SUPPLEMENTARY DATA

Supplementary Table 1. Inclusion and exclusion criteria

Inclusion criteria:	
Eligible subjects had to fulfil all the inclusion criteria below.	
1	Informed consent obtained before any trial-related activities. Trial-related activities are any procedures that are carried out as part of the trial, including activities to determine suitability for the trial.
2	Male or female subjects aged 18–64 years.
3	Type 2 diabetes mellitus (diagnosed clinically) for ≥24 weeks prior to screening.
4	Currently treated with any anti-diabetic treatment (incl. diet and exercise) except insulin with a stable dose for more than 4 weeks prior to screening.
5	HbA_{1c} ≤10.0% by local laboratory analysis.
6	Body mass index (BMI) <35 kg/m^2.
7	Ability and willingness to adhere to the protocol including three overnight in-house stays with polysomnographic and glycaemic monitoring.
Exclusion criteria	
Eligible subjects were not allowed to meet any of the exclusion criteria below.	
1	Previous participation in this trial. Participation is defined as screened.
2	Receipt of any investigational medicinal product within 3 month prior to screening in this trial.
3	Severe hypoglycemic event during the past 6 months or hospitalization for diabetic ketoacidosis during the previous 6 months.
4	Hypoglycemic unawareness as judged by the investigator.
5	Any other chronic disorder or severe disease by clinical information (including chronic obstructive pulmonary disease [COPD], stroke and epilepsy) which, in the opinion of the investigator might jeopardise subject's sleep, safety or compliance with the protocol, or subjects with mental incapacity or language barriers precluding adequate understanding or co-operation or who, in the opinion of the investigator, should not participate in the trial.
6	Presence of sleep apnoea with an apnoea hypopnea index (AHI) ≥10 per hour of sleep or periodic limb movement (PLM) index with arousals ≥15 per hour of sleep identified by ambulatory PSG.
7	Use of antidepressants, antipsychotics, hypnotics, sedatives, other drugs or over the counter drugs known to influence sleep within 8 weeks prior to screening.
8	Hemoglobin <8.0 mmol/L (male) or <6.4 mmol/L (female), total leukocyte count <3.0 x 10^9/L, thrombocytes <100 x 10^9/L, serum creatinine levels ≥126 µmol/L (male) or ≥111 µmol/L (female), alanine aminotransferase (ALAT) >2 x the upper limit of normal (ULN), bilirubin >3 x ULN.
9	Significant history of alcoholism or drug/chemical abuse as per investigator's judgement.
10	Tobacco users who are not able or not willing to refrain from smoking during the in-patient periods.
11	Cardiac problems defined as decompensated heart failure (New York Heart Association [NYHA] class III and IV) at any time and/or angina pectoris within the last 12 months and/or acute myocardial infarction at any time.
12	Supine blood pressure at screening (after resting for 5 min) ≥180 mmHg for systolic and/or ≥100 mmHg for diastolic excluding white-coat hypertension. This exclusion criterion also pertains to subjects being on antihypertensives.
13	Clinically significant abnormal electrocardiogram (ECG) at screening, as judged by the investigator, or clinical relevant arrhythmia as judged by the investigator, including current treatment with propafenone ("Rytmonorm").
14	Proliferative retinopathy or maculopathy and/or severe neuropathy, including autonomic neuropathy, as judged by the investigator.
15	Subjects who has donated any blood or plasma in the past month or more than 500 mL within 3 months prior to screening.
16	Surgery or trauma with significant blood loss (more than 500 mL) within the last 3 months prior to screening.
17	Current treatment with systemic (oral or i.v.) corticosteroids, non-selective beta-blockers. Furthermore, thyroid hormones are not allowed unless the use of these has been stable during the past 3 months.
18	Female of childbearing potential (menopause: at least 12 months without menstrual cycle) who is pregnant, breast-feeding or intend to become pregnant during the trial or are not using adequate contraceptive methods (adequate contraceptive measures include sterilisation, hormonal intrauterine devices, oral contraceptives, spiral, transdermal depot plaster and depot injection).
Adaption night visit exclusion criteria	
Subjects who met one or more of the following adaptation night visit exclusion criteria prior to Day 1 of Visit 2 were excluded from adaptation night visit:	
1	Abnormal sleeping patterns as determined by the ActiGraph.
2	Episodes of hypoglycemia within 48 hours prior to Day 1 of Visit 2.
3	Episodes of *severe* hypoglycemia occurring within 72 hours prior to Day 1 of Visit 2.
4	Consumption of coffee, tea, alcohol, cola or other caffeine-containing beverages after 16:00 hours on Day 1 of Visit 2.
5	Any condition that the investigator feels may interfere with the subject's sleep or other at Visit 2.
Experimental night visit exclusion criteria	
Subjects who met one or more of the following experimental night visit exclusion criteria prior to Day 1 of Visit 3 and 4 were excluded from the experimental night visit:	
1	Any condition that, in the opinion of the investigator, could interfere with sleep pattern or hormonal responses.
2	Use of systemic (oral or i.v.) corticosteroids, non-selective beta-blockers. Furthermore, thyroid hormones are not allowed

	unless the use of these has been stable during the past 3 months.
3	Abnormal sleeping patterns as determined by the ActiGraph.
4	Episodes of hypoglycemia within 48 hours prior to Day 1 of Visit 3 and 4.
5	Episodes of *severe* hypoglycemia occurring 72 hours prior to Day 1 of Visit 3 and 4.
6	Consumption of coffee, tea, alcohol, cola or other caffeine-containing beverages after 16:00 hours on Day 1 of Visit 3 and 4.
7	Any condition that the investigator feels may interfere with the subject's sleep or other at Day 1 of Visit 3 and 4.
Withdrawal criteria	
The subject could withdraw consent at any time. The subject could be withdrawn from the trial at the discretion of the investigator due to a safety concern or if judged non-compliant with trial procedures:	
1	If a protocol deviation or concurrent illness occurred, which, in the clinical judgement of the investigator, may invalidate the trial by interfering with sleep patterns or otherwise, the subject was to be withdrawn by the investigator.
2	Adverse events (AEs) which are considered unacceptable by the investigator.
3	Pregnancy or intention of becoming pregnant.
4	Blood donation during the trial.

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Supplementary Table 2. Experimental night visits blood sampling scheme

<table>
<tr><th rowspan="2">Seq. no.</th><th colspan="2">Nominal time</th><th rowspan="2">Day no.</th><th rowspan="2">Scheduled time[1]</th><th rowspan="2">Intervention</th><th colspan="3">Parameter</th><th>Assessment</th></tr>
<tr><th>Hours</th><th>Minutes</th><th>Hormones[2]</th><th>IGFBP-1</th><th>Glucose[3]</th><th>Polysomnography</th></tr>
<tr><td>1</td><td>-03</td><td>00</td><td>1</td><td>20:00</td><td rowspan="2">Initiation of clamp/PG stabilization Target: 5-7 mmol/L</td><td></td><td></td><td rowspan="12"></td><td></td></tr>
<tr><td>2</td><td>-02</td><td>00</td><td>1</td><td>21:00</td><td></td><td></td><td></td></tr>
<tr><td rowspan="2">3</td><td rowspan="2">-01</td><td rowspan="2">00</td><td rowspan="2">1</td><td rowspan="2">22:00</td><td rowspan="2">Lights are turned off between 22:00 and 23:00 hours[3]</td><td></td><td></td><td></td></tr>
<tr><td>X (pre-sample, prior to reaching sleep stage N2 or deeper)[3]</td><td>X</td><td rowspan="17">PSG monitoring throughout the night until 07:00 hours[4]</td></tr>
<tr><td>5</td><td>00</td><td>00</td><td>1</td><td>23:00</td><td>Sleep stage N2 or deeper reached. Initiation of hypoglycemia or normoglycemia</td><td>X</td><td></td></tr>
<tr><td>6</td><td>00</td><td>15</td><td>1</td><td>23:15</td><td></td><td>X</td><td></td></tr>
<tr><td>7</td><td>00</td><td>30</td><td>1</td><td>23:30</td><td></td><td>X</td><td>X</td></tr>
<tr><td>8</td><td>00</td><td>45</td><td>1</td><td>23:45</td><td></td><td>X</td><td></td></tr>
<tr><td>9</td><td>01</td><td>00</td><td>2</td><td>00:00</td><td></td><td>X</td><td></td></tr>
<tr><td>10</td><td>01</td><td>15</td><td>2</td><td>00:15</td><td></td><td>X</td><td></td></tr>
<tr><td>11</td><td>01</td><td>30</td><td>2</td><td>00:30</td><td></td><td>X</td><td></td></tr>
<tr><td>12</td><td>01</td><td>45</td><td>2</td><td>00:45</td><td></td><td>X</td><td></td><td rowspan="9">Glucose: every 5-30 min
↓
↓
↓
↓
↓</td></tr>
<tr><td>13</td><td>02</td><td>00</td><td>2</td><td>01:00</td><td></td><td>X</td><td>X</td></tr>
<tr><td>14</td><td>02</td><td>30</td><td>2</td><td>01:30</td><td></td><td>X</td><td></td></tr>
<tr><td>15</td><td>03</td><td>00</td><td>2</td><td>02:00</td><td></td><td>X</td><td>X</td></tr>
<tr><td>16</td><td>04</td><td>00</td><td>2</td><td>03:00</td><td></td><td>X</td><td></td></tr>
<tr><td>17</td><td>05</td><td>00</td><td>2</td><td>04:00</td><td></td><td>X</td><td></td></tr>
<tr><td>18</td><td>06</td><td>00</td><td>2</td><td>05:00</td><td></td><td>X</td><td></td></tr>
<tr><td>19</td><td>07</td><td>00</td><td>2</td><td>06:00</td><td></td><td>X</td><td></td></tr>
<tr><td>20</td><td>08</td><td>00</td><td>2</td><td>07:00</td><td>Clamp termination. Lights turned on. Subjects woken up[4]</td><td>X</td><td></td></tr>
<tr><td></td><td></td><td></td><td></td><td colspan="3">Vital signs check after subject had been woken up.</td><td></td><td></td></tr>
<tr><td></td><td></td><td></td><td></td><td colspan="3">Light breakfast served.
At approximately 08:00 hours the subject was released from the clinical trial unit.</td><td></td><td></td></tr>
</table>

[1] Actual starting time was approximate but assessments were to be performed in accordance with the nominal times stated.
[2] Blood sampling for concentrations of adrenaline, noradrenaline, ACTH, cortisol, growth hormone, glucagon, insulin, C-peptide, pancreatic polypeptide and melatonin.
[3] Pre-sample was to be taken immediately before lights were turned off.
[4] The subject was woken up at 07:00 hours. In case the subject woke up prematurely, the PSG assessments were stopped.
ACTH: adrenocorticotropic hormone; IGFBP-1: insulin-like growth factor-binding protein 1; PG: plasma glucose; PSG: polysomnography

SUPPLEMENTARY DATA

Supplementary Table 3. Miscellaneous polysomnography results

Endpoint (min)	N	Hypoglycemia Mean (SD)	Normoglycemia Mean (SD)	Estimated difference	95% CI	p value
TST, 0-4 h	20	191.6 (42.4)	186.6 (41.3)	ND[a]	ND[a]	ND[a]
TST, 4-8 h	20	176.6 (55.0)	164.3 (35.5)	ND[a]	ND[a]	ND[a]
TST, 0-8 h	20	366.1 (78.9)	348.5 (54.3)	20.91	[-5.26; 47.08]	0.1105
WASO	20	76.8 (43.5)	91.4 (47.9)	-16.4	[-33.1; 0.37]	0.0548
Latency to N1	20	23.8 (18.9)	22.4 (28.2)	2.12	[-6.64; 10.87]	0.6178
Latency to N2	20	29.5 (19.8)	26.7 (28.6)	2.63	[-4.83; 10.09]	0.4683
Latency to N3	20	78.0 (68.8)	84.0 (73.5)	-8.11	[-39.2; 23.01]	0.5907
Latency to REM	20	163.5 (93.7)	154.7 (57.7)	-3.35	[-40.5; 33.78]	0.8514

Arithmetic means (SD) are shown for descriptive statistics and estimated differences, p values and 95% CIs are based on a linear mixed model with type of night (hypoglycaemic or normoglycaemic) and period as fixed effects, and subject as a random effect. The analysis was based on the completers analysis set.
CI: confidence interval; h: hour; SD: standard deviation; REM: rapid eye movement; TST: total sleep time; WASO: wake time after sleep onset
[a] No statistical analysis on TST, 0−4h and TST, 4−8h was performed because it was not an endpoint described in the protocol.

SUPPLEMENTARY DATA

Supplementary Table 4. Variation in counter-regulatory hormonal response

Hormone	Timepoint	Hypoglycemia Geometric Mean (CV)	Min; max	Normoglycemia Geometric Mean (CV)	Min; max
Adrenaline	0 h	0.04 (64.03)	0.01; 0.12	0.04 (52.34)	0.01; 0.10
(nmol/L)	1 h	0.18 (143.75)	0.02; 2.90	0.04 (63.06)	0.01; 0.12
	2 h	0.18 (178.20)	0.03; 3.54	0.04 (57.38)	0.01; 0.15
	3 h	0.08 (93.11)	0.01; 0.44	0.04 (63.65)	0.01; 0.12
	4 h	0.06 (76.58)	0.01; 0.25	0.04 (48.86)	0.01; 0.10
ACTH	0 h	1.71 (52.00)	0.65; 4.12	1.71 (56.50)	0.31; 4.45
(pmol/L)	1 h	3.92 (162.58)	0.81; 50.76	2.23 (57.68)	0.96; 6.33
	2 h	6.55 (107.56)	1.39; 40.41	2.76 (52.02)	0.87; 5.93
	3 h	2.74 (58.18)	0.95; 8.03	2.85 (48.73)	0.83; 7.20
	4 h	1.71 (62.85)	0.56; 5.62	3.32 (45.87)	1.43; 8.00
Cortisol	0 h	84.61 (58.47)	37.31; 271.20	86.79 (80.40)	40.18; 377.40
(nmol/L)	1 h	145.94 (83.93)	32.41; 571.50	92.91 (82.79)	45.48; 462.40
	2 h	370.13 (54.85)	55.57; 864.40	150.08 (60.61)	42.22; 362.20
	3 h	301.13 (54.43)	88.55; 783.40	198.27 (31.15)	70.40; 292.40
	4 h	211.05 (57.45)	93.60; 650.70	216.34 (42.37)	96.79; 513.90
Growth Hormone	0 h	0.17 (248.65)	0.10; 4.40	0.16 (77.25)	0.10; 0.62
(ug/L)	1 h	1.24 (130.08)	0.10; 12.70	0.76 (159.81)	0.10; 11.90
	2 h	2.59 (81.00)	0.34; 10.90	0.45 (131.80)	0.10; 4.80
	3 h	0.92 (170.46)	0.13; 16.30	0.64 (134.63)	0.10; 5.90
	4 h	0.45 (122.65)	0.10; 3.50	0.31 (104.29)	0.10; 1.80

Data are reported for the completers analysis set
CV: coefficient of variation; h: hour

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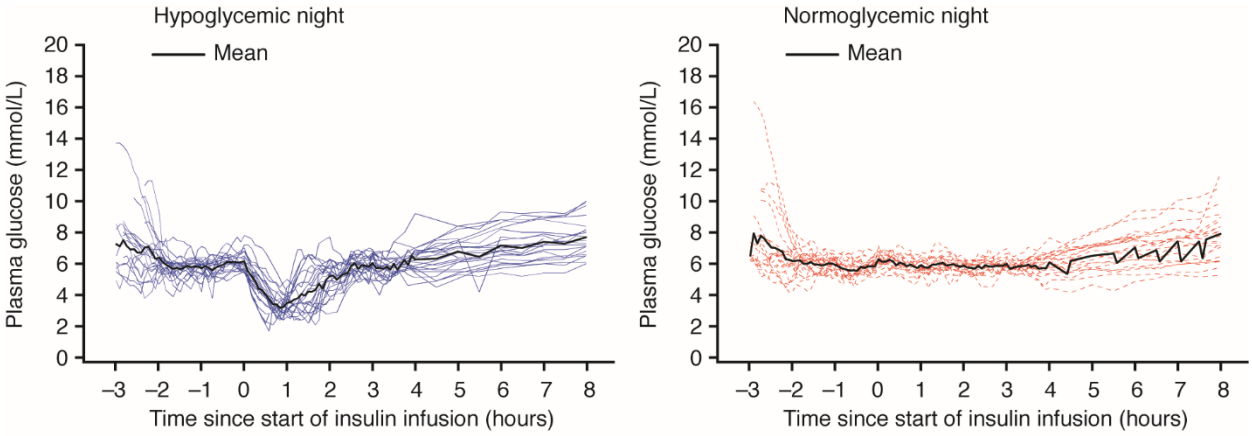
Supplementary Figure 1. Plasma glucose profiles



SUPPLEMENTARY DATA

Supplementary Figure 2. Counter-regulatory hormonal responses for noradrenaline, ACTH, glucagon, C-peptide, melatonin and IGFBP-1