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Size at birth, morning cortisol and cardiometabolic risk markers in healthy Indian children

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Summary

Objective—Prenatal programming of the hypothalamic-pituitary-adrenal (HPA) axis may link reduced fetal growth with higher adult chronic disease risk. South Asians have a high prevalence of low birth weight and a thin-fat phenotype which is associated with subsequent type 2 diabetes and the metabolic syndrome. Altered HPA activity could be one of the pathological processes underlying this link.

Methods—Plasma morning cortisol and corticosteroid binding globulin (CBG) concentrations were determined in 528 children aged 9.5 years from a prospective birth cohort in India. They had detailed anthropometry at birth, and current measurements of anthropometry, plasma glucose, insulin and lipid concentrations and blood pressure. Insulin resistance (Homeostasis Model Assessment) and insulin secretion (the 30-minute insulin increment) were also assessed.

Results—None of the birth measurements were associated with cortisol concentrations, but both birth weight (P=0.03) and length (P=0.004) were inversely associated with CBG concentrations. Cortisol concentrations were inversely associated with current body mass index (P=0.02), and positively associated with glucose (fasting: P<0.001; 30-minute: P=0.002) concentrations, and systolic blood pressure (P=0.005) but not insulin resistance or the insulin increment.

Conclusion—Higher morning cortisol is associated with higher cardiometabolic risk markers in Indian children. Although cortisol concentrations did not appear to be related to birth size, small size at birth was associated with higher CBG levels, and may be one of the processes by which fetal undernutrition affects adult health. The findings suggest a need for dynamic testing of HPA axis activity (such as measuring stress responses).

Keywords

Cortisol; CBG; birth size; India

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Introduction

Epidemiological studies have shown that low weight at birth, a marker of fetal undernutrition, is associated with a higher risk of central adiposity, adult type 2 diabetes and cardiovascular disease in later life.[1-3] Animal studies[4] and limited human data[5] have suggested that the link between fetal growth retardation and adult non-communicable disease could be explained in part by altered hypothalamic-pituitary-adrenal (HPA) axis activity. Studies in Caucasian populations have reported links between measures of fetal growth and adrenocortical activity in adults as well as children[5,6], and elevated cortisol levels are shown to be associated with the features of metabolic syndrome even in children.[7] However, this mechanism has not been examined systematically in South Asian populations where low birth weight is common and is linked with a high and rising prevalence of cardiometabolic disease.[8] In India, a characteristic thin-fat phenotype: more total and truncal body fat than Caucasians of similar body weight, and lower lean body mass, which is present at birth and persists into adult life appears to predispose to the above diseases.[8] This phenotype itself has been attributed to widespread fetal undernutrition combined with a recent economic transition resulting in enhanced postnatal adiposity.

In a study in Mysore, India, we showed strong positive associations between fasting cortisol concentrations and components of the metabolic syndrome including blood pressure (BP), fasting glucose and triglyceride concentrations, insulin resistance and insulin secretion in 40-60 year old adults, especially in the presence of higher BMI.[9] This study suggested that high HPA axis activity and maintenance of high cortisol levels in the face of higher adiposity may be an underlying cause for the increased cardiovascular risk in south Asian populations.

The Mysore Parthenon birth cohort was established to examine associations of early life factors with later risk of chronic disease.[10] As part of the study, detailed anthropometric measurements were obtained at birth using carefully standardised techniques. Using these measurements we have tested whether lower birth size and higher adiposity at birth are associated with alterations in adrenocortical function at the age of 9.5 years. We also examined the current associations between plasma cortisol concentrations, and anthropometry, adiposity and cardiometabolic risk markers.

Materials and Methods

Between 1997 and 1998, we performed oral glucose tolerance tests (OGTT) in 830 women attending the antenatal clinics of the CSI Holdsworth Memorial Hospital (HMH), Mysore, and meeting our eligibility criteria (gestational age 30±2 weeks, singleton pregnancy, no known history of diabetes, plan to deliver at HMH); 49 women were diagnosed with gestational diabetes mellitus (GDM) using the Carpenter-Coustan criteria.[11] Six-hundred and sixty-three women delivered live, normal babies at HMH. Neonatal anthropometry was performed within 72-hours of birth by one of 4 trained measurers, using standardised techniques, including weight (Seca digital weighing scales, Germany), crown-heel length (Harpenden neonatal stadiometer, CMS instruments, London, UK), and mid-upper arm

(MUAC) circumference (blank tapes and ruler), and subscapular and triceps skinfold thickness measurements (Harpenden callipers, CMS instruments).

Excluding 25 deaths and 8 children with major medical conditions, all available children were measured annually until 5 years of age, and 6 monthly thereafter. Five-hundred and thirty-nine children were available for follow-up at 9.5 years of age (35 offspring of GDM mothers).

Detailed anthropometry was carried out in all children including the measurement of weight (Salter, Tonbridge, Kent, UK), height (Microtoise, CMS instruments), MUAC and waist circumference (anthropometric tape) and triceps and subscapular skinfold thickness measurements (Harpenden callipers, CMS instruments). Percentage body fat (fat%) was measured using bioimpedance (Bodystat, Quadscan 4000, Isle of Mann, UK). Waist-height ratio (waist circumference/ height) was calculated as a measure of central adiposity. Systolic and diastolic BP were measured in the left arm using an automated BP monitor (Dinamap 8100, Criticon, FL, USA), using appropriate-sized cuffs based on the MUAC. Measurers were standardised by regular intra- and inter-observer variation studies. The socio-economic status (SES) of the family was determined by the Standard of Living Index designed by the National Family Health Survey-2.[12] The pubertal stage was classified as the stage of breast development (in girls) or genital development (in boys) using Tanner's method.[13]

Plasma glucose and insulin concentrations were measured in all children from a 2-hour OGTT after an overnight fast. An intravenous cannula was inserted after anaesthetising the skin with EMLA cream™ (AstraZeneca LP, Wilmington, DE, USA). Blood samples were collected immediately after cannulation for fasting plasma glucose and insulin concentrations (between 0800 h and 1100 h), and 30 and 120 minutes after a 1.75 g/kg body weight load of anhydrous glucose in 150 ml of water. Fasting samples were also used for measuring plasma total cholesterol, triglyceride and HDL-cholesterol concentrations. Laboratory assays were carried out at the Diabetes Research Centre, KEM Hospital, Pune, India, whose laboratory is a member of the UK (NEQAS) quality control programme for insulin assays. Plasma glucose and lipid concentrations were measured on an autoanalyzer (Alcyon 300, Abbott laboratories, Abbott Park, IL, USA) by standard enzymatic methods. Insulin was measured using a time-resolved, fluoroimmunoassay (Delfia, Wallac QY, Turku, Finland). The inter-assay coefficients of variation (CV) were 12.5% at <45 pmol/l, 9.6% at 45–90 pmol/l and 4.3% at >90 pmol/l.

Insulin resistance was estimated using the Homeostasis Model Assessment equation (HOMA).[14] Triglycerides-HDL-cholesterol ratio (TG/HDL) has been shown to be a reliable index to identify insulin resistant individuals in both adults and children.[15,16] Considering the biological variability of insulin during early puberty, we calculated TG/HDL as another surrogate marker of insulin resistance. Insulin increment was calculated as a measure of insulin secretion using the formula: (30-min insulin-fasting insulin)/(30-minute glucose).[17]

Cortisol assay

From the stored fasting samples (N=528, stored at −80° C for 2.5 years), total plasma cortisol concentrations were measured by ELISA method (Diagnostic Biochem Canada Inc.,

Ontario, Canada) using Victor Multi label Counter (Wallac Oy. Turku Finland) at the KEM Hospital, Pune, India. The sensitivity of the assay was 11 nmol/l and the inter- and intra-batch CV was <7.7%. Corticosteroid binding globulin (CBG) concentration was measured by Radioimmunoassay (Bioline S.A, Bruxelles, Belgium) using PC-RIA Counter (STRATEC Biomedical Systems AG, Birkenfeld, Germany). The sensitivity of the assay was 0.2 μg/ml and the inter- and intra-batch CV was <8%. Free cortisol index was calculated as a ratio of total cortisol to CBG.

The HMH ethics committee approved the study, and informed written consent was obtained from the parents and assent from the children.

Statistical methods

Offspring body mass index (BMI), skinfold measurements, insulin concentrations, HOMA, insulin increment, cortisol concentrations and free cortisol index had skewed distributions. These variables were log-transformed where required. The association between anthropometry at birth and cortisol measurements, and the association between cortisol and anthropometry and risk factors at 9.5 years were analysed using multiple linear regression. We converted the exposure and outcome variables into standard deviation scores (SDS) for analysis, and reported SD change in outcome per SD change in exposure variable. All analyses were adjusted for gestational age (for birth parameters) or current age (for 9.5 year parameters) and sex. Additional adjustments were made for children's current BMI, pubertal stage, the cortisol sampling time, maternal GDM status and SES where relevant. P values of <0.05 were considered significant.

Data were analysed using SPSS v 18 (SPSS Inc, Chicago, USA).

Results

Table 1 describes the characteristics of the study children at birth and at 9.5 years of age. Newborn male babies were heavier and longer than females. Fifty-five (10.4%) babies were born small-for-gestational age (SGA, birth weight<2500g). At 9.5 years of age, girls were more adipose (skinfolds, fat%), and had significantly higher insulin concentrations, insulin increment, insulin resistance (HOMA) and fasting triglyceride concentrations. Systolic BP was higher in boys. The prevalence of overweight/obesity according to the WHO cut-off[18] was 4.5% (N=24); 22.4% (N=121) of the children were underweight (BMI <-2 SD of the WHO reference). Pubertal staging was done in 510 children. The majority of the children were in the pre-pubertal stage (N=366, 72%); 140 (27%) children were in stage 2 and only 4 girls (1%) were in stage 3 of the pubertal growth.

Median (IQR) cortisol concentrations at 9.5 years were 256.6 nmol/l (191.5, 359.8), CBG concentrations were 54.3 mg/l (44.5, 65.4) and free cortisol index was 5.0 nmol/mg (3.4, 7.0). The cortisol measurements were not associated with the children's gender, pubertal stage, SES, religion or maternal GDM status.

Children who were lost to follow-up (N=135) had higher SES as measured at the time of pregnancy (P=0.01) compared to those who were followed up (N=528) at 9.5 years. There

were no differences between those who were followed-up and who were lost to follow up in gestational age, birth measurements, and maternal age, parity, BMI and GDM status.

Size at birth and cortisol measures

Although the cortisol concentrations tended to decrease as measurements at birth increased, (Table 2) the associations with birth weight or birth length were not statistically significant. There was a marginally significant inverse association between triceps skinfold at birth and cortisol concentrations at 9.5 years of age but this did not persist after adjusting for current BMI, pubertal stage, sample time and SES (Table 2). Lower birth weight and shorter length at birth were, however, associated with higher CBG concentrations at 9.5 years of age (Table 2). These associations remained significant after adjusting for the child's current BMI, pubertal stage, sample time and SES. The associations between birth size and cortisol or CBG measurements did not differ by gender.

Cortisol measures and antropometry and adiposity at 9.5 years

Plasma cortisol concentrations were inversely associated with current, weight, height, BMI and MUAC; these associations remained significant after adjusting for age, sex, pubertal staging, SES, maternal GDM status and the sample time (Table 3). There was no association between cortisol concentrations and measurements of adiposity including skinfold thickness, waist circumference, fat% and waist-height ratio. The CBG concentrations were inversely associated with current weight and height, which became non-significant after full adjustment.

Cortisol measures and risk factors at 9.5 years

At 9.5 years, higher plasma cortisol concentrations were associated with higher values for fasting, 30-minute and 120-minute glucose concentrations and lower values for the insulin increment (Table 3). However, the association with 120-minute glucose and insulin increment became non-significant after adjusting for current BMI, pubertal staging, SES, the sample time and maternal GDM status (Table 3). There was a borderline significant positive association between cortisol concentration and systolic BP; this association became statistically significant after full adjustment. There were no associations between cortisol concentrations and HOMA or TG/HDL. There was no significant interaction between cortisol concentrations and BMI for these outcomes (P>0.3, after full adjustment). The free cortisol index paralleled the associations of cortisol concentrations with the risk factors. The children's CBG concentrations were not associated with any of the risk factors at 9.5 years (Table 3).

As reported previously, birth weight was inversely associated with the fasting glucose concentrations (P=0.01 adjusted for age and sex) at 9.5 years.[10] After additionally adjusting for current weight, birth weight was also associated with insulin concentrations at 0-, 30-minutes, HOMA, triglyceride concentrations and systolic and diastolic BP. There was no change in the associations after adjusting for either cortisol or CBG concentrations.

Discussion

We have measured a range of cardiometabolic risk markers as well as cortisol and CBG concentrations in Indian children who have had detailed anthropometry carried out at birth. Although we found current relationships between both cortisol concentrations and free cortisol index and measurements of blood pressure and glucose concentrations, there were few consistent relationships between size or adiposity at birth and these measures of HPA function. We did observe, however, that babies of lower birth weight or who were short at birth had higher levels of CBG.

The Parthenon Study is a large and representative study of children in urban India. The blood samples were taken at varying time points between 8 and 11 in the morning, which is a limitation in our study as is the lack of data on maternal stress during pregnancy. The mean cortisol concentrations in this study were generally lower than those reported for healthy, non-obese children in studies elsewhere.[19-21] Although, the wide variation in the sampling time may have contributed to this (as cortisol concentrations continue to decrease as the day progresses), low cortisol concentrations may be a feature of South Asian populations as they have been observed in studies of adults in Mysore[9] and in the UK.[22] Cortisol concentrations were inversely related to body size (weight, height, BMI, MUAC), a consistent finding in many studies, possibly resulting from increased peripheral metabolism of cortisol due to increased action of 5 α-reductase and impaired re-activation of cortisol by 11β-HSD1 in the liver in adipose individuals.[23] The associations between serum cortisol concentrations and metabolic risk factors in our children parallel findings in Indian adults both in India and the UK.[9,22]

Our main hypothesis was that lower size at birth and higher adiposity in Mysore children would be associated with higher cortisol concentrations. Because of the detailed neonatal measurements available in the Mysore Study, it was possible to examine a wider range of neonatal measurements in relation to subsequent cortisol concentrations than has been available elsewhere. While there were trends of decreasing cortisol concentrations with increasing birth weight and length, the associations were not significant, and contrary to our hypothesis, we did not find any clear associations between measures of fetal body composition (MUAC, subscapular and triceps skinfold thicknesses) and either cortisol or free cortisol index. A number of studies report that low birth weight was associated with elevated fasting cortisol concentrations. A metanalysis of 11 studies in Caucasian populations suggests a modest but significant inverse association between birthweight and cortisol concentrations of 25.3 nmol/l per kg.[24] However, subsequent studies have shown associations between low birth weight and a heightened stress response[6] rather than the underlying non-stimulated cortisol profile[25] suggesting that the fasting cortisol associations described previously reflect a stress response resulting from the combination of fasting, venepuncture and the novel clinic setting in which the blood samples were obtained. It is not clear, therefore, whether the absence of birth weight and cortisol associations in our study is due to differences in study technique or results from racial differences in fetal HPA programming, and suggests the need for more detailed studies particularly evaluating the dynamic response of the HPA axis.

An unexpected finding in this study was an association between low weight or shortness at birth and raised CBG concentrations, which has not been previously described in human studies, although a recent small study in Caucasians reported no difference in CBG concentrations between SGA and appropriate-for-gestational age (N=35 each) individuals at 20 years of age.[26] Nevertheless, this finding is biologically plausible as it is known that CBG levels in the adult are imprinted by the neonatal hormonal environment as it has been shown that neonatal exposure to androgens is a determinant of the gender differences in CBG levels in rats.[27] CBG is present in the fetal liver and its expression is regulated by fetal exposure to cortisol. In sheep, prenatal infusions of cortisol increase CBG mRNA in the fetal liver.[28] Thus it is possible that fetal growth restriction and the resulting fetal stress could affect CBG levels in the adult. However, it is not clear whether fetally induced changes in CBG could persist in to adult life as studies in animal models are few and somewhat contradictory. Klemcke et al reported that low birth weight in pigs following unilateral hysterectomy resulted in a 75% increase in CBG binding in the low birth weight piglets at three days of age compared with the larger controls.[29] In another porcine model based on maternal restraint stress, Kanitz et al were able to show that the prenatally stressed piglets had raised CBG levels on the third postnatal day.[30] However, subsequent studies by the same group found that piglets exposed to maternal hypercortisolaemia had lower CBG levels on postnatal day 1 but higher levels in male offspring on day 28 as compared with the untreated controls.[31] Yet another porcine model based on rough handling or ACTH administration between days 42 to 77 of gestation produced no change in CBG concentrations in the offspring.[32] However comparisons of these studies and extrapolation to human populations are difficult because of differences in the nature and timing of the stressors.

The significance of the changes in CBG concentrations is not clear. As higher CBG implies lower free, physiologically active cortisol, our findings weigh against our initial hypothesis. The principle function of CBG is the high affinity binding and transport of cortisol and raised CBG concentrations would tend to blunt stress-induced peaks in cortisol concentrations. However, CBG may have a number of secondary roles. These include actively delivering cortisol to target tissues especially at sites of inflammation where activated neutrophils secrete serine proteases which cleave CBG.[33] Higher CBG levels also appear to reduce the rate of cortisol clearance.[34] Finally, CBG may have a dynamic role in regulating cortisol responses after stress. Recently, it was observed in rats that acute stress induced rapid elevation of circulating CBG levels ahead of the free corticosterone concentration in blood.[35] The authors suggested that pre-release of CBG delays the rise in circulating free hormone levels/ action. Though our findings are consistent with a stress-induced rise in CBG consequent upon blood taking which is more marked in children born small, a corresponding rise in cortisol concentrations were not observed. These considerations based on animal models are speculative, and reinforce the need for dynamic studies of the glucocorticoid and CBG responses to stress using salivary cortisol in this population.

In conclusion, our study shows no associations between birth size and cortisol concentrations in childhood but demonstrates that physiological variations in HPA axis

activity may be associated with higher levels of cardiovascular risk markers even in non-obese, pre-pubertal children in India.

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Table 1
Characteristics of the study participants at birth and at 9.5 years of age

Measurements	N	Boys (N= 250)	Girls (N= 278)	P
Birth				
Gestational age (weeks)	528	39.0(1.6)	39.2(1.6)	0.07
Birth weight (g)	528	2910 (453)	2827 (412)	0.03
SGA (N)	528	19 (7.6%)	36 (12.9%)	0.045
Crown-heel-length (cm)	528	48.9 (2.3)	48.3 (2.2)	0.002
MUAC (cm)	528	10.3 (1.0)	10.3 (1.0)	0.5
Subscapular skinfold (mm)*	528	4.3 (3.9,4.8)	4.4 (3.9,5.0)	0.056
Triceps skinfold (mm)*	528	4.0 (3.5,4.6)	4.2 (3.7,4.8)	0.02
9.5 years				
Anthropometry				
Weight (kg)	528	25.2 (4.3)	25.0 (4.8)	0.6
Height (cm)	528	131.1 (0.3)	130.4 (5.9)	0.1
BMI (kg/m^2)	528	14.6(1.8)	14.6 (2.0)	0.99
MUAC (cm)	528	17.9 (2.0)	18.1 (2.0)	0.3
Waist-height ratio	528	0.41 (0.04)	0.42 (0.04)	0.1
Subscapular skinfold (mm)*	528	6.7 (5.3,8.0)	8.5 (6.4,10.4)	<0.001
Triceps skinfold (mm)*	528	8.4 (6.6,9.9)	10.6 (8.5,13.2)	<0.001
Body Fat (%)	528	24.2(6.1)	30.3 (5.6)	<0.001
Risk factors				
Glucose^0(mmol/L)	528	4.7 (0.4)	4.7 (0.4)	0.1
Glucose^{30} (mmol/L)	516	6.7 (1.3)	6.9 (1.2)	0.08
Glucose^{120} (mmol/L)	504	5.0 (0.9)	5.2 (0.8)	0.04
Insulin^0 (pmol/L)*	528	18.3 (12.0,28.5)	25.7 (18.6,36.0)	<0.001
Insuli^{30} (pmol/L)*	516	197.7 (124.8,343.2)	255.8 (166.8,409.2)	0.001
Insulin^{120} (pmol/L)*	504	82.9 (54.0,138.6)	119.8 (79.5,173.7)	<0.001
Insulin resistance (HOMA)*	528	0.6 (0.4,1.0)	0.9 (0.6,1.3)	<0.001
Insulin increment (pmol/mmol)*	516	31.5 (16.6,45.5)	37.8 (14.1,50.5)	0.001
Systolic blood pressure (mmHg)	528	102.3 (8.6)	100.1 (8.4)	0.003
Diastolic blood pressure (mmHg)	528	58.9 (7.0)	58.1 (6.6)	0.2
Total cholesterol (mmol/L)	528	3.8 (0.6)	3.9 (0.7)	0.2
HDL cholesterol (mmol/L)	528	1.11 (0.22)	1.08(0.21)	0.0503
Triglycerides (mmol/L)	528	0.9 (0.3)	1.0 (0.4)	<0.001
Triglyceride-HDL ratio	528	0.8 (0.4)	1.0(0.5)	<0.001
Cortisol (nmol/L)*	528	274.5 (199.2,367.8)	262.6 (184.3,356.7)	0.3
CBG (mg/L)	528	56.7 (15.2)	55.1 (14.8)	0.2
Free cortisol index (nmol/mg)*	528	5.0 (3.5,7.0)	4.9 (3.4,7.0)	0.7

Values given are mean (SD) or *geometric mean (IQR) P value for the difference between boys and girls was calculated using independent t-tests.

Table 2

Association between measurements at birth and total Cortisol, CBG, and free Cortisol index

Quartiles of birth measurements		N Max	Cortisol (nmol/L)*	CBG (mg/L)	Free cortisol index (nmol/mg)*
Weight (kg)	**<2.60**	131	277.5 (199.5,366.9)	57.3 (15.3)	5.0 (3.6,7.4)
	2.60-2.85	133	275.0 (184.0,386.3)	56.7 (16.5)	5.1 (3.3,7.2)
	2.86-3.14	132	263.4 (191.5,352.3)	55.4 (13.0)	4.9 (3.4,6.8)
	>3.14	132	256.8 (187.6,318.3)	54.1 (14.9)	4.9 (4.9,6.6)
β (95% CI)			**−0.06 (−0.15,0.04)**	**−0.12 (−0.21,−0.03)**	**0.03 (−0.07, 0.12)**
P1			**0.2**	**0.01**	**0.6**
P2			**0.6**	**0.03**	**0.3**
Length (cm)	**<47.5**	128	265.7 (192.6,358.2)	57.7 (15.4)	4.8 (3.3,7.1)
	47.5-48.6	136	281.6 (198.4,388.5)	56.5 (13.2)	5.1 (3.3,7.2)
	48.9-50.0	133	271.8 (188.9,360.3)	55.5 (16.3)	5.1 (3.6,6.9)
	>50.0	131	253.2(184.6,312.3)	53.8 (14.7)	4.9 (3.4,6.8)
β (95% CI)			**−0.05 (−0.13,0.04)**	**−0.13 (−0.21,−0.05)**	**0.03 (−0.05,0.11)**
P1			**0.3**	**0.002**	**0.5**
P2			**0.2**	**0.004**	**0.6**
MUAC (cm)	**<9.7**	133	272.8 (195.9,366.4)	56.5 (15.5)	4.9 (3.3,6.9)
	9.7-10.2	127	274.5 (201.1,376.6)	56.0 (14.7)	5.2 (3.8,7.6)
	10.3-10.9	144	278.3 (187.7,389.6)	54.9 (14.7)	5.1 (3.4,7.2)
	>10.9	124	245.7 (183.7,311.6)	55.9 (15.2)	4.6 (3.4,6.5)
β (95% CI)			**−0.07 (−0.16,0.02)**	**−0.05 (−0.15,0.04)**	**−0.01 (−0.10,0.08)**
P1			**0.1**	**0.2**	**0.8**
P2			**0.3**	**0.3**	**0.9**
Subscapular skinfold (mm)	**<3.9**	112	281.3 (196.2,378.3)	57.8 (15.6)	5.1 (3.4,7.1)
	3.9-4.3	170	271.4 (193.0,367.3)	56.2 (14.2)	4.9 (3.3,7.1)
	4.4-4.9	123	256.3 (181.3,329.7)	55.0 (15.6)	4.9 (3.5,6.9)
	>4.9	123	263.8 (195.1,346.5)	54.32(14.6)	5.1 (3.6,7.0)
β (95% CI)			**−0.06 (−0.15,0.03)**	**−0.09 (−0.18,−0.005)**	**0.02 (−0.07,0.11)**
P1			**0.2**	**0.04**	**0.7**
P2			**0.6**	**0.06**	**0.3**
Triceps skinfold (mm)	**<3.6**	123	290.3 (204.2,387.9)	56.1 (15.2)	5.3 3.6,7.6)
	3.6-4.0	126	274.4 (193.0,376.9)	54.4 (15.0)	5.3 (3.9,7.4)
	4.1-4.7	150	259.8 (195.0,338.3)	58.0 (15.3)	4.7 (3.2,6.5)
	>4.7	129	251.7 (182.4,322.1)	55.0 (14.3)	4.8 (3.2,6.7)
β (95% CI)			**−0.09 (−0.18,−0.003)**	**−0.01 (−0.10,0.08)**	**−0.06 (−0.15,0.03)**
P1			**0.04**	**0.8**	**0.2**
P2			**0.09**	**0.99**	**0.3**

Values given are Mean (SD) or *geometnc mean (IQR) according to quartiles of birth measurements. P values were derived by using exposure and outcome variables as continuous, Standard Deviation Scores (SDS) in linear regression analyses; β represents SDS change in outcome per SDS change in exposure; p^1adjusted for gestational age and sex; P^2 adjusted for gestational age, sex, current BMI, pubertal stage, sample time and socio-economic status

Table 3
Association between total cortisol, CBG and free cortisol index and outcomes at 9.5 years

	Total Cortisol (SDS)			CBG (SDS)			Free cortisol index (SDS)		
SDS	**β(95% CI)**	***P*[1]**	***P*[2]**	**β(95% CI)**	***P*[1]**	***P*[2]**	**β(95% CI)**	***P*[1]**	***P*[2]**
Anthropometry									
Weight	−0.14 (−7.5,−0.06)	<0.001	0.002	−0.10 (−0.18,−0.02)	0.02	0.09	−0.08 (−0.16,0.002)	0.06	0.07
Height	−0.12 (−0.21,−0.03)	0.006	0.02	−0.12 (−0.20,−0.03)	0.008	0.051	−0.04 (−0.12,0.05)	0.4	0.4
BMI	−0.12 (−0.20,−0.04)	0.004	0.02	−0.06 (−0.14,0.02)	0.1	0.3	−0.08 (−0.16,−0.001)	0.049	0.08
MUAC	−0.12 (−0.20,−0.04)	0.005	0.02	−0.07 (−0.16,0.01)	0.09	0.3	−0.07 (−0.16,0.01)	0.1	0.1
Waist circumference	−0.09 (−0.17,−0.003)	0.04	0.2	−0.04 (−0.12,0.05)	0.4	0.97	−0.05 (−0.14,0.04)	0.3	0.4
Subscapular skinfold	−0.03 (−0.11,0.06)	0.6	0.96	−0.06 (−0.14,0.03)	0.2	0.4	0.004 (−0.08,0.09)	0.9	0.8
Triceps skinfold	−0.02 (−0.04,0.008)	0.2	0.3	−0.01 (−0.03,0.02)	0.5	0.9	−0.01 (−0.04,0.01)	0.4	0.3
Waist-height ratio	−0.05 (−0.13,0.04)	0.3	0.8	0.02 (−0.07,0.10)	0.7	0.996	−0.04 (−0.13,0.04)	0.3	0.5
Risk Factors *									
Glucose									
0 minutes	0.25 (0.16,0.33)	<0.001	<0.001	0.08 (−0.006,0.16)	0.07	0.2	0.20 (0.12,0.29)	<0.001	<0.001
30 minutes	0.12 (0.04,0.21)	0.005	0.002	0.02 (−0.07,0.10)	0.7	0.5	0.10 (0.01,0.18)	0.02	0.02
120 minutes	0.10 (0.01,0.19)	0.03	0.07	0.03 (−0.05,0.12)	0.5	0.5	0.06 (−0.03,0.15)	0.2	0.3
Insulin									
0 minutes	0.001 (−0.08,0.09)	0.97	0.4	−0.02 (−0.10,0.06)	0.6	0.8	0.008 (−.08,0.09)	0.9	0.4
30 minutes	−0.06 (−0.15,0.02)	0.1	0.9	−0.03 (−0.12,0.06)	0.5	0.7	−0.05 (−0.13,0.04)	0.3	0.8
120 minutes	0.09 (−0.002,0.17)	0.06	0.06	−0.04 (−0.13,0.05)	0.4	0.1	0.09 (0.0004,0.18)	0.049	0.01
IR-HOMA	0.04 (−0.05,0.12)	0.4	0.1	−0.01 (−0.10,0.07)	0.8	0.9	0.04 (−0.05,0.12)	0.4	0.1
Insulin increment	−0.09 (−0.18,−0.008)	0.03	0.4	−0.03 (−0.12,0.05)	0.4	0.6	−0.07 (−0.16,0.02)	0.1	0.6
SBP	0.08 (−0.003,0.17)	0.06	0.005	−0.01 (−0.10,0.07)	0.8	0.9	0.08 (−0.001,0.17)	0.054	0.007
DBP	0.07 (−0.01,0.16)	0.1	0.09	0.06 (−0.03,0.15)	0.2	0.4	0.05 (−0.04,0.13)	0.3	0.2
Total cholesterol	−0.02 (−0.10,0.07)	0.7	0.8	−0.02 (−0.10,0.07)	0.7	0.7	−0.01 (−0.09,0.08)	0.9	0.8
Triglycerides	0.08 (−0.07,0.16)	0.07	0.4	−0.02 (−0.10,0.07)	0.7	0.6	0.08 (−0.0003,0.17)	0.051	0.06
HDL-cholesterol	−0.001(−0.09,0.08)	0.98	0.8	−0.02 (−0.11,0.06)	0.6	0.5	0.03 (−0.05,0.12)	0.5	0.3
Triglyceride-HDL ratio	0.06 (−0.03,0.14)	0.2	0.3	0.01 (−0.07,0.10)	0.7	0.9	0.04 (−0.05,0.1)	0.4	0.4

SDS: Standard Deviation Score; P values were derived by using exposure and outcome variables as SDS in linear regression analyses; β represents SDS change in outcome per SDS change in exposure; p^1adjusted for age and sex; P^2 adjusted for gestational age, sex, pubertal stage, sample time, socio-economic status and maternal gestational diabetes status.

*Additionally adjusted for child's current BMI.