



# Influences of Breakfast on Clock Gene Expression and Postprandial Glycemia in Healthy Individuals and Individuals With Diabetes: A Randomized Clinical Trial

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## OBJECTIVE

The circadian clock regulates glucose metabolism by mediating the activity of metabolic enzymes, hormones, and transport systems. Breakfast skipping and night eating have been associated with high HbA<sub>1c</sub> and postprandial hyperglycemia after lunch and dinner. Our aim was to explore the acute effect of breakfast consumption or omission on glucose homeostasis and clock gene expression in healthy individuals and individuals with type 2 diabetes.

## RESEARCH DESIGN AND METHODS

In a crossover design, 18 healthy volunteers and 18 volunteers with  $14.5 \pm 1.5$  years diabetes, BMI  $30.7 \pm 1.1$  kg/m<sup>2</sup>, and HbA<sub>1c</sub>  $7.6 \pm 0.1\%$  ( $59.6 \pm 0.8$  mmol/mol) were randomly assigned to a test day with breakfast and lunch (YesB) and a test day with only lunch (NoB). Postprandial clock and clock-controlled gene expression, plasma glucose, insulin, intact glucagon-like peptide 1 (iGLP-1), and dipeptidyl peptidase IV (DPP-IV) plasma activity were assessed after breakfast and lunch.

## RESULTS

In healthy individuals, the expression level of *Per1*, *Cry1*, *Rora*, and *Sirt1* was lower ( $P < 0.05$ ) but *Clock* was higher ( $P < 0.05$ ) after breakfast. In contrast, in individuals with type 2 diabetes, *Per1*, *Per2*, and *Sirt1* only slightly, but significantly, decreased and *Rora* increased ( $P < 0.05$ ) after breakfast. In healthy individuals, the expression level of *Bmal1*, *Rora*, and *Sirt1* was higher ( $P < 0.05$ ) after lunch on YesB day, whereas the other clock genes remained unchanged. In individuals with type 2 diabetes, *Bmal1*, *Per1*, *Per2*, *Rev-erba*, and *Ampk* increased ( $P < 0.05$ ) after lunch on the YesB day. Omission of breakfast altered clock and metabolic gene expression in both healthy and individuals with type 2 diabetes.

## CONCLUSIONS

Breakfast consumption acutely affects clock and clock-controlled gene expression leading to normal oscillation. Breakfast skipping adversely affects clock and clock-controlled gene expression and is correlated with increased postprandial glycemic response in both healthy individuals and individuals with diabetes.

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The circadian clock controls the activity of most enzymes, hormones, and transport systems involved in glucose metabolism (1,2). The central circadian clock, located in the suprachiasmatic nuclei of the anterior hypothalamus, generates endogenous ~24-h rhythms. Similar clocks are found in peripheral tissues, such as the liver,  $\beta$ -cells, muscle, and adipose tissue (3–5). The core clock mechanism in the brain and peripheral tissues comprises two loops. The positive loop consists of the CLOCK and BMAL1 heterodimer that mediates transcription of tissue-specific genes and those of the negative feedback loop. The negative feedback loop consists of the period (PER) and cryptochrome (CRY) proteins that inhibit CLOCK:BMAL1-mediated transcription (6).

Unlike the suprachiasmatic nuclei clock, which responds mainly to the light-dark cycle, peripheral clocks respond to meal content and timing, leading to coordinated regulation of digestive and absorptive functions and hormone secretion, thereby preventing metabolic dysregulation (1–4). CLOCK:BMAL1 heterodimer plays a critical role in mediating the transcription of coactivators that regulate the circadian synthesis of most of the enzymes and hormones involved in glucose homeostasis, i.e., regulation of hepatic gluconeogenesis and pancreatic  $\beta$ -cell insulin secretion (7). CRY proteins inhibit gluconeogenic gene expression by regulating CREBP activity and their hepatic depletion increases circulating glucose, and CRY overexpression reduces fasting blood glucose and improves whole-body insulin sensitivity in obese mice (7). CLOCK:BMAL1 heterodimer regulates the expression of *Rev-erb $\alpha$*  and *Ror $\alpha$*  (8–10). REV-ERB $\alpha$ , the negative regulator of *Bmal1* expression (8), is induced during normal adipogenesis and mediates a suppressive effect on hepatic gluconeogenesis and glucose output by regulating the expression of PEPCK and glucose-6-phosphatase (G6pase). Depletion of REV-ERB $\alpha$  leads to hyperglycemia (2,7). In contrast, ROR $\alpha$ , the positive regulator of *Bmal1* expression (10), activates the hepatic gluconeogenic enzyme G6pase and regulates lipogenesis and lipid storage in skeletal muscle (2,10).

AMPK, the cellular energy sensor, also plays a key role in the clock mechanism by enhancing degradation of PER and CRY proteins (11). Upregulation of AMPK signaling significantly enhances GLUT4 translocation and muscular glucose uptake, ensuring

metabolic efficiency and improving postprandial glucose and insulin responses (12). AMPK exerts a positive effect on SIRT1, associated with beneficial effects on  $\beta$ -cell viability and insulin sensitivity, which interacts directly with CLOCK and deacetylates BMAL1 and PER2 (12–15).

Reduced glucose-stimulated insulin secretion, insulin resistance, diminished  $\beta$ -cell proliferation, and apoptosis have been associated with asynchrony or deficiencies in clock genes (16). Moreover, lower transcripts of *Bmal1* and *Cry2* are inversely correlated with HbA<sub>1c</sub> levels (17,18). Furthermore, higher risk of obesity, metabolic alterations, and type 2 diabetes have been found in shift workers and individuals who underwent acute or chronic forced circadian misalignment (19,20), supporting the notion that the clock plays an essential role in the preservation of insulin sensitivity and  $\beta$ -cell function.

It has recently been found that secretion of glucagon-like peptide 1 (GLP-1), a key incretin hormone that regulates glucose-dependent insulin secretion from intestinal L cells, shows a rhythmic pattern in rats and humans in vivo. In addition, its secretion is altered by circadian disruptors, such as constant light exposure, consumption of a Western diet, and feeding during the inactivity hours. The alterations in the rhythm of GLP-1 secretory response parallel changes in the pattern of insulin responses (5). This would suggest that it is important to preserve clock functionality in subjects with type 2 diabetes. In fact, recent studies suggest that breakfast skipping and night eating disrupt circadian rhythms and, as a result, impair glucose metabolism and  $\beta$ -cell function (16,21,22).

We have recently shown that omission of breakfast in patients with type 2 diabetes led to increased postprandial glycemia and attenuated insulin and GLP-1 responses after lunch and dinner (23). Although the mechanism underlying this effect is unclear, we hypothesize that breakfast consumption triggers proper cyclic clock gene expression, leading to improved postprandial glycemia and insulin and GLP-1 responses. Since the acute effect of meals and specifically breakfast on clock gene expression is not known in humans, we compared clock and clock-controlled gene expression in association with glucose, insulin, and GLP-1 responses and plasma

dipeptidyl peptidase IV (DPP-IV) activity in healthy individuals and individuals with type 2 diabetes consuming or skipping breakfast.

## RESEARCH DESIGN AND METHODS

### Participants

This was a randomized, open-label, crossover-within-subject clinical trial. The study population included 18 subjects with type 2 diabetes and 18 healthy subjects. All participants habitually ate breakfast, did not work shifts within the last 5 years, and did not cross time zones within the last month prior to the study. Participants usually woke up at 0600–0700 h and went to sleep at 2200–2400 h. None of the participants had impaired thyroid, renal, or liver function; anemia; pulmonary disease; psychiatric, immunological, or neoplastic diseases; or severe diabetic complications or underwent bariatric surgery. All participants were insulin naïve, and only patients taking metformin were included. Patients taking oral hypoglycemic agents, such as GLP-1 analogs, anorectic drugs, or steroids, were excluded. Those patients on metformin were told to stop taking the medication 2 days before test days. All participants gave their informed consent. The study was registered at clinicaltrials.gov, NCT01939782. The Helsinki Committee of the Wolfson Medical Center, where the trial was performed, approved the study.

### Study Design

There were two groups of participants: a group with type 2 diabetes and a group of healthy control subjects. Both groups underwent two separate test days in the clinic, with two different experimental conditions: 1) the participants ate breakfast and lunch (YesB), or 2) the participants did not eat breakfast, i.e., continued the overnight fast until lunchtime and then had lunch (NoB). The two test days were separated by 1–2 weeks of washout. A person not involved in the study, using a coin flip, randomized participants within their group to start with the YesB or NoB day. Participants ingested their last oral therapy 18 h before the test days. The subjects were instructed to eat a standardized meal in the evening, between 2100 and 2200 h, prior to test day, consisting of two white/wheat bread slices and an optional drink, such as tea or coffee with an artificial sweetener or diet soda. Eating a standardized meal the night before the test

prevents long overnight fasting in the YesB group and more so in the NoB group. Additionally, participants were instructed to avoid alcohol and excessive physical activity 6 days preceding each test day. On the YesB day, participants had two identical standard meals that were provided in the clinic as breakfast at 0830 h and lunch at 1200 h. On the NoB day, breakfast was omitted and the individuals continued their overnight fast until lunch at 1200 h. The energy and content of each test meal were identical ( $572 \pm 8$  kcal; 19.4% fat, 49% carbohydrates, and 32% protein). The primary outcome was to compare clock and clock-controlled gene expression (*Clock*, *Bmal1*, *Per1*, *Per2*, *Cry1*, *Rev-erba*, *Rora*, *Ampk*, and *Sirt1*) in white blood cells (WBCs) on YesB versus NoB days in healthy individuals and individuals with type 2 diabetes. Gene expression profiling in the WBCs has been shown to reflect food-related metabolic changes, even in the postprandial state (24). Gene expression was relative to actin, a housekeeping gene, normalizing variations in WBC count. The secondary outcome was to compare postprandial plasma glucose, insulin, intact GLP-1 (iGLP-1), and DPP-IV plasma activity.

### Meal Tests

On the day of the meal tests, each participant reported to the laboratory at 0730 h after an overnight fast. Anthropometric data were collected in the morning. At 0800 h, a catheter was placed in the vein of the nondominant arm and remained there until 1530 h. Venous blood samples for quantification of plasma glucose, insulin, and iGLP-1 were collected before breakfast (at 0830 h), at 60 and 120 min after breakfast, and at 210 min (at 1200 h, just before lunch). After lunch (at 1200 h), samples were collected at 60, 120, and 210 min (420 min including breakfast). On the NoB day, blood samples were taken at the same time points as on the YesB day. The samples for the assessment of clock and clock-controlled gene expression and DPP-IV activity were taken after the overnight fast (at 0830 h), before lunch (at 1200 h), and 210 min after lunch (at 1530 h).

### Biochemical and Hormonal Blood Analyses

Plasma glucose was immediately analyzed with hexokinase using a Cobas analyzer (Roche Diagnostics, Madison, WI). Blood samples for determining iGLP-1 were

collected into chilled tubes containing EDTA and diprotin A (0.1 mmol/L). Samples were centrifuged at 3,000 rpm at 4°C for 10 min and stored at  $-80^{\circ}\text{C}$ . Insulin was determined by electrochemiluminescence immunoassay using a Cobas analyzer (Roche Diagnostics) according to the manufacturer's instructions. iGLP-1 was quantified using ELISA (IBL America, Minneapolis, MN). DPP-IV plasma activity was analyzed as previously described (25).

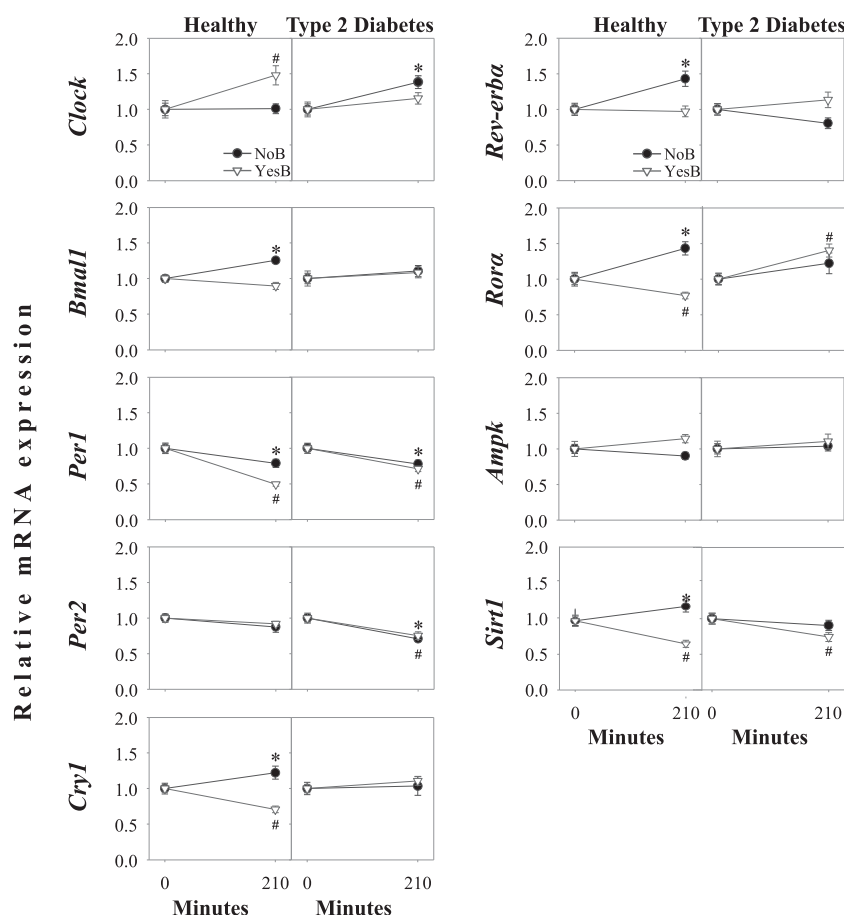
### Analysis of Gene Expression in Leukocytes

Blood for gene expression was collected in Tempus tubes (Applied Biosystems, Foster City, CA), and total RNA was extracted according to the manufacturer's instructions. Total RNA was DNase I treated using RQ1 DNase (Promega, Madison, WI) for 2 h at 37°C, as previously described (26). Two micrograms of DNase

I-treated RNA was reverse transcribed using M-MuLV reverse transcriptase and random hexamers (Promega). One-twentieth of the reaction was then subjected to quantitative real-time PCR using primers spanning exon-exon boundaries and the ABI Prism 7300 Sequence Detection System (Applied Biosystems). The fold change in target gene expression was calculated by the  $2^{-\Delta\Delta\text{Ct}}$  relative quantification method (Applied Biosystems).

### Sample Size and Power Analysis

A sample size of  $n = 16$  healthy participants and  $n = 16$  participants with type 2 diabetes in crossover was determined to have  $>90\%$  power to detect a true, between-group difference of  $40 \pm 25\%$  relative mRNA expression levels of clock genes with a  $P$  value = 0.05. The current study provided 80% power to detect 5% difference between-group in overall postprandial plasma area under the curve



**Figure 1**—Gene expression of healthy participants and participants with type 2 diabetes with or without breakfast. Blood samples were collected after overnight fasting (time point 0), after which breakfast was given (YesB) or fasting continued (NoB). #Statistical differences ( $P < 0.05$ ) between time point 0 and time point 210 min, 3.5 h after breakfast or no breakfast in the healthy group. \*Statistical differences ( $P < 0.05$ ) between time point 0 and time point 210 min, 3.5 h after breakfast or no breakfast in the group with type 2 diabetes. Data are means  $\pm$  SE.

(AUC) for iGLP-1, insulin, and glucose. To allow discontinuation, 36 participants were recruited.

### Statistical Analysis

Thirty-six subjects were enrolled in the study and four subjects dropped out. They were excluded from the analyses; therefore, the results are based on  $n = 32$  subjects. AUC was calculated by the trapezoidal rule. For time series, a two-way ANOVA (time  $\times$  diet) was performed and a least significant difference paired Student  $t$  test post hoc analysis was used for comparison between the YesB and NoB test days at each time point. For clock gene expression, fasting (time point 0) was determined as baseline for the YesB and NoB groups 3.5 h after (time point 210 min). Time point 210 min was determined as the baseline for the postlunch (time point 420 min) gene expression. Student  $t$  test analyses were performed to evaluate differences between time points within each group and treatment. The results are expressed as mean  $\pm$  SEM. A  $P$  value  $\leq 0.05$  was considered statistically significant. Statistical analysis was performed with JMP software (version 12; SAS Institute Inc., Cary, NC).

## RESULTS

### Participants

Thirty-six individuals (18 with type 2 diabetes and 18 healthy) were enrolled in the study, with 32 participants completing (16 with type 2 diabetes and 16 healthy). During the first meal test day, four dropped out (two healthy and two with diabetes) because of problems in the installation of the intravenous catheter. Dropouts did not differ significantly from those who continued the study. The patients with type 2 diabetes were  $66.8 \pm 1.9$  years of age and had the disease for  $14.5 \pm 1.5$  years with  $59.6 \pm 0.8$  mmol/mol ( $7.6 \pm 0.1\%$ ) HbA<sub>1c</sub> and BMI  $30.7 \pm 1.1$  kg/m<sup>2</sup> (Supplementary Table 1). Ten patients were treated with diet alone, whereas six were treated with diet and metformin. Seven patients had a history of hypertension and were treated with ACE inhibitors and/or calcium channel antagonists. The participants in the healthy group were  $44.3 \pm 2.9$  years of age with  $4.9 \pm 0.1\%$  ( $30.1 \pm 0.6$  mmol/mol) HbA<sub>1c</sub> and BMI  $23.1 \pm 0.4$  kg/m<sup>2</sup> (Supplementary Table 1). They were not taking any medications.

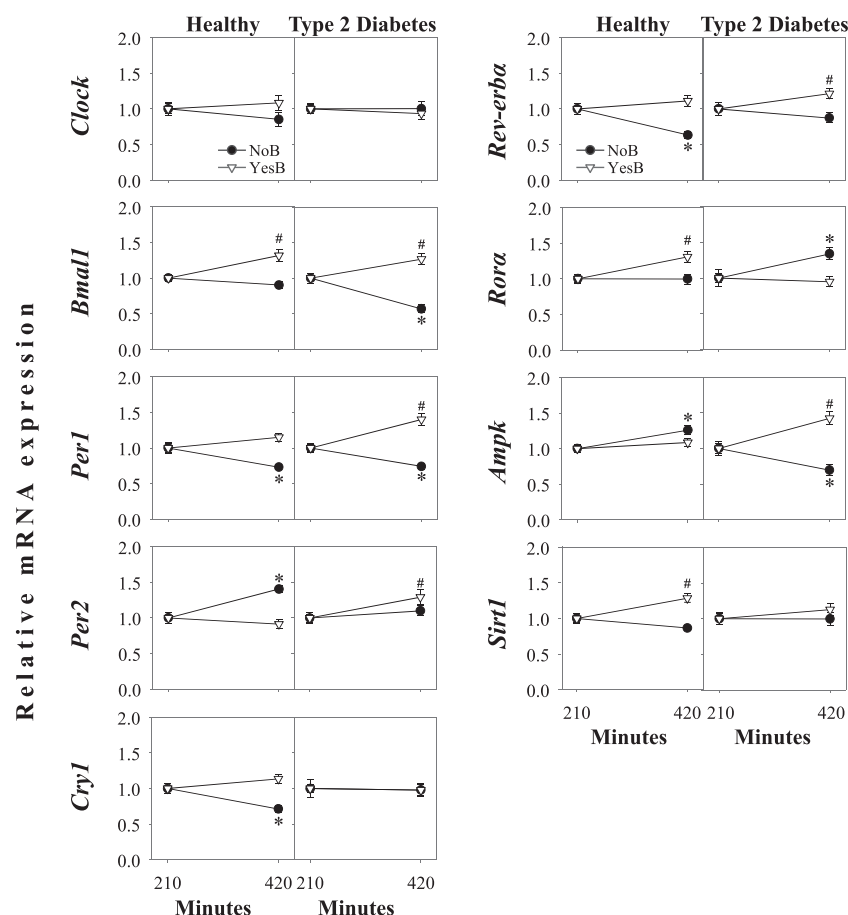
### Clock Gene Expression on YesB and NoB Days in Healthy Individuals Versus Individuals With Diabetes Before Lunch

In healthy individuals, the expression level of *Per1*, *Cry1*, *Rora*, and *Sirt1* was lower (Student  $t$  test,  $P < 0.05$ ), but *Clock* was higher (Student  $t$  test,  $P < 0.05$ ) after breakfast (Fig. 1). In contrast, in individuals with type 2 diabetes, *Per1*, *Per2*, and *Sirt1* only slightly, but significantly, decreased and *Rora* increased (Student  $t$  test,  $P < 0.05$ ) after breakfast. Omission of breakfast altered clock and metabolic gene expression in both healthy people and individuals with type 2 diabetes. In healthy individuals, there was an increase in the expression level of *Bmal1*, *Cry1*, *Rev-erba*, *Rora*, and *Sirt1* (Student  $t$  test,  $P < 0.05$ ), whereas in individuals with type 2 diabetes, *Clock* expression increased (Student  $t$  test,  $P < 0.05$ ) (Fig. 1). In both healthy individuals and individuals

with diabetes, *Per1* and *Per2* expression was not affected whether breakfast was consumed or not (Fig. 1).

### Clock Gene Expression on YesB and NoB Days in Healthy Individuals Versus Individuals With Diabetes After Lunch

In healthy individuals, the expression level of *Bmal1*, *Rora*, and *Sirt1* was higher (Student  $t$  test,  $P < 0.05$ ) after lunch on the YesB day, whereas the other clock genes remained unchanged (Fig. 2). In individuals with type 2 diabetes, *Bmal1*, *Per1*, *Per2*, *Rev-erba*, and *Ampk* increased (Student  $t$  test,  $P < 0.05$ ) after lunch on the YesB day. Omission of breakfast altered clock and metabolic gene expression after lunch in both healthy individuals and individuals with type 2 diabetes. In healthy individuals, *Per2* and *Ampk* expression increased and *Per1*, *Cry1*, and *Rev-erba* decreased (Student  $t$  test,  $P < 0.05$ ) (Fig. 2),



**Figure 2**—Gene expression of healthy participants and participants with type 2 diabetes after lunch. Blood samples were collected 3.5 h after breakfast or no breakfast (time point 210 min) and 3.5 h after lunch (time point 420 min). #Statistical differences ( $P < 0.05$ ) between time point 210 min and time point 420 min in the healthy group. \*Statistical differences ( $P < 0.05$ ) between time point 210 min and time point 420 min in the group with type 2 diabetes. Data are means  $\pm$  SE.

whereas in individuals with type 2 diabetes, *Bmal1*, *Per1*, and *Ampk* expression decreased and *Rora* expression increased (Student *t* test,  $P < 0.05$ ) after lunch on the NoB day (Fig. 2). Taken together, breakfast and lunch led to increased expression of the positive loop and down-regulation of the negative feedback loop, whereas breakfast skipping altered this expression pattern. Lunch, as the first meal of the day, could not rectify the altered clock expression. In addition, individuals with type 2 diabetes who skip breakfast have a greater disruption of their circadian rhythms compared with those who consume breakfast.

### Glucose, Insulin, and iGLP-1 Levels and DPP-IV Activity on YesB and NoB Days

In the healthy group and group with diabetes, the  $AUC_{\text{glucose}}$  after lunch was 15–18% higher during the NoB day versus the YesB day ( $P < 0.001$ ) (Fig. 3 and Supplementary Table 2). In healthy participants,  $AUC_{\text{insulin}}$  after lunch was not significantly different between the tests ( $P = 0.5$ ). In contrast, in the group with diabetes, the  $AUC_{\text{insulin}}$  after lunch was 25% lower on the NoB day compared with the YesB day ( $P < 0.004$ ) (Fig. 3 and Supplementary Table 2). In both the healthy group and group with diabetes, the  $AUC_{\text{iGLP-1}}$  after lunch was ~35% lower on the NoB day compared with the YesB day ( $P < 0.0001$ ) (Fig. 3 and Supplementary Table 2). No significant change in plasma DPP-IV activity was found after lunch between the groups on YesB or NoB day.

### CONCLUSIONS

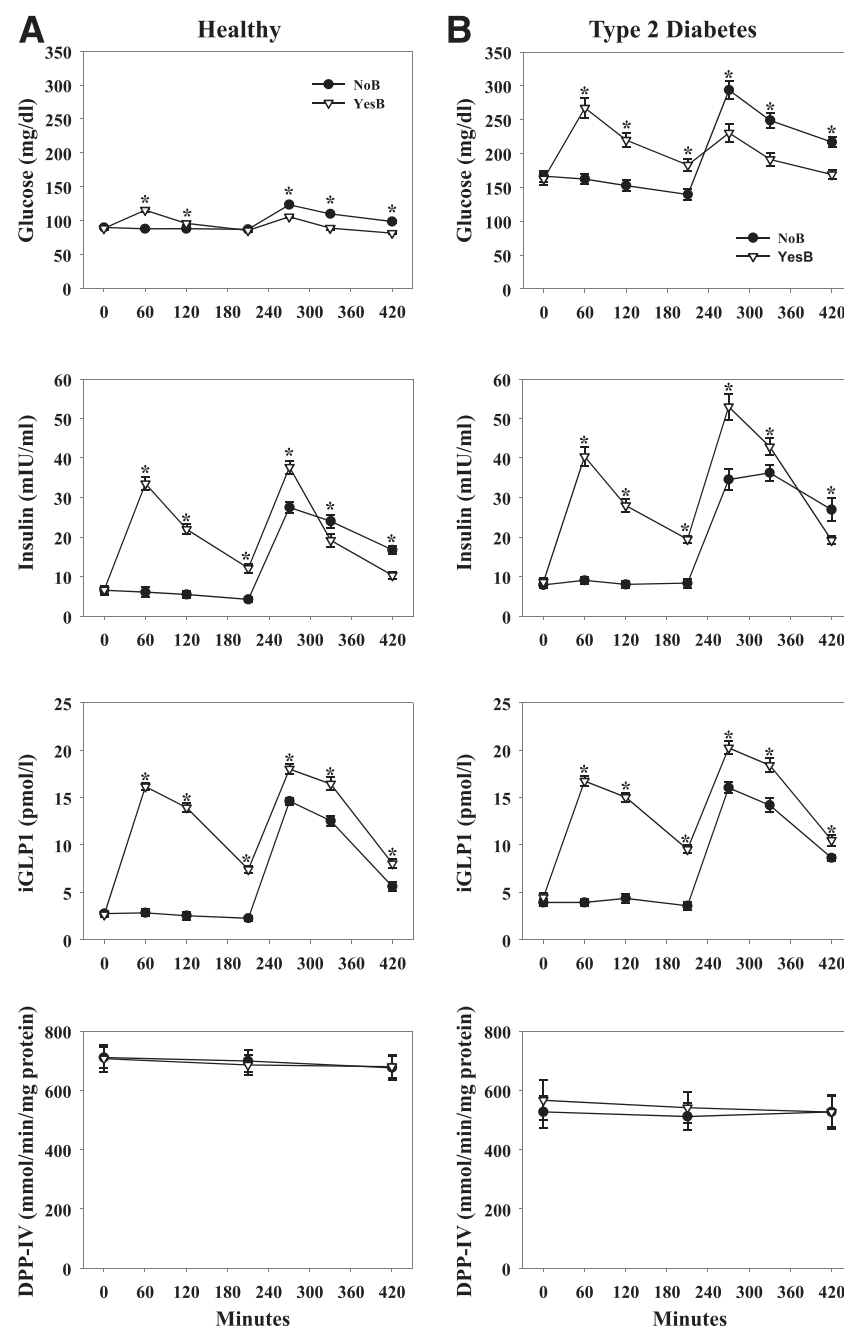
In this study, we report for the first time an acute postprandial effect on clock and clock-controlled gene expression in both healthy individuals and individuals with type 2 diabetes. We showed that breakfast skipping acutely disrupts circadian rhythms in both healthy individuals and people with type 2 diabetes. Furthermore, clock gene expression after lunch was different and depended on whether breakfast was consumed or not. Thus, the study results suggest that breakfast consumption is of high relevance for preserving clock gene activity, and this is true for both healthy subjects and subjects with diabetes.

Several reports have suggested that meal timing exerts a pivotal influence on peripheral clocks and clock output

systems involved in the regulation of metabolic pathways (1,26–29). Breakfast skipping (22,30) and/or eating at hours designed to sleep (21), for the long run, lead to disruption of clock gene expression and are associated with weight gain and diabetes (31,32). In contrast, acclimation to timed feeding, high-energy breakfast, and fasting during the hours designed for sleep reversed impaired clock gene expression and enhanced

AMPK expression, resulting in a more efficient weight loss, insulin sensitivity, and reduction of lipid accumulation (21,22,30–32).

In healthy people, breakfast and lunch acutely led to an overall increased expression of the positive loop of the core clock mechanism, whereas the expression of the negative feedback loop was down-regulated (Fig. 4). This pattern maintains the normal cyclic expression of output



**Figure 3**—Line charts of healthy participants (A) and participants with type 2 diabetes (B) on YesB and NoB days for glucose, insulin, iGLP-1, and DPP-IV. Breakfast was given to the YesB group at time point 0. Lunch was given to both groups at time point 210 min. \*Statistical difference between YesB and NoB at a specific time point. Data are means  $\pm$  SE.

systems (Fig. 4). In contrast, breakfast skipping in healthy people led to an altered clock gene expression pattern. After lunch on the NoB day, *Sirt1*, *Clock*, *Bmal1*, and *Rora* were upregulated and *Per1* was downregulated, similarly to the effect on the YesB day (Fig. 4). However, unlike on the YesB day, after lunch, *Per2* and *Ampk* were upregulated and *Cry1* did not change, disrupting the normal cyclic expression (Fig. 4).

In patients with type 2 diabetes, breakfast and lunch eventually led to an overall altered clock gene expression (Fig. 4). However, *Ampk* expression was upregulated after lunch. As AMPK activation leads to glucose uptake, this upregulation may indicate improved glycemic control, as indeed was found herein. In contrast, breakfast skipping in people with diabetes further imparted a greater disruption, as it also led to reduced *Ampk* levels, accentuating blood glucose dysregulation (Fig. 4).

Our results show that the effect of breakfast is acute, as only 1 day of skipping breakfast led to such a deleterious effect on clock and clock-controlled gene expression. The rapid change in clock gene expression on YesB versus NoB days is consistent with reports in animal models, in which a 30-min feeding stimulus altered clock gene expression within 2–4 h (33,34). As feeding poses such an acute effect, it is clear why long-term abnormal patterns of clock gene expression are manifested in obese individuals and individuals with diabetes.

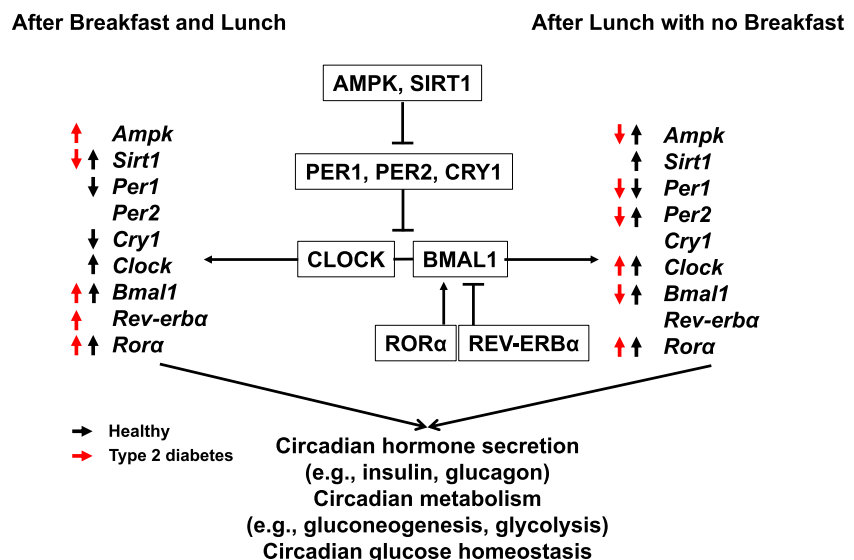
A change in clock and clock-controlled gene expression, due to breakfast omission, is expected to yield altered metabolism. Impaired circadian rhythms have been closely associated with the pathophysiology of type 2 diabetes (4). Reduced glucose-stimulated insulin secretion, insulin resistance, diminished  $\beta$ -cell proliferation, and apoptosis have been associated with asynchrony or deficiencies in clock genes (16). Indeed, in people with diabetes, omission of breakfast led to a significant increase in postprandial glucose excursions with reduced insulin and iGLP-1 responses after lunch compared with YesB day. Furthermore, this deleterious effect of breakfast skipping was also observed in healthy participants, although with a lower magnitude.

The reduction of hyperglycemia and enhanced insulin response after lunch with prior consumption of breakfast has been described as the second meal

phenomenon and has been previously reported in several studies in healthy individuals and individuals with type 2 diabetes (23,35). This has been explained by enhanced  $\beta$ -cell responsiveness at the second meal induced by the first meal (36,37). This explanation is based on the findings that both the first and the second phase of insulin release are influenced by  $\beta$ -cell memory, and the magnitude of insulin release is enhanced significantly by previous glucose exposure (37). The absence of glucose elevation due to fasting until noon may diminish  $\beta$ -cell responsiveness and memory, leading to a reduced and delayed insulin response after lunch on the NoB day. Indeed, it was recently reported that lower insulin release by  $\beta$ -cells in response to nutrient depletion or starvation induces lysosomal degradation of nascent secretory insulin granules, and this is controlled by protein kinase D (PKD), a key player in secretory granule biogenesis (38). The impaired insulin secretion at lunch on the NoB day may also be due to perturbed incretin regulation, since both  $\beta$ -cell memory and sensitivity to glucose are enhanced by GLP-1. Therefore, the higher levels of GLP-1 on the YesB day may explain both the enhanced insulin secretion and the

reduced glycemic response after lunch. At the cellular level, the reduction of postprandial glycemia after lunch on the YesB day can be explained by the fact that breakfast triggers correct cyclic expression of clock genes, which, in turn, assures metabolic efficiency, leading to normal glucose, insulin, and GLP-1 responses after lunch. *Ampk* upregulation in participants with type 2 diabetes may enhance GLUT4 translocation and muscular glucose uptake, leading to improved postprandial glycemia.

At baseline, clock gene levels were different between the healthy group and the group with type 2 diabetes, presumably due to differences related to age, body weight, and/or HbA<sub>1c</sub> levels. Although the age and body weight of the group with type 2 diabetes was significantly different from the healthy group, and may be a limitation in our study, changes in clock gene expression as a result of breakfast skipping cannot be attributed to these parameters, as each group was normalized to its own baseline. In addition, the crossover study assured that the same person had both YesB and NoB treatments, reiterating the effect of breakfast skipping. Another limitation of our study is that it was performed until after lunch.



**Figure 4**—Effect of lunch with or without prior breakfast on the interrelations between the clock mechanism and metabolic proteins. CLOCK and BMAL1 mediate the expression of clock and clock-controlled genes regulating circadian hormone secretion (insulin and glucagon), metabolism (gluconeogenesis and glycolysis), and glucose homeostasis. PER1, PER2, and CRY1 serve as the negative feedback loop that inhibits CLOCK:BMAL1-mediated expression. SIRT1 and AMPK, when activated under low cellular energy levels, relieve the inhibition mediated by the negative feedback loop. BMAL1 expression is positively regulated by ROR $\alpha$  and negatively regulated by REV-ERB $\alpha$ . Breakfast consumption followed by lunch assures this overall cyclic regulation is maintained. Omission of breakfast disrupts this cyclic regulation in both healthy people and patients with type 2 diabetes.

Analyses of subsequent dinner and other time points during the nighttime are required in order to determine overall daily rhythms and how each meal affects this oscillatory pattern. In summary, our study demonstrates that breakfast consumption exerts a rapid influence on clock and clock-controlled genes involved in glucose regulation, such as *Ampk*, and this effect is associated with a significant reduction in postprandial glycemia and enhanced insulin and GLP-1 responses after subsequent lunch. In contrast, breakfast skipping adversely affects the expression of clock and clock-controlled genes involved in glucose metabolism in both healthy individuals and individuals with diabetes. This study emphasizes the consumption of breakfast as an important strategy when targeting glycemic control in type 2 diabetes. As the circadian clock also regulates blood pressure, heart rate, and cardiovascular activity, as well as adipose tissue and other metabolic organs (39), meal timing may affect overall metabolism and influence chronic complications of obesity and type 2 diabetes.

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

**Author Contributions.** D.J. and O.F. contributed to the conception and design of the study; acquired, analyzed, and interpreted data; and drafted and revised the manuscript. J.W. contributed to the conception and design of the study, acquired and interpreted data, and drafted the manuscript. Z.L., N.C., T.G., M.M., and Y.B.-D. contributed to the conception and design of the study, acquired and interpreted data, organized the randomization, and drafted the manuscript. I.R. and B.A. researched data, contributed to the interpretation of the data, and drafted and revised the manuscript. M.B. analyzed and interpreted data. D.J. 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.** This study was presented orally at the 77th Scientific Sessions of the American Diabetes Association, San Diego, CA, 9–13 June 2017.

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