Greased lighting : implications of circadian lipid metabolism for cardiometabolic health

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PART II

Circadian rhythms in human studies: implications for metabolic health

Chapter 6

A single night of sleep curtailment increases plasma acylcarnitines: novel insights in the relationship between sleep and insulin resistance

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ABSTRACT

We have previously shown that acute sleep curtailment induces insulin resistance, both in healthy individuals as well as in patients with type 1 diabetes, suggesting a causal role for sleep disturbances in pathogenesis of insulin resistance, independent of endogenous insulin production. However, the underlying mechanisms remain unclear. This study aimed to explore the metabolic pathways affected by sleep loss using targeted metabolomics in human fasting plasma samples. Healthy individuals (n = 9) and patients with type 1 diabetes (n = 7) were studied after a single night of short sleep (4 hours) versus normal sleep (8 hours) in a cross-over design. Strikingly, one night of short sleep specifically increased the plasma levels of acylcarnitines, essential intermediates in mitochondrial fatty acid oxidation (FAO). Specifically, short sleep increased plasma levels of tetradecenoyl-L-carnitine (C14:1) (+32%, $p=2.67*10^{-4}$), octadecanoyl-L-carnitine (C18:1) (+22%, $p=1.92*10^{-4}$) and octadecadienyl-L-carnitine (C18:2) (+27%, $p=1.32*10^{-4}$). Since increased plasma acylcarnitine levels could be a sign of disturbed FAO, it is possible that sleep curtailment acutely induces inefficient mitochondrial function. Our observations provide a basis for further research into the role of acylcarnitines as a potential mechanistic pathway by which sleep deprivation – even short term – causes adverse metabolic effects, such as insulin resistance.

INTRODUCTION

Diabetes mellitus (DM) is characterized by either an absolute (type 1; DM1) or relative (type 2; DM2) deficiency of insulin. Both DM1 and DM2 are associated by increased morbidity and increased cardiovascular risk [1, 2]. Peripheral insulin resistance precedes the development of DM2 and recently it has been recognized that a certain degree of insulin resistance is also present in DM1 [3]. Therefore, uncovering modifiable risk factors in an early stage of insulin resistance development is of crucial importance to reduce the number of patients with DM2 and improve glycemic control in DM1. Interestingly, the DM2 epidemic coincides with a reduction in the average sleep duration, which has gradually declined with ~1.5 hours per night [4] over the past decades. In fact, large epidemiological cohorts have documented an association between sleep duration and increased insulin resistance [5]. Furthermore, short sleep has been associated with poor glycemic control in DM1 [6]. Both short and long duration of sleep are associated with an increased risk for insulin resistance, implying that there might be an optimal sleep duration of approximately 8 hours [7-10]. Several human intervention studies showed that decreased sleep duration causes insulin resistance. Repeated sleep curtailment during more than 6 nights increased insulin resistance in healthy individuals [11-13]. Moreover, we previously published that even one single night with partial sleep loss, i.e. 4 hours sleep allowed, a condition representative for incidental daily life sleep habits, is sufficient to induce peripheral insulin resistance in both healthy young individuals [14] as well as patients with DM1 [15].

The mechanism by which acute sleep curtailment induces insulin resistance has not been fully elucidated. Plasma metabolomics is considered a valuable approach to assess underlying biological processes, complementary to genomics and transcriptomics. Strikingly, metabolite levels reflect biological activity of the encoded proteins and are thus closer to the clinical endpoints [16]. Indeed, metabolomics has previously been demonstrated to be a powerful tool in investigating insulin resistance and DM2 [17]. Thus far, the effects of sleep loss on the human metabolome are poorly characterized. Prolonged sleep deprivation during 5 days has been shown to induce metabolite changes in lipid, carbohydrate, amino acid and protein pathways [18, 19]. In contrast, Davies *et al.* [20] subjected healthy individuals to complete sleep restriction of 24 hours. This extreme sleep deprivation resulted in increased plasma levels of glycerophospholipids, acylcarnitines, sphingolipids and amino acids. However, the sleep intervention and control sleep occurred on consecutive days in all individuals. Differences between metabolite levels were also observed between the wake periods, suggesting that the study conditions were not fully comparable. In addition, none of these previous studies included measurements of insulin resistance. Therefore, the aim of the present study was to use metabolomics to explore pathways involved in the relationship between sleep and insulin resistance in a cohort with proven insulin resistance upon short sleep duration [14, 15]. To this end, we examined 163 metabolites in 16 individuals (healthy individuals and individuals with DM1) subjected to a night of normal sleep duration (8 hours) and one night of short sleep duration (4 hours). Here, we report that one night of sleep curtailment specifically increases the metabolic class of

acylcarnitines in plasma, suggesting that increased acylcarnitines are associated with the observed relationship between sleep curtailment and induction of insulin resistance.

MATERIALS AND METHODS

Protocol

Two studies were previously performed, to study the effect of one night of short sleep duration (4 hours) compared to normal sleep duration (8 hours) on peripheral insulin resistance [14, 15]. The studies applied the same study design in two different populations, namely healthy individuals and patients [14] with type 1 diabetes (DM1) [15]. Healthy individuals were studied to determine the effects of a single night of short sleep duration on insulin resistance. The second study assessed the effects of short sleep duration on insulin resistance in DM1 patients on stable insulin pump therapy. DM1 patients do not have endogenous insulin production and therefore cannot compensate for fluctuations in insulin resistance. We hypothesized that variations in sleep duration could contribute the intra-individual variations in glucoregulation. In both healthy individuals and individuals with DM1, decreased sleep duration induced insulin resistance. Therefore, we reasoned that a single night of short sleep duration may increase peripheral insulin resistance via a common metabolic pathway. To investigate which pathways could be involved, we analyzed metabolites from both studies and pooled the data.

Subjects

The study was approved by the medical ethical committee of the Leiden University Medical Center and all subjects gave written informed consent. We recruited a total of 18 individuals. Briefly, nine healthy individuals were recruited by advertisement and nine individuals with DM1 with stable continuous subcutaneous insulin pump therapy were included from our outpatient clinic. Exclusion criteria for all individuals were BMI>26 kg/m^2, history of sleep disorders, psychiatric disorders and use of sleep medication, β-blocking drugs and prokinetic drugs. All individuals had a stable weight in the past 3 months and had regular and non-extreme sleeping habits. Habitual sleep duration was assessed by 7 days of actigraphy (Actiwatch AW7; Cambridge Neurotechnology, Cambridge, UK) prior to both study days and sleep questionnaires (Epworth Sleepiness Scale, Pittsburg Sleep Quality Index and Berlin Questionnaire). Subjects were instructed to maintain a regular dietary, activity and sleep regiments 3 days prior to both study days, fitting their habits, which they recorded in a diary. DM1 patients were instructed to keep a stable insulin pump setting. Of the 18 recruited individuals, 2 individuals with DM1 were excluded from all analyses, one due to previously undiagnosed sleep apnea and one due to nocturnal hypoglycemia.

Experimental design

Subjects were subjected to in-hospital sleep registration for 3 days, of which study day 1 was for basal measurements and habituation to hospital conditions. Sleep duration and quality (of parameters) was assessed by polysomnography as described previously [14, 15]. All subjects underwent both a normal sleep night of at least 8 hours and one night of 4 hours sleep, the order of which was determined by balanced assignment, in a cross-over design with at least 3 weeks interval between measurements. In both sleep conditions, subjects spent 8.5 hours (from 23:00 to 7:30) in bed and were fasting from 22:00 onwards. During sleep curtailment, subjects were allowed to sleep from 01:00 to 05:00, the remaining time they were allowed to read or watch movies in upward position in dim light. Their wakefulness was monitored. After the night of normal or short sleep, a fasting plasma sample was obtained at 8:30 am, after which a hyperinsulinemic euglycemic clamp was performed as described in detail previously [15] to establish peripheral insulin sensitivity, endogenous glucose production and hepatic insulin sensitivity. Briefly, a primed (17.6 µmol*kg^{-1}) continuous (0.22 µmol*kg^{-1}*min^{-1}) infusion of [6,6-$^{2}H_{2}$]glucose (Cambridge Isotope laboratory, Andover, MA) was administered via a catheter. Infusion of insulin (Actrapid, Novo Nordisk, Alphen a/d Rijn) occurred simultaneously according to DeFronzo [21]. Blood samples were obtained every 5 minutes from the contralateral arm for glucose measurements to adjust variable infusion of 20% glucose with 3% [6,6-$^{2}H_{2}$]glucose to maintain euglycemia (i.e. 5.0 mM), which was started 4 min after start of insulin infusion. Free fatty acids were determined in basal fasting plasma samples as by enzymatic colorimetric assay [14, 15].

Metabolomics

Metabolomics analysis was performed on fasting plasma samples in all individuals using the Biocrates Absolute*IDQ*™ p150 kit (Biocrates, Life Science AG, Innsbruck, Austria) in the Genome Analysis Center at the Helmholtz Zentrum, Munich, Germany. The assay procedures of the Absolute*IDQ*™ p150 kit as well as the metabolite nomenclature have been described in detail previously [22, 23]. Briefly, 10 µL of each plasma sample was pipetted into a 96 well sandwich plate containing an inserted filter with previously applied stable isotope labeled internal standards. The filters in the wells were dried using a stream of nitrogen. Amino acids were derivatized with 5% phenylisothiocyanate reagent (PITC) and the filters were dried again. Metabolites as well as internal standards were extracted with 5 mM ammonium acetate in methanol and the solutions were centrifuged through the filter membrane into the lower deep well plate. The extracts were diluted with MS running solvent and analyzed. Flow injection analysis (FIA) tandem mass spectrometry (MS/MS) method was used to quantify 163 metabolites, including free carnitine, 40 acylcarnitines, 14 amino acids (13 proteinogenic + ornithine), hexoses (sum of hexoses), 92 glycerophospholipids (15 lysophosphatidylcholines (lysoPC) and 77 phosphatidylcholines (PC), and 15 sphingolipids. Internal standards served as reference for the calculation of metabolite concentrations (µM). The complete list of analyzed metabolites grouped by metabolite class is presented in supplementary material (Table S3).

Statistical analysis

For all metabolites, differences between short and normal sleep were calculated by subtracting plasma levels obtained after short sleep from those obtained after normal sleep. Paired Students T-tests for were performed comparing normal and short sleep (SPSS statistical package edition 20) with Bonferroni post-hoc correction for multiple testing. $P< 3.07*10^{-4}$ (=0.05/163; after correction) was considered statistically significant. Calculations for hyperinsulinemic euglycemic clamp analysis were described previously [14, 15]. Since we aimed to investigate the effect of short sleep on metabolite levels, individuals of both groups (healthy individuals and individuals with DM1) were pooled to determine effects of sleep duration. Two way repeated measure ANOVA was performed to analyze interaction effects of subgroup (healthy *vs.* DM1) with sleep duration. Data are presented as means ± SD. Since baseline characteristics and insulin sensitivity data were published for healthy individuals and individuals with DM1 separately, in this paper these data are shown for the two groups together. To allow comparison between subgroups, the baseline characteristics, sleep indices and insulin sensitivity data are included in the supplemental tables and were compared using Student's t-test.

RESULTS

Basal clinical characteristics

Metabolites were measured in sixteen individuals after a night of short sleep (4 hours) versus after a night of normal sleep (8 hours) duration. Subjects had a mean age of 44 ± 14 years and included 8 women. Individuals were lean, with an average BMI of 23.7 ± 2.2 kg/m^2 and a waist hip ratio of 0.85 ± 0.08 (Table 1). The study population consisted of nine healthy individuals (56%) and seven individuals with type 1 diabetes mellitus (DM1) (44%). Sleep duration prior to the study days did not differ healthy individuals (mean recorded sleep duration prior to study day 1 and 2: 420 ± 20 min *vs.* 476 ± 11 min; p=0.19) nor in individuals with DM1 (mean recorded sleep duration prior to study day 1 and 2: 475 ± 8 min *vs.* 490 ± 7 min; p=0.12). Results of healthy individuals and individuals with DM1 were reported previously separately [14, 15]. Age, sex distribution, BMI and waist-hip ratio were comparable between these two subgroups (Table S1).

	Subjects (n = 16)
Females (%)	8 (50%)
Age (years)	44 ± 14
BMI (kg/m^2)	23.7 ± 2.2
WHR	0.85 ± 0.08

Table 1: Study population characteristics[1]. *BMI = body mass index. WHR = waist hip ratio. Data is presented as mean (SD or percentage). [1]Data are pooled from two previously published studies [14;15].*

	Subjects (n = 16)		
Sleep parameters	**Normal sleep**	**Short sleep**	**p**
TST (min)	461 ± 25	225 ± 24	**<0.001**
Stage 1 (% of TST)	10 ± 3	10 ± 6	0.798
Stage 2 (% of TST)	43 ± 7	37 ± 9	**0.002**
Stage 3 (% of TST) (SWS)	24 ± 7	34 ± 10	**<0.001**
REM sleep (% of TST)	23 ± 4	18 ± 8	**0.025**
Sleep efficiency (%)	93 ± 4	91 ± 7	0.418
Plasma parameter			
Free fatty acids (mmol/l)	0.65 ± 0.24	0.61 ± 0.19	0.24
Insulin sensitivity parameters			
EGP (µmol * kg LBM^{-1} * min^{-1})	4.7 ± 1.9	5.5 ± 1.7	0.087
GDR (µmol * kg LBM^{-1} * min^{-1})	34.1 ± 13.8	27.9 ± 9.8	**0.001**
GIR (µmol * kg LBM^{-1} * min^{-1})	29.0 ± 14.7	22.1 ± 10.7	**0.001**

Table 2: Effects of short sleep on sleep parameters and insulin sensitivity[1]. *Insulin sensitivity parameters were determined by hyperinsulinemic euglycemic clamp. EGP = endogenous glucose production, GDR = glucose disposal rate (glucose Rd), GIR = glucose infusion rate. LBM = lean body mass. Sleep characteristics were determined by polysomnography. TST = total sleep time. SWS = slow wave sleep. Data is presented as means (SD). Effect of sleep intervention was tested with paired Students T-test, significant differences shown in bold. [1] Data are pooled from two previously published studies [14;15].*

Short sleep increases insulin resistance

Short sleep intervention was effective in reducing total sleep time (TST) by -51% (461 ± 25 *vs* 225 ± 26 min, p < 0.001). The reduction of sleep duration was due to decreased sleep duration of both non-REM (stage 2 and stage 3) and REM sleep (Table 2). Fasting plasma free fatty acids did not differ between sleep conditions (Table 2) or between subgroups (Table S2). Next, the effect of short sleep on insulin resistance was investigated by hyperinsulinemic euglycemic clamp studies. Interestingly, a single night of short sleep increased peripheral insulin resistance, as indicated by a decreased glucose disposal rate (GDR) (34.1 ± 13.8 *vs* 27.9 ± 9.8 µmol*kg LBM^{-1}*min^{-1}, p = 0.001) and decreased glucose infusion rate (GIR) (29.0 ± 14.7 *vs* 22.1 ± 10.7 µmol*kg LBM^{-1}*min^{-1}, p=0.001). Short sleep tended to increase endogenous glucose production (EGP) by the liver in all subjects (4.7 ± 1.9 vs 5.5 ± 1.6 µmol*kg LBM^{-1}*min^{-1}, p=0.08; Table 2). This was mainly due to increased endogenous glucose production in the subset of healthy individuals (Table S2; previously published in [14]). Expectedly, individuals with DM1 displayed higher baseline insulin resistance than in healthy individuals [3] (EGP 6.2 ± 1.9 *vs*. 3.6 ± 0.6, p=0.003; GDR 25.5 ± 6.4 *vs.* 40.7 ± 14.3, p=0.028; GIR 19.0 ± 7.0 *vs*. 36.9 ± 14.4, p=0.014, Table S2). Moreover, short sleep increased peripheral insulin resistance irrespective of this difference in baseline insulin sensitivity, suggesting a that short sleep may induce insulin resistance in healthy individuals and individuals with DM1 via a common pathway. Therefore, the effect of short sleep was investigated for healthy individuals and individuals with DM1 together.

	All subjects			*Healthy*			*DM1*		
Metabolite	**Mean Difference[1]**	**Change (%)[2]**	**P-Value**	**Mean Difference[1]**	**Change (%)[2]**	**P-Value**	**Mean Difference[1]**	**Change (%)[2]**	**P-Value**
C0	0.191	0.7%	$8.81*10^{-1}$	0.398	1.3%	$8.47*10^{-1}$	-0.075	-0.3%	$9.61*10^{-1}$
C2	**0.662$**	**16.8%**	**2.28*10-2**	**0.184**	**4.4%**	**5.40*10-1**	**1.276$**	**35.6%**	**1.33*10-2**
C3	-0.006	-2.6%	$6.76*10^{-1}$	-0.002	-0.7%	$9.33*10^{-1}$	-0.012	-6.2%	$6.03*10^{-1}$
C3:1	-0.001	-5.9%	$4.16*10^{-1}$	0.000	-0.1%	$9.93*10^{-1}$	-0.001	-12.3%	$1.58*10^{-1}$
C3-DC (C4-OH)	0.007	11.3%	$4.55*10^{-1}$	0.006	8.0%	$7.20*10^{-1}$	0.009	17.7%	$2.87*10^{-1}$
C3-OH	0.001	3.3%	$5.03*10^{-1}$	0.002	8.5%	$5.91*10^{-2}$	-0.001	-2.1%	$8.24*10^{-1}$
C4	0.011	10.3%	$1.22*10^{-1}$	0.008	7.2%	$4.95*10^{-1}$	0.015	14.4%	$8.68*10^{-2}$
C4:1	0.002	6.7%	$2.90*10^{-1}$	0.002	8.7%	$2.55*10^{-1}$	0.001	4.8%	$6.58*10^{-1}$
C5	0.011	10.2%	$2.80*10^{-1}$	0.012	10.4%	$4.59*10^{-1}$	0.010	9.8%	$4.30*10^{-1}$
C5:1	0.000	1.0%	$8.74*10^{-1}$	-0.001	-2.5%	$7.86*10^{-1}$	0.001	5.0%	$6.20*10^{-1}$
C5:1-DC	0.000	2.6%	$6.75*10^{-1}$	-0.001	-4.8%	$5.96*10^{-1}$	0.002	12.5%	$1.27*10^{-1}$
C5-DC (C6-OH)	-0.001	-4.1%	$4.87*10^{-1}$	-0.001	-8.3%	$2.56*10^{-1}$	0.000	0.7%	$9.45*10^{-1}$
C5-M-DC	0.000	0.3%	$9.32*10^{-1}$	0.000	-1.1%	$8.45*10^{-1}$	0.001	1.9%	$7.11*10^{-1}$
C5-OH (C3-DC-M)	0.000	0.3%	$9.40*10^{-1}$	0.000	-1.5%	$6.89*10^{-1}$	0.001	2.4%	$7.40*10^{-1}$
C6 (C4:1-DC)	0.005	8.7%	$1.22*10^{-1}$	0.001	1.9%	$7.82*10^{-1}$	0.011	18.8%	$6.20*10^{-2}$
C6:1	0.001	2.6%	$3.99*10^{-1}$	0.001	3.5%	$3.35*10^{-1}$	0.000	1.5%	$8.05*10^{-1}$
C7-DC	**0.005$**	**20.0%**	**5.41*10-4**	**0.002**	**8.4%**	**8.50*10-2**	**0.008$**	**34.8%**	**3.21*10-4**
C8	0.003	1.9%	$6.06*10^{-1}$	-0.005	-3.1%	$5.56*10^{-1}$	0.013$	11.0%	$4.38*10^{-3}$
C8:1	0.012	15.4%	$1.62*10^{-1}$	0.008	11.8%	$3.93*10^{-1}$	0.017	18.9%	$3.09*10^{-1}$
C9	0.000	-0.4%	$9.47*10^{-1}$	-0.001	-4.7%	$5.73*10^{-1}$	0.001	5.4%	$5.51*10^{-1}$
C10	0.014	5.8%	$1.84*10^{-1}$	0.003	1.1%	$8.62*10^{-1}$	0.027*	15.1%	$4.54*10^{-3}$
C10:1	0.008	6.9%	$2.68*10^{-1}$	-0.004	-3.1%	$6.68*10^{-1}$	0.022*	23.9%	$2.72*10^{-2}$
C10:2	0.000	-0.7%	$8.65*10^{-1}$	0.000	-0.1%	$9.79*10^{-1}$	0.000	-1.3%	$8.51*10^{-1}$

	All subjects			*Healthy*			*DM1*		
Metabolite	**Mean Difference[1]**	**Change (%)[2]**	**P-Value**	**Mean Difference[1]**	**Change (%)[2]**	**P-Value**	**Mean Difference[1]**	**Change (%)[2]**	**P-Value**
C12	**0.012$**	**17.2%**	**1.70*10-3**	**0.010**	**12.0%**	**9.00*10-2**	**0.015$**	**27.5%**	**3.30*10-3**
C12:1	**0.017$**	**23.6%**	**1.74*10-3**	**0.014**	**17.0%**	**8.32*10-2**	**0.021$**	**35.2%**	**5.85*10-3**
C12-DC	0.002	2.4%	$1.53*10^{-1}$	0.002	2.1%	$3.78*10^{-1}$	0.003	2.8%	$3.01*10^{-1}$
C14	**0.004$**	**15.6%**	**1.21*10-2**	**0.002**	**8.5%**	**2.42*10-1**	**0.006$**	**28.0%**	**1.97*10-2**
C14:1	**0.020#**	**32.4%**	**2.67*10-4**	**0.017$**	**23.5%**	**2.34*10-2**	**0.024$**	**49.1%**	**6.55*10-3**
C14:1-OH	0.001	6.2%	$3.79*10^{-1}$	0.000	-1.7%	$8.10*10^{-1}$	0.001	16.9%	$2.36*10^{-1}$
C14:2	**0.006$**	**26.1%**	**5.48*10-4**	**0.005**	**17.3%**	**5.01*10-2**	**0.008$**	**41.5%**	**4.17*10-3**
C14:2-OH	0.000	5.0%	$3.64*10^{-1}$	0.000	6.4%	$2.91*10^{-1}$	0.000	3.3%	$7.48*10^{-1}$
C16	**0.007$**	**10.8%**	**1.94*10-2**	**0.005**	**6.7%**	**2.05*10-1**	**0.010**	**17.1%**	**5.91*10-2**
C16:1	**0.005$**	**8.8%**	**9.60*10-3**	**0.005$**	**8.3%**	**3.06*10-2**	**0.005**	**9.6%**	**1.55*10-1**
C16:1-OH	**0.001$**	**20.8%**	**5.71*10-3**	**0.001**	**11.8%**	**1.90*10-1**	**0.002$**	**33.8%**	**1.10*10-2**
C16:2	**0.001$**	**21.3%**	**9.72*10-3**	**0.001**	**9.6%**	**2.39*10-1**	**0.002$**	**39.5%**	**1.91*10-2**
C16:2-OH	0.000	2.1%	$6.29*10^{-1}$	0.000	3.8%	$5.45*10^{-1}$	0.000	-0.1%	$9.83*10^{-1}$
C16-OH	0.000	0.9%	$8.89*10^{-1}$	0.000	-3.4%	$5.84*10^{-1}$	0.000	6.1%	$6.49*10^{-1}$
C18	0.003	10.8%	$7.34*10^{-2}$	0.002	9.6%	$3.04*10^{-1}$	0.003	12.5%	$1.18*10^{-1}$
C18:1	**0.016#**	**22.3%**	**1.92*10-4**	**0.015***	**20.1%**	**2.96*10-3**	**0.019$**	**25.1%**	**2.84*10-2**
C18:1-OH	0.001	12.8%	$6.02*10^{-2}$	0.001	5.7%	$3.93*10^{-1}$	0.002	22.3%	$1.07*10^{-1}$
C18:2	**0.007#**	**27.0%**	**1.32*10-4**	**0.005$**	**20.7%**	**9.01*10-4**	**0.010$**	**34.4%**	**1.38*10-2**

Table 3: (caption on next page).

Table 3: (previous pages) Difference between short sleep and normal sleep duration in acylcarnitine levels.

[1]Difference in metabolite levels (μM) as measured by BiocratesIDQTM p150 kit between short and normal sleep duration. Positive mean difference indicates an increase after short sleep duration. Negative mean difference indicates a decrease after short sleep duration.

[2]Change (%) represents percentage of change in metabolite level in short compared to normal sleep (metabolite level (short sleep) – metabolite level (normal sleep)) / metabolite level (normal sleep).

DM1 = individuals with type 1 diabetes. P-values are based on paired Students t-tests. N= 16 (healthy: n=9, DM1: n=7). Full results table is shown in Supplemental Table S2. Abbreviations of acylcarnitines are shown in Supplemental Table S3.

*$: Significant difference (p<0.05). #: Significant difference after Bonferroni correction ($p<3.0*10^{-4}$ (=0.05/163)). Significant differences metabolites in all subjects are displayed in bold.*

Short sleep specifically increases plasma acylcarnitines

To investigate possible pathways which could be involved in the increased of insulin resistance by short sleep duration, we performed metabolomics analysis on fasting morning plasma samples. A total of 163 metabolites representing 5 different classes were measured (Table S3). Short sleep increased thirteen metabolites ($p<0.05$) (Table 3). Strikingly, all of these are acylcarnitines. After stringent post-hoc correction, short sleep significantly increased plasma levels of tetradecenoyl-L-carnitine (C14:1) by +32% (plasma level difference: +0.017 μM, $p=2.67*10^{-4}$), octadecenoyl-L-carnitine (C18:1) by +22% (plasma level difference: +0.015 μM, $p=1.92*10^{-4}$) and octadecadienyl-L-carnitine (C18:2) by +27% (plasma level difference: +0.005 μM, $p=1.32*10^{-4}$). Short sleep duration increased acylcarnitines in both subgroups, indicating that the effect of short sleep on acylcarnitines was not dependent on having DM1 or being healthy. There was no interaction effect of the subgroup (healthy *vs.* DM1) with the sleep duration (short *vs.* normal) for the 13 increased acylcarnitines. Baseline acylcarnitine levels (i.e. after normal sleep) did not differ between healthy individuals and DM1, except for a higher level of C:12-DC in DM1 (0.087 ± 0.005 vs 0.101 ± 0.005 μM, $p<0.0001$) (Table S5). Acylcarnitines levels did not differ between healthy individuals and DM1 after short sleep (Table S6). We therefore conclude that a single night of short sleep specifically increased plasma acylcarnitines (Table 4).

DISCUSSION

The present study aimed to explore the metabolic pathways affected by sleep curtailment using targeted plasma metabolomics in individuals (healthy individuals and individuals with type 1 diabetes (DM1)) subjected to both short sleep (4 hours) and normal sleep (8 hours). As part of the same study, we previously reported that this short sleep intervention increased peripheral insulin resistance in both study groups as determined by hyperinsulinemic

Metabolite	Interaction P-value	Effect of sleep P-value	Effect of DM1 status P-value
C2	3.24 * 10-2	6.70 * 10-3$	8.33 * 10-1
C7-DC	1.10 * 10-3	<1.0 * 10-4#	4.53 * 10-1
C12	4.48 * 10-1	1.80 * 10-3#	2.35 * 10-1
C12:1	4.43 * 10-1	1.90 * 10-3#	2.25 * 10-1
C14	2.24 * 10-1	8.70 * 10-3#	1.01 * 10-1
C14:1	4.05 * 10-1	3.00 * 10-4#	1.68 * 10-1
C14:2	2.30 * 10-1	4.00 * 10-4#	4.22 * 10-1
C16	3.48 * 10-1	1.68 * 10-2$	2.09 * 10-1
C16:1	8.94 * 10-1	1.25 * 10-2$	3.82 * 10-1
C16:1-OH	1.42 * 10-1	3.30 * 10-3#	8.79 * 10-1
C16:2	8.34 * 10-2	4.30 * 10-3$	6.62 * 10-1
C18:1	5.97 * 10-1	3.00 * 10-4#	7.64 * 10-1
C18:2	1.35 * 10-1	<1.00 * 10-4#	1.81 * 10-1

Table 4. Interaction effects of diabetes status and short sleep on increased acylcarnitine levels. *DM1 = individuals with type 1 diabetes. Abbreviations of acylcarnitines are shown in Supplemental Table S3. $p<0.05, #p<0.004 (0.05/13) (two way repeated measure ANOVA).*

euglycemic clamp analysis [14, 15]. We now show that one night of short sleep specifically increases plasma levels of acylcarnitines, in both healthy individuals and DM1 patients.

Our study is the first to show that short sleep duration increased plasma acylcarnitines in concert with increased insulin resistance in both healthy individuals and individuals with DM1. This indicates that short sleep duration affects metabolism irrespective of pre-existing insulin producing capacity. The relationship between increased plasma acylcarnitine levels and increased insulin resistance is supported by association studies. Human studies showed increased plasma levels of acylcarnitines in individuals with impaired fasting glucose and with type 2 diabetes (DM2), compared to healthy controls [24, 25]. The significance of this association is still a matter of debate, since human intervention studies are lacking [26].

It is interesting to speculate about the biological relevance of increased plasma levels of acylcarnitines. Acylcarnitines are vital to energy homeostasis. They are esters of fatty acids and carnitine, which are transported over the outer and inner mitochondrial membranes by carnitine palmitoyl transferases (CPTs). Thus, acylcarnitines are essential to shuttle fatty acids from the cytoplasm into mitochondria were they can be oxidized and enter the tricarboxylic acid (TCA) cycle to generate ATP. An excess of acylcarnitines is generally viewed as a result from a mismatch between TCA flux and fatty acid oxidation (FAO) [27]. Previously reported causes of this mismatch include prolonged fasting and excessive muscle activity [28-30]. The present study, in which subjects participated in a protocol that controlled for food intake and physical activity, adds sleep deprivation as a provoking event. A mismatch between FAO and TCA flux has been related to mitochondrial dysfunction. Patients with inborn errors of FAO have increased plasma levels of especially

long chain acylcarnitines [31]. Interestingly, altered mitochondrial parameters have been frequently linked to insulin resistance in the context of both DM1 and DM2 [32-38]. Moreover, mitochondrial dysfunction in mice induces skeletal muscle insulin resistance [27] while TCA-FAO mismatch predisposes mice to diet-induced obesity and insulin resistance [39]. It is therefore tempting to speculate that in our model of insulin resistance due to short sleep deprivation, the increased plasma acylcarnitine levels are a sign of inefficient mitochondrial function.

The tissue distribution of acylcarnitines coincides with important targets of insulin, i.e. muscle and liver. The majority of the body's L-carnitine is stored in muscle (~ 97% of the body's L-carnitine), followed by liver which contains 1% of the total L-carnitine pool [40]. Acylcarnitine results from the acylation of L-carnitine, and is therefore dependent on the fatty acid pool of the tissue. Interestingly, animal studies demonstrate the distribution of acylcarnitines is different between metabolic organs. In mice, the muscle tissue contains relatively more long-chain acylcarnitines, including C14:1 and C18:1, while liver is richer in free carnitines and short-chain carnitines [41]. Collectively, these data suggest that plasma short-chain acyl- and free carnitines are mainly derived from the liver, as indeed demonstrated in pigs [42], while plasma long-chain acylcarnitines in plasma presumably originate from muscle tissue. These data thus suggest that the increase in long-chain acylcarnitine that we observe after a single night of short sleep is likely derived from muscle.

Mechanistically, increased acylcarnitine levels after short sleep duration could be a marker of altered metabolic processes: increased fatty acid oxidation (FAO), inefficient mitochondrial function or a disturbed metabolism of the branched-chain amino acids (BCAA) valine, isoleucine or leucine. Although disturbed BCAA metabolism has been associated with insulin resistance in humans [43], our data do not support a role of BCAA metabolism as short sleep duration did not increase BCAA plasma levels or short-chain acylcarnitines. Increased acylcarnitine levels due to increased FAO can be caused by either increased energy demand and/or prolonged fasting. In the present study, the length of fasting was equal; however energy expenditure was not measured. Therefore, we cannot exclude that the increased acylcarnitines after short sleep are due to increased FAO. Sleep is accompanied by lower resting energy expenditure than wakefulness [44] and therefore short sleep duration may increase energy demand. In fact, complete (24 h) sleep deprivation increases energy demand by 7% [45]. However, the effects of short sleep duration on energy expenditure are inconclusive [46]. A recent study shows that short sleep intervention for five consecutive days increased long-chain plasma acylcarnitines [19]. Interestingly, after one night of recovery sleep, plasma acylcarnitines did not normalize. Likely, the increased acylcarnitines were not due to differences in overnight energy expenditure. Besides being a marker of insulin resistance and/or mitochondrial processes, acylcarnitines could also play a causal role in development of insulin resistance. *In vitro* studies have shown that acylcarnitines have bioactive properties and indeed have pro-inflammatory effects [47, 48]. Of note, treatment of both rodent and human myotubes with acylcarnitines in a physiological concentration caused decreased insulin signaling and glucose uptake in response to insulin

[49]. Although this finding needs to be confirmed *in vivo*, it provides a putative causal link between acylcarnitines and insulin resistance.

Taken all these data together, it is interesting to speculate on a mechanistic model for the relationship between sleep curtailment and insulin resistance. Upon sleep curtailment, the energy demands of peripheral tissues increases at a time conflicting with the physiological circadian rhythm. The energy homeostasis is adapted to anticipate the changing energy need and availability throughout the day. Indeed, muscle tissue is also under circadian control [50]. These clock genes are also important in driving rhythmicity in energy producing capacity of the mitochondria, as evidenced by mice studies [51]. We hypothesize that the mismatch in energy producing capacity and demand could be the cause of incomplete FAO, leading to accumulation of intermediates of FAO. Acylcarnitine levels increase, which may increase insulin resistance either through direct interaction with insulin signaling or through increased inflammatory pathways.

Our findings are supported by three studies which have investigated the effects of sleep on the human metabolome. Davies *et al.* [20] subjected 12 healthy individuals to an extreme sleep deprivation of 24 hours and reported nine increased short and medium-chain acylcarnitines, including tetradecenoyl-L-carnitine. Bell *et al.* [18] reported a trend towards increased acylcarnitines after prolonged mild sleep curtailment of 8 consecutive nights of 5.5 hours sleep in 11 young individuals with family history of DM2. Weljie et al [19] also reported increased C18:1, C10:0 and C12:0 acylcarnitines upon five consecutive nights of 4 hours sleep. Strikingly, despite the difference in study populations and sleep curtailment protocols of the present and previous studies used, the acylcarnitines invariably increase after sleep curtailment.

In conclusion, the present study shows that a single night of 4 hours short sleep, which induces insulin resistance [14, 15], also increases plasma levels of acylcarnitines, in particular tetradecenoyl-L-carnitine, octadecenoyl-L-carnitine and octadecadienyl-L-carnitine. We propose that sleep curtailment impairs mitochondrial function, which coincides with insulin resistance. Our findings provide a basis for mechanistic studies to further elucidate the role of acylcarnitines in the complex relationship between short sleep and increased insulin resistance.

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SUPPLEMENTARY APPENDIX

	Healthy	DM1
Females (%)	4 (44%)	4 (57%)
Age (years)	45 ± 14	43 ± 16
BMI (kg/m^2)	23.8 ± 2.2	23.5 ± 2.2
WHR	0.88 ± 0.05	0.81 ± 0.09

Table S1. General population characteristics of healthy individuals and patients with type 1 diabetes[1]. *DM1 = individuals with type 1 diabetes. BMI = body mass index. WHR = waist hip ratio. Healthy individuals n= 9, DM n= 7. Data are represented as mean ± SD (percentage). [1]Data previously published separately [14;15].*

	Healthy			DM1			Healthy vs. DM1	
	Normal sleep	Short sleep	Effect of sleep	Normal sleep	Short sleep	Effect of sleep	Normal sleep	Short sleep
Sleep characteristics			P-value			P-value	P-value	P-value
TST (min)	454 ± 26	228 ± 32	**<0.0001**	469 ± 22	222 ± 19	**<0.0001**	0.237	0.761
Stage 1 (% of TST)	10 ± 3	11 ± 6	0.490	11 ± 3	10 ± 6	0.868	0.577	0.664
Stage 2 (% of TST)	41 ± 7	35 ± 10	**0.006**	44 ± 7	41 ± 6	0.160	0.533	0.327
Stage 3 (% of TST) (SWS)	25 ± 5	33 ± 9	**0.007**	23 ± 9	35 ± 12	**0.006**	0.570	0.447
REM sleep (% of TST)	24 ± 5	21 ± 8	0.364	22 ± 4	14 ± 6	**0.038**	0.684	0.069
Sleep efficiency (%)	91 ± 5	90 ± 7	0.699	94 ± 2	93 ± 8	0.484	0.199	0.464
Plasma parameter								
Free fatty acids (mmol/l)	0.63 ± 0.15	0.62 ± 0.14	0.76	0.67 ± 0.31	0.59 ± 0.24	0.23	0.74	0.81
Insulin sensitivity parameters								
EGP (µmol*kg LBM^{-1}*min^{-1})	3.57 ± 0.6	4.43 ± 0.8	**0.017**	6.21 ± 1.9	6.88 ± 1.4	0.505	**0.003**	**0.001**
GDR (µmol*kg LBM^{-1}*min^{-1})	40.7 ± 14.3	32.5 ± 10.2	**0.009**	25.5 ± 6.4	22.1 ± 5.1	**0.039**	**0.028**	**0.035**
GIR (µmol*kg LBM^{-1}*min^{-1})	36.9 ± 14.4	27.8 ± 10.5	**0.006**	19.0 ± 7.0	14.9 ± 5.0	**0.041**	**0.014**	**0.014**

Table S2. Sleep and insulin sensitivity parameters of healthy individuals and patients with type 1 diabetes[1]. *DM1= individuals with type 1 diabetes. Insulin sensitivity parameters were determined by hyperinsulinemic euglycemic clamp. EGP = Endogenous glucose production, GDR = glucose disposal rate (glucose Rd), GIR = Glucose infusion rate. Sleep characteristics were determined by polysomnography. TST = total sleep time. SWS = slow wave sleep. Free fatty acids were measured in basal fasting plasma samples. Effect of sleep intervention was tested with paired Students T-test, significant differences shown in bold. Healthy individuals n= 9, DM1 n = 7. Data is presented as means ± SD. [1]Data previously published in separately [14;15].*

Table S3. (below and next pages) Metabolites determined by BiocratesIDQ™ p150 kit.

Metabolite Class	Short name	Biochemical Name
Acylcarnitines	C0	DL-Carnitine
	C2	Acetyl-L-carnitine
	C3	Propionyl-L-carnitine
	C3:1	Propenyl-L-carnitine
	C3-DC / C4-OH	Malonyl-L-carnitine / hydroxybutyryl-L-carnitine
	C3-DC-M / C5-OH	Methylmalonyl-L-carnitine / hydroxyvaleryl-L-carnitine
	C3-OH	Hydroxypropionyl-L-carnitine
	C4	Butyryl-L-carnitine
	C4:1	Butenyl-L-carnitine
	C4:1-DC / C6	Fumaryl-L-carnitine/Hexanoyl-L-carnitine
	C5	Valeryl-L-carnitine
	C5:1	Tiglyl-L-carnitine
	C5:1-DC	Glutaconyl-L-carnitine
	C5-DC / C6-OH	Glutaryl-L-carnitine/Hydroxyhexanoyl-L-carnitine
	C5-M-DC	Methylglutaryl-L-carnitine
	C6:1	Hexenoyl-L-carnitine
	C7-DC	Pimelyl-L-carnitine
	C8	Octanoyl-L-carnitine
	C8:1	Octenoyl-L-carnitine
	C9	Nonayl-L-carnitine
	C10	Decanoyl-L-carnitine
	C10:1	Decenoyl-L-carnitine
	C10:2	Decadienyl-L-carnitine
	C12	Dodecanoyl-L-carnitine
	C12:1	Dodecenoyl-L-carnitine
	C12-DC	Dodecanedioyl-L-carnitine
	C14	Tetradecanoyl-L-carnitine
	C14:1	Tetradecenoyl-L-carnitine
	C14:1-OH	Hydroxytetradecenoyl-L-carnitine
	C14:2	Tetradecadienyl-L-carnitine
	C14:2-OH	Hydroxytetradecadienyl-L-carnitine
	C16	Hexadecanoyl-L-carnitine
	C16:1	Hexadecenoyl-L-carnitine
	C16:1-OH	Hydroxyhexadecenoyl-L-carnitine
	C16:2	Hexadecadienyl-L-carnitine
	C16:2-OH	Hydroxyhexadecadienyl-L-carnitine
	C16-OH	Hydroxyhexadecanoyl-L-carnitine
	C18	Octadecanoyl-L-carnitine
	C18:1	Octadecenoyl-L-carnitine
	C18:1-OH	Hydroxyoctadecenoyl-L-carnitine
	C18:2	Octadecadienyl-L-carnitine

Metabolite Class	Short name	Biochemical Name
Sugars	H1	Hexose
Amino acids	Arg	Arginine
	Gln	Glutamine
	Gly	Glycine
	His	Histidine
	Met	Methionine
	Orn	Ornithine
	Phe	Phenylalanine
	Pro	Proline
	Ser	Serine
	Thr	Threonine
	Trp	Tryptophan
	Tyr	Tyrosine
	Val	Valine
	xLeu	xLeucine
Glycerophospholipids	lysoPC a C14:0	lysoPhosphatidylcholine acyl C14:0
	lysoPC a C16:0	lysoPhosphatidylcholine acyl C16:0
	lysoPC a C16:1	lysoPhosphatidylcholine acyl C16:1
	lysoPC a C17:0	lysoPhosphatidylcholine acyl C17:0
	lysoPC a C18:0	lysoPhosphatidylcholine acyl C18:0
	lysoPC a C18:1	lysoPhosphatidylcholine acyl C18:1
	lysoPC a C18:2	lysoPhosphatidylcholine acyl C18:2
	lysoPC a C20:3	lysoPhosphatidylcholine acyl C20:3
	lysoPC a C20:4	lysoPhosphatidylcholine acyl C20:4
	lysoPC a C24:0	lysoPhosphatidylcholine acyl C24:0
	lysoPC a C26:0	lysoPhosphatidylcholine acyl C26:0
	lysoPC a C26:1	lysoPhosphatidylcholine acyl C26:1
	lysoPC a C28:0	lysoPhosphatidylcholine acyl C28:0
	lysoPC a C28:1	lysoPhosphatidylcholine acyl C28:1
	lysoPC a C6:0	lysoPhosphatidylcholine acyl C6:0
	PC aa C24:0	Phosphatidylcholine diacyl C 24:0
	PC aa C26:0	Phosphatidylcholine diacyl C 26:0
	PC aa C28:1	Phosphatidylcholine diacyl C 28:1
	PC aa C30:0	Phosphatidylcholine diacyl C 30:0
	PC aa C30:2	Phosphatidylcholine diacyl C 30:2
	PC aa C32:0	Phosphatidylcholine diacyl C 32:0
	PC aa C32:1	Phosphatidylcholine diacyl C 32:1
	PC aa C32:2	Phosphatidylcholine diacyl C 32:2
	PC aa C32:3	Phosphatidylcholine diacyl C 32:3
	PC aa C34:1	Phosphatidylcholine diacyl C 34:1
	PC aa C34:2	Phosphatidylcholine diacyl C 34:2

Metabolite Class	Short name	Biochemical Name
Glycerophospholipids	PC aa C34:3	Phosphatidylcholine diacyl C 34:3
	PC aa C34:4	Phosphatidylcholine diacyl C 34:4
	PC aa C36:0	Phosphatidylcholine diacyl C 36:0
	PC aa C36:1	Phosphatidylcholine diacyl C 36:1
	PC aa C36:2	Phosphatidylcholine diacyl C 36:2
	PC aa C36:3	Phosphatidylcholine diacyl C 36:3
	PC aa C36:4	Phosphatidylcholine diacyl C 36:4
	PC aa C36:5	Phosphatidylcholine diacyl C 36:5
	PC aa C36:6	Phosphatidylcholine diacyl C 36:6
	PC aa C38:0	Phosphatidylcholine diacyl C 38:0
	PC aa C38:1	Phosphatidylcholine diacyl C 38:1
	PC aa C38:3	Phosphatidylcholine diacyl C 38:3
	PC aa C38:4	Phosphatidylcholine diacyl C 38:4
	PC aa C38:5	Phosphatidylcholine diacyl C 38:5
	PC aa C38:6	Phosphatidylcholine diacyl C 38:6
	PC aa C40:1	Phosphatidylcholine diacyl C 40:1
	PC aa C40:2	Phosphatidylcholine diacyl C 40:2
	PC aa C40:3	Phosphatidylcholine diacyl C 40:3
	PC aa C40:4	Phosphatidylcholine diacyl C 40:4
	PC aa C40:5	Phosphatidylcholine diacyl C 40:5
	PC aa C40:6	Phosphatidylcholine diacyl C 40:6
	PC aa C42:0	Phosphatidylcholine diacyl C 42:0
	PC aa C42:1	Phosphatidylcholine diacyl C 42:1
	PC aa C42:2	Phosphatidylcholine diacyl C 42:2
	PC aa C42:4	Phosphatidylcholine diacyl C 42:4
	PC aa C42:5	Phosphatidylcholine diacyl C 42:5
	PC aa C42:6	Phosphatidylcholine diacyl C 42:6
	PC ae C30:0	Phosphatidylcholine acyl-alkyl C 30:0
	PC ae C30:1	Phosphatidylcholine acyl-alkyl C 30:1
	PC ae C30:2	Phosphatidylcholine acyl-alkyl C 30:2
	PC ae C32:1	Phosphatidylcholine acyl-alkyl C 32:1
	PC ae C32:2	Phosphatidylcholine acyl-alkyl C 32:2
	PC ae C34:0	Phosphatidylcholine acyl-alkyl C 34:0
	PC ae C34:1	Phosphatidylcholine acyl-alkyl C 34:1
	PC ae C34:2	Phosphatidylcholine acyl-alkyl C 34:2
	PC ae C34:3	Phosphatidylcholine acyl-alkyl C 34:3
	PC ae C36:0	Phosphatidylcholine acyl-alkyl C 36:0
	PC ae C36:1	Phosphatidylcholine acyl-alkyl C 36:1
	PC ae C36:2	Phosphatidylcholine acyl-alkyl C 36:2
	PC ae C36:3	Phosphatidylcholine acyl-alkyl C 36:3

Metabolite Class	Short name	Biochemical Name
Glycerophospholipids	PC ae C36:4	Phosphatidylcholine acyl-alkyl C 36:4
	PC ae C36:5	Phosphatidylcholine acyl-alkyl C 36:5
	PC ae C38:0	Phosphatidylcholine acyl-alkyl C 38:0
	PC ae C38:1	Phosphatidylcholine acyl-alkyl C 38:1
	PC ae C38:2	Phosphatidylcholine acyl-alkyl C 38:2
	PC ae C38:3	Phosphatidylcholine acyl-alkyl C 38:3
	PC ae C38:4	Phosphatidylcholine acyl-alkyl C 38:4
	PC ae C38:5	Phosphatidylcholine acyl-alkyl C 38:5
	PC ae C38:6	Phosphatidylcholine acyl-alkyl C 38:6
	PC ae C40:0	Phosphatidylcholine acyl-alkyl C 40:0
	PC ae C40:1	Phosphatidylcholine acyl-alkyl C 40:1
	PC ae C40:2	Phosphatidylcholine acyl-alkyl C 40:2
	PC ae C40:3	Phosphatidylcholine acyl-alkyl C 40:3
	PC ae C40:4	Phosphatidylcholine acyl-alkyl C 40:4
	PC ae C40:5	Phosphatidylcholine acyl-alkyl C 40:5
	PC ae C40:6	Phosphatidylcholine acyl-alkyl C 40:6
	PC ae C42:0	Phosphatidylcholine acyl-alkyl C 42:0
	PC ae C42:1	Phosphatidylcholine acyl-alkyl C 42:1
	PC ae C42:2	Phosphatidylcholine acyl-alkyl C 42:2
	PC ae C42:3	Phosphatidylcholine acyl-alkyl C 42:3
	PC ae C42:4	Phosphatidylcholine acyl-alkyl C 42:4
	PC ae C42:5	Phosphatidylcholine acyl-alkyl C 42:5
	PC ae C44:3	Phosphatidylcholine acyl-alkyl C 44:3
	PC ae C44:4	Phosphatidylcholine acyl-alkyl C 44:4
	PC ae C44:5	Phosphatidylcholine acyl-alkyl C 44:5
	PC ae C44:6	Phosphatidylcholine acyl-alkyl C 44:6
Sphingolipids	SM (OH) C14:1	Hydroxysphingomyeline C 14:1
	SM (OH) C16:0	Hydroxysphingomyeline C 16:0
	SM (OH) C22:1	Hydroxysphingomyeline C 22:1
	SM (OH) C22:2	Hydroxysphingomyeline C 22:2
	SM (OH) C24:1	Hydroxysphingomyeline C 24:1
	SM C16:0	Sphingomyeline C 16:0
	SM C16:1	Sphingomyeline C 16:1
	SM C18:0	Sphingomyeline C 18:0
	SM C18:1	Sphingomyeline C 18:1
	SM C20:2	Sphingomyeline C 20:2
	SM C22:3	Sphingomyeline C 22:3
	SM C24:0	Sphingomyeline C 24:0
	SM C24:1	Sphingomyeline C 24:1
	SM C26:0	Sphingomyeline C 26:0
	SM C26:1	Sphingomyeline C 26:1

Table S4. (below and next pages) Metabolite changes after one night of short sleep duration.[1] *Difference in metabolite levels (μM) as measured by BiocratesIDQ™ p150 kit between short and normal sleep duration. Positive mean difference indicates an increase after short sleep duration. Negative mean difference indicates a decrease after short sleep duration.* [2]*Change (%) represents percentage of change in metabolite level in short compared to normal sleep (metabolite level (short sleep) – metabolite level (normal sleep)) / metabolite level (normal sleep). DM1 = individuals with type 1 diabetes. P-values are based on paired Students t-tests. Abbreviations of all metabolites are shown in Supplemental Table S3. N= 16 (healthy: n=9, DM1: n=7).*

	All subjects			Healthy			DM1		
Metabolite	**Mean difference**[1]	**Change (%)2**	**P-value**	**Mean difference**[1]	**Change (%)2**	**P-value**	**Mean difference**[1]	**Change (%)2**	**P-value**
C0	0.191	0.6%	$8.81*10^{-1}$	0.398	1.2%	$8.47*10^{-1}$	-0.075	-0.3%	$9.61*10^{-1}$
C2	0.662	16.8%	$2.28*10^{-2}$	0.184	4.3%	$5.40*10^{-1}$	1.276	35.6%	$1.33*10^{-2}$
C3	-0.006	-2.6%	$6.76*10^{-1}$	-0.002	-0.7%	$9.33*10^{-1}$	-0.012	-6.2%	$6.03*10^{-1}$
C3-DC (C4-OH)	0.007	11.3%	$4.55*10^{-1}$	0.006	8.0%	$7.20*10^{-1}$	0.009	17.7%	$2.87*10^{-1}$
C3-OH	0.001	3.3%	$5.03*10^{-1}$	0.002	8.5%	$5.91*10^{-2}$	-0.001	-2.1%	$8.24*10^{-1}$
C3:1	-0.001	-5.9%	$4.16*10^{-1}$	0.000	-0.1%	$9.93*10^{-1}$	-0.001	-12.3%	$1.58*10^{-1}$
C4	0.011	10.3%	$1.22*10^{-1}$	0.008	7.2%	$4.95*10^{-1}$	0.015	14.4%	$8.68*10^{-2}$
C4:1	0.002	6.7%	$2.90*10^{-1}$	0.002	8.7%	$2.55*10^{-1}$	0.001	4.8%	$6.58*10^{-1}$
C5	0.011	10.2%	$2.80*10^{-1}$	0.012	10.4%	$4.59*10^{-1}$	0.010	9.8%	$4.30*10^{-1}$
C5-DC (C6-OH)	-0.001	-4.1%	$4.87*10^{-1}$	-0.001	-8.3%	$2.56*10^{-1}$	0.000	0.7%	$9.45*10^{-1}$
C5-M-DC	0.000	0.3%	$9.32*10^{-1}$	0.000	-1.0%	$8.45*10^{-1}$	0.001	1.8%	$7.11*10^{-1}$
C5-OH (C3-DC-M)	0.000	0.3%	$9.40*10^{-1}$	0.000	-1.5%	$6.89*10^{-1}$	0.001	2.4%	$7.40*10^{-1}$
C5:1	0.000	1.0%	$8.74*10^{-1}$	-0.001	-2.5%	$7.86*10^{-1}$	0.001	5.0%	$6.20*10^{-1}$
C5:1-DC	0.000	2.6%	$6.75*10^{-1}$	-0.001	-4.8%	$5.96*10^{-1}$	0.002	12.5%	$1.27*10^{-1}$
C6 (C4:1-DC)	0.005	8.7%	$1.22*10^{-1}$	0.001	1.9%	$7.82*10^{-1}$	0.011	18.8%	$6.20*10^{-2}$
C6:1	0.001	2.6%	$3.99*10^{-1}$	0.001	3.5%	$3.35*10^{-1}$	0.000	1.5%	$8.05*10^{-1}$
C7-DC	0.005	19.9%	$5.41*10^{-4}$	0.002	8.4%	$8.50*10^{-2}$	0.008	34.8%	$3.21*10^{-4}$
C8	0.003	1.9%	$6.06*10^{-1}$	-0.005	-3.1%	$5.56*10^{-1}$	0.013	11.0%	$4.38*10^{-3}$

	All subjects			Healthy			DM1		
Metabolite	**Mean difference[1]**	**Change (%)2**	**P-value**	**Mean difference[1]**	**Change (%)2**	**P-value**	**Mean difference[1]**	**Change (%)2**	**P-value**
C8:1	0.012	15.4%	$1.62*10^{-1}$	0.008	11.8%	$3.93*10^{-1}$	0.017	18.9%	$3.09*10^{-1}$
C9	0.000	-0.4%	$9.47*10^{-1}$	-0.001	-4.7%	$5.73*10^{-1}$	0.001	5.3%	$5.51*10^{-1}$
C10	0.014	5.8%	$1.84*10^{-1}$	0.003	1.1%	$8.62*10^{-1}$	0.027	15.1%	$4.54*10^{-3}$
C10:1	0.008	6.9%	$2.68*10^{-1}$	-0.004	-3.1%	$6.68*10^{-1}$	0.022	23.9%	$2.72*10^{-2}$
C10:2	0.000	-0.7%	$8.65*10^{-1}$	0.000	-0.1%	$9.79*10^{-1}$	0.000	-1.3%	$8.51*10^{-1}$
C12	0.012	17.2%	$1.70*10^{-3}$	0.010	12.0%	$9.00*10^{-2}$	0.015	27.5%	$3.30*10^{-3}$
C12-DC	0.002	2.4%	$1.53*10^{-1}$	0.002	2.1%	$3.78*10^{-1}$	0.003	2.8%	$3.01*10^{-1}$
C12:1	0.017	23.6%	$1.74*10^{-3}$	0.014	17.0%	$8.32*10^{-2}$	0.021	35.2%	$5.85*10^{-3}$
C14	0.004	15.6%	$1.21*10^{-2}$	0.002	8.5%	$2.42*10^{-1}$	0.006	28.0%	$1.97*10^{-2}$
C14:1	0.020	32.4%	$2.67*10^{-4}$	0.017	23.5%	$2.34*10^{-2}$	0.024	49.1%	$6.55*10^{-3}$
C14:1-OH	0.001	6.2%	$3.79*10^{-1}$	0.000	-1.7%	$8.10*10^{-1}$	0.001	16.9%	$2.36*10^{-1}$
C14:2	0.006	26.1%	$5.48*10^{-4}$	0.005	17.3%	$5.01*10^{-2}$	0.008	41.5%	$4.17*10^{-3}$
C14:2-OH	0.000	5.0%	$3.64*10^{-1}$	0.000	6.4%	$2.91*10^{-1}$	0.000	3.3%	$7.48*10^{-1}$
C16	0.007	10.8%	$1.94*10^{-2}$	0.005	6.7%	$2.05*10^{-1}$	0.010	17.1%	$5.91*10^{-2}$
C16-OH	0.000	0.9%	$8.89*10^{-1}$	0.000	-3.4%	$5.84*10^{-1}$	0.000	6.0%	$6.49*10^{-1}$
C16:1	0.005	8.8%	$9.60*10^{-3}$	0.005	8.3%	$3.06*10^{-2}$	0.005	9.6%	$1.55*10^{-1}$
C16:1-OH	0.001	20.8%	$5.71*10^{-3}$	0.001	11.7%	$1.90*10^{-1}$	0.002	33.8%	$1.10*10^{-2}$
C16:2	0.001	21.3%	$9.72*10^{-3}$	0.001	9.6%	$2.39*10^{-1}$	0.002	39.5%	$1.91*10^{-2}$
C16:2-OH	0.000	2.1%	$6.29*10^{-1}$	0.000	3.8%	$5.45*10^{-1}$	0.000	-0.1%	$9.83*10^{-1}$
C18	0.003	10.8%	$7.34*10^{-2}$	0.002	9.6%	$3.04*10^{-1}$	0.003	12.5%	$1.18*10^{-1}$
C18:1	0.016	22.3%	$1.92*10^{-4}$	0.015	20.1%	$2.96*10^{-3}$	0.019	25.1%	$2.84*10^{-2}$
C18:1-OH	0.001	12.8%	$6.02*10^{-2}$	0.001	5.7%	$3.93*10^{-1}$	0.002	22.3%	$1.07*10^{-1}$
C18:2	0.007	27.0%	$1.32*10^{-4}$	0.005	20.7%	$9.01*10^{-4}$	0.010	34.4%	$1.38*10^{-2}$
Arg	4.297	6.0%	$2.74*10^{-1}$	6.988	10.0%	$2.66*10^{-1}$	0.838	1.1%	$8.57*10^{-1}$
Gln	13.186	4.5%	$2.52*10^{-1}$	10.500	3.6%	$4.88*10^{-1}$	16.641	5.7%	$4.00*10^{-1}$

	All subjects			Healthy			DM1		
Metabolite	**Mean difference[1]**	**Change (%)2**	**P-value**	**Mean difference[1]**	**Change (%)2**	**P-value**	**Mean difference[1]**	**Change (%)2**	**P-value**
Gly	-9.002	-4.7%	$2.84*10^{-1}$	-3.264	-1.9%	$7.57*10^{-1}$	-16.380	-7.4%	$2.69*10^{-1}$
His	0.533	1.0%	$8.27*10^{-1}$	-0.174	-0.3%	$9.57*10^{-1}$	1.441	3.0%	$7.32*10^{-1}$
Met	0.264	1.3%	$7.98*10^{-1}$	0.557	2.6%	$6.91*10^{-1}$	-0.113	-0.6%	$9.48*10^{-1}$
Orn	3.038	8.3%	$1.48*10^{-1}$	5.466	15.2%	$1.05*10^{-1}$	-0.083	-0.2%	$9.70*10^{-1}$
Phe	2.588	6.2%	$1.06*10^{-1}$	3.131	7.6%	$1.16*10^{-1}$	1.890	4.5%	$5.13*10^{-1}$
Pro	-3.161	-1.9%	$6.20*10^{-1}$	-0.909	-0.5%	$9.10*10^{-1}$	-6.057	-4.0%	$5.93*10^{-1}$
Ser	3.273	3.6%	$4.27*10^{-1}$	2.253	2.7%	$7.18*10^{-1}$	4.585	4.6%	$4.29*10^{-1}$
Thr	4.527	4.7%	$5.04*10^{-1}$	4.930	5.1%	$5.55*10^{-1}$	4.010	4.1%	$7.47*10^{-1}$
Trp	1.178	1.9%	$5.56*10^{-1}$	2.563	4.2%	$3.30*10^{-1}$	-0.602	-1.0%	$8.58*10^{-1}$
Tyr	4.638	9.8%	$1.05*10^{-1}$	6.966	14.7%	$1.30*10^{-1}$	1.644	3.5%	$6.12*10^{-1}$
Val	3.933	2.6%	$4.86*10^{-1}$	6.183	3.9%	$5.06*10^{-1}$	1.039	0.7%	$8.67*10^{-1}$
xLeu	9.916	6.4%	$1.56*10^{-1}$	9.156	5.7%	$3.55*10^{-1}$	10.892	7.3%	$3.24*10^{-1}$
lysoPC a C14:0	-0.047	-1.4%	$5.83*10^{-1}$	-0.022	-0.6%	$8.67*10^{-1}$	-0.079	-2.4%	$5.04*10^{-1}$
lysoPC a C16:0	-1.774	-2.4%	$5.84*10^{-1}$	0.872	1.3%	$8.39*10^{-1}$	-5.176	-6.6%	$3.34*10^{-1}$
lysoPC a C16:1	-0.144	-7.2%	$1.58*10^{-1}$	-0.061	-3.1%	$6.72*10^{-1}$	-0.249	-12.3%	$1.02*10^{-1}$
lysoPC a C17:0	-0.015	-1.3%	$7.88*10^{-1}$	0.005	0.5%	$9.46*10^{-1}$	-0.042	-3.4%	$6.57*10^{-1}$
lysoPC a C18:0	-0.003	0.0%	$9.97*10^{-1}$	1.077	6.4%	$3.22*10^{-1}$	-1.392	-6.9%	$4.33*10^{-1}$
lysoPC a C18:1	-0. 7	-2.9%	$5.91*10^{-1}$	-0.210	-1.6%	$7.91*10^{-1}$	-0.728	-4.2%	$6.67*10^{-1}$
lysoPC a C18:2	0.575	1.9%	$8.14*10^{-1}$	-1.447	-5.5%	$4.00*10^{-1}$	3.176	8.9%	$5.59*10^{-1}$
lysoPC a C20:3	0.008	0.4%	$9.43*10^{-1}$	0.080	4.0%	$5.80*10^{-1}$	-0.085	-4.5%	$6.19*10^{-1}$
lysoPC a C20:4	-0.248	-4.7%	$3.27*10^{-1}$	-0.302	-6.3%	$2.31*10^{-1}$	-0.179	-3.1%	$7.32*10^{-1}$
lysoPC a C24:0	-0.003	-0.5%	$9.28*10^{-1}$	0.014	3.1%	$6.56*10^{-1}$	-0.024	-4.7%	$6.56*10^{-1}$
lysoPC a C26:0	-0.009	-0.9%	$9.02*10^{-1}$	0.014	1.4%	$8.63*10^{-1}$	-0.039	-4.1%	$7.87*10^{-1}$
lysoPC a C26:1	-0.004	-1.1%	$8.81*10^{-1}$	-0.002	-0.6%	$9.42*10^{-1}$	-0.007	-1.7%	$9.03*10^{-1}$
lysoPC a C28:0	0.001	0.1%	$9.84*10^{-1}$	0.012	1.5%	$8.49*10^{-1}$	-0.013	-1.7%	$8.84*10^{-1}$

	All subjects			Healthy			DM1		
Metabolite	**Mean difference[1]**	**Change (%)2**	**P-value**	**Mean difference[1]**	**Change (%)2**	**P-value**	**Mean difference[1]**	**Change (%)2**	**P-value**
lysoPC a C28:1	-0.006	-0.6%	$9.28*10^{-1}$	0.010	1.1%	$8.84*10^{-1}$	-0.025	-2.6%	$8.31*10^{-1}$
lysoPC a C6:0	-0.002	-8.4%	$4.94*10^{-1}$	0.002	10.9%	$6.26*10^{-1}$	-0.007	-26.5%	$1.14*10^{-2}$
PC aa C24:0	-0.005	-1.8%	$7.93*10^{-1}$	-0.005	-1.9%	$8.42*10^{-1}$	-0.005	-1.8%	$8.78*10^{-1}$
PC aa C26:0	-0.028	-2.4%	$6.22*10^{-1}$	-0.040	-3.4%	$5.37*10^{-1}$	-0.013	-1.1%	$9.09*10^{-1}$
PC aa C28:1	0.061	2.7%	$3.74*10^{-1}$	0.089	3.9%	$2.47*10^{-1}$	0.026	1.1%	$8.47*10^{-1}$
PC aa C30:0	0.081	2.7%	$6.62*10^{-1}$	0.099	3.1%	$6.76*10^{-1}$	0.058	2.1%	$8.60*10^{-1}$
PC aa C30:2	0.002	1.2%	$9.33*10^{-1}$	0.010	7.0%	$7.71*10^{-1}$	-0.009	-4.3%	$8.40*10^{-1}$
PC aa C32:0	0.352	4.0%	$2.19*10^{-1}$	0.490	5.8%	$5.12*10^{-2}$	0.174	1.9%	$7.76*10^{-1}$
PC aa C32:1	0.370	3.7%	$6.36*10^{-1}$	1.063	9.4%	$3.68*10^{-1}$	-0.521	-6.5%	$6.17*10^{-1}$
PC aa C32:2	0.020	0.8%	$9.12*10^{-1}$	-0.038	-1.3%	$8.84*10^{-1}$	0.095	4.1%	$7.38*10^{-1}$
PC aa C32:3	0.001	0.2%	$9.68*10^{-1}$	-0.005	-1.3%	$7.93*10^{-1}$	0.007	1.9%	$7.90*10^{-1}$
PC aa C34:1	2.395	1.9%	$5.09*10^{-1}$	5.489	4.3%	$1.84*10^{-1}$	-1.583	-1.3%	$8.16*10^{-1}$
PC aa C34:2	7.304	3.7%	$1.04*10^{-1}$	6.544	3.3%	$1.31*10^{-1}$	8.282	4.1%	$3.78*10^{-1}$
PC aa C34:3	0.353	3.0%	$5.32*10^{-1}$	0.435	3.5%	$5.90*10^{-1}$	0.247	2.2%	$7.78*10^{-1}$
PC aa C34:4	-0.013	-1.2%	$8.47*10^{-1}$	-0.032	-2.7%	$7.55*10^{-1}$	0.012	1.3%	$8.98*10^{-1}$
PC aa C36:0	0.017	0.7%	$9.23*10^{-1}$	-0.046	-2.1%	$8.05*10^{-1}$	0.099	3.3%	$7.81*10^{-1}$
PC aa C36:1	1.673	5.8%	$1.72*10^{-1}$	2.555	8.7%	$1.40*10^{-1}$	0.538	1.9%	$7.75*10^{-1}$
PC aa C36:2	6.029	4.8%	$1.46*10^{-1}$	6.133	4.9%	$1.96*10^{-1}$	5.894	4.7%	$4.61*10^{-1}$
PC aa C36:3	3.808	4.5%	$1.64*10^{-1}$	6.521	7.5%	$5.27*10^{-2}$	0.320	0.4%	$9.47*10^{-1}$
PC aa C36:4	1.441	1.3%	$6.29*10^{-1}$	1.110	1.0%	$7.39*10^{-1}$	1.866	1.9%	$7.49*10^{-1}$
PC aa C36:5	-0.045	-0.3%	$9.65*10^{-1}$	0.559	4.1%	$7.51*10^{-1}$	-0.821	-6.8%	$3.50*10^{-1}$
PC aa C36:6	-0.010	-1.5%	$8.53*10^{-1}$	0.004	0.5%	$9.69*10^{-1}$	-0.028	-4.4%	$6.29*10^{-1}$
PC aa C38:0	0.050	2.0%	$6.68*10^{-1}$	0.039	1.7%	$8.06*10^{-1}$	0.065	2.3%	$7.41*10^{-1}$
PC aa C38:1	0.041	2.3%	$6.99*10^{-1}$	0.151	9.3%	$2.19*10^{-1}$	-0.101	-5.0%	$5.98*10^{-1}$
PC aa C38:3	2.020	7.5%	$6.46*10^{-2}$	3.788	12.9%	$1.68*10^{-2}$	-0.254	-1.1%	$8.48*10^{-1}$
PC aa C38:4	1.113	2.0%	$5.25*10^{-1}$	1.760	3.0%	$4.88*10^{-1}$	0.280	0.6%	$9.16*10^{-1}$
PC aa C38:5	0.060	0.2%	$9.55*10^{-1}$	0.609	2.3%	$7.31*10^{-1}$	-0.645	-2.6%	$5.62*10^{-1}$

	All subjects			Healthy			DM1		
Metabolite	**Mean difference[1]**	**Change (%)2**	**P-value**	**Mean difference[1]**	**Change (%)2**	**P-value**	**Mean difference[1]**	**Change (%)2**	**P-value**
PC aa C38:6	-0.622	-1.6%	$7.12*10^{-1}$	-0.987	-2.3%	$7.03*10^{-1}$	-0.154	-0.4%	$9.47*10^{-1}$
PC aa C40:1	0.020	2.5%	$6.90*10^{-1}$	0.060	8.0%	$3.34*10^{-1}$	-0.031	-3.5%	$7.38*10^{-1}$
PC aa C40:2	0.078	5.4%	$4.46*10^{-1}$	0.149	11.7%	$2.16*10^{-1}$	-0.012	-0.7%	$9.52*10^{-1}$
PC aa C40:3	0.068	4.6%	$4.70*10^{-1}$	0.136	10.5%	$2.55*10^{-1}$	-0.020	-1.2%	$9.04*10^{-1}$
PC aa C40:4	0.154	4.6%	$2.35*10^{-1}$	0.290	8.9%	$1.09*10^{-1}$	-0.021	-0.6%	$9.16*10^{-1}$
PC aa C40:5	0.232	4.1%	$3.01*10^{-1}$	0.540	9.3%	$9.49*10^{-2}$	-0.164	-3.0%	$5.89*10^{-1}$
PC aa C40:6	0.033	0.3%	$9.48*10^{-1}$	0.413	3.3%	$5.51*10^{-1}$	-0.454	-4.4%	$5.85*10^{-1}$
PC aa C42:0	0.028	4.0%	$2.80*10^{-1}$	0.040	6.2%	$2.00*10^{-1}$	0.012	1.6%	$8.01*10^{-1}$
PC aa C42:1	0.009	1.9%	$7.04*10^{-1}$	0.041	10.0%	$1.49*10^{-1}$	-0.033	-6.2%	$3.74*10^{-1}$
PC aa C42:2	0.020	3.1%	$6.55*10^{-1}$	0.054	9.5%	$3.06*10^{-1}$	-0.024	-3.1%	$7.75*10^{-1}$
PC aa C42:4	0.031	5.6%	$2.44*10^{-1}$	0.053	10.6%	$1.88*10^{-1}$	0.002	0.4%	$9.49*10^{-1}$
PC aa C42:5	0.029	5.5%	$1.84*10^{-1}$	0.047	9.7%	$1.34*10^{-1}$	0.005	0.8%	$8.81*10^{-1}$
PC aa C42:6	0.019	3.4%	$5.38*10^{-1}$	0.049	9.3%	$1.60*10^{-1}$	-0.020	-3.5%	$7.21*10^{-1}$
PC ae C30:0	0.002	0.5%	$9.20*10^{-1}$	0.012	3.5%	$6.07*10^{-1}$	-0.012	-3.6%	$5.95*10^{-1}$
PC ae C30:1	-0.003	-1.2%	$8.94*10^{-1}$	-0.003	-1.2%	$9.07*10^{-1}$	-0.004	-1.3%	$9.43*10^{-1}$
PC ae C30:2	0.006	2.5%	$6.96*10^{-1}$	0.011	5.1%	$5.28*10^{-1}$	-0.001	-0.3%	$9.81*10^{-1}$
PC ae C32:1	0.046	2.4%	$4.28*10^{-1}$	0.035	1.9%	$6.30*10^{-1}$	0.060	2.9%	$5.66*10^{-1}$
PC ae C32:2	0.011	2.2%	$4.36*10^{-1}$	0.017	3.7%	$3.50*10^{-1}$	0.003	0.6%	$8.99*10^{-1}$
PC ae C34:0	0.006	0.7%	$8.25*10^{-1}$	0.015	1.6%	$6.90*10^{-1}$	-0.005	-0.5%	$9.25*10^{-1}$
PC ae C34:1	0.159	2.7%	$3.75*10^{-1}$	0.202	3.3%	$4.23*10^{-1}$	0.105	1.8%	$7.15*10^{-1}$
PC ae C34:2	0.274	4.1%	$4.06*10^{-1}$	0.029	0.4%	$9.36*10^{-1}$	0.589	8.7%	$3.55*10^{-1}$
PC ae C34:3	0.213	3.9%	$3.04*10^{-1}$	0.093	1.8%	$7.10*10^{-1}$	0.368	6.3%	$3.34*10^{-1}$
PC ae C36:0	0.048	6.1%	$1.66*10^{-1}$	0.037	4.6%	$3.13*10^{-1}$	0.063	8.0%	$3.68*10^{-1}$
PC ae C36:1	0.832	6.7%	$3.11*10^{-1}$	0.792	6.9%	$3.45*10^{-1}$	0.883	6.4%	$5.98*10^{-1}$
PC ae C36:2	0.308	2.9%	$3.51*10^{-1}$	0.152	1.4%	$7.39*10^{-1}$	0.508	4.7%	$3.39*10^{-1}$
PC ae C36:3	0.143	2.8%	$5.30*10^{-1}$	0.044	0.9%	$8.77*10^{-1}$	0.272	5.4%	$5.13*10^{-1}$

	All subjects			Healthy			DM1		
Metabolite	**Mean difference[1]**	**Change (%)2**	**P-value**	**Mean difference[1]**	**Change (%)2**	**P-value**	**Mean difference[1]**	**Change (%)2**	**P-value**
PC ae C36:4	0.257	2.7%	$5.65*10^{-1}$	-0.112	-1.2%	$7.36*10^{-1}$	0.732	7.4%	$4.57*10^{-1}$
PC ae C36:5	0.079	1.2%	$7.36*10^{-1}$	-0.063	-0.9%	$8.10*10^{-1}$	0.260	3.7%	$5.64*10^{-1}$
PC ae C38:0	0.054	2.8%	$6.14*10^{-1}$	0.049	2.4%	$7.92*10^{-1}$	0.061	3.4%	$5.34*10^{-1}$
PC ae C38:1	0.263	6.4%	$4.50*10^{-1}$	0.411	11.8%	$3.06*10^{-1}$	0.073	1.5%	$9.13*10^{-1}$
PC ae C38:2	0.414	6.9%	$3.07*10^{-1}$	0.631	11.3%	$1.77*10^{-1}$	0.134	2.1%	$8.61*10^{-1}$
PC ae C38:3	0.776	7.3%	$1.96*10^{-1}$	0.799	7.6%	$2.04*10^{-1}$	0.745	6.9%	$5.38*10^{-1}$
PC ae C38:4	0.189	2.2%	$5.31*10^{-1}$	0.199	2.3%	$6.11*10^{-1}$	0.176	2.0%	$7.39*10^{-1}$
PC ae C38:5	0.132	1.3%	$7.42*10^{-1}$	-0.130	-1.3%	$7.68*10^{-1}$	0.470	4.4%	$5.50*10^{-1}$
PC ae C38:6	0.096	2.5%	$5.50*10^{-1}$	-0.033	-0.9%	$8.70*10^{-1}$	0.261	6.8%	$3.48*10^{-1}$
PC ae C40:0	0.010	0.3%	$9.35*10^{-1}$	0.044	1.1%	$8.20*10^{-1}$	-0.032	-0.9%	$8.54*10^{-1}$
PC ae C40:1	0.040	1.8%	$7.94*10^{-1}$	0.031	1.5%	$8.49*10^{-1}$	0.051	2.2%	$8.67*10^{-1}$
PC ae C40:2	0.144	5.2%	$3.60*10^{-1}$	0.233	8.5%	$2.19*10^{-1}$	0.031	1.1%	$9.15*10^{-1}$
PC ae C40:3	0.349	7.0%	$1.55*10^{-1}$	0.441	9.1%	$1.16*10^{-1}$	0.231	4.5%	$6.22*10^{-1}$
PC ae C40:4	0.223	4.5%	$3.41*10^{-1}$	0.332	7.1%	$2.64*10^{-1}$	0.082	1.6%	$8.42*10^{-1}$
PC ae C40:5	0.278	4.4%	$3.21*10^{-1}$	0.241	3.8%	$5.41*10^{-1}$	0.325	5.2%	$4.65*10^{-1}$
PC ae C40:6	0.051	1.8%	$6.17*10^{-1}$	0.057	2.0%	$7.26*10^{-1}$	0.044	1.5%	$7.36*10^{-1}$
PC ae C42:0	0.028	4.0%	$2.93*10^{-1}$	0.049	7.1%	$1.08*10^{-1}$	0.000	0.1%	$9.94*10^{-1}$
PC ae C42:1	0.044	4.5%	$4.52*10^{-1}$	0.075	8.4%	$2.37*10^{-1}$	0.004	0.3%	$9.75*10^{-1}$
PC ae C42:2	0.023	2.3%	$6.72*10^{-1}$	0.057	6.1%	$4.38*10^{-1}$	-0.020	-1.8%	$8.26*10^{-1}$
PC ae C42:3	0.047	3.4%	$5.37*10^{-1}$	0.090	7.0%	$3.21*10^{-1}$	-0.007	-0.5%	$9.59*10^{-1}$
PC ae C42:4	0.060	4.2%	$2.59*10^{-1}$	0.120	8.7%	$1.16*10^{-1}$	-0.017	-1.1%	$8.27*10^{-1}$
PC ae C42:5	0.119	4.3%	$1.96*10^{-1}$	0.149	5.4%	$2.06*10^{-1}$	0.081	2.9%	$6.20*10^{-1}$
PC ae C44:3	0.011	3.0%	$6.00*10^{-1}$	0.022	6.8%	$4.11*10^{-1}$	-0.003	-0.8%	$9.26*10^{-1}$
PC ae C44:4	0.016	3.3%	$2.93*10^{-1}$	0.040	8.1%	$5.12*10^{-2}$	-0.014	-2.7%	$5.62*10^{-1}$
PC ae C44:5	0.041	2.8%	$3.86*10^{-1}$	0.058	3.9%	$3.78*10^{-1}$	0.018	1.2%	$8.06*10^{-1}$

	All subjects			Healthy			DM1		
Metabolite	**Mean difference[1]**	**Change (%)2**	**P-value**	**Mean difference[1]**	**Change (%)2**	**P-value**	**Mean difference[1]**	**Change (%)2**	**P-value**
PC ae C44:6	0.021	1.9%	$6.18*10^{-1}$	0.060	5.7%	$2.69*10^{-1}$	-0.031	-2.7%	$6.51*10^{-1}$
SM (OH) C14:1	0.146	2.6%	$2.81*10^{-1}$	0.213	4.0%	$1.67*10^{-1}$	0.059	1.0%	$8.19*10^{-1}$
SM (OH) C16:1	0.096	3.7%	$1.60*10^{-1}$	0.084	3.3%	$3.39*10^{-1}$	0.111	4.2%	$3.54*10^{-1}$
SM (OH) C22:1	0.271	2.4%	$5.34*10^{-1}$	0.730	6.9%	$1.15*10^{-1}$	-0.320	-2.7%	$7.02*10^{-1}$
SM (OH) C22:2	-0.036	-0.4%	$8.93*10^{-1}$	0.084	1.0%	$7.57*10^{-1}$	-0.191	-2.0%	$7.29*10^{-1}$
SM (OH) C24:1	0.021	1.8%	$7.53*10^{-1}$	0.075	6.5%	$9.51*10^{-2}$	-0.049	-4.0%	$7.43*10^{-1}$
SM C16:0	3.468	3.9%	$1.56*10^{-1}$	4.587	5.4%	$8.66*10^{-2}$	2.030	2.1%	$6.71*10^{-1}$
SM C16:1	0.585	4.0%	$1.77*10^{-1}$	0.568	4.3%	$1.42*10^{-1}$	0.606	3.8%	$5.14*10^{-1}$
SM C18:0	0.859	5.0%	$9.13*10^{-2}$	0.375	2.1%	$5.47*10^{-1}$	1.482	8.9%	$9.81*10^{-2}$
SM C18:1	0.265	3.2%	$3.15*10^{-1}$	0.014	0.2%	$9.65*10^{-1}$	0.588	6.8%	$2.07*10^{-1}$
SM C20:2	0.024	5.5%	$3.80*10^{-1}$	0.010	2.3%	$7.28*10^{-1}$	0.042	9.9%	$4.41*10^{-1}$
SM C22:3	-0.017	-5.8%	$7.54*10^{-1}$	-0.021	-7.4%	$8.15*10^{-1}$	-0.061	-19.9%	$2.00*10^{-1}$
SM C24:0	1.132	5.0%	$1.61*10^{-1}$	2.201	9.9%	$3.84*10^{-2}$	-0.242	-1.0%	$8.48*10^{-1}$
SM C24:1	2.222	4.7%	$6.72*10^{-2}$	2.991	6.6%	$3.74*10^{-2}$	1.234	2.5%	$5.82*10^{-1}$
SM C26:0	0.032	51.0%	$1.31*10^{-1}$	0.023	24.4%	$3.09*10^{-1}$	0.050	139.3%	$9.71*10^{-2}$
SM C26:1	0.017	5.1%	$4.07*10^{-1}$	-0.008	-2.5%	$7.53*10^{-1}$	0.048	14.8%	$1.48*10^{-1}$
H1	419.618	7.8%	$3.58*10^{-1}$	126.200	2.8%	$3.61*10^{-1}$	796.870	12.3%	$4.65*10^{-1}$

	Healthy		DM1		Healthy *vs.* DM1
Metabolite	**Mean**	**SD**	**Mean**	**SD**	**P-value**
C0	31.9	5.8	26.6	4.8	0.073
C2	4.24	0.57	3.58	1.06	0.133
C3	0.273	0.043	0.192	0.042	0.002
C3:1	0.010	0.002	0.012	0.003	0.139
C3-DC (C4-OH)	0.074	0.062	0.049	0.011	0.304
C3-OH	0.020	0.003	0.024	0.005	0.020
C4	0.105	0.019	0.105	0.057	0.979
C4:1	0.022	0.003	0.031	0.007	0.005
C5	0.120	0.032	0.097	0.027	0.153
C5:1	0.022	0.003	0.024	0.004	0.148
C5:1-DC	0.020	0.004	0.019	0.002	0.628
C5-DC (C6-OH)	0.017	0.003	0.019	0.005	0.273
C5-M-DC	0.035	0.003	0.039	0.004	0.047
C5-OH (C3-DC-M)	0.023	0.003	0.025	0.004	0.405
C6 (C4:1-DC)	0.066	0.022	0.058	0.014	0.385
C6:1	0.027	0.003	0.026	0.002	0.828
C7-DC	0.025	0.008	0.024	0.008	0.975
C8	0.172	0.126	0.121	0.066	0.356
C8:1	0.065	0.009	0.088	0.043	0.151
C9	0.025	0.010	0.024	0.005	0.817
C10	0.274	0.228	0.181	0.073	0.321
C10:1	0.124	0.073	0.093	0.039	0.324
C10:2	0.027	0.006	0.028	0.004	0.593
C12	0.084	0.051	0.055	0.015	0.179
C12:1	0.080	0.035	0.059	0.018	0.163
C12-DC	0.087	0.005	0.101	0.005	**0.000#**
C14	0.029	0.008	0.022	0.004	0.044
C14:1	0.071	0.028	0.049	0.015	0.080
C14:1-OH	0.009	0.002	0.009	0.001	0.684
C14:2	0.027	0.016	0.020	0.008	0.300
C14:2-OH	0.007	0.001	0.007	0.001	0.277
C16	0.072	0.018	0.060	0.010	0.136
C16:1	0.059	0.004	0.056	0.006	0.231
C16:1-OH	0.006	0.001	0.005	0.001	0.174
C16:2	0.007	0.002	0.006	0.001	0.164
C16:2-OH	0.010	0.001	0.010	0.002	0.684
C16-OH	0.006	0.001	0.007	0.003	0.714
C18	0.025	0.007	0.024	0.007	0.798
C18:1	0.073	0.009	0.074	0.019	0.949
C18:1-OH	0.009	0.001	0.009	0.001	0.668
C18:2	0.025	0.006	0.028	0.006	0.467

Table S5: Acylcarnitine levels after normal sleep duration. *Mean = mean plasma metabolite level (μM). DM1 = individuals with type 1 diabetes. #P<0.001 (0.05/41). P-values are based on independent Students t-tests. Abbreviations of all metabolites are shown in Supplemental Table S3. Healthy individuals n=9, DM1 n=7.*

	Healthy		DM1		Healthy *vs.* DM1
Metabolite	**Mean**	**SD**	**Mean**	**SD**	**P-value**
C0	32.3	7.0	26.6	6.4	0.113
C2	0.287	0.212	0.208	0.078	0.434
C3	0.120	0.057	0.115	0.037	0.839
C3:1	0.027	0.005	0.028	0.005	0.680
C3-DC (C4-OH)	0.094	0.056	0.070	0.018	0.309
C3-OH	0.094	0.034	0.080	0.023	0.356
C4	0.089	0.006	0.104	0.011	0.004
C4:1	0.032	0.008	0.028	0.006	0.300
C5	0.087	0.032	0.073	0.026	0.336
C5:1	0.009	0.001	0.010	0.003	0.261
C5:1-DC	0.032	0.016	0.028	0.008	0.595
C5-DC (C6-OH)	0.007	0.001	0.008	0.002	0.447
C5-M-DC	0.077	0.016	0.070	0.014	0.398
C5-OH (C3-DC-M)	0.063	0.006	0.061	0.011	0.577
C6 (C4:1-DC)	0.007	0.002	0.007	0.002	0.610
C6:1	0.008	0.002	0.008	0.003	0.715
C7-DC	0.010	0.001	0.010	0.001	0.937
C8	0.006	0.001	0.007	0.002	0.213
C8:1	0.027	0.009	0.027	0.006	0.932
C9	0.088	0.014	0.092	0.024	0.664
C10	0.010	0.002	0.011	0.002	0.225
C10:1	0.031	0.006	0.037	0.009	0.103
C10:2	4.421	1.169	4.859	1.478	0.517
C12	0.271	0.065	0.180	0.039	0.006
C12:1	0.010	0.002	0.010	0.003	0.810
C12-DC	0.080	0.047	0.057	0.015	0.241
C14	0.021	0.003	0.024	0.005	0.255
C14:1	0.113	0.028	0.120	0.072	0.793
C14:1-OH	0.024	0.004	0.032	0.007	0.012
C14:2	0.132	0.038	0.106	0.036	0.188
C14:2-OH	0.021	0.005	0.026	0.006	0.121
C16	0.019	0.004	0.021	0.004	0.209
C16:1	0.015	0.003	0.019	0.003	0.028
C16:1-OH	0.034	0.005	0.040	0.007	0.086
C16:2	0.023	0.003	0.025	0.005	0.222
C16:2-OH	0.068	0.016	0.069	0.025	0.919
C16-OH	0.027	0.003	0.027	0.004	0.637
C18	0.027	0.008	0.033	0.008	0.159
C18:1	0.166	0.109	0.135	0.063	0.507
C18:1-OH	0.073	0.028	0.104	0.048	0.128
C18:2	0.024	0.007	0.025	0.005	0.645

Table S6: Acylcarnitine levels after short sleep duration. *Mean = mean plasma metabolite level (μM). DM1 = individuals with type 1 diabetes. P-values are based on independent Students t-tests. Abbreviations of all metabolites are shown in Supplemental Table S3. Healthy individuals n=9, DM1 n=7.*