ± 1.87 μmol/kgFFM/min, $P = 0.0362$). Postprandial EGP iAUC was lower ($P < 0.01$) in subjects with T2D than in ND during B, L, and D.

Within the group with T2D, the EGP iAUC was significantly lower after L than after B ($P = 0.02$) (Fig. 3, right panel). However, there was no statistically significant difference in iAUC between B and D or L and D meals. Within the ND group, there was no statistically significant difference between the EGP iAUC between the three meals, but B had a numerically lower iAUC compared with L and D. R_d was lower in T2D than in ND after all three meals ($P < 0.006$), and the subjects with T2D had lower R_d at D relative to B ($P = 0.043$).

Indices of Insulin Action, ϕ, SI, HE, and Extracted Insulin

SI was significantly lower in subjects with T2D after B and D but not after L compared with ND subjects ($P < 0.04$) (Table 1). The cohort differences along with differences among meals for each cohort are shown in Fig. 4. Within the ND group, SI was significantly higher at B than D ($P = 0.018$), but the SI for the three meals did not differ in the group with T2D. Total Φ (Φ_{total}) was significantly lower for all three meals in subjects with T2D compared with ND subjects ($P < 0.001$). Within the group with T2D, Φ_{total} did not differ between meals. Within the ND group, Φ_{total} was significantly higher after B compared with L and D ($P = 0.01$). Within the ND group, HE percentage (HE%) was significantly higher after B than after D ($P = 0.009$); however, there was no significant difference between meals in subjects with in T2D (Figs. 4 and 5). HE was significantly lower after B ($P = 0.023$) in subjects T2D compared with ND subjects but did not differ significantly for L and D. Insulin secretion rate (Fig. 5*A*) was significantly higher for all meals in subjects with ND compared with T2D ($P < 0.01$). Within-group difference was noted for B versus L meal in T2D ($P = 0.034$). HE, calculated as pmol/min over the time course of the meals (Fig. 5*B*), showed the same trend as fractional HE, with more insulin extracted (Fig. 5*C*) at B, followed by L and D in ND. In T2D, insulin extraction during the meals tended to be higher at B and L but lower at D. Of note, as expected insulin extraction over the duration

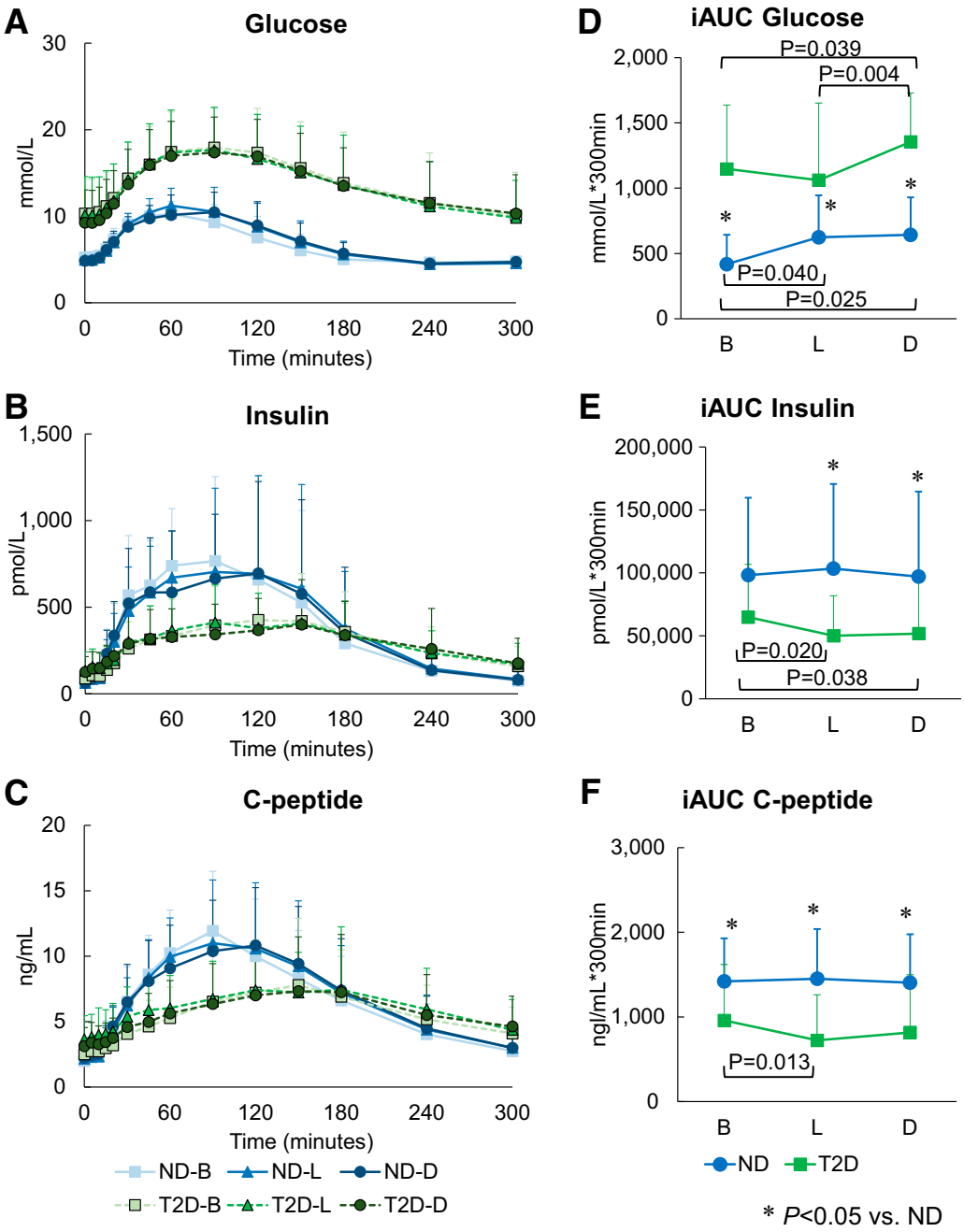


Figure 2—Plasma glucose concentration (*A*), insulin concentrations (*B*), and C-peptide concentrations (*C*) in subjects with T2D (n = 19) and ND subjects (n = 19) for B (0700 h), L (1300 h), and D (1900 h). Plots show mean ± SD. iAUC for plasma glucose concentration (*D*), insulin concentrations (*E*), and C-peptide concentrations (*F*). Plots show mean ± SD. The mixed meal was fed at time 0. *P* values are reported for within-group differences and between-group differences. *$P < 0.05$ vs. ND.

of the meals was approximately fourfold higher in ND compared with T2D and significantly higher for each corresponding meal in ND versus T2D ($P < 0.01$). DI was significantly lower for all three meals in subjects with T2D than in those with ND ($P < 0.001$).

DISCUSSION

This study characterizes postprandial glucose tolerance throughout the day in overweight/obese individuals with T2D and contrasts parameters of Φ to glucose, insulin action, and post–mixed-meal glucose turnover with anthropometrically matched ND subjects. Meal sizes and macronutrient contents did not differ between meals for all subjects.

We observed that while the DI was higher at B than at L and D in ND individuals, it was unchanging and low through B, L, and D in subjects with T2D. This demonstrates that the diurnal pattern of carbohydrate tolerance varies between obese ND individuals and individuals with T2D.

Table 1—Comparison of meal fluxes between subjects with and without T2D after ingestion of mixed meal

	ND (n = 19)			T2D (n = 19)			Estimated cohort difference		Estimated B difference		Estimated L difference		Estimated D difference	
	B	L	D	B	L	D	Est (95% CI)	P value	Est (95% CI)	P value	Est (95% CI)	P value	Est (95% CI)	P value
Glucose														
Baseline (mmol/L)	5.3 (0.5)	5.0 (0.4)	4.9 (0.5)	10.3 (4.0)	10.1 (4.5)	9.3 (3.8)	4.9 (3.0 to 6.7)	**<0.0001**	5.0 (3.1 to 6.9)	**<0.0001**	5.2 (3.3 to 7.1)	**<0.0001**	4.4 (2.5 to 6.3)	**<0.0001**
iAUC (0–300 min)	418.0 (225.4)	623.3 (321.6)	642.8 (286.3)	1,147.6 (488.7)	1,061.0 (591.4)	1,354.7 (374.2)	626 (419 to 834)	**<0.0001**	730 (471 to 989)	**<0.0001**	438 (179 to 697)	**0.0012**	712 (453 to 971)	**<0.0001**
Insulin														
Baseline (pmol/L)	62.1 (31.6)	62.1 (35.5)	61.9 (31.6)	88.3 (44.4)	133.4 (108.1)	125.4 (89.6)	53.7 (17.8 to 89.5)	**0.0044**	26.3 (−15.5 to 68.0)	0.2136	71.2 (29.5 to 113)	**0.0011**	63.5 (21.7 to 105.0)	**0.0035**
iAUC (0–300 min)	98,166.0 (61,493.0)	103,348.4 (67,202.7)	96,996.6 (67,554.5)	64,854.0 (41,886.4)	50,058.7 (31,840.9)	51,740.8 (46,164.6)	−43,953 (−78,243 to −9,662)	**0.0134**	−33,312 (−68,920 to 2,296)	0.066	−53,290 (−88,898 to −17,682)	**0.0043**	−45,256 (−80,864 to −9,648)	**0.014**
C-peptide														
Baseline (ng/mL)	2.0 (0.9)	2.2 (1.0)	2.2 (0.9)	2.5 (0.8)	3.7 (1.8)	3.1 (1.4)	0.95 (0.23 to 1.67)	**0.0113**	0.49 (−0.29 to 1.26)	0.2174	1.48 (0.70 to 2.25)	**0.0004**	0.88 (0.10 to 1.66)	**0.0276**
iAUC (0–300 min)	1,420.9 (508.3)	1,450.1 (588.5)	1,405.6 (571.0)	958.4 (660.2)	723.8 (539.0)	817.3 (680.5)	−592 (−953 to −232)	**0.002**	−462 (−850 to −75)	**0.0203**	−726 (−1,114 to −339)	**0.0004**	−588 (−976 to −201)	**0.0037**
MR_a (μmol/kgFFM/min)														
Total AUC (0–300 min)	8,455.9 (2,007.2)	9,121.5 (2,290.5)	8,750.9 (2,267.8)	8,213.1 (2,481.4)	7,928.5 (2,112.0)	7,667.0 (1,979.4)	−840 (−2,151 to 472)	0.2022	−243(−1,673 to 1,187)	0.7347	−1,193 (−2,623 to 237)	0.1002	−1,084 (−2,514 to 346)	0.1344
EGP														
Baseline (μmol/kgFFM/min)	14.4 (1.9)	15.4 (1.8)	15.8 (1.9)	18.2 (4.6)	20.2 (6.9)	18.7 (5.2)	3.8 (1.5 to 6.2)	**0.0023**	3.8 (1.1 to 6.5)	**0.0071**	4.8 (2.1 to 7.5)	**0.0008**	2.9 (0.2 to 5.7)	**0.0362**
iAUC (0–300 min)	−2,956.4 (625.4)	−3,248.5 (825.3)	−3,323.1 (774.9)	−4,026.7 (1,597.6)	−4,559.4 (1,803.6)	−4,360.4 (1,750.2)	−1,139 (−1929 to −350)	**0.0059**	−1,070 (−1,933 to −207)	**0.016**	−1,311 (−2,174 to −448)	**0.0036**	−1,037 (−1,900 to −175)	**0.0194**
R_d														
Baseline (μmol/kgFFM/min)	14.3 (1.9)	15.4 (1.8)	15.8 (1.9)	18.2 (4.6)	20.2 (6.9)	18.7 (5.2)	3.9 (1.5, 6.2)	**0.0022**	3.84 (1.11 to 6.57)	**0.0065**	4.79 (2.06 to 7.52)	**0.0008**	2.92 (0.19 to 5.65)	**0.0362**
iAUC (0–300 min)	5,772.3 (1,892.8)	6,204.4 (2,005.7)	5,447.7 (1,749.0)	3,638.8 (2,872.4)	3,017.8 (2,475.0)	2,663.2 (2,638.3)	−2,702 (−4,004 to −1,399)	**0.0002**	−2,133 (−3,631 to −636)	**0.006**	−3187 (−4684 to −1684)	**0.0001**	−2784 (−4282 to −1287)	**0.0004**
SI (10^{-5} dL/kg/min per pmol/L)	9.0 (5.2)	6.9 (5.3)	6.7 (4.9)	4.3 (3.7)	3.7 (2.4)	3.8 (4.1)	−3.5 (−6.1 to −0.8)	**0.0141**	−4.48 (−7.51 to −1.45)	**0.0046**	−2.86 (−5.97 to 0.25)	0.0703	−3.26 (−6.29 to −0.23)	**0.0355**
Φ_{total} (10^{-9}/min)	49.1 (17.3)	42.1 (16.4)	42.0 (17.1)	19.0 (13.4)	19.7 (19.0)	14.8 (10.5)	−26.7 (−35.9 to −17.5)	**<0.0001**	−30.9 (−41.2 to −20.6)	**<0.0001**	−21.7 (−32.1 to −11.4)	**0.0001**	−27.4 (−37.7 to −17.1)	**<0.0001**
DI														
Total (10^{-14} dL/kg/min^2 per pmol/L)	422.2 (252.6)	321.3 (333.8)	268.1 (230.3)	92.2 (115.9)	84.8 (80.3)	44.9 (49.2)	−266 (−390 to −143)	**0.0001**	−328 (−463 to −193)	**<0.0001**	−232 (−369 to −95)	**0.0013**	−238 (−372 to −104)	**0.0008**
HE, %	0.670 (0.253)	0.567 (0.274)	0.476 (0.239)	0.432 (0.273)	0.420 (0.280)	0.375 (0.202)	−0.16 (−0.28 to −0.04)	**0.0111**	−0.24 (−0.41 to −0.07)	**0.0053**	−0.14 (−0.31 to 0.03)	0.1097	−0.1 (−0.27 to 0.07)	0.2314

Data are mean (SD), unless otherwise indicated. Bold P values are statistically significant (<0.05).

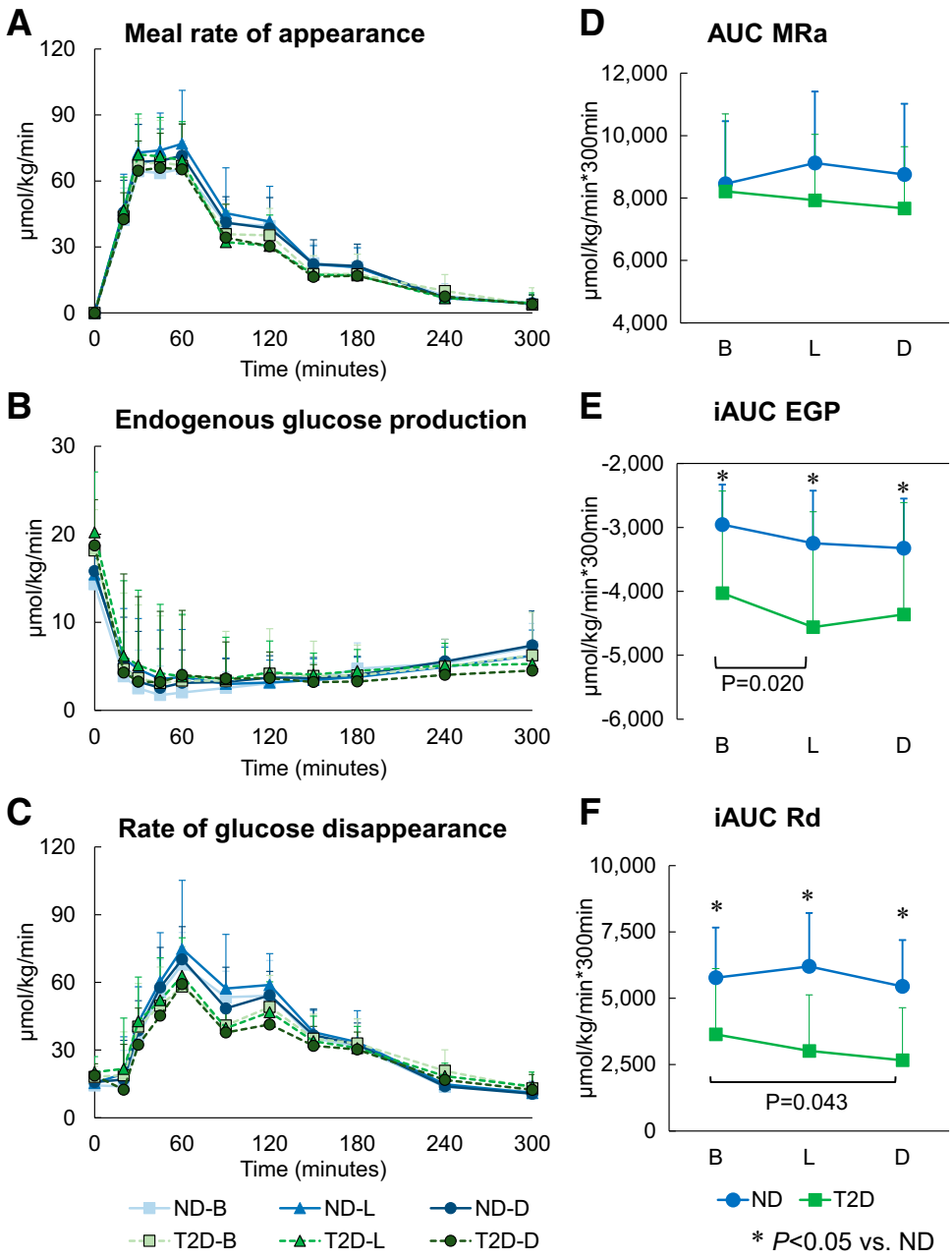


Figure 3—Rates of MR_a (*A*), EGP (*B*), and R_d (C) in subjects with type 2 diabetes (T2D; *n* = 19) and ND subjects (*n* = 19) for B (0700 h), L (1300 h), and D (1900 h). Plots show mean ± SD. Total AUC for rates of MR_a (*D*) and iAUC for rates of EGP (*E*), and R_d (*F*). Plots show mean ± SD. The mixed meal was fed at time 0. *P* values are reported for within-group differences and between-group differences. *$P < 0.05$ vs. ND.

Examining this in more detail, in both groups postprandial glucose excursions followed a diurnal pattern and were higher at D than B, with values being the lowest at B in ND subjects and highest at D in subjects with T2D. Furthermore, as expected, postprandial glucose excursions were significantly higher in subjects with T2D than in ND subjects for all three meals. In ND subjects, the decline in glucose tolerance from B to D was due to lower SI, Φ, and DI, similar to that observed in our previous study in lean ND subjects (7). In contrast, each of the above parameters—SI, Φ, and DI—were substantially (40–75%) lower in subjects with T2D than in ND subjects throughout the day, such that there were no differences between meals for any of these parameters in the subjects with T2D;—they remained low and flat throughout the day. In other words, these indices were substantially impaired at the start of the day in subjects with T2D and they never recovered as the day progressed, hence confirming significant deterioration in insulin action and β-cell functions throughout the day in this cohort. This is all the more important since our cohort with T2D had a wide span of glucose control (HbA_{1c} 5.5–9.6%), diabetes duration (1–19 years), on lifestyle therapy (*n* = 4), monotherapy

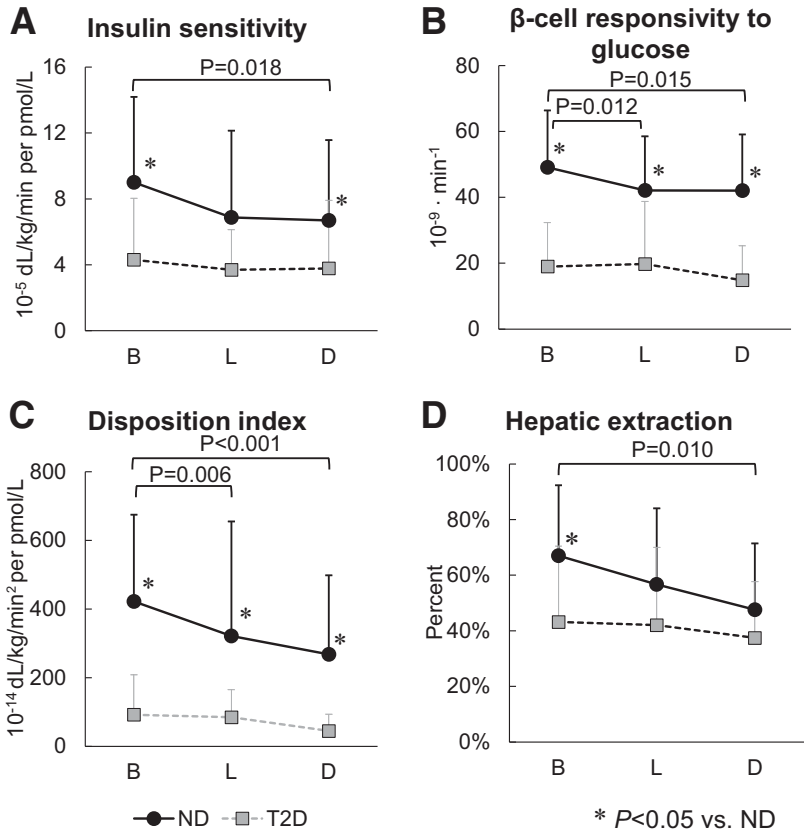


Figure 4—SI (*A*), Φ (*B*), DI (*C*), and HE (*D*) in subjects with T2D (n = 19) and ND subjects (n = 19) for B (0700 h), L (1300 h), and D (1900 h). Plots show mean ± SD. *P* values are reported for within-group differences and between-group differences. *$P < 0.05$ vs. ND.

(n = 9), and combination therapy (n = 6). Taken together, these observations imply that across the spectrum of non–insulin-treated individuals with T2D, substantial abnormalities exist in SI and action throughout the day. Additional longitudinal assessments of diurnal patterns of insulin action and β-cell function are needed to characterize the natural history and progression and the impact of antecedent glucose control of these parameters in cohorts with T2D matched for duration of diabetes or glycemia. Studies are also needed in those with prediabetes to determine the extent of diurnal changes to these parameters.

Regarding postprandial glucose turnover, MR_a neither differed between meals nor between cohorts. This important observation is in line with our prior studies in lean ND subjects and individuals with type 1 diabetes (7,8). The pattern of MR_a between groups remained unchanged whether meal carbohydrate content varied from 75 g (1) or 50 g, as in our prior studies (7,8). Taken together, these observations demonstrate that meal glucose absorption and its systemic appearance does not explain differences in postprandial glucose excursions between individuals with ND, T2D, and type 1 diabetes. The primary cause of higher postprandial glucose excursions for all three meals throughout the day in T2D appears to be entirely due to an almost 50% reduction in whole-body R_a.

We measured HE in this study and observed interesting differences between meals and cohorts. In general, HE was lower in subjects with T2D than in ND subjects throughout the day, especially at B. This implies that larger proportions of secreted insulin reached the peripheral circulation in subjects with T2D than in ND subjects for all three meals. Likewise, HE was lower at dinner than breakfast in ND subjects. Taken together, these intriguing observations suggest that reductions in HE could posit a compensatory protective phenomenon in an attempt to overcome lower SI in subjects with T2D for all three meals, and in ND subjects where SI was lower at D than at B. This observation appears to corroborate recent observations, albeit during an oral glucose challenge (18). It is also possible that reduced hepatic insulin clearance could be due to increased hepatic fat in individuals with T2D, although we did not measure hepatic fat content in this study.

Few studies have investigated the existence of a diurnal pattern in postprandial glucose tolerance in T2D. Peter et al. (19) observed higher postprandial insulin excursions at B than at L and D, similar to our findings. However, in contrast to this study, lower glucose excursions were observed at L and D. Importantly, their study design was limited to 1 day and was therefore not representative of daily living as it necessitates the subjects to avoid physical activity the entire day. Our observations of higher postprandial

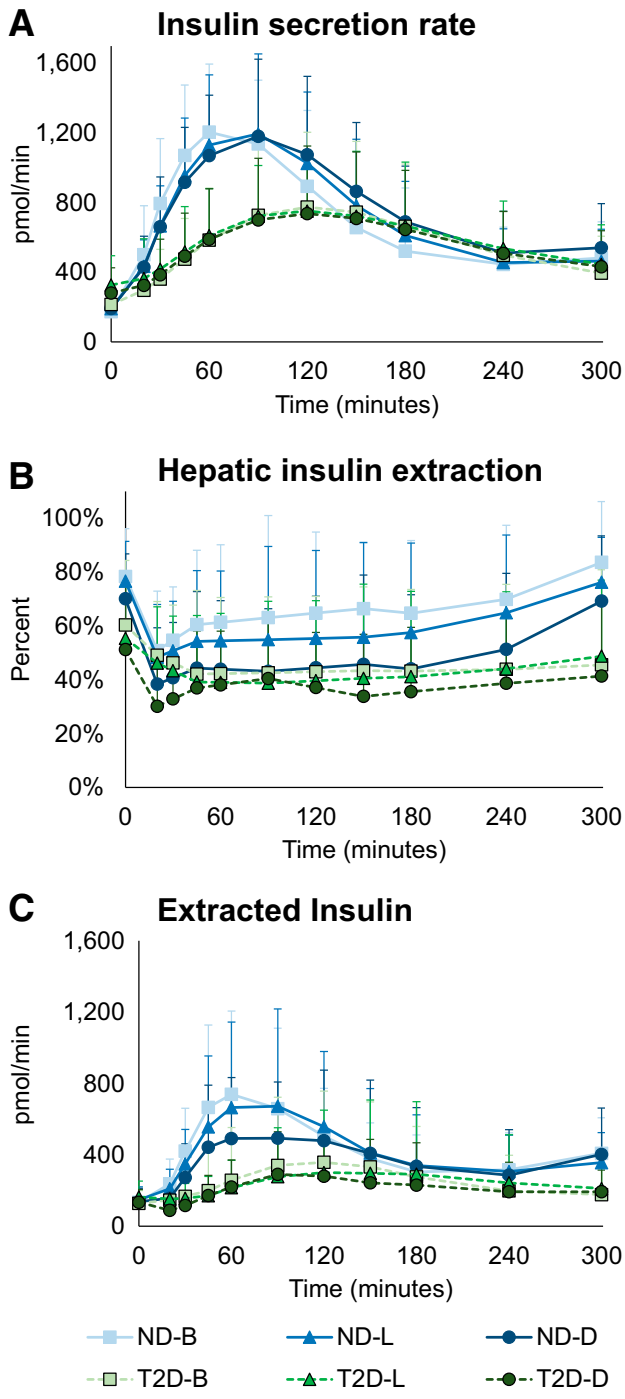


Figure 5—Insulin secretion rate (*A*) and time course of HE (*B*) and extracted insulin (*C*) in subjects with T2D (n = 19) and ND (n = 19) for B (0700 h), L (1300 h), and D (1900 h). Plots show mean ± SD. The mixed meal was fed at time 0.

glucose excursion at D in subjects with T2D corroborates another report (20) where postprandial glucose excursions were compared, after subjects consumed or skipped the B meal, during the day. The pattern of postprandial glucose and insulin excursions was similar to our study. Of note, the meals were consumed in only 1 day and glucose turnover and β-cell function were not measured.

We have observed that glucose tolerance was worse at D in T2D compared with the other two meals of the day. The fact that SI is identical with different iAUC glucose depends on the fact that insulin concentrations were lower, leading to lower iAUC insulin. In some sense, SI (and consequently DI) normalizes glucose to circulating insulin. Glucose can be higher because SI is lower and/or because insulin is lower. In this case, we found that iAUC glucose is higher and iAUC insulin is lower at D in type 2 diabetes compared with B. This is supported also by the result that R_d is lower at D than B (P = 0.043). Similarly, in ND subjects, iAUC glucose was higher at L and D, with similar iAUC insulin, thus implying lower SI at D than at B in ND subjects.

Like all studies, our study has limitations. First, while we strove to recruit individuals with T2D and healthy ND subjects that were generally alike with respect to a range of clinical characteristics, the individuals with T2D were somewhat older and heavier than the ND subjects. These slight but statistically insignificant variances may confound differences between individuals with T2D and ND individuals; however, these differences would not impact comparisons associated with the diurnal patterns within the subject cohort.

Next, we incorporated mixed meals that had comparable and equal proportion of macronutrients and energy in each meal. This approach was taken to minimize the confounding effects of variability in meal composition to permit precise estimation of the outcome variables. However, the effect of variations in meal composition and content that usually occur in the day-to-day free-living state will need to be evaluated in future experiments.

Lastly, these studies were performed on 3 consecutive days instead of on a single day, which would have minimized intraindividual and interday variability. However, this alternative approach would have confounded the results, with unequal labeling of hepatic glycogen leading to erroneous estimates of glucose turnover. Hence, the Latin square design was ultimately preferred as in our previous studies (7,8).

To summarize, these data demonstrate that under carefully controlled conditions of identical meal composition, the diurnal pattern of carbohydrate tolerance in T2D differs from overweight glucose-tolerant individuals. These findings have potentially important practical and therapeutic implications. Given deteriorating glucose tolerance as the day progresses, mitigating strategies could involve recommending smaller meal sizes in both cohorts and/or use of insulin secretagogues or sensitizers in the later part of the day rather than in the morning in T2D. Last, but not least, physical exercise during the afternoon/evening pre-D may help improve dinner time glucose tolerance in both groups.

Acknowledgments. The authors are deeply indebted to the research participants. The authors sincerely thank the following University of Virginia staff: Dr. Safia Sawleh and Alexandra Weaver (study coordinators) for the

conduct of the studies, Benjamin Gran (lead technologist), Benjamin Paysour (research technologist), Nirmal Bhandari (research technologist), and David Fulkerson (research technologist) for sample analyses, and Rajia Arbab (research assistant) for technical assistance.

Funding. The work received support through funds from the National Institute of Diabetes and Digestive and Kidney Diseases R01 DK029953 to R.B. and R01 DK085516 to A.B. Sample processing performed at Vanderbilt University received support from the National Institutes of Health, the National Institute of Diabetes and Digestive and Kidney Diseases grants DK059637 (Mouse Metabolic Phenotyping Center) and DK020593 (Diabetes Research and Training Center). Support was received from the Ministero dell'Universit e della Ricerca (MIUR; Italian Minister for Education) under the initiative "Departments of Excellence" (Law 232/2016) to D.R. and C.D.M.

Duality of Interest. No potential conflicts of interest relevant to this article were reported.

Author Contributions. Y.Y. and D.R. assisted with the study, analyzed data, and helped in the conduct of the study. C.C. and C.D.M. analyzed the data and reviewed the manuscript. R.C. analyzed the data and edited the manuscript. A.B. and R.B. designed and conducted the studies, analyzed data, and wrote the manuscript. R.B. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Prior Presentation. Parts of this study were published in abstract form at the 80th Scientific Sessions of the American Diabetes Association, virtual meeting, 12–16 June 2020, published in Diabetes 2020;69(Suppl. 1):313-OR, and at the 57th EASD Annual Meeting of the European Association for the Study of Diabetes, virtual meeting, 28 September–1 October 2021, published in Diabetologia 2021;64(Suppl. 1):S129–S130.

References

1. Basu A, Dalla Man C, Basu R, Toffolo G, Cobelli C, Rizza RA. Effects of type 2 diabetes on insulin secretion, insulin action, glucose effectiveness, and postprandial glucose metabolism. Diabetes Care 2009;32:866–872

2. Sheehan JP. Fasting hyperglycemia: etiology, diagnosis, and treatment. Diabetes Technol Ther 2004; 6:525–533

3. Basu R, Dalla Man C, Campioni M, et al. Effects of age and sex on postprandial glucose metabolism: differences in glucose turnover, insulin secretion, insulin action, and hepatic insulin extraction. Diabetes 2006;55:2001–2014

4. Basu R, Schwenk WF, Rizza RA. Both fasting glucose production and disappearance are abnormal in people with "mild" and "severe" type 2 diabetes. Am J Physiol Endocrinol Metab 2004;287:E55–E62

5. Basu R, Chandramouli V, Dicke B, Landau B, Rizza R. Obesity and type 2 diabetes impair insulin-induced suppression of glycogenolysis as well as gluconeogenesis. Diabetes 2005;54:1942–1948

6. Basu A, Basu R, Shah P, et al. Type 2 diabetes impairs splanchnic uptake of glucose but does not alter intestinal glucose absorption during enteral glucose feeding: additional evidence for a defect in hepatic glucokinase activity. Diabetes 2001;50:1351–1362

7. Saad A, Dalla Man C, Nandy DK, et al. Diurnal pattern to insulin secretion and insulin action in healthy individuals. Diabetes 2012; 61:2691–2700

8. 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

9. Basu R, Di Camillo B, Toffolo G, et al. Use of a novel triple-tracer approach to assess postprandial glucose metabolism. Am J Physiol Endocrinol Metab 2003;284: E55–E69

10. Ciucanu I, Kerek F. A simple and rapid method for the permethylation of carbohydrates. Carbohydr Res 1984;131:209–217

11. Beylot M, Previs SF, David F, Brunengraber H. Determination of the ^{13}C-labeling pattern of glucose by gas chromatography-mass spectrometry. Anal Biochem 1993;212:526–531

12. Laine RA, Sweeley CC. Analysis of trimethylsilyl *O*-methyloximes of carbohydrates by combined gas-liquid chromatography-mass spectrometry. Anal Biochem 1971;43:533–538

13. Dalla Man C, Caumo A, Basu R, Rizza R, Toffolo G, Cobelli C. Measurement of selective effect of insulin on glucose disposal from labeled glucose oral test minimal model. Am J Physiol Endocrinol Metab 2005; 289:E909–E914

14. Dalla Man C, Caumo A, Cobelli C. The oral glucose minimal model: estimation of insulin sensitivity from a meal test. IEEE Trans Biomed Eng 2002;49:419–429

15. Campioni M, Toffolo G, Basu R, Rizza RA, Cobelli C. Minimal model assessment of hepatic insulin extraction during an oral test from standard insulin kinetic parameters. Am J Physiol Endocrinol Metab 2009;297:E941–E948

16. Cobelli C, Toffolo GM, Dalla Man C, et al. Assessment of beta-cell function in humans, simultaneously with insulin sensitivity and hepatic extraction, from intravenous and oral glucose tests. Am J Physiol Endocrinol Metab 2007;293:E1–E15

17. Basu A, Joshi N, Miles J, Carter RE, Rizza RA, Basu R. Paradigm shifts in nocturnal glucose control in type 2 diabetes. J Clin Endocrinol Metab 2018;103: 3801–3809

18. Gastaldelli A, Abdul Ghani M, DeFronzo RA. Adaptation of insulin clearance to metabolic demand is a key determinant of glucose tolerance. Diabetes 2021;70:377–385

19. Peter R, Dunseath G, Luzio SD, Chudleigh R, Roy Choudhury S, Owens DR. Daytime variability of postprandial glucose tolerance and pancreatic B-cell function using 12-h profiles in persons with Type 2 diabetes. Diabet Med 2010;27:266–273

20. Jakubowicz D, Wainstein J, Ahren B, Landau Z, Bar-Dayan Y, Froy O. Fasting until noon triggers increased postprandial hyperglycemia and impaired insulin response after lunch and dinner in individuals with type 2 diabetes: a randomized clinical trial. Diabetes Care 2015;38:1820–1826