

Metabolic response to 36 hours of fasting in young men born small vs appropriate for gestational age

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Abstract

Aims/hypothesis Being born small for gestational age (SGA) is associated with an increased risk of type 2 diabetes in an affluent society, but could confer an improved chance of survival during sparse living conditions. We studied whether insulin action and other metabolic responses to prolonged fasting differed between 21 young adults born SGA and 18 matched controls born appropriate for gestational age (AGA). **Methods** A frequently sampled IVGTT and indirect calorimetry measurements were performed after a 36 h fast. Endogenous glucose production, insulin sensitivity (S_I), first-phase insulin secretion and glucose effectiveness were estimated by stable isotope tracer techniques and minimal

modelling. Muscle and fat biopsies were obtained after 35 h of fasting.

Results During fasting, SGA individuals experienced a more pronounced decrease in serum insulin and lower plasma triacylglycerol levels compared with AGA individuals. In addition, energy expenditure decreased in SGA but increased in AGA individuals. After fasting, SGA individuals displayed lower fat oxidation than AGA individuals. S_G was reduced in SGA compared with AGA individuals, whereas hepatic or whole body insulin action (S_I) did not differ between groups. SGA individuals had increased muscle *PPARGC1A* DNA methylation. We found no differences in adipose tissue *PPARGC1A* DNA methylation, muscle and adipose tissue *PPARGC1A* mRNA expression, or muscle glycogen levels between the groups.

Conclusion Compared with AGA individuals, SGA individuals displayed a more energy-conserving and energy-conserving cardiometabolic response to 36 h fasting. The role of increased muscle *PPARGC1A* DNA methylation in mediating this response requires further study.

Dr Les Bluck died on 14 May 2014 before publication of this work

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Keywords DNA methylation · Insulin resistance · Low birthweight · Prolonged fasting · Small for gestational age

Abbreviations

AGA	Appropriate for gestational age
EE	Energy expenditure
EGP	Endogenous glucose production
FPIR	First-phase insulin response
K_G	Glucose tolerance
R_a	Rate of appearance
SAT	Subcutaneous adipose tissue
S_G	Glucose effectiveness
SGA	Small for gestational age
S_I	Insulin sensitivity

Introduction

Individuals born small for gestational age (SGA) have an increased risk of type 2 diabetes and cardiovascular disease later in life [1–5]. We and others have previously documented several metabolic defects in young adult SGA individuals, including altered fat tissue distribution [6], muscle and hepatic insulin resistance [7], reduced beta cell function [8], increased lipolysis [9], reduced expression of key insulin signalling proteins in fat and muscle tissue [10, 11], abnormal epigenetic regulation of the peroxisome proliferator-activated receptor gamma, coactivator 1 alpha (*PPARGC1A*) transcription factor in muscle and fat [12, 13] and immature muscle fibre composition [14]. The development of peripheral insulin resistance may represent a beneficial response to fasting by facilitating sufficient energy delivery to vital organs, including the brain and heart [9]. We hypothesised that the observed adverse phenotype of SGA individuals in adulthood may be primarily driven by a programmed enforced tolerance of fasting. Thus, SGA individuals may cope with fasting in a different and more energy-conserving manner compared with those born appropriate for gestational age (AGA), potentially including increased insulin resistance and/or lower total energy expenditure.

We studied young men born SGA and age- and BMI-matched AGA controls during 36 h of fasting. A frequently sampled IVGTT was then performed to test the hypothesis that SGA individuals may respond to energy deprivation in a different and potentially more energy-conserving manner than AGA individuals. Furthermore, we hypothesised that improved tolerance to fasting might unmask or influence adverse metabolic and epigenetic changes previously shown to occur in the muscle and adipose tissue of SGA individuals, including disproportionately increased DNA methylation and correspondingly reduced *PPARGC1A* gene expression and muscle glycogen content [12, 13, 15]. The *PPARGC1A* gene influences mitochondrial function and is thought to be involved in the control of both insulin action and energy metabolism [16]. These properties, together with its known aberrant regulation in type 2 diabetes [17, 18] and in SGA individuals [12, 13], suggest that this gene could contribute to a putative altered energy-conserving response to fasting in SGA vs AGA individuals.

Methods

Study population

Thirty-nine young healthy white men born at term (week 39–41) were recruited from the Danish National Birth Register. SGA was defined as a birthweight lower than the 10th percentile, and AGA as the 50th to 75th percentile. Exclusion criteria were diabetes in two generations of the

family, a BMI >30 kg/m², exercise for >10 h/week or the use of medication known to affect metabolism. Written informed consent was obtained from all participants. The study protocol was in accordance with the Helsinki II declaration and approved by the local ethics committee.

Thirty-six hour fasting protocol

Participants received standardised, precooked meals and abstained from exercise, alcohol and soft drinks for 3 days prior to the study (electronic supplementary material (ESM) Fig. 1). During this period, the calorie intake was fixed at 10 MJ/day, and comprised 35% fat, 15% protein and 50% carbohydrate. Participants arrived at the laboratory at 19:30, received a standardised meal and fasted thereafter. Water was allowed ad libitum. Smokers were allowed to use a 10 mg Nicorette inhaler (McNeil, Birkerød, Denmark).

Day 1 Anthropometry was determined and an intravenous cannula was placed in an antecubital vein. Blood samples were drawn at 08:00 and 14:00. All participants spent two 10-min sessions on an ergometer bicycle. A dual energy x-ray absorption scan was performed at 14:30 (Hologic Discovery QDR Series, Bedford, MA, USA). At 20:00, a second intravenous antecubital cannula was placed in the other arm. Participants were instructed to go to sleep at 22:00.

Overnight Blood samples were drawn every 15 min from 20:00 throughout the night until 07:59 the next morning to test for growth hormone (not shown), and hourly to test for glucose, insulin and NEFAs. At 05:00 (33 h fast), a primed infusion of [6,6-²H₂]glucose (4 mg/kg followed by 0.04 mg/kg/min) and [1,1,2,3,3-²H₅]glycerol (0.15 mg/kg followed by 0.01 mg/kg/min) was administered. Tracers had been tested for pyrogenicity and prepared in saline in ampoules at the Department of Pharmacy and Medicines Management, the Ipswich Hospital NHS Trust, Ipswich, UK. Infusions were given for 180 min to allow equilibration with endogenous glucose and glycerol pools, and then blood samples were taken at 5 min intervals from 07:30 to 07:59 hours to determine basal endogenous glucose production and lipolysis. At 06:45, each participant was transferred to the laboratory by wheelchair. Biopsies were obtained between 07:05 and 07:20. Biopsies were obtained from the vastus lateralis muscle and abdominal subcutaneous adipose tissue (SAT) under local anaesthesia using a Bergström needle, snap frozen in liquid nitrogen and stored at −80°C.

Day 2 At 08:00 hours (36 h fast), a bolus of 270 mg/kg naturally abundant and 30 mg/kg [6,6-²H₂]glucose as a 20% solution was administered intravenously over a period of 2 min. Blood samples for measuring plasma glucose, serum insulin and serum C-peptide were collected after 0, 2, 3, 4, 5,

6, 8, 10, 15, 19, 22, 23, 25, 27, 30, 35, 40, 60, 90, 120, 150 and 180 min. Additional samples for plasma NEFA determination were collected at 0, 10, 19, 30 and 180 min. A bolus of 0.02 IU insulin/kg (Actrapid; Novo Nordisk, Bagsværd, Denmark) was administered intravenously at 08:20 hours to improve estimates of insulin sensitivity. For each participant, a hand and forearm was placed in a heated Plexiglas box to arterialise the blood. Indirect calorimetry was performed for 30 min at baseline (12 h fast) and after 31 h and 34 h of fasting to assess energy expenditure (EE) and substrate oxidation rates (Deltatrac monitor; Datex instruments, Baldwin Park, CA, USA). Urine samples were collected after 12 and 36 h of fasting. All samples were stored at -80°C except for those for cholesterol testing, which were analysed the same day.

Biochemical analysis

Blood ketone levels were measured using one-touch, insulin and C-peptide levels were analysed by fluoroimmunoassay and lipids, glucose and carbamide were analysed on a Vitros 5600. HbA_{1c} was measured using high performance liquid chromatography. LDL-cholesterol was calculated as described in the [ESM](#). NEFA levels were measured using a human NEFA kit. Isotopic analysis of glucose and glycerol were performed using GC/MS, see [ESM](#) for further details.

Muscle glycogen content was determined by a fluorometric system, *PPARGC1A* DNA methylation levels were measured using PyroSequencing and gene expression was determined by quantitative real-time PCR as described in further details in the [ESM](#).

Calculations

Total AUC for glucose, insulin and C-peptide was determined using the trapezoid rule. The RQ was calculated as expired CO₂ divided by consumed O₂. Net lipid and glucose oxidation rates were calculated using the non-protein RQ [19]. The first-phase insulin response (FPIR) was calculated as the baseline-adjusted AUC of insulin from 0 to 10 min of the IVGTT, and the Phil index as FPIR divided by the baseline-adjusted glucose response from 0 to 10 min of the IVGTT. Hepatic insulin clearance was calculated by dividing serum C-peptide levels by serum insulin levels from 12 to 36 h of fasting [20]. Basal endogenous glucose production (EGP) was estimated from the quotient of the labelled glucose infusion rate and the tracer:trace ratio. Hepatic insulin resistance was calculated as the product of EGP multiplied by the serum insulin level at 06:00 [21].

The insulin sensitivity index (S_I) and glucose effectiveness (S_G) were calculated from the minimal model using MinMod Millennium v 6.02 (Richard N. Bergman, Los Angeles, CA, USA), an implementation of the minimal model of Bergman [22]. Glucose tolerance (K_G) was determined as the rate

constant for glucose clearance after the insulin bolus, expressed as an exponential function.

Statistical analysis

Paired and unpaired Student's *t* tests were performed to compare single measurements. An unpaired *t* test with Welch's correction and a paired Wilcoxon signed rank test were used to analyse groups with unequal variance.

Development of peripheral insulin resistance was defined a priori as the primary endpoint. We had a power of >90% to detect a 50% difference in S_I between groups with a significance level of 0.05 including at least 14 individuals in each group.

Correlation analyses were performed using the Spearman's rank correlation test. A mixed effects model was used to analyse the longitudinal measurements of plasma and serum, with an appropriate choice of correlation model depending on the data structure, variable numbers and residual variances. In the statistical analysis, all insulin and triacylglycerol data from the fasting period as well as C-peptide and glucose data from the IVGTT period were logarithmically transformed. The reported *p* values indicate the overall differences in effect over time between groups. Since waking up led to large fluctuations in all values measured after 06:00, mixed effects models were only conducted until this time point except for blood ketones and lipids. A *p* value of ≤ 0.05 was considered statistically significant. Analyses were performed using SAS version 9.2 (SAS Institute, Cary, NC, USA) and GraphPad Prism version 5.01 (GraphPad Software, San Diego, CA, USA).

Results

Characteristics of SGA and AGA groups

SGA individuals were lighter at birth ($p < 0.0001$), shorter ($p = 0.01$) and had reduced lean body mass ($p = 0.03$) than AGA individuals. The proportion of fat mass was similar in the two groups. Baseline lipid and glucose profiles did not differ between the groups (Table 1).

Response to 12–36 h of fasting

Indirect calorimetry There were no differences in EE between groups at baseline (Fig. 1a–f). The RQ reached a stable level after 31 h of fasting in the SGA group but decreased continuously in the AGA group, resulting in a tendency towards a lower RQ ($p = 0.055$) after 34 h of fasting in the AGA group. This difference resulted from a significantly higher fat oxidation rate (Fig. 1d) in AGA individuals after a 34 h fast

Table 1 Clinical characteristics of the study participants

Characteristic	AGA (n=18)	SGA (n=21)	p value
Birthweight (g)	3643±185	2811 (205)	<0.0001
Gestational age (weeks)	39.9±0.7	40±0.9	0.83
Age (years)	24.6±1.2	24.8±1.4	0.65
Height (cm)	185.3±5.3	180.7±5.7	0.01
Weight (kg)	78.5±10.7	73.5±10	0.14
BMI (kg/m ²)	22.9±3.2	22.5±2.6	0.65
Waist–hip ratio	0.89±0.05	0.9±0.07	0.76
Truncal fat (kg)	5.5±2.6	5.6±2.6	0.90
Truncal fat (%)	15.3±5.2	16.6±5.8	0.46
Subtotal fat (kg)	12.4±5.4	12.2±5.1	0.90
Subtotal fat (%)	16.3±5.1	17.1±5.2	0.61
Subtotal lean tissue (kg)	59.2±6.7	54.6±5.8	0.03
Subtotal lean tissue (%)	80.6±4.8	79.7±5.0	0.58
HbA _{1c} (%)	5.1±0.2	5.1±0.3	0.87
HbA _{1c} (mmol/mol)	31.99±2.43	32.13±2.81	0.87
Total cholesterol (mmol/l)	4.3±0.7	4.0±0.6	0.20
HDL-cholesterol (mmol/l)	1.13±0.27	1.11±0.23	0.80
LDL-cholesterol (mmol/l)	2.7±0.6	2.5±0.6	0.28
Triacylglycerol (mmol/l)	1.04±0.34	0.95±0.24	0.35
NEFA (μmol/l)	220±95	190±68	0.26
Plasma glucose (mmol/l)	4.8±0.4	4.7±0.3	0.41
Serum insulin (pmol/l)	23.3±13.8	25.6±10.1	0.55
Serum C-peptide (pmol/l)	440.8±156.3	441.9±122.5	0.98

Data are presented as means ± SD

Subtotal, without the head; lean, without fat and bone. Lipids, plasma glucose, serum insulin and serum C-peptides were measured after 12 h of fasting subsequent to 3 days of standardised meals. HbA_{1c} was measured before the standardisation period

($p=0.02$). There was no difference in glucose oxidation at any time point between the two groups (Fig. 1c).

From 12 to 31 h of fasting, the EE decreased slightly in SGA individuals; in contrast, it increased significantly ($p=0.02$) in AGA individuals (Fig. 1b). Therefore, SGA individuals had a lower EE after 31 h ($p=0.02$) and 34-h fast ($p=0.03$) compared with AGA individuals. This was related to a reduced amount of exhaled CO₂ in the SGA group ($p=0.0004$; Fig. 1e) and increased O₂ consumption in the AGA group ($p=0.005$; Fig. 1f).

Plasma and serum samples Plasma glucose and serum C-peptide levels decreased equally during fasting in both groups ($p=0.72$ and $p=0.21$ for the effect over time; Fig. 2a, c). There was a significant difference in the change in serum insulin concentrations over time (12–34 h fasting) between the two groups ($p=0.05$), with a 42% reduction in the AGA group compared with a 60% reduction in the SGA group (Fig. 2b). A borderline significant difference in hepatic insulin clearance over time between the two groups was

observed ($p=0.07$; Fig. 2d). Baseline-adjusted AUC of hepatic insulin clearance from 12 to 34 h of fasting was significantly higher in the SGA group (116±85) than in the AGA group (−5.96±230; $p=0.045$).

During the fasting period, plasma NEFA concentrations increased significantly ($p<0.001$) and to the same extent (effect over time $p=0.53$) in both groups (Fig. 3a). Levels of total cholesterol (Fig. 3b) increased from 12 to 24 h of fasting in the AGA group only ($p<0.05$), but no difference in effect over time was found between the groups from 12 to 36 h of fasting ($p=0.47$). Plasma HDL-cholesterol and LDL-cholesterol (Fig. 3c, d) levels increased slightly but significantly from 12 to 24 h of fasting in both groups ($p<0.04$ and $p=0.001$), and decreased again during sleep, but with no differences in effect over time between the groups (HDL-cholesterol $p=0.46$, LDL-cholesterol $p=0.42$). Plasma triacylglycerol levels (Fig. 3e) decreased steeply from 12 to 24 h of fasting in both groups (AGA $p=0.0003$, SGA $p<0.0001$), but increased again in the morning. The SGA group did not return to the baseline level, resulting in a significantly lower triacylglycerol level after 36 h of fasting compared with the AGA group ($p=0.02$); in fact, the triacylglycerol level was significantly lower after 36 h than after 12 h ($p=0.008$). There was no difference in the effect of time between groups ($p=0.11$). Blood ketone levels (Fig. 3f) increased ($p<0.0001$) from 12 to 36 h of fasting in both groups, to a similar extent ($p=0.19$).

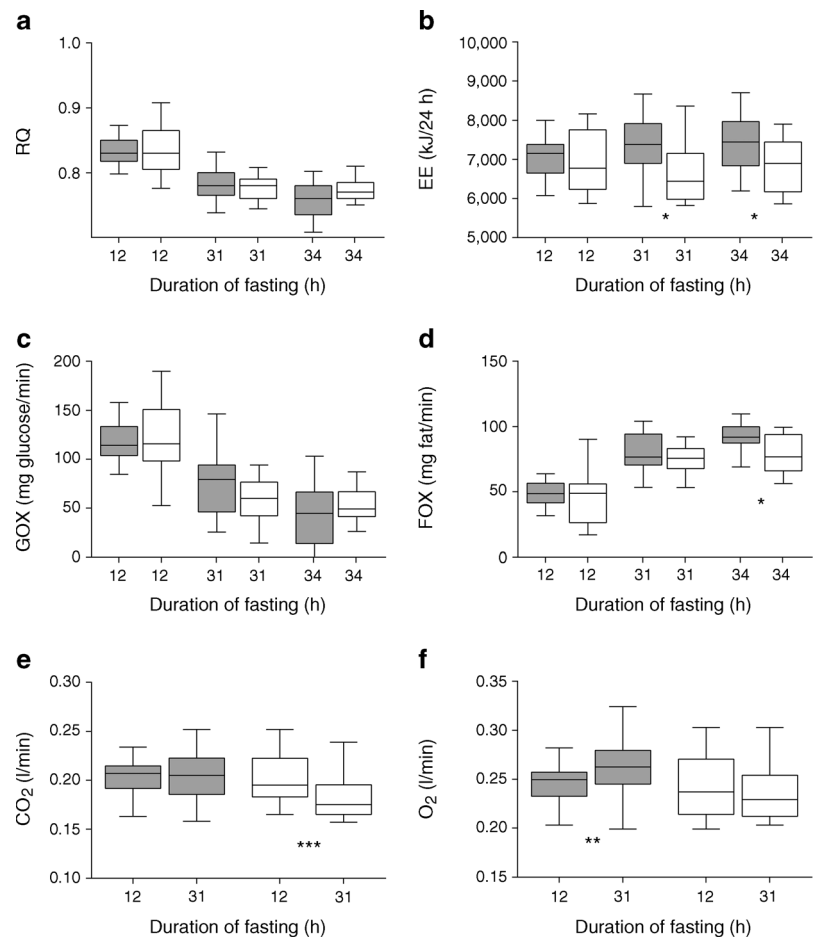
Stable isotopes The absolute rate of EGP, expressed as the rate of appearance (R_a) of glucose was lower in SGA individuals than in AGA individuals after 36 h of fasting ($p=0.046$; Table 2). However, no difference in EGP was found between groups when expressed in relation to total or lean body mass (Table 2). There was no difference between the groups in lipolysis expressed as R_a of glycerol ($p=0.95$).

Frequently sampled IVGTT

We observed no differences in plasma glucose, serum insulin or serum C-peptide levels in response to injected insulin during the IVGTT between groups (Fig. 4a–c).

Plasma NEFA levels were suppressed by 56% in the AGA group and by 63% in the SGA group ($p=0.15$) during the first 30 min of the IVGTT; however, 3 h after the glucose bolus plasma NEFA levels had returned to pre-IVGTT levels in both groups (Fig. 4d). Blood ketone levels were approximately halved in both groups after the IVGTT (Fig. 4e). There was no difference between the two groups in effect over time for any of the above-mentioned variables, and the calculated FPIR and Phil values did not differ (Table 2).

Fig. 1 (a) RQ, (b) EE, (c) glucose oxidation (GOX), (d) fat oxidation (FOX), (e) CO₂ excretion and (f) O₂ consumption from 12 to 31 h of fasting. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Grey boxes, AGA group; white boxes, SGA group. Bars represent 90th percentiles



Stable isotopes S_G estimated using the glucose tracer (hot S_G) was lower in the SGA group than the AGA group after 36 h of fasting ($p = 0.05$). In contrast, S_G estimated from glucose and

insulin levels during the IVGTT (cold S_G) did not differ ($p = 0.83$; Table 2). There was no difference in S_I or K_G values between groups (Table 2).

Fig. 2 (a) Glucose, (b) insulin, (c) C-peptide and (d) hepatic insulin clearance after 12–36 h of fasting. Changes during the fasting period within groups were analysed using paired t tests; differences between time points were analysed using unpaired t tests and differences in changes over time were determined using a mixed effects model. Solid line, AGA group; dotted line, SGA group. Data are presented as means \pm SEM

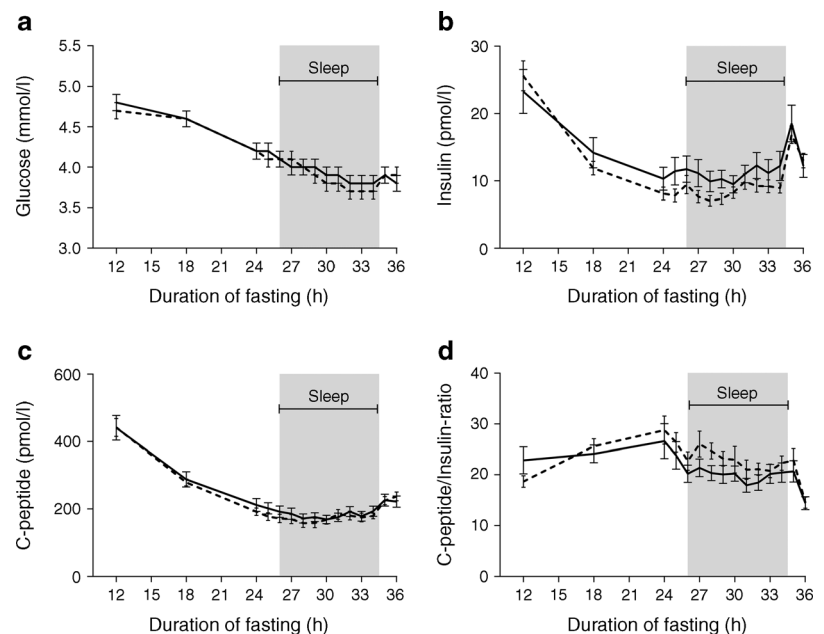
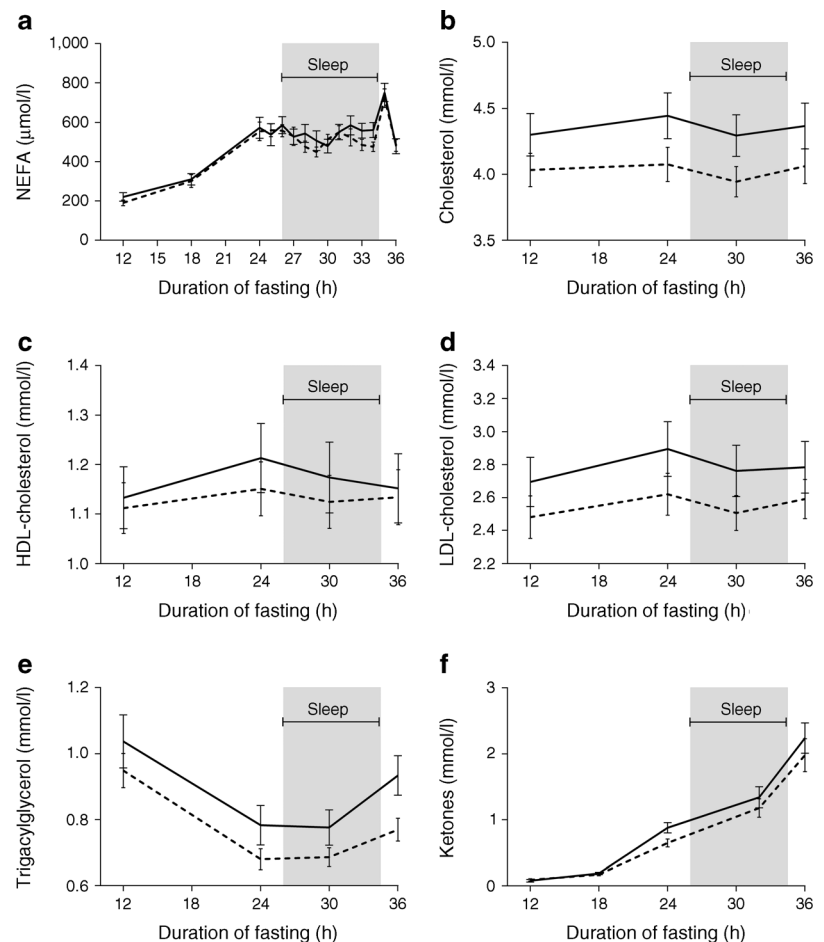


Fig. 3 Lipid and ketone levels after 12 to 36 h of fasting. **(a)** NEFA, **(b)** cholesterol, **(c)** HDL-cholesterol, **(d)** LDL-cholesterol, **(e)** triacylglycerol and **(f)** ketones. Changes during the fasting period within groups were analysed using paired *t* tests, differences between time points were analysed using unpaired *t* tests, and differences in changes over time were determined using a mixed effects model. Solid line, AGA group; dotted line, SGA group; data are presented as means \pm SEM



Muscle glycogen content

No difference in muscle glycogen content was found after fasting between SGA (535 ± 142 nmol glucose/mg) and AGA individuals (488 ± 98 nmol glucose/mg; $p=0.27$; ESM Fig. 2).

PPARGC1A DNA methylation and gene expression

The degree of muscle *PPARGC1A* DNA methylation was higher (4.5%) in SGA individuals than in AGA individuals after fasting at two out of the three CpG sites investigated (CpG-816, $p=0.03$; CpG-783, $p=0.02$; Fig. 5a). The average

Table 2 Measurements of glucose and insulin metabolism in AGA and SGA individuals

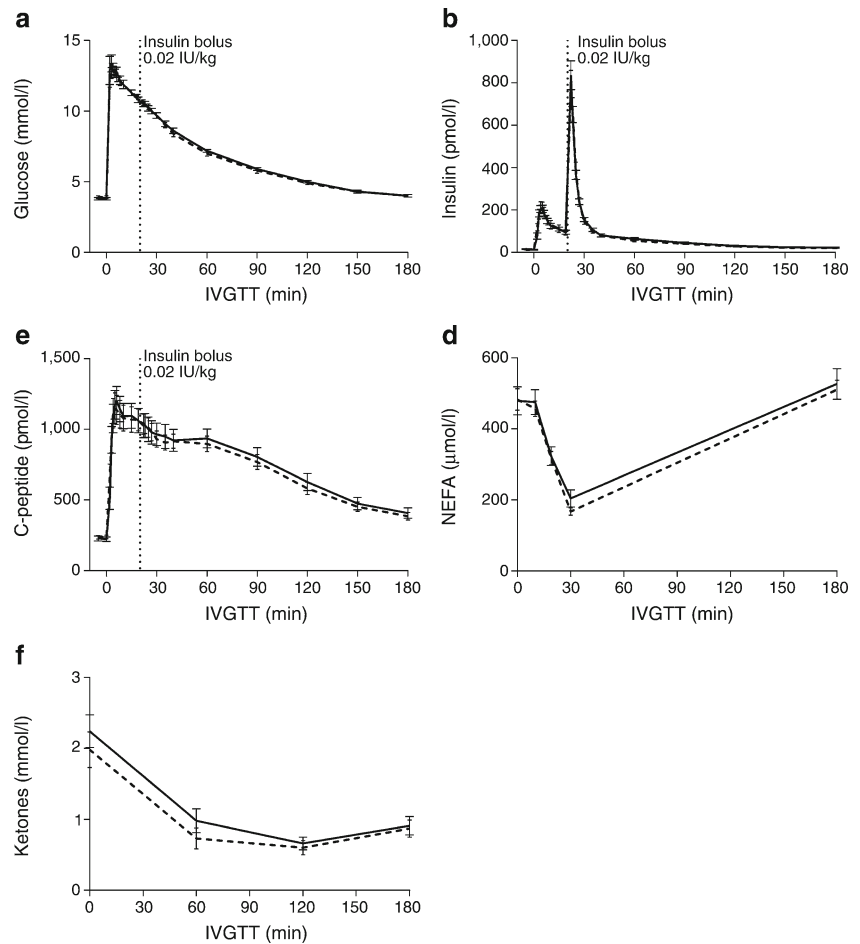
Measurement	AGA ($n=18$)	SGA ($n=21$)	<i>p</i> value
R_a Glucose (mg min^{-1})	113.4 ± 12.4	104.8 ± 13.3	0.046
R_a Glucose ($\text{mg min}^{-1} \text{kg}^{-1}$)	1.45 ± 0.14	1.45 ± 0.17	0.94
R_a Glycerol (mmol h^{-1})	21.44 ± 12.2	21.22 ± 10.0	0.95
R_a Glycerol ($\mu\text{mol kg}^{-1} \text{min}^{-1}$)	4.77 ± 3.16	5.03 ± 2.70	0.79
FPIR	1297.7 ± 433.2	1374.2 ± 816.8	0.73
Phi1	16.8 ± 6.1	17.6 ± 9.3	0.76
Hepatic IR	1368.6 ± 1037.3	929.7 ± 355.9	0.11
S_G [hot] (h^{-1})	0.491 ± 0.186	0.367 ± 0.199	0.05
S_G [cold] (h^{-1})	0.676 ± 0.357	0.702 ± 0.396	0.83
S_I [hot] ($\text{pM}^{-1} \text{h}^{-1}$)	0.0021 ± 0.0029	0.0033 ± 0.0034	0.27
S_I [cold] ($\text{pM}^{-1} \text{h}^{-1}$)	0.0087 ± 0.0156	0.0022 ± 0.0032	0.10
K_G [hot] (min^{-1})	0.631 ± 0.10	0.625 ± 0.09	0.82
K_G [cold] (min^{-1})	0.55 ± 0.06	0.57 ± 0.07	0.40

Data are means \pm SD

Hot, with tracer kinetics; cold, without tracer kinetics; Phi1 = $\text{AUC}_{\text{glucose}0-10 \text{ min}} / \text{FPIR}$

IR, insulin resistance

Fig. 4 (a) Glucose, (b) insulin, (c) C-peptide, (d) NEFA and (e) ketone levels during the IVGTT. An i.v. bolus of insulin was given after 20 min. Solid line, AGA group; dotted line, SGA group; data are presented as means \pm SEM



extent of DNA methylation across the three sites was also significantly higher in SGA individuals ($p=0.05$; Fig. 5a). The degree of *PPARGC1A* DNA methylation in SAT did not differ between groups at any of the CpG sites studied (Fig. 5b). *PPARGC1A* expression levels were not different in muscle ($p=0.82$) or SAT ($p=0.99$; Fig. 5C + D), and we did not observe any significant associations between *PPARGC1A* expression levels and average degree of DNA methylation across the three CpG sites in either muscle ($p=0.16$) or SAT ($p=0.58$; ESM Fig. 3).

Discussion

In the current study, SGA and AGA individuals displayed a similar degree of insulin resistance, as determined by minimal modelling of IVGTT data after fasting. However, SGA individuals showed a more pronounced decline in serum insulin and plasma triacylglycerol levels during 36 h of fasting. Interestingly, SGA individuals showed a significantly greater decrease in the total rate of EE during fasting compared with AGA controls.

A number of studies have confirmed an association between SGA and an increased risk of type 2 diabetes [23–26], as well as the presence of multiple metabolic abnormalities

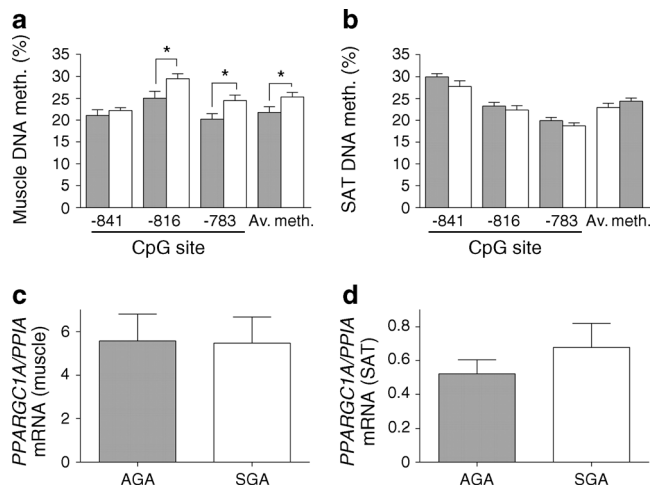


Fig. 5 Effect of birthweight on *PPARGC1A* DNA methylation in (a) skeletal muscle tissue and (b) SAT, and on *PPARGC1A* gene expression levels in (c) skeletal muscle ($n=18$ SGA individuals, 16 AGA individuals) and (d) SAT ($n=20$ SGA individuals, 13 AGA individuals). $*p<0.05$. Grey bars, AGA group; white bars, SGA group; data are presented as means \pm SEM. Av., average; meth., methylation

that can precede diabetes, including hepatic and peripheral insulin resistance in healthy SGA individuals [1]. However, we are unaware of any human experimental studies that have provided evidence to support the thrifty phenotype hypothesis, which claims that SGA is detrimental during conditions of affluent living conditions and potentially beneficial during sparse living conditions. Indeed, our finding that energy deprivation is associated with differential energy saving metabolic responses including lower EE, as well as a tendency towards lower glucose effectiveness in SGA individuals is in accordance with the thrifty phenotype hypothesis.

In contrast to our *a priori* hypothesis, we did not find any differential response in SGA individuals regarding whole body S_I . That is, both study groups responded to 36 h of fasting by developing severe insulin resistance approaching the detection limit for whole body S_I . The finding of a steeper decline in serum insulin and lower plasma triacylglycerol levels during fasting in SGA individuals than in AGA individuals, with no difference in S_I , should be seen in the context of previous consistent reports that SGA is associated with increased fasting insulin levels [2], insulin resistance [3, 7, 9] and elevated plasma triacylglycerol levels after an 8–10 h overnight fast [4]. Whether the observed alterations during prolonged fasting in our study are associated with beneficial changes in other cardiometabolic risk factors is currently unknown.

In line with the lower serum insulin and triacylglycerol levels in SGA individuals during fasting, the calculated hepatic insulin resistance value tended to be lower after 36 h fasting in the SGA group. This contrasts with the otherwise consistent finding of increased hepatic insulin resistance in SGA individuals after an 8 h overnight fast [7]. However, the exact role of insulin vs other metabolic and hormonal substances in controlling hepatic glucose production during prolonged fasting is unclear, and the importance of estimated hepatic insulin action may be questioned in this metabolic condition. Studies have shown that SGA individuals, despite commonly having slightly elevated fasting insulin levels, display a disproportionate reduction in insulin secretion in relation to their ambient degree of peripheral insulin action [8]. Furthermore, animal studies have consistently shown that fetal energy deprivation is associated with impaired pancreatic development and reduced postnatal pancreatic insulin secretion [27]. Accordingly, our finding of lower serum insulin levels in SGA individuals during fasting conditions could be a reflection of their primary reduction in basal pancreatic insulin secretion. However, the reduced insulin levels in SGA individuals could also be caused by an increased ability to extract insulin in the liver during fasting, when assessed as the ratio between serum C-peptide and insulin levels. However, this needs to be documented using more direct assessments of *in vivo* insulin clearance rates. A similar FPIR was found during the IVGTT, suggesting that the

capability of pancreatic beta cells to sense and respond to glucose is conserved in SGA individuals.

Several lines of evidence suggest that the plasma triacylglycerol level is an independent risk factor for cardiovascular disease in people with and without diabetes [28]. Hepatic VLDL production represents an important source of fasting plasma triacylglycerols, and hepatic VLDL production is elevated in people with hepatic insulin resistance [29]. Accordingly, it is likely that lower fasting plasma triacylglycerol levels in SGA individuals may be caused by lower hepatic VLDL production associated with a trend towards improved hepatic insulin action.

EE has previously been shown to increase during acute fasting [30], and this has been suggested to result from increased plasma noradrenaline levels [31]. The lower nocturnal EE in fasting SGA individuals was associated with a lower lipid oxidation rate. We previously found a disproportionately elevated rate of lipid oxidation in SGA individuals during the night after ingestion of an isocaloric diet including an evening meal [32], as well as after 5 days of high fat overfeeding [7]. Thus, our current finding of a disproportionately lower fat oxidation rate in SGA individuals after 36 h of fasting is another example of a metabolic alteration in SGA individuals that is reversed in response to fasting.

We previously found an elevated rate of whole body and SAT lipolysis in SGA individuals after overnight fasting [9, 32]. Recent studies suggest that individuals born SGA display significant alterations to their SAT functions, of which impaired expandability and capacity to store excess fat represent core metabolic changes [33, 34]. It is likely that the finding of increased lipolysis in SGA individuals after an 8 h overnight fast may be a metabolic manifestation of their impaired capability to accumulate fat, and that “excess fat” is therefore released at an increased rate after an overnight fast. In contrast, upon examination after 36 h of fasting, we did not find an increased rate of whole body lipolysis among SGA compared with AGA individuals. These data support our overall conclusion that SGA individuals may have a preferential metabolic response to long-term fasting.

Interestingly, the lower lipid oxidation rate was not reflected in an increased glucose oxidation rate, as otherwise predicted from the glucose-fatty acid cycle [35]. This may represent another manifestation of a preferential energy-conserving phenotype in SGA individuals with respect to glucose turnover rates. However, it should be mentioned that the RQ is also to some extent influenced by ketogenesis, and that the increased rate of oxygen consumption with no change in carbon dioxide production seen in SGA individuals during fasting could theoretically be explained by an increased rate of ketogenesis, which may not be reflected in any changes in plasma ketone body levels.

The notion of an energy-conserving metabolic state in fasted SGA individuals was further supported by our finding

of reduced S_G reflecting glucose-mediated glucose uptake and suppression of hepatic glucose production during the IVGTT (Table 2).

We previously showed that SGA individuals tend to exhibit lower muscle glycogen levels after 3 months of exercise training compared with healthy AGA individuals [15]. The finding in the present study of similar muscle glycogen levels in SGA and AGA individuals provides some support for the idea that SGA individuals at least do not exhibit a reduced capability to conserve energy during fasting.

Increased *PPARGC1A* DNA methylation has been reported in skeletal muscle, SAT and isolated pancreatic islets from type 2 diabetic patients [13, 36, 37]. We previously reported increased muscle *PPARGC1A* DNA methylation in SGA compared with AGA individuals after overnight fasting, which subsequently disappeared after 5 days of high fat overfeeding because of an increased *PPARGC1A* DNA methylation response to overfeeding in healthy AGA controls [12]. Interestingly, in the present study fasting did not normalise the increased *PPARGC1A* DNA methylation in skeletal muscle biopsies from SGA individuals despite reversal of the dysmetabolic traits. *PPARGC1A* is thought to play a key role in the control of mitochondrion content and function, as well as in the control of energy metabolism and insulin action [18, 38]. To the best of our knowledge, increased *PPARGC1A* methylation represents the most consistent epigenetic alteration in the skeletal muscle of SGA individuals [39]. Transcriptional repression of *PPARGC1A* among SGA individuals during fasting could therefore theoretically explain the decreased mitochondrial EE in SGA participants during fasting. However, in accordance with previous studies of our group [12, 13], no correlation was found between *PPARGC1A* DNA methylation and gene expression during fasting, and *PPARGC1A* mRNA expression was not significantly reduced in SGA individuals. Therefore, alternative mechanisms and/or more data are needed to explain the extent to which differential changes in response to fasting in SGA individuals may be influenced by their altered skeletal muscle *PPARGC1A* methylation levels. The differential response to fasting was not associated with any differences in adipose tissue *PPARGC1A* DNA methylation or mRNA expression between SGA and AGA individuals (Fig. 5).

Taken together, our data provide further support for the thrifty phenotype hypothesis, including a preferential metabolic and energy-conserving phenotype as well as unchanged muscle glycogen content in young adults born SGA compared with AGA individuals when exposed to 36 h of fasting. The extent to which the consistent finding of increased muscle *PPARGC1A* DNA methylation during fasting may contribute mechanistically to the more energy-conserving phenotype of SGA individuals needs further exploration.

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Duality of interest CB, KF, MF and AV are shareholders in Novo Nordisk. All other authors declare that there is no duality of interest associated with their contribution to this manuscript.

Contribution statement SWJ designed and performed the study, researched data, wrote the first draft of the manuscript, and reviewed and edited the manuscript. LB analysed the isotopes and performed the mathematical modeling of the IVGTT data, and contributed to, reviewed and edited the manuscript. CB, DBD and AAV designed the study, contributed to discussions, and reviewed and edited the manuscript. LH collected data, conducted the mixed effects model analysis and analysed the biopsies. LG analysed the biopsies. KF, AT, LH, LG and MF contributed to data interpretation and discussions, and reviewed and edited the manuscript. SWJ had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. All authors, except for LB, who died on 14 May 2014, approved the final version of the manuscript.

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