



The Impact of Nocturnal Hypoglycemia on Sleep in Subjects With Type 2 Diabetes

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OBJECTIVE

The aim of this trial was to investigate the impact of nocturnal hypoglycemia on sleep patterns (assessed by polysomnography) and counterregulatory hormones.

RESEARCH DESIGN AND METHODS

In this single-blinded, crossover trial, 26 subjects with type 2 diabetes attended two experimental night visits (one normoglycemic and one hypoglycemic) in randomized order. Plasma glucose (PG) levels were controlled by hyperinsulinemic glucose clamping. On the hypoglycemic night, hypoglycemia was induced after reaching sleep stage N2 by turning off glucose infusion until the PG target of 2.7–2.8 mmol/L was reached and maintained for 15 min. Thereafter, subjects were brought back to normoglycemia for the rest of the night. On the normoglycemic night, PG was maintained at 5.0–7.0 mmol/L throughout the night.

RESULTS

During the first 4 h of sleep (0–4 h; after reaching sleep stage N2), no difference between experimental nights was observed in the rate of electroencephalography-identified arousals or awakenings, but the rate of awakenings was 27% lower during 4–8 h and 20% lower during 0–8 h on the hypoglycemic night than on the normoglycemic night (both statistically significant). Total sleep time tended to be longer on the hypoglycemic night (observed means 366 vs. 349 min, *P* non-significant). Statistically significantly higher counterregulatory hormonal responses (adrenaline, growth hormone, and cortisol) to hypoglycemia were observed compared with normoglycemia.

CONCLUSIONS

Nocturnal hypoglycemia in patients with type 2 diabetes caused a decrease in awakening response in the 4–8-h period following the event. These findings underscore the risks associated with nocturnal hypoglycemia because nocturnal hypoglycemia potentially affects the patient's ability to wake up and respond with an adequate intake of carbohydrates.

In the treatment of diabetes, good glycemic control decreases the risk of long-term complications, but iatrogenic hypoglycemia, particularly nocturnal hypoglycemia, is a major barrier to achieving these targets (1–3). The frequency of nocturnal hypoglycemia is not negligible (4), and patients experiencing nocturnal hypoglycemia often report poor quality of sleep, fatigue, and impaired next-day performance (5). Furthermore, substantial evidence indicates that acute hypoglycemia alters

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A slide set summarizing this article is available online.

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cognitive function (6). As of today, however, the consequences of nocturnal hypoglycemia on objective measures of sleep are poorly understood.

Polysomnography (PSG) is considered the gold standard for the assessment of sleep and sleep architecture. Sleep architecture is the cyclical pattern of sleep as it shifts among the various sleep stages, including non-rapid eye movement (NREM) sleep, which is subdivided into sleep stages N1, N2, and N3, and rapid eye movement (REM) sleep (7). Arousals are sudden transient elevations of the vigilance state that allow the individual to process relevant incoming information so that an adaptation to internal or external stimuli can occur. Awakenings are more definitive, irreversible interruptions of sleep (8). Hypoglycemia may induce changes in electrophysiological patterns, such as slowing of electroencephalographic (EEG) frequencies (9), but the impact of hypoglycemia on sleep patterns, including arousability, remains to be established (10). The aim of the present trial was to investigate the impact of nocturnal hypoglycemia on sleep and counterregulatory hormonal responses during sleep in subjects with type 2 diabetes.

RESEARCH DESIGN AND METHODS

This randomized, single-blinded, two-period, crossover trial investigated the impact of nocturnal hypoglycemia on sleep (as determined by PSG) and counterregulatory hormonal responses. The trial was conducted from 18 January 2013 to 26 September 2013 at the Danish Center for Sleep Medicine, Copenhagen

University Hospital, Glostrup, Denmark. Written informed consent was obtained from all subjects before any trial-related activities. The trial was carried out in accordance with the Declaration of Helsinki (11) and Good Clinical Practice (12) and was approved according to local regulations by an independent ethics committee.

Subjects

The study included 26 insulin-naïve men and women aged 18–64 years with type 2 diabetes (clinically diagnosed ≥ 24 weeks before screening). All subjects had an $HbA_{1c} \leq 10.0\%$ (86 mmol/mol) and a BMI < 35 kg/m² and were receiving any antidiabetic treatment (including diet and exercise) except insulin at a stable dose for > 4 weeks before screening. Key exclusion criteria were sleep apnea (assessed by a Trackit 32 ambulatory EEG recorder), recent manifestations of macrovascular disease, and concomitant illnesses or use of medications (e.g., antidepressants, antipsychotics, hypnotics, sedatives) that could affect their sleep. Hypoglycemic unawareness was also an exclusion criterion identified by asking subjects, “Do you recognize symptoms when you have a hypo,” with answers categorized as “always,” “usually,” “occasionally,” “never,” or “don’t know,” according to previous research (13,14). Only subjects scoring “always” were included. Additional exclusion criteria at each experimental visit were an abnormal sleeping pattern (assessed by an actigraph device worn on the wrist to assess sleep/wake behavior), hypoglycemia within 48 h (self-assessed by diary), severe hypoglycemia within 72 h before the first day of the visit and

consumption of caffeine-containing beverages or alcohol after 4 P.M. on the first day of the visit. A full list of inclusion and exclusion criteria is presented in Supplementary Table 1.

Trial Design and Randomization

The trial consisted of five visits (Fig. 1): a screening visit, an adaptation night visit, two experimental night visits (one hypoglycemic and one normoglycemic in randomized order), and a follow-up visit (a phone call). Subjects had to complete both experimental nights for their data to be analyzed. The randomization scheme was provided by Novo Nordisk A/S, and subjects were blinded to the treatment sequence (hypoglycemia followed by normoglycemia or vice versa).

During the adaptation night visit, subjects were equipped with electrodes and catheters in accordance with procedures conducted during the experimental night visits but without PSG monitoring and blood sampling. Thereafter, those fulfilling all inclusion criteria and no exclusion criteria were randomized 1:1 to one of the treatment sequences (Fig. 1).

Clamp Procedures

On the experimental nights, plasma glucose (PG) levels were controlled by hyperinsulinemic glucose clamping, and PG was continuously monitored using a YSI model 2300 Stat Plus (YSI Inc., Yellow Springs, OH) (Supplementary Table 2). On both nights, an intravenous infusion of human insulin (Actrapid; Novo Nordisk A/S) was initiated at a constant rate (either 20 or 40 mU/m²/min) at ~ 8 P.M. Additionally, intravenous glucose (Glucose Intravenous Infusion BP

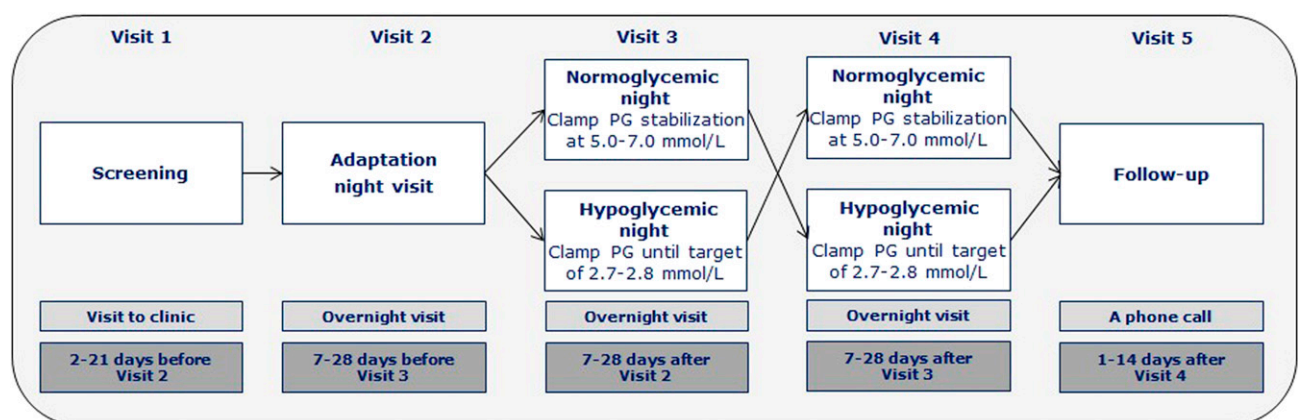


Figure 1—Trial design.

20% w/v Solution for Infusion 1,000 mL; Baxter) was administered at a variable rate to obtain the desired clamp target. An insulin rate of 20 mU/m²/min was used for the first few subjects but turned out to be too low to induce hypoglycemia without an additional bolus insulin infusion, so the protocol was amended. A rate of 40 mU/m²/min was used for subjects enrolled after the amendment was issued. Of note, the same rate was used at both experimental visits for each subject.

On the hypoglycemic night, hypoglycemia was induced when subjects had reached sleep stage N2 or deeper for at least 3 min by turning off the glucose infusion until the PG target of 2.7–2.8 mmol/L (48.6–50.4 mg/dL) was reached and maintained for 15 min. Thereafter, subjects were brought back to normoglycemia for the rest of the night (Supplementary Table 2). On the normoglycemic night, PG was maintained at 5.0–7.0 mmol/L (90.0–126.0 mg/dL) throughout the night. On both experimental nights, insulin infusion was discontinued 3 h after reaching sleep stage N2, and glucose infusion was discontinued 30–60 min later; no insulin or glucose was infused for the remainder of the night.

PSG Assessments

Sleep patterns were assessed by supervised PSG with simultaneous video recording. Between 10 P.M. and 11 P.M., the light was turned off and subjects went to sleep. PSG recordings were scored offline according to American Academy of Sleep Medicine criteria (7) by PSG technicians and supervised by an experienced sleep physician blinded to the treatment allocation. Arousals were defined as abrupt shifts of the EEG frequency, including alpha, theta, and/or frequencies >16 Hz that lasted >3 s, with >10 s stable sleep preceding the change. Awakenings were defined as the presence of alpha rhythm (8.5–13 Hz) in >50% of a 30-s sleep epoch.

Counterregulatory Hormonal Responses

Blood samples were drawn at predefined time points from indwelling venous tubes placed in the distal part of the hand or forearm (Supplementary Table 2) for the assessment of counterregulatory hormonal response (adrenaline, noradrenaline, ACTH, cortisol, growth

hormone, glucagon, insulin, C-peptide, insulin-like growth factor-binding protein 1 [IGFBP-1], pancreatic polypeptide, and melatonin). To ensure minimal disturbance of the subjects' sleep during blood sampling, a room divider (a sound-absorbing black blanket) was installed. Through a hole in the blanket, a physician took blood samples using an extended tube (15 cm).

Hormones were analyzed according to manufacturers' instructions. Plasma adrenaline and noradrenaline were measured by radioimmunoassay (RIA) using the 2-CAT RIA Fast Track (BA R-6500; Labor Diagnostika Nord GmbH & Co KG, Nordhorn, Germany), with an intra-assay coefficient of variation (CV%) of 14% (adrenaline) and 5% (noradrenaline) and an interassay variation of 6% (adrenaline) and 10% (noradrenaline). ACTH and cortisol were measured using an automated method (cobas e411 analyzer; Roche Diagnostics, Hvidovre, Denmark), with an interassay CV% of 2% (ACTH) and 3% (cortisol). Growth hormone was analyzed using an automated method (LIAISON; DiaSorin, Saluggia, Italy), with an interassay CV% of 5.4%. Glucagon was measured using a Glucagon EURIA (Euro Diagnostica, Malmö, Sweden), with an interassay variation of 9%. Insulin and C-peptide were analyzed on the automated cobas c501 module (Roche Diagnostics), with interassay variations of 4% (insulin) and 8% (C-peptide). IGFBP-1 was measured with ELISA (Mediagnost, Reutlingen, Germany), with an interassay variation of 13%. Pancreatic polypeptide was measured with a PP EURIA (Euro Diagnostica), with an intra-assay variation of 2.6% and an interassay variation of 3.5%. Melatonin was determined using the Melatonin Direct RIA (Labor Diagnostika), with an interassay variation of 14%.

End Points and Statistical Analyses

To evaluate sleep changes during hypoglycemia, all PSG end points (except wake time after sleep onset and latency to sleep) were analyzed by period (0–4, 4–8, and 0–8 h where time 0 is when sleep stage N2 has been reached for at least 3 min). The primary end point was number of EEG-identified arousals during 0–4 h.

According to the protocol, the numbers of EEG-identified arousals,

awakenings, and sleep transitions were to be compared between the hypoglycemic night and the normoglycemic night by a Poisson regression model including type of night (hypoglycemic or normoglycemic) and period as fixed factors and subject as a random effect. However, when data were analyzed using the predefined model described in the protocol, overdispersion was detected. Hence, a negative binomial regression model was used instead.

Time spent in various sleep stages (N1, N2, N3, and REM), total sleep time, latency to sleep, and wake time after sleep onset were analyzed using a linear mixed model with type of night (hypoglycemic or normoglycemic) and period as fixed factors and subject as a random effect. Hormonal response profile data were analyzed using a mixed-effects model including type of night (hypoglycemic or normoglycemic), period, time, and interaction between type of night and time as fixed factors and subject as a random effect. After unblinding, the distribution of the data were observed to be skewed, and hence, the analysis was performed on log-transformed data. Because this trial was exploratory in nature, no multiplicity control was performed.

Sample Size Calculation

Sample size was calculated using SAS statistical software version 9.3. Assuming that the probability of more arousals during the hypoglycemic night than during the normoglycemic night is 0.8 (15), a sample size of 20 subjects completing the protocol would provide ~81% power to detect a difference between the nights. To account for dropouts (estimated dropout rate 15%), a total of 24 subjects were to be randomized.

RESULTS

A total of 42 patients were screened, and 26 were randomized (18 men and 8 women). Sixteen patients failed the screening, including 6 with sleep apnea and 2 taking excluded concomitant medications. Mean (SD) age in the full analysis set was 55.2 (5.0) years. Mean (SD) BMI was 29.6 (3.2) kg/m²; duration of diabetes, 5.4 (3.4) years; and HbA_{1c} at baseline, 6.7% (0.8%), corresponding to 49.7 mmol/mol. At screening, 73.1% of subjects were receiving treatment with metformin. Other commonly used

antidiabetic treatments were GLP-1 receptor agonists and sulfonylureas. One subject received only diet and exercise as treatment. Twenty subjects completed both nights (i.e., hypoglycemic, normoglycemic) of the study; therefore, data are presented for this sample only.

Individual plasma glucose profiles during the hypoglycemic and normoglycemic nights are shown in Supplementary Fig. 1. The profiles show that the clamp targets were kept within the pre-specified limits in all subjects. Because some subjects required additional bolus insulin infusion to reach the glycemic targets on both experimental nights, we examined whether a correlation existed between the insulin concentration and the number of awakenings in the 4–8-h period. We found no association between these two variables.

During 0–4 h after reaching sleep stage N2, there was no clinically relevant or statistically significant difference between the hypoglycemic and normoglycemic night in the number of awakenings or arousals (Table 1). During 4–8 h after reaching sleep stage N2, subjects had on average 10 awakenings (geometric mean) on the hypoglycemic night (range 2–26) and 14 on the normoglycemic night (range 6–31). The rate of awakenings was 27% lower on the hypoglycemic night than on the normoglycemic night. This difference was statistically significant (estimated rate ratio 0.73 [95% CI 0.56; 0.95]). During 4–8 h, subjects had on average 20 arousals on the hypoglycemic night (range 1–183) and 30 on the normoglycemic night (range 2–99). This difference was not statistically significant.

During 0–8 h after reaching sleep stage N2, subjects had on average 25

awakenings on the hypoglycemic night (range 11–54) and 30 on the normoglycemic night (range 12–55). The rate of awakenings was 20% lower on the hypoglycemic night than on the normoglycemic night. This difference was statistically significant (estimated rate ratio 0.80 [95% CI 0.68; 0.95]). During 0–8 h, subjects had on average 54 arousals on the hypoglycemic night (range 4–328) and 66 on the normoglycemic night (range 6–231). This difference was not statistically significant.

When comparing data across the time periods of the night, the number of awakenings during 0–4 and 4–8 h was similar on the normoglycemic night, as was the number of arousals. In contrast, on the hypoglycemic night, the number of awakenings decreased by 29% during 4–8 h compared with 0–4 h, and the number of arousals decreased by 35% during 4–8 h compared with 0–4 h (Table 1).

There was no difference in the number of sleep transitions between the hypoglycemic and the normoglycemic nights during 0–4, 4–8, and 0–8 h. Additionally, there was no difference between experimental nights in latency to sleep or in time spent in the various sleep stages. Total sleep time tended to be longer on the hypoglycemic night than on the normoglycemic night (mean [SD] 366.1 [78.9] vs. 348.5 [54.3] min), and subjects tended to spend less time awake on the hypoglycemic night than on the normoglycemic night (mean wake time after sleep onset [SD] 76.8 [43.5] vs. 91.4 [47.9] min) (Supplementary Table 3). However, none of these differences were statistically significant.

The prehypoglycemia levels of the counterregulatory hormones assessed

varied among subjects, and the geometric mean and range values for these during time 0–4 h are reported in Supplementary Table 4. A counterregulatory hormonal response was seen on the hypoglycemic night compared with the normoglycemic night (see Fig. 2 for adrenaline, growth hormone, cortisol, and pancreatic polypeptide and Supplementary Fig. 2 for noradrenaline, ACTH, glucagon, C-peptide, melatonin, and IGFBP-1), with statistically significant increases in adrenaline, glucagon, growth hormone, cortisol, ACTH, pancreatic polypeptide, and melatonin (Table 2). Contrary to expectations, noradrenaline concentration was statistically significantly lower on the hypoglycemic night than on the normoglycemic night (Supplementary Fig. 2). C-peptide concentration was also statistically significantly lower on the hypoglycemic night. For IGFBP-1, no statistically significant difference in responses between experimental nights was found.

CONCLUSIONS

To our knowledge, this study is the first patient-blinded, controlled glucose clamp trial investigating the impact of nocturnal hypoglycemia on sleep in type 2 diabetes. The PG target (2.7–2.8 mmol/L) and duration of the hypoglycemic clamp (15 min) were chosen to ensure that subject safety was not compromised. Severe hypoglycemia has been associated with neuronal death in susceptible brain areas, such as the hippocampus (6). Furthermore, hypoglycemia is associated with seizures, cognitive dysfunction, and an increased risk of cardiovascular events, including stroke and arrhythmias (16–18). Therefore, the hypoglycemic episode was

Table 1—Polysomnography results

		Hypoglycemia		Normoglycemia		Estimated rate ratio	95% CI	P value
End point	n	Geometric mean (CV)	Range	Geometric mean (CV)	Range			
Number of arousals								
0–4 h	20	31 (88)	3–145	34 (67)	4–132	0.92	0.64; 1.33	0.6547
4–8 h	20	20 (113)	1–183	30 (61)	2–99	0.81	0.53; 1.24	0.3070
0–8 h	20	54 (96)	4–328	66 (62)	6–231	0.86	0.59; 1.24	0.3955
Number of awakenings								
0–4 h	20	14 (44)	3–30	15 (37)	5–26	0.87	0.72; 1.06	0.1694
4–8 h	20	10 (54)	2–26	14 (51)	6–31	0.73	0.56; 0.95	0.0237*
0–8 h	20	25 (41)	11–54	30 (39)	12–55	0.80	0.68; 0.95	0.0112*

P values and 95% CIs are based on the negative binomial regression model using logarithm of total sleep time (h) during the corresponding exposure hours as an offset. The model included type of night and period as fixed effects and subject as a random effect. The analysis was based on the completers analysis set. *Statistically significant.

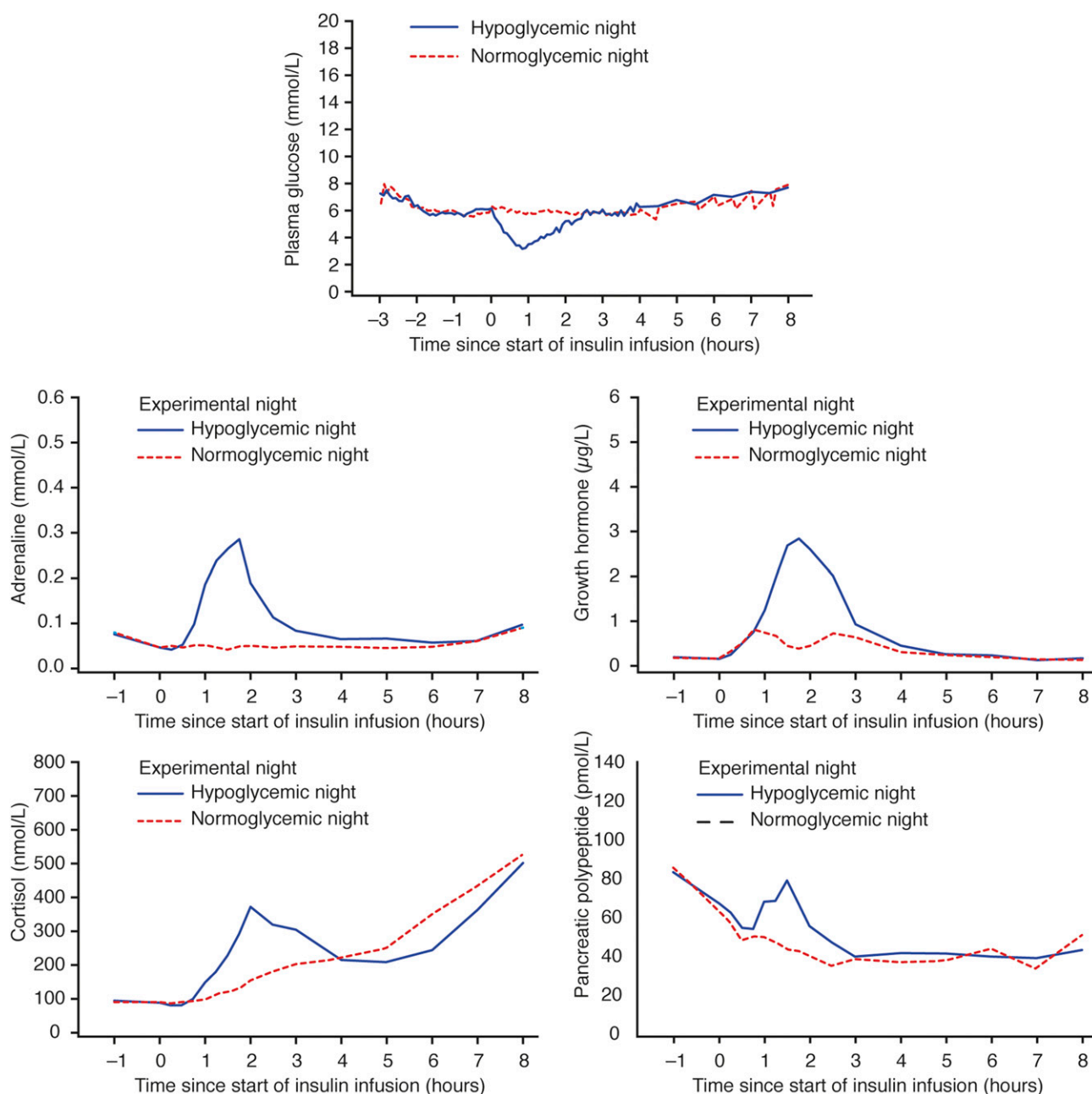


Figure 2—Plasma glucose levels and corresponding counterregulatory hormonal responses.

restricted in terms of both PG level and duration.

Patients with type 2 diabetes often present with sleep disturbances (19) and subsequent increased daytime sleepiness (20). One of the most common sleep disorders in patients with type 2 diabetes is sleep apnea (21), which leads to sleep architecture disruptions and nocturnal arousals. Whether hypoglycemia induces or worsens sleep apnea is not currently known; however, hypoxemia due to sleep apnea in combination with the induction of

hypoglycemia would impose a considerable risk on the trial subjects. Patients with sleep apnea were therefore excluded (6 of the 16 screening failures were due to sleep apnea). The prevalence of sleep disorders (mainly sleep apnea) increases with increasing BMI (22); therefore, patients with a BMI ≥ 35 kg/m² were also excluded, as were those with other concomitant illnesses or who were taking medications that could affect sleep (two patients). Hypoglycemia induction should not inflict unnecessary risk to study subjects;

hence, patients with recent manifestations of macrovascular disease were excluded. In summary, patients eligible for this trial had better physical health than the general type 2 diabetes population. It remains to be established how hypoglycemia affects sleep in patients with obesity, sleep apnea (i.e., suffering from severe hypoxemia), or severe cardiovascular disease.

During the first 4 h (i.e., 0–4 h) after reaching sleep stage N2, no difference was observed between the hypoglycemic and normoglycemic nights in the

Table 2—Counterregulatory hormonal response: statistical analysis

Hormone	n	Estimated hypoglycemia/ normoglycemia ratio	95% CI	P value
Adrenaline	20	2.01	1.80; 2.24	<0.0001*
Noradrenaline	20	0.93	0.89; 0.97	0.0005*
ACTH	20	1.20	1.10; 1.31	<0.0001*
Cortisol	20	1.21	1.11; 1.31	<0.0001*
Growth hormone	20	1.70	1.44; 2.00	<0.0001*
Glucagon	20	1.04	1.00; 1.09	0.0495*
C-peptide	20	0.84	0.81; 0.87	<0.0001*
Pancreatic polypeptide	20	1.24	1.16; 1.32	<0.0001*
Melatonin	20	1.05	1.00; 1.09	0.0290*
IGFBP-1	20	1.08	0.95; 1.22	0.2299

The log-transformed hormonal response profiles were analyzed using a mixed-effects model with type of night (hypoglycemic or normoglycemic), period, time, and interaction between type of night and time as fixed effects and subject as a random effect. The analysis was based on the completers analysis set. *Statistically significant.

number of EEG-identified arousals or awakenings, but the rate of awakenings was 27% lower during 4–8 h and 20% lower during 0–8 h on the hypoglycemic night than on the normoglycemic night (both statistically significant). A significant counterregulatory hormonal response was observed following induction of hypoglycemia, including an immediate increase in glucagon and decrease in C-peptide. Likewise, an immediate sympathetic response occurred with an increase in adrenaline, the role of which is to alert the individual of the hypoglycemia so that he or she can compensate behaviorally with an intake of carbohydrates (23). Large increases in growth hormone and cortisol were also seen; these hormones also play a role in counterregulation, although less importantly than glucagon and adrenaline (24). An increase in pancreatic polypeptide confirmed activation of the parasympathetic nervous system (25). Taken together, the hormone profile data demonstrate that the hypoglycemic event (PG target 2.7–2.8 mmol/L for 15 min) was of sufficient magnitude to induce a clinically relevant and statistically significant counterregulatory hormonal response.

In healthy individuals, nocturnal hypoglycemia causes a counterregulatory hormonal response as described herein, and most wake up upon the hypoglycemic stimulus (13,26). In contrast, in patients with type 1 diabetes, the identical stimulus very rarely causes awakening (13,27), and in the great majority of the patients, no clear-cut counterregulatory

hormonal response to hypoglycemia is observed (26). In the present trial in type 2 diabetes, a reduction in the awakening response was observed after hypoglycemia, although the effect was not immediate (occurring several hours after the hypoglycemic event) and the counterregulatory hormonal response was preserved.

It is noteworthy that a reduced awakening response was observed in this population, despite the relatively recent diagnosis (mean time since type 2 diagnosis 5.4 years) and that the subjects were insulin-naïve. Although not fully understood, arousals and arousal responses are known to occur in response to certain stressful internal and external stimuli (e.g., sleep apnea) and are mediated by cortical and sympathetic activations (28). For patients experiencing life-threatening physiological changes during sleep, the arousal response can be critical for survival (29). In contrast, the reduced awakening response seen following nocturnal hypoglycemia in this study could potentially be harmful by rendering patients unable to compensate behaviorally (i.e., by food ingestion) and, thereby, prolonging and worsening the hypoglycemic episode. An observational study investigating the correlation between hypoglycemia and cardiac arrhythmia in patients with diabetes with established cardiovascular disease showed that hypoglycemic episodes lasted longer at night (170 min) than during the day (62 min). In terms of cardiovascular effects, bradycardia was observed 40–50 min after

glucose nadir, and atrial and ventricular ectopic beats were higher during nocturnal hypoglycemia compared with euglycemia (hypothesized to be due to excessive compensatory vagal activation). These findings indicate that prolonged, undetected episodes of nocturnal hypoglycemia (exacerbated by a reduced awakening response) may increase the risk of impaired cardiac autonomic activity and cardiac arrhythmias, which may in turn lead to an increased risk of cardiovascular mortality (16).

In conclusion, the number of PSG-identified arousals did not differ between experimental nights, but the rate of awakenings was 27% lower on the hypoglycemic night than on the normoglycemic night during 4–8 h and 20% lower during 0–8 h. Hence, a reduction in hypoglycemic episodes, especially during the night, is an important therapeutic goal for future insulin treatment regimens. Of interest would be an assessment of how sleep responses to nocturnal hypoglycemia are specifically influenced by factors such as current drug regimens (e.g., insulin, sulfonylureas), recent daytime hypoglycemia, and hypoglycemic unawareness. In addition, the present findings call for further studies to examine the pathophysiology of hypoglycemia during sleep. Here, we used the gold standard macro sleep evaluation (i.e., PSG) (7). However, macro sleep evaluation is not sensitive to discrete physiologic changes. These include micro sleep events (e.g., sleep spindles) (30), the sleep-wake transition (31), and variables such as autonomic responses due to arousability (32). Although a major contributory factor is obesity, the contribution of hypoglycemia to sleep apnea remains to be fully elucidated.

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Author Contributions. P.J. and S.M. contributed to the trial concept and design, data collection and interpretation, discussion of the results, and review and editing of the manuscript. P.J. was the principal investigator responsible for the sleep assessments, and S.M. was the coinvestigator responsible for the clamp assessments. K.S.-P. and R.R. contributed to the trial concept and design, data interpretation, discussion of the results, and review and editing of the manuscript. N.R.J. contributed to the data collection and interpretation, discussion of the results, and review and editing of the manuscript. P.-L.C. performed the statistical analyses and contributed to the data interpretation, discussion of the results, and review and editing of the manuscript. P.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.

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