Endocrine and metabolic diurnal rhythms in young adult men born small vs appropriate for gestational age

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Abstract

Objective: Sleep disturbances and alterations of diurnal endocrine rhythms are associated with increased risk of type 2 diabetes (T2D). We previously showed that young men born small for gestational age (SGA) and with increased risk of T2D have elevated fat and decreased glucose oxidation rates during nighttime. In this study, we investigated whether SGA men have an altered diurnal profile of hormones, substrates and inflammatory markers implicated in T2D pathophysiology compared with matched individuals born appropriate for gestational age (AGA).

Methods: We collected hourly blood samples for 24 h, to measure levels of glucose, free fatty acids (FFA), triglycerides (TG), insulin, C-peptide, leptin, resistin, ghrelin, plasminogen activator inhibitor-1 (PAI-1), incretins (GLP-1 and GIP), and inflammatory markers (TNF- α and IL-6) in 13 young men born SGA and 11 young men born AGA.

Results: Repeated measurements analyses were used to analyze the diurnal variations and differences between groups. The SGA subjects had increased 24-h glucose ($P=0.03$), glucagon ($P=0.03$) and resistin ($P=0.003$) levels with no difference in diurnal rhythms compared with AGA controls. We found significant diurnal variations in levels of blood glucose, plasma TG, FFA, insulin, C-peptide, GLP-1, GIP, leptin, visfatin, TNF- α , IL-6 and PAI-1. The variation in FFA levels differed between the groups during the evening. Plasma ghrelin and glucagon levels did not display diurnal variations.

Conclusions: Young men born SGA exhibit elevated 24-h blood glucose, and plasma glucagon and resistin levels with no major differences in diurnal rhythms of these or other key metabolic hormones, substrates or inflammatory markers implicated in the origin of adiposity and T2D.

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Introduction

Disruption of biological rhythms, as seen for example with night-shift work and sleep deficiency or a disruption of sleep in general, is associated with an increased risk of developing obesity and type 2 diabetes (T2D) (1, 2, 3, 4). The development of T2D is due to complicated interactions between genetic and pre- and postnatal environmental factors. There is mounting evidence supporting that impaired fetal growth, associated with

being small for gestational age (SGA), represents an independent risk factor for T2D (5).

We and others have shown that individuals born SGA have reduced adult height and increased abdominal obesity as well as numerous metabolic impairments such as reduced protein expression of key insulin signaling molecules in muscle and adipose tissue, increased lipolysis, hepatic and peripheral insulin resistance, and

disproportionately reduced insulin secretion (6, 7, 8, 9). In strong support of the 'thrifty phenotype' hypothesis as proposed by Hales and Baker (10), we have previously shown that young SGA men exhibit decreased energy expenditure when subjected to 36-h fasting (11). Furthermore, recent data suggest that young healthy SGA men may have an altered diurnal rhythm including diurnal variations in metabolic hormones compared with control individuals born appropriate for gestational age (AGA), since they exhibit pronounced differences in substrate oxidation rates in particular during nighttime (12, 13). A central clock located in the suprachiasmatic nucleus (SCN) in the hypothalamus, generating a circadian (24-h) rhythm, regulates most physiological functions. The SCN plays a key role in controlling daily blood glucose homeostasis and regulates a part of the metabolism during the day through neural and/or humeral signaling (14, 15). It is synchronized to the 24-h day primarily by daily light–darkness cycles; however, for many central diurnal processes, it is the timing and feedback from nutrient intake that is the most important stimulus for synchronizing diurnal oscillators (16, 17). A disruption of endocrine rhythms may result in hormonal dysfunctions affecting glucose homeostasis, thereby influencing the risk of developing metabolic disease including T2D (15).

The primary aim of this study was to examine whether individuals born SGA, compared with matched control individuals born AGA, exhibit an altered metabolic profile with regard to selected key hormones, substrates and inflammatory markers involved in the glucose and lipid metabolism. It includes change in plasma insulin, C-peptide, glucagon, ghrelin, leptin, resistin and visfatin; incretins glucagon-like peptide-1 (GLP-1) and gastric inhibitory polypeptide (GIP); cytokines TNF- α , IL-6 and plasminogen activator inhibitor-1 (PAI-1) levels, as well as glucose, triglycerides (TG) and free fatty acid (FFA) levels. We hypothesized that altered diurnal variation in the selected key hormones and substrates could explain the disproportionally increased nocturnal fat oxidation and decreased glucose oxidation recently reported in young men born SGA (12, 13).

Subjects and methods

Subjects

The participants were recruited using data from the Danish Medical Birth Registry. All of them were males born singletons at term (Week 39–41) in Copenhagen County. Small for gestational age was defined as a birth

weight in the lowest 10th percentile and AGA was defined as a birth weight between the 50th and 90th percentile. In total, 48 healthy Caucasian men were recruited for the study. To avoid major genetic confounding, subjects with a family history of diabetes in two generations were excluded. Further, participants having BMI > 30 kg/m², those performing physical activity for ≥ 10 h per week, and those taking medication that influences the outcome measures were excluded.

Blood samples from the first participant were hemolyzed upon addition of a protease inhibitor, thus reducing the number of participants to 47. Furthermore, because of the instability of samples analyzed in the first batch, we chose to exclude all uncertain measures with unacceptable high coefficient of variation (CV) values defined *a priori* – in this case to include CV values > 25% in the duplicate samples. Therefore, in an unbiased and blinded manner, data from 12 AGA and 11 SGA men were excluded, subsequently resulting in valid data from a total of 13 SGA and 11 AGA individuals.

Informed written consent was obtained from all subjects after fully explaining the purpose and nature of the study. The study protocol was approved by the Local Ethics Committee, and procedures were performed according to the principles of the Helsinki Declaration.

Study protocol

All subjects were asked to refrain from strenuous physical activity and from alcohol intake for three days before the examination. All meals, 10 MJ/day in accordance with current recommendations (18), were provided to the participants for 3 days before and during examinations. The study activities were carried out over 3 days. The subjects were admitted to Steno Diabetes Center at 18:00 h the day before the examination. Height and weight were measured and body composition was assessed with dual-energy X-ray absorptiometry (DXA) scanning. Participants had to go to sleep at 23:00 h.

On the day of examination, the participants exercised on a bike at 60% of their maximum pulse for 10 min in the morning and afternoon to imitate a regular day. Similar to the preceding days, three main meals and three snacks were served. Breakfast was served at 07:30 h, lunch at 12:10 h, dinner at 18:10 h, and snacks were served in between meals (Fig. 1). Coffee/tea was offered with breakfast, lunch, and afternoon snack, and a glass of milk and a sugar-free soft drink was offered for dinner. Water was allowed *ad libitum*. Besides the bike ride, quiet indoor activities, except sleeping, were allowed during daytime.

Figure 1

Overview of the study activities.

Smokers were offered sugar-free nicotine chewing gum. The participants were instructed to go to bed and sleep at 23:00h, and the last blood sample was taken at 07:00h the following morning. Hemoglobin levels were measured and iron supplements were handed out to all participants. After breakfast was served, the participants had to answer a questionnaire concerning normal sleep habits inspired by the Pittsburgh Sleep Quality Index (19), and they also had to score sleep quality during the night of the experiment. The participants were observed during the entire duration of the examination. The evening (postprandial) period was from 18:00 to 23:00h and nighttime (sleep) period was from 01:00 to 07:00h.

24-h blood sampling

Examinations started at 06:30h. An intravenous cannula (Venflon) was placed in the antecubital fossa in each arm through which 25 blood samples were collected from 07:00h until 07:00h the next day. Blood samples were taken every 30min for 2h after breakfast and dinner, every hour during the rest of the day, and every 2h during nighttime. To avoid clotting of the Venflon, 2mL saline

was infused after sampling and 2mL blood/saline was aspirated and discarded before every sampling. All samples were immediately centrifuged and stored at -80°C until analysis. A small flashlight was used for nighttime sampling, as the SCN is affected by light, especially at night. If participants woke up, they were instructed to keep quiet and turn their arm to a position at which the sample could be easily taken.

Analysis of blood samples

The Diabetes Pro Hu 12-plex assays from Luminex xMAP (Luminex, Austin, TX, USA) were used to measure plasma levels of insulin, C-peptide, glucagon, GLP-1, GIP, ghrelin, leptin, resistin, visfatin, IL-6, TNF- α , and PAI-1. The assay had a precision intra-assay CV of $\leq 20\%$, an inter-assay CV of $\leq 30\%$, a cross-reactivity of $< 0.5\%$, and an accuracy (recovery range) of 70–130% according to the manufacturer. The 12 analytes were measured simultaneously, and all procedures were performed according to the manufacturer's instructions. Thirty-six samples were loaded in duplicate on each plate along with a standard curve. After a 2-h incubation period, the

plates were washed and analyzed using the Luminex 200 system.

Blood glucose levels were measured using an Ascensia Contour blood glucose meter (LifeScan, Milpitas, CA, USA) as it requires a low sample volume and has a CV less than 5%. The same instrument was used on all subjects throughout the entire experimental period to avoid confounding. HbA1c was determined on the sampling day using high-pressure chromatography (Tosoh G7, Tokyo, Japan). Total plasma cholesterol, HDL cholesterol, and TG samples were analyzed using enzymatic colorimetric analysis on a Hitachi 912 (Roche Diagnostics). Plasma FFAs were analyzed using spectrometry on a Hitachi 912 (Roche Diagnostics). All above assays were analyzed collectively at Steno Diabetes Center, Gentofte, Denmark.

Statistics

The Bio-Plex Manager software (version 5.0; Bio-Rad, Hercules, CA, USA) used values from the standard dilution series to create a standard curve for each analyte; based on these curves, concentrations of the 12 analytes were determined using logistic regression. The standard curve was manually fitted by removing outliers at the low and/or high end. The best curve fit was defined as one where the accuracy of all standard points were as close as possible to 100% of the expected concentration. When a standard curve was not optimal to detect the low concentration of a hormone, the standard curve from the other plate analyzed on the same day was used. Out-of-range (OOR) data as determined by the Bio-Plex Manager software were not included in the analyses. CV values were determined by the Bio-Plex Manager software for each analyte on a plate and used to assess intra-assay variance; if a duplicate sample had a CV > 25%, it was considered an outlier and excluded. Data points outside the standard curve were also excluded.

We fitted a longitudinal linear mixed effects model with a random intercept and errors with an exponential covariance structure to account for correlation between measurements within subjects. The class variables group and time and their interaction were included as fixed effects. Based on the residual plots for normality, the response variables were log-transformed before analysis to make the residuals approximately normally distributed. Analyses were performed using Proc Mixed in SAS 9.3 (SAS Institute, Cary, NC, USA).

Repeated measurements were determined for 3 periods: the 24-h period (07:00–07:00h), the evening

(postprandial) period (18:00–23:00h), and the nighttime (sleep) period (01:00–07:00h).

The total area under the curve (AUC) was calculated using the trapezoidal rule and the incremental AUC by subtracting the baseline area from AUC for the postprandial phase after dinner. Unpaired Student's *t*-test was used to detect differences between the birth weight groups, and the Kolmogorov–Smirnov test was used to check for normality. Non-normally distributed data were analyzed using nonparametric tests, whereas normally distributed data were analyzed using parametric tests. A *P* value ≤ 0.05 was considered significant. Data and figures in the text are given as mean ± s.d., unless otherwise stated.

Results

Subject characteristics

As shown in Table 1, the SGA subjects had significantly lower birth weight than the AGA controls (*P* = 7.8E-11). There were no differences in age, height, weight, BMI, or blood pressure. The two groups did not differ with regard to fat distribution and there were no differences in either fasting blood glucose levels, HbA1c, or fasting plasma concentrations of insulin, C-peptide, cholesterol, triglycerides, and FFAs. Quality of sleep did not differ between the two groups (AGA: 6.41 ± 1.99 vs SGA: 6.54 ± 1.98, *P* = 0.88).

Table 1 Subject characteristics and baseline plasma concentrations for men born AGA and SGA. Data are presented as mean ± s.d.

	AGA (n = 11)	SGA (n = 13)	P value
Birth weight (g)	3802 ± 163	2778 ± 251	7.8E-11
Age (year)	23.2 ± 0.9	23.6 ± 0.8	0.21
Height (cm)	181 ± 9	178 ± 4	0.30
Weight (kg)	77.1 ± 7.3	78.5 ± 10.5	0.71
BMI (kg/cm ²)	23.6 ± 2.4	24.8 ± 2.7	0.29
BP systolic (mgHb)	124 ± 9	131 ± 22	0.26
BP diastolic (mgHb)	76 ± 11	73 ± 7	0.70
Proportional fat distribution			
Trunk/total fat mass (g)	0.44 ± 0.05	0.42 ± 0.06	0.51
Leg/total fat mass (g)	0.36 ± 0.03	0.38 ± 0.04	0.16
Trunk/leg fat mass (%)	0.91 ± 0.15	0.87 ± 0.16	0.53
Baseline plasma concentrations			
Glucose (mmol/L)	4.96 ± 0.37	5.07 ± 0.35	0.48
HbA1c (%)	5.11 ± 0.28	5.13 ± 0.22	0.82
Insulin (pmol/L)	283 ± 122	370 ± 313	0.40
C-peptide (pmol/L)	317 ± 90	376 ± 88	0.12
Cholesterol (mmol/L)	4.44 ± 1.06	4.14 ± 0.63	0.40
HDL cholesterol (mmol/L)	1.13 ± 0.19	1.13 ± 0.25	0.99
Triglyceride (mmol/L)	1.02 ± 0.55	1.05 ± 0.44	0.91
FFA (mmol/L)	473 ± 258	379 ± 158	0.29

BP, blood pressure; FFA, free fatty acids.

Area under the curve

The SGA individuals had a significantly higher 24-h ($P=0.02$), evening (postprandial) ($P=0.01$), and nighttime ($P=0.02$) AUC for plasma resistin levels compared with the AGA controls (Table 2). The SGA individuals had a significantly higher postprandial ($P=0.03$) as well as borderline increased 24-h ($P=0.06$) AUC for plasma glucagon compared with the AGA control subjects. There were no other significant AUC differences between the two groups.

Repeated measurements

During the 24-h period, applying repeated measurements analyses revealed that the SGA and AGA group differed significantly with regard to blood glucose ($P=0.03$), glucagon ($P=0.03$), and resistin ($P=0.003$) levels (Fig. 2 and Table 3). There was a significant difference in 24-h diurnal variation in blood glucose, plasma TG, FFA, C-peptide, GIP, leptin, IL-6, and PAI-1 (all $P<0.0001$), insulin ($P=0.0007$), GLP-1 ($P=0.03$), visfatin ($P=0.007$), and TNF- α ($P=0.004$) plasma concentrations (Figs 2 and 3; Table 3). There was no difference in the Time $\times$ Group interaction for any of the measured hormones or metabolites.

In the postprandial evening period (18:00–23:00h), there was a difference between the two groups in blood glucose ($P=0.01$), glucagon ($P=0.03$), and resistin ($P=0.005$) levels (Table 3). Time exhibited an effect on the concentrations of blood glucose ($P=0.01$); plasma TG, FFA, and C-peptide (all $P<0.0001$); and GIP ($P=0.0002$) during the postprandial period. There was a significant interaction between time and group for plasma FFA levels only ($P=0.05$) (Figs 2 and 3; Table 3).

During nighttime (01:00–07:00h), there was a significant difference between the two groups in plasma resistin concentrations ($P=0.002$). A variability in concentrations during the nighttime was found for blood glucose ($P=0.001$), TG ($P=0.01$), FFA ($P<0.0001$), insulin ($P=0.04$), C-peptide ($P<0.0001$), GLP-1 ($P=0.005$), GIP ($P<0.0001$), leptin ($P=0.04$), resistin ($P=0.002$), visfatin ($P=0.003$), TNF- α ($P=0.006$), IL-6 ($P=0.008$), and PAI-1 ($P=0.0008$) plasma concentrations. There was no difference in the Time $\times$ Group interaction for any of the hormones or metabolites.

Discussion

The aim of this study was to explore whether an altered metabolic diurnal rhythm could play a role in the link between being born SGA and risk of developing T2D. The

Table 2 Area under the curve (AUC) for the 24-h, evening, and nighttime periods for the AGA and SGA groups. Data are presented as mean $\pm$ s.d.

	24h			Evening (18:00–23:00 h)			Night (01:00–07:00 h)		
	AGA (n=11)	SGA (n=13)	P	AGA (n=11)	SGA (n=13)	P	AGA (n=11)	SGA (n=13)	P
Glucose (mM)	114 $\pm$ 7	120 $\pm$ 11	0.12	24.0 $\pm$ 2.0	26.0 $\pm$ 2.0	0.09	30.5 $\pm$ 2.8	31 $\pm$ 3	0.52
TG (mM)	26.6 $\pm$ 14.0	27.6 $\pm$ 11.0	0.85	6.4 $\pm$ 3.0	6.6 $\pm$ 2.5	0.80	6.8 $\pm$ 3.4	6.5 $\pm$ 2.3	0.77
FFA (mM)	4210 $\pm$ 1269	4214 $\pm$ 1177	0.99	568 $\pm$ 161	596 $\pm$ 229	0.74	1637 $\pm$ 544	1665 $\pm$ 453	0.89
Insulin (pg/mL)	50159 $\pm$ 15990	53279 $\pm$ 21174	0.70	10402 $\pm$ 3135	10746 $\pm$ 3325	0.80	14629 $\pm$ 7501	13632 $\pm$ 3441	0.70
C-peptide (pg/mL)	32335 $\pm$ 5314	37080 $\pm$ 7835	0.10	7020 $\pm$ 1245	8454 $\pm$ 2384	0.08	5360 $\pm$ 1290	6048 $\pm$ 1276	0.20
Glucagon (pg/mL)	16615 $\pm$ 1762	18404 $\pm$ 4193	0.06	3575 $\pm$ 393	4030 $\pm$ 653	0.03	4433 $\pm$ 834	4840 $\pm$ 946	0.20
GLP-1 (pg/mL)	24540 $\pm$ 7384	24278 $\pm$ 6351	0.93	5344 $\pm$ 1513	5244 $\pm$ 1191	0.86	6440 $\pm$ 2678	6030 $\pm$ 1448	0.66
GIP (pg/mL)	4855 $\pm$ 2071	4907 $\pm$ 1200	0.94	2531 $\pm$ 13387	1316 $\pm$ 337	0.78	154 $\pm$ 68	859 $\pm$ 160	0.61
Ghrelin (pg/mL)	1246 $\pm$ 649	1171 $\pm$ 542	0.77	259 $\pm$ 141	258 $\pm$ 127	0.97	342 $\pm$ 191	323 $\pm$ 148	0.79
Leptin (pg/mL)	42181 $\pm$ 23918	42504 $\pm$ 9836	0.96	9076 $\pm$ 4594	9312 $\pm$ 2419	0.87	12157 $\pm$ 5646	12540 $\pm$ 2911	0.84
Resistin (pg/mL)	15803 $\pm$ 3751	19953 $\pm$ 3978	0.02	3359 $\pm$ 743	4360 $\pm$ 929	0.01	4145 $\pm$ 1087	5349 $\pm$ 1201	0.02
Visfatin (pg/mL)	212369 $\pm$ 128994	184139 $\pm$ 50528	0.51	45563 $\pm$ 24696	39884 $\pm$ 11214	0.46	56929 $\pm$ 26833	49182 $\pm$ 10969	0.39
TNF- α (pg/mL)	672 $\pm$ 237	599 $\pm$ 130	0.35	152 $\pm$ 58	145 $\pm$ 35	0.70	157 $\pm$ 70	153 $\pm$ 63	0.88
IL-6 (pg/mL)	347 $\pm$ 110	341 $\pm$ 60	0.87	82 $\pm$ 26	81 $\pm$ 18	0.94	93 $\pm$ 34	97 $\pm$ 19	0.72
PAI-1 (pg/mL)	36144 $\pm$ 11977	39140 $\pm$ 11083	0.53	6582 $\pm$ 1891	7229 $\pm$ 2155	0.45	8758 $\pm$ 4019	9412 $\pm$ 2606	0.64

TG, triglyceride; FFA, free fatty acids.

Figure 2

Selected diurnal rhythms of the SGA (red) and AGA (black) individuals for the 24 h and evening period respectively. (A and B) glucose, (C and D) glucagon, (E and F) FFA, (G and H) resistin.

experimental conditions in our study were designed to reflect general daily life conditions, and the participants were neither sleep-deprived nor exposed to forced dyssynchrony (manipulating with the 24-h day) as seen in other studies (16, 20, 21).

We did not find any statistically significant differences in the clinical baseline characteristics between the two

groups as reported earlier (6, 7, 22). However, this may be due to fewer study participants compared with our previous studies. Despite this, we were able to confirm the prior results showing significantly elevated blood glucose levels in the young SGA men compared with AGA controls in the 24-h repeated measurements analysis. This is in full agreement with the general consensus of SGA being a

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Table 3 Repeated measurements for group, time and time×group during the 24-h, evening, and nighttime period. Data are presented as *P* values and significant values are in bold.

	24 h			Evening (18:00–23:00 h)			Night (01:00–07:00 h)		
	Group	Time	Time×group	Group	Time	Time×group	Group	Time	Time×group
Glucose (mM)	0.03	< 0.0001	0.90	0.01	0.01	0.87	0.24	0.001	0.75
TG (mM)	0.63	< 0.0001	0.13	0.72	< 0.0001	0.57	0.80	0.01	0.09
FFA (mM)	0.61	< 0.0001	0.14	0.66	< 0.0001	0.05	0.77	< 0.0001	0.60
Insulin (pg/mL)	0.84	0.0007	0.59	0.86	0.20	0.43	0.81	0.04	0.36
C-peptide (pg/mL)	0.12	< 0.0001	0.58	0.07	< 0.0001	0.64	0.38	< 0.0001	0.12
Glucagon (pg/mL)	0.03	0.43	0.75	0.03	0.41	0.36	0.12	0.62	0.59
GLP-1 (pg/mL)	0.90	0.03	0.74	0.83	0.87	0.20	0.62	0.005	0.35
GIP (pg/mL)	0.54	< 0.0001	0.56	0.59	0.0002	0.66	0.86	< 0.0001	0.06
Ghrelin (pg/mL)	0.93	0.11	0.58	0.87	0.55	0.30	0.96	0.08	0.35
Leptin (pg/mL)	0.54	< 0.0001	0.41	0.58	0.96	0.53	0.53	0.04	0.46
Resistin (pg/mL)	0.003	0.59	0.80	0.005	0.10	0.25	0.002	0.002	0.64
Visfatin (pg/mL)	0.94	0.007	0.81	0.62	0.98	0.51	0.71	0.003	0.75
TNF- α (pg/mL)	0.47	0.004	0.61	0.93	0.78	0.06	0.69	0.006	0.54
IL-6 (pg/mL)	0.73	< 0.0001	0.54	0.82	0.67	0.17	0.49	0.008	0.07
PAI-1 (pg/mL)	0.73	< 0.0001	0.87	0.49	0.17	0.61	0.54	0.0008	0.08

prediabetic state associated with increased risk of developing T2D (5). This finding illustrates the strength and statistical power of having multiple measurements over a 24-h period, in a limited number of subjects in contrast to having only one or few measurements in each subject in a larger sample size. Indeed, this also provides some assurance of an appropriate statistical power comparing the levels of other hormones and substrates between the two groups.

Interestingly, the increased concentration of blood glucose among the SGA subjects became even more significant in the evening, which is consistent with impaired glucose tolerance being more obvious at this time point (22). Using AUC comparisons and repeated measurements, we furthermore found that the individuals born SGA displayed elevated plasma concentrations of glucagon during the entire 24-h period. Elevated plasma glucagon levels is a hallmark of T2D and may play a key role in the development of elevated hepatic glucose production rate and elevated fasting blood glucose levels in T2D as well as in prediabetic subjects (23). We, therefore, also speculate that the elevated plasma glucagon levels, documented for the first time in SGA subjects in this study, may contribute to the disproportionately elevated hepatic glucose production rate in SGA subjects as reported previously (24). The physiological explanation for why we are finding a higher concentration of glucagon in the SGA men may be due to an over-activity/expression of pancreatic α -cells due to impaired β -cell function, or because of insulin resistance in the α -cells, and thus a lack of negative feedback from insulin (25, 26, 27).

Also, at all times, the SGA men displayed markedly elevated levels of plasma resistin compared with the controls born AGA. We did not find any variation in plasma resistin concentration during daytime and evening, but during the nighttime, plasma resistin concentrations increased toward the morning in both groups (Fig. 2G and H). Human resistin is a proinflammatory cytokine expressed in and secreted by monocytic cells, and a recent meta-analysis provided evidence for an association between higher circulating resistin levels and increased mortality (28, 29). Furthermore, resistin gene expression is positively correlated with insulin resistance in humans. Serum resistin level is elevated in human obesity, which has been associated with the development of hepatic insulin resistance in mice (30, 31, 32). Although the exact physiological and metabolic function(s) of resistin remains inconclusive in humans, it has been suggested to play a key role in the link between obesity and insulin resistance (33). In support of the current results, a study reported increased cord blood resistin levels in SGA newborns (34). Furthermore, young and healthy shift workers exhibit increased levels of resistin suggested to be associated with a misaligned diurnal rhythm and an early marker of the metabolic syndrome (35). Elevated plasma resistin levels have also been associated with increased inflammation including increased plasma IL-6 and TNF- α levels (36). Our finding of elevated plasma resistin levels in the face of normal plasma IL-6 and TNF- α level in prediabetic SGA men suggest that elevated plasma resistin may precede the development of overt inflammation with elevated inflammation markers. Plasma resistin could, therefore, potentially serve as an

Figure 3

The diurnal rhythms of the non-significant findings, all during the 24-h period. (A) insulin, (B) C-peptide, (C) ghrelin, (D) leptin, (E) triglycerides, (F) TNF- α , (G) IL-6, (H) PAI-1, (I) GLP-1, (J) GIP, (K) visfatin (SGA (red) and AGA (black)).

early marker of insulin resistance, T2D, and associated cardiometabolic disease in SGA individuals. However, further studies are needed to understand the origin, physiological function(s), and the potential adverse cardiometabolic consequences of the increased plasma resistin levels in human subjects born SGA.

Most of the hormones, substrates, and inflammatory markers showed a significant diurnal rhythmicity. Surprisingly, no significant diurnal variations in plasma glucagon, ghrelin, or resistin concentrations were observed during the 24-h period in any of the study groups, as would otherwise be expected in relation to food intake (37) (Figs 2C, G and 3C; Table 3). Similarly, the expected findings of diurnal rhythmicity of blood glucose, plasma TG, FFA insulin, and C-peptide levels support the dynamic variation in relation to food intake during the day and fasting during nighttime, as also shown in other studies (15, 16, 22). The secretion of the gut incretin hormones GLP-1 and GIP is enhanced by food intake and decreased during fasting as also shown in the current study (38) (Fig. 3I and J; Table 3). Reduced plasma GLP-1 levels (39, 40) and impaired insulinotropic effect of the incretin hormone GIP are commonly seen in T2D patients (38, 41). We found, however, identical levels of both GLP-1 and GIP in the two groups, which are in agreement with previous studies showing similar fasting and postprandial incretin hormone levels in young and healthy SGA and AGA men (24, 42).

Ghrelin is a 'hunger' hormone and its levels are, therefore, highest right before a meal and lowest right after a meal (43). The meal-related regulation of plasma ghrelin is thought to be controlled by nutrient sensing either in the intestine or at postabsorptive sites. Interestingly, when applying repeated measurements, we found no diurnal fluctuation in plasma ghrelin concentrations, as would otherwise have been expected in response to food intake. However, when looking at the curve, it can be seen that, although not statistically significant, some fluctuation in plasma ghrelin levels is present in both groups, in particular during the daytime (Fig. 3C). One possible explanation for not finding large daily variations could be that the study participants were provided with meals and snacks throughout the day according to a set schedule, which may be the reason why the secretion of the 'hunger' hormone was not stimulated. We previously studied fasting plasma ghrelin levels in SGA and AGA subjects and found no effect due to 5 days of high-fat overfeeding compared with a control diet between the two groups (24).

Leptin acts as the hormonal counterpart to ghrelin as a satiety hormone suppressing appetite. We found a 24-h

dynamic, as well as a nocturnal, rhythm in plasma leptin levels. The diurnal rhythm of leptin is also thought to be altered by meal timing and a nocturnal rise in leptin as has been shown previously, likely suppressing appetite while sleeping (44, 45). We previously showed that, in response to high-fat overfeeding, only AGA control subjects had significant increase in their plasma leptin, suggesting that the SGA individuals do not experience the same degree of appetite inhibition when exposed to overfeeding (24).

The diurnal variations in blood glucose, FFA, TG, insulin, C-peptide, GLP-1, and GIP levels are all well known and can be explained by meal-related fluctuations. However, the diurnal variations in plasma visfatin, TNF- α , IL-6, or PAI-1 levels, as seen in both the SGA and AGA subjects in this group, may not be well known or easily explained in the context of meals. There was a small decrease in visfatin, TNF- α , and IL-6 concentrations (Fig. 3F, G and K), whereas PAI-1 concentration increased (Fig. 3H) during nighttime. Visfatin is an adipokine that has been proposed to play a role in the development of obesity, insulin resistance, and/or T2D (46). Interestingly, people with sleep apnea syndrome have been reported to exhibit a disproportionately increased rise of plasma PAI-1 and visfatin levels from their nighttime nadir values to the peak day values (47). However, in this study, we were unable to demonstrate that SGA subjects showed any altered diurnal variation in plasma visfatin or PAI-1 levels compared with the AGA control subjects.

TNF- α and IL-6 are key markers and mediators of systemic inflammation, which in turn have been implicated in the development of insulin resistance, T2D, cardiovascular disease, as well as in sleep disturbances (48, 49). However, we did not demonstrate any differences in plasma levels or in diurnal variations in plasma TNF- α or IL-6 levels in SGA subjects with a known increased risk of developing the above-mentioned diseases. The explanation for the diurnal variations in these inflammatory markers is unknown, but could be explained as being secondary to meal effects and perhaps the rise in plasma insulin levels in connection with meals (50, 51). However, further studies are needed to understand these variations over the day.

The link between T2D and diurnal disruption is primarily caused by extrinsic factors controlling the metabolism (i.e. change in sleep pattern and lack of sleep), which increases appetite, decreases leptin levels, and, therefore, leads to obesity and hence increased postprandial levels of glucose, increased insulin levels, and impaired glucose tolerance (17, 20). Thus, the increased risk of T2D due to

diurnal disruption appears to be mediated primarily through weight gain. We did not find any differences between the diurnal rhythms of the SGA and the AGA (Table 3) groups that could explain the link between being born SGA and T2D, because the two groups were matched for weight.

We hypothesized that plasma FFA and/or TG levels might differ between the groups during nighttime. This hypothesis was based on the studies of 24-h substrate oxidation rates in SGA and AGA controls, the finding of increased nocturnal fat oxidation in SGA men (12, 13), as well as on our previously reported increased rate of lipolysis as determined using a stable isotope glycerol tracer in the fasting state among young men born SGA (7). However, in this study, no difference in plasma FFA levels between the groups was documented during nighttime. However, the evening differences between SGA and AGA lipid profiles with the FFA level falling after dinner and rising during nighttime, when the participants were fasting (Fig. 2E and F; Table 3), could support the notion of a disproportionately elevated rate of lipolysis driving the increased fat oxidation rate (6, 12, 24).

Limitations of the study include low number of study participants and lack of information regarding catch-up growth. Indeed, SGA subjects commonly display increased catch-up growth early in life, and increased catch-up growth *per se* has been proposed as an independent risk factor of cardiometabolic diseases including T2D later in life (52).

In conclusion, young and healthy adult men born SGA do not exhibit any major changes in hormonal or metabolic plasma diurnal rhythms compared with AGA control individuals. Increased plasma resistin and glucagon levels may contribute to the development of peripheral and hepatic insulin resistance and thereby elevated glucose levels in SGA subjects. More studies on human are needed to understand the origin and metabolic implications of the elevated plasma resistin level among the subjects born SGA.

Declaration of interest

The authors declare no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

C B designed the study, analyzed and interpreted data, and wrote the manuscript. P N interpreted data and drafted manuscript. M F and Y C analyzed and interpreted data. A V designed the study and interpreted data. All authors critically revised the manuscript.

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