SUPPLEMENTARY METHODS

Study protocol

Baseline measurements

At baseline, participants recorded food intake and sleep times for 3 days. Subsequently participants visited the clinical research unit on the morning of day 1. A fasting blood sample was obtained, resting energy expenditure was measured with a ViaSys Vmax Encore 29 (CareFusion, San Diego, CA, USA), and body fat percentage was measured by bio-electrical impedance analysis (BIA) with a Maltron BF-906 body fat analyser (Maltron International, Rayleigh, Essex, UK). The presence of cardiac autonomic neuropathy was defined as described previously (1), and assessed with continuous non-invasive finger cuff arterial blood pressure measurements (Nexfin, Edwards Lifesciences BMEYE, Amsterdam, The Netherlands). A FreeStyle Navigator continuous glucose measurement (CGM) sensor (Abbott Diabetes Care, Alameda, CA, USA) was applied to the laterodorsal non-dominant upper arm, and a GENEActiv accelerometer (Activinsights, Kimbolton, UK, measurement frequency 50 Hz) was connected to the non-dominant wrist.

Sleep-wake rhythm, diet and medication

For each participant, an individual zeitgeber time (ZT) 0:00 was determined by the average wake up time from the baseline sleep log. Food intake diaries were analysed using food analysis software based on the Dutch Food Composition Database (http://www.eetmeter.nl, Netherlands Nutrition Centre Foundation). Eating episodes were defined as food intake of at least 209 kJ, with 15 minutes between separate episodes (2), and the food intake period was defined as the duration between the first and the last eating episode.

Meal frequency

The individuals with type 2 diabetes were instructed to pause metformin use from two days prior to the measurements until study end (in total 5 days). We provided participants with three identical liquid meals per day at evenly spaced fixed time points, starting from lunch on day 1. The daily amount of calories was set at 105 kJ/kg bodyweight. The daily amount of calories was divided into three equal portions of Ensure Plus (6.3 kJ/ml, 54% energy from carbohydrates, 29% from fat and 17% from protein; Abbott Nutrition, Columbus, Ohio, USA), and participants were instructed to consume an exact amount of the liquid meal within 15 minutes at three equally distanced times corresponding to ZT 0:30, ZT 6:00, and ZT 11:30.

After the visit to the clinical research unit on day 1, participants returned home. They were instructed to adhere to ZT 0:00 as wake-up time, and average baseline sleep time as sleep time. Participants recorded food intake and sleep-wake behaviour in a diary.

Clinical research unit measurements

Participants came back to the clinical research unit at the evening of day 2. A cannula was inserted in a peripheral arm vein. Participants slept undisturbed in darkness (0 lux) during their habitual sleep times. On day 3 at ZT 0:00, room lights were turned on at ~150 lux at eye level. Participants remained in a semi-recumbent position. Superficial subcutaneous adipose tissue samples were obtained in a random order from the four peri-umbilical quadrants on day 2 at ZT 15:30, and on day 3 at ZT 0:15, ZT 5:45, and ZT 11:15. After local anesthesia with 5 ml 2% lidocaine, continuous vacuum suction was applied with a 15 gauge needle. Samples were directly rinsed with 0.9% sodium chloride, subsequently added to 1 ml Tripure Isolation Reagent (Roche, Basel, Switzerland) shaken on a TissueLyser (Qiagen, Hilden, Germany) and stored at -80°C within 15 minutes after tissue sampling.

Starting from ZT 0:00 on day 3, blood samples were obtained with 30-min intervals (15-min intervals in the first postprandial hour) until ZT 15:00. Blood plasma was separated from the cells by centrifugation. Glucose was determined immediately and aliquots for other assays were stored at -20°C.

Adipose tissue RNA sequencing

RNA isolation was performed according to the RNeasy-Mini protocol for animal tissues and cells on the Qiacube (Qiagen) including an RNAse-free-DNAse digestion step. RNA concentration was measured with the Nanodrop 2000 Spectrophotometer (Thermo Fisher Scientific) and quality checked with the 2100 Bioanalyzer (Agilent Technologies). RIN values were >7.5 for all samples except for 5 samples: 1 sample had a RIN of 6.9, and for 4 samples (3 healthy control samples, 1 patient sample), no RIN could be obtained due to low RNA concentration (range 8-14 ng/ul). However, visual inspection of the electrophoresis results of these samples revealed clear 18s and 28s peaks without additional peaks, indicating good RNA integrity, and therefore these samples were included for the cDNA library preparation.

cDNA was constructed with the Ovation RNA-Seq System V2 (NuGen, Manchester, UK) and the product was purified with the MinElute PCR Purification kit (Qiagen). cDNA concentration was determined by fluormetric measurement with Qubit dsDNA BR (Invitrogen, Thermo Fisher Scientific) and quality checked with the Bioanalyzer DNA1000 (Agilent Technologies).

2 ug cDNA was sheared using a Covaris sonicator (Covaris, Woburn, MA, USA). Sheared cDNA was size selected to maintain fragments of 140 to 180 bp, by double AMPure XP beads purification (Agencourt, Beckman Coulter, Brea, CA, USA). Sizing and concentration were checked using Bioanalyzer and Qubit.

Barcoded adaptor ligated library construction was performed using the 5500 SOLiD Fragment Library Core Kit (Life Technologies, Thermo Fisher Scientific, Waltham, MA, USA). Each sample was generated using a separate 10bp barcode incorporated in the adaptor sequence. After 8 cycles of amplification, samples were purified twice using AMPure (ratio of 1.5) and pooled equimolar based on concentration and average sample size. Sequencing of this equimolar pool was performed on the Solid 5500 wildfire sequencer (Life technologies, Thermo Fisher Scientific) generating an average of 40 million 50bp fragment and 10bp barcode reads per sample.

The relative expressions of *PER1*, *PER2*, *PER3*, and *ARNTL* (*BMAL1*) were validated by qPCR using a reaction mix consisting of forward primer, reverse primer (for sequences see ESM Table 3**)**, SensiFAST SYBR NO-ROX Mix (Bioline, Singapore) and purified water in a ratio of 1:1:20:10. qPCR reactions were performed in the Lightcycler 480 (Roche). mRNA expression was quantified with the LinRegPCR software (Heart Failure Center, AMC, Amsterdam) (3) and normalized against the housekeeping gene *EEF1A1*.

GEO datasets

The following datasets can be downloaded via NCBI Gene Expression Omnibus (GEO series accession number GSE104674):

- Raw sequencing data
- Dataset 1: Rhythmic expression analysis
    - Tab 1: Diurnal rhythmicity
    - Tab 2: Up or down regulation
    - Tab 3: Rhythm both groups (184 genes)
- Dataset 2: Ingenuity Pathway Analysis
    - Tab 1: Nomenclature
    - Tab 2: Enrichment gene groups
    - Tab 3: Enrichment up or down
    - Tab 4: Upstream all timepoints

SUPPLEMENTARY REFERENCES

1. Kim YS, Immink RV, Stok WJ, et al.: Dynamic cerebral autoregulatory capacity is affected early in type 2 diabetes. Clinical science 2008;115:255-262

2. St-Onge MP, Ard J, Baskin ML, et al.: Meal Timing and Frequency: Implications for Cardiovascular Disease Prevention: A Scientific Statement From the American Heart Association. Circulation 2017;135:e96-e121

3. Ramakers C, Ruijter JM, Deprez RH, Moorman AF: Assumption-free analysis of quantitative real-time polymerase chain reaction (PCR) data. Neuroscience letters 2003;339:62-66

SUPPLEMENTARY TABLES

ESM Table 1: Primers used for qPCR.

Gene	Forward primer (5' - 3')	Reverse primer (5' - 3')
ARNTL1 (BMAL1)	CATTAAGAGGTGCCACCAATCC	TCATTCTGGCTGTAGTTGAGGA
PER1	AGTCCGTCTTCTGCCGTATCA	AGCTTCGTAACCCGAATGGAT
PER2	GCGTGTTCCACAGTTTCACC	GCGGATTTCATTCTCGTGGC
PER3	CAAGACATGAGGGTATTCTACGC	CAGCTCTTTGGGTCCAGTTGT
EEF1A1	TTTTCGCAACGGGTTTGCC	TTGCCCGAATCTACGTGTCC

ESM Table 2. Compliance and sleep quality.

	Control	Type 2 diabetes	P value
Sleep log[a]			
Night before day 1			
Delta bedtime (min)	15 (8-48)	30 (8-86)	0.56
Delta wake up time (min)	40 (11-51)	15 (5-45)	0.43
Night between day 1 and day 2			
Delta bedtime (min)	15 (8-30)	0 (0-13)	0.10
Delta wake up time (min)	35 (23-52)	10 (3-48)	0.43
Diet log			
Day 1			
Delta lunch time (min)	0 (0-9)	18 (0-31)	0.18
Delta dinner time (min)	0 (0-0)	0 (0-9)	0.39
Day 2			
Delta breakfast time (min)	0 (0-0)	10 (2-38)	<0.01
Delta lunch time (min)	8 (0-23)	0 (0-0)	0.24
Delta dinner time (min)	0 (0-12)	1 (0-26)	0.662
Actigraphy			
Night between day 1 and day 2			
Sleep onset latency (min)	7 (4-37)	0 (0-8)	0.07
Sleep efficiency (%)	89 (85-91)	93 (87-96)	0.24
Snooze time (min)	7 (0-15)	3 (1-16)	1.00
Night between day 2 and day 3			
Sleep onset latency (min)	10 (4-26)	17 (9-58)	0.39
Sleep efficiency (%)	86 (75-91)	87 (77-89)	1.00
Snooze time (min)	6 (0-17)	6 (0-17)	0.82

Compliance to the prescribed sleep-wake rhythm is expressed as the absolute difference (delta) between prescribed sleep times and reported sleep times. Compliance to the diet is expressed as the absolute difference (delta) between prescribed meal times and reported meal times. Sleep quality was assessed by actigraphy (te Lindert et al, Sleep 2013, ref 23 in main text). 'Sleep onset latency' represents the difference (delta) between reported lights off time and actigraphic sleep onset. 'Sleep efficiency' represents the percentage of the analysis period (between actigraphic sleep onset time and actigraphic final wake time) spent asleep according to the actigraph. 'Snooze time' represents the time between reported wake time and actigraphic wake

time. Data are presented as medians (interquartile range). Between group differences are assessed with a Mann Whitney U test for non-normally distributed variables. The only significant between group difference was breakfast time at day 2 which was reported to be on time for healthy controls, but on average 10 minutes off-target by the individuals with type 2 diabetes.

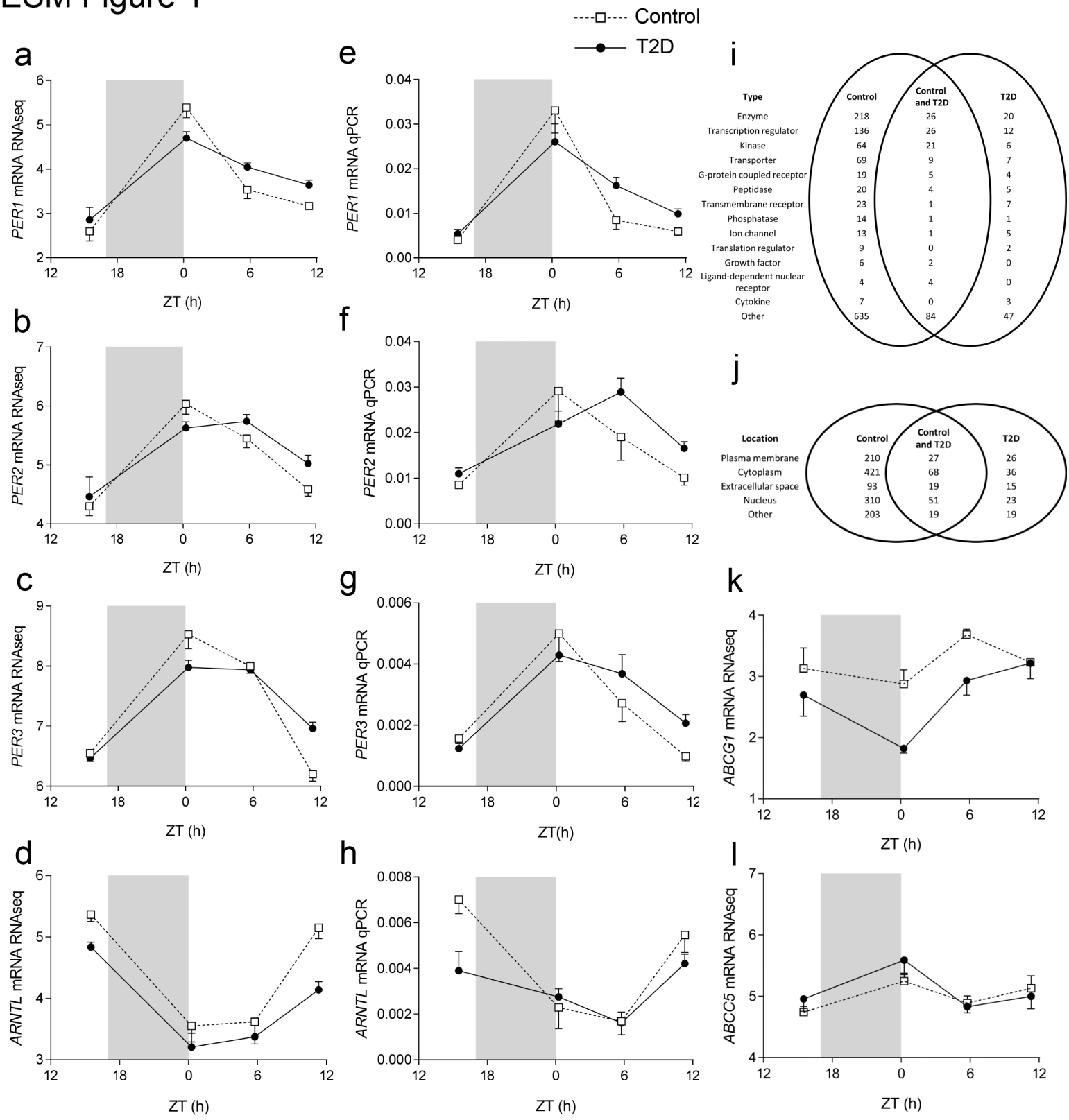
[a]for 7 time points (15%), data were missing due to incomplete sleep diaries

ESM Table 3. Non-esterified fatty acid levels.

		Preprandial NEFA (mmol/l)				Postprandial trough NEFA (mmol/l)			
					P				P
Control	Breakfast	0.43	±	0.07	0.188	0.07	±	0.01	0.018
	Lunch	0.50	±	0.08	0.391	0.08	±	0.01	0.015
	Dinner	0.49	±	0.09	0.320	0.07	±	0.01	0.005
Type 2 diabetes	Breakfast	0.56	±	0.06		0.14	±	0.02	
	Lunch	0.41	±	0.06		0.13	±	0.02	
	Dinner	0.39	±	0.04		0.16	±	0.02	

Data are means ± SEM. Between group differences were assessed with an independent samples two-sided t-test.

ESM Figure 1


Control
T2D
a
PER1 mRNA RNAseq
ZT (h)
e
PER1 mRNA qPCR
ZT (h)
i
Type
Control
Control and T2D
T2D
Enzyme 218 26 20
Transcription regulator 136 26 12
Kinase 64 21 6
Transporter 69 9 7
G-protein coupled receptor 19 5 4
Peptidase 20 4 5
Transmembrane receptor 23 1 7
Phosphatase 14 1 1
Ion channel 13 1 5
Translation regulator 9 0 2
Growth factor 6 2 0
Ligand-dependent nuclear receptor 4 4 0
Cytokine 7 0 3
Other 635 84 47
b
PER2 mRNA RNAseq
ZT (h)
f
PER2 mRNA qPCR
ZT (h)
j
Location
Control
Control and T2D
T2D
Plasma membrane 210 27 26
Cytoplasm 421 68 36
Extracellular space 93 19 15
Nucleus 310 51 23
Other 203 19 19
c
PER3 mRNA RNAseq
ZT (h)
g
PER3 mRNA qPCR
ZT(h)
k
ABCG1 mRNA RNAseq
ZT (h)
d
ARNTL mRNA RNAseq
ZT (h)
h
ARNTL mRNA qPCR
ZT (h)
l
ABCC5 mRNA RNAseq
ZT (h)

ESM Fig. 1. (**a-h**) Validation of the expression of four clock genes using qPCR. (**a-d**) Expression as determined by RNA sequencing (*n*=6 per time point). (**e-h**) Expression as determined by qPCR. The negative clock genes (**ae**) *PER1* (qPCR: *n*=4 for 1 time point (control ZT 0:15), *n*=6 for 7 time points), (**bf**) *PER2* (qPCR: *n*=5 for 1 time point (control ZT 0:15), *n*=6 for 7 time points), and (**cg**) *PER3* (qPCR: *n*=5 for 2 time points (control ZT 0:15 and T2D ZT 15:30), *n*=6 for 6 time points) as determined by qPCR replicated the pattern of a decrease over the day with a tendency towards a decreased amplitude for *PER1* and *PER3* in individuals with type 2 diabetes as determined by RNA sequencing. (**dh**) The positive clock gene *ARNTL* as determined by qPCR (qPCR: *n*=5 for 1 time point (control ZT 0:15), *n*=6 for 7 time points) replicated the increase over the day and the tendency towards a lower amplitude in individuals with type 2 diabetes as determined by RNA sequencing. (**i**) Annotated functions of rhythmic genes (number of genes), determined with IPA. (**j**) Annotated cellular locations of rhythmic genes (number of genes) determined with IPA. (**kl**) Examples of genes showing a significant rhythm in individuals with type 2 diabetes but not in healthy controls: the genes encoding for (**k**) ATP binding cassette subfamily G member 1 (*ABCG1*) and (**l**) ATP binding cassette subfamily C member 5 (*ABCC5*).

The units for RNA sequencing data are $\log_2$ CPM normalised expression values. The units for qPCR data are relative expression to the housekeeping gene. Data are means ± SEM.