Antenatal Synthetic Glucocorticoid Exposure at Human Therapeutic Equivalent Doses Predisposes Middle-Age Male Offspring Baboons to an Obese Phenotype That Emerges With Aging

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Abstract

Introduction: Women threatening premature delivery receive synthetic glucocorticoids (sGC) to accelerate fetal lung maturation, reducing neonatal mortality and morbidity. Few investigations have explored potential long-term offspring side effects. We previously reported increased pericardial fat and liver lipids in 10-year-old (human equivalent 40 years) male baboons exposed to 3 antenatal sGC courses. We hypothesized middle-aged sGC male offspring show obesity-related morphometric changes. **Methods:** Pregnant baboons received courses of 2 betamethasone injections ($175 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{d}^{-1}$ intramuscular) at 0.6, 0.64, and 0.68 gestation. At 10 to 12.5 years, we measured morphometrics and serum lipids in 5 sGC-exposed males and 10 age-matched controls. We determined whether morphometric parameters predicted amount of pericardial fat or lipids. Life-course serum lipids were measured in 25 males (7-23 years) providing normal regression formulas to compare sGC baboons' lipid biological and chronological age. **Results:** Birth weights were similar. When studied, sGC-exposed males showed a steeper weight increase from 8 to 12 years and had increased waist and hip circumferences, neck and triceps skinfolds, and total and low-density lipoprotein cholesterol. Triceps skinfold correlated with apical and midventricular pericardial fat thickness, hip and waist circumferences with insulin. **Conclusions:** Triceps skinfold and waist and hip circumferences are useful biomarkers for identifying individuals at risk for obesity and metabolic dysregulation following fetal sGC exposure. Prenatal sGC exposure predisposes male offspring to internal adiposity, greater body size, and increased serum lipids. Results provide further evidence for developmental programming by fetal sGC exposure and call attention to potential emergence of adverse life-course effects.

Keywords

developmental programming, skinfold, betamethasone, nonhuman primate, metabolic syndrome, adiposity, biomarkers

Introduction

Pregnant women at risk of preterm delivery are treated with synthetic glucocorticoids (sGC) to accelerate fetal lung development, thereby reducing prematurity-related neonatal mortality and morbidity.^{1,2} Because of the treatment efficacy and frequent clinical need of sGC, it is important to determine potential long-term, unwanted, persistent offspring side effects. Animal models indicate that exposure to multiple courses of glucocorticoids leads to a decrease in placental transport³ and postnatal alterations in multiple organ systems including adipose issue⁴ and cardiovascular function resulting from developmental programming.^{5,6} Various mediating mechanisms have been proposed,⁷ including altered DNA methylation,⁸ increased oxidative stress,⁹ and reduced stem cell number.¹⁰ However, there are few investigations of long-term effects of

fetal exposure to sGC in nonhuman primate models. We developed a baboon model (*Papio* species) that mimics fetal exposure to courses of sGC equivalent to those administered

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therapeutically in pregnant women. We have used this model to explore long-term consequences and mechanisms of fetal sGC exposure,^{4,11,12} an essential step for development of preventative and therapeutic options.¹¹

In sGC-exposed baboon fetuses, we have reported evidence of increased fetal blood pressure,¹³ altered function of the fetal musculoskeletal insulin signaling pathways,¹⁴ and changes in neuronal cytoskeleton and synapses indicating functional neuronal disturbances.¹⁵ Juvenile sGC-exposed offspring showed differences in cognition and decreased motivation compared to controls (CTR).¹² Recently, we reported magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS) data indicating 150% increase in pericardial fat thickness and 432% increase in liver lipids in adult (10 year old) sGC-exposed male baboons compared to CTR.⁴ These findings suggest the development of dysfunction of lipid handling in sGC males.

Studies from rodents, sheep, and nonhuman primates are in agreement with our findings, with alterations seen in multiple organ systems. However, most animal studies of later life effects of programming by glucocorticoids have been conducted in juveniles or young adults, and few studies have examined effects of sGC therapy beyond young adulthood. Studies have shown increased body weight and adipose tissue in glucocorticoid-exposed juvenile rats,¹⁶ and increased hepatic triglycerides,¹⁷ glucose intolerance,¹⁸ and altered adipose tissue metabolism¹⁹ in young adult rats. Studies over 2 generations in antenatal sGC-exposed juvenile sheep (F1: age 2.5-3.5 years) and their progeny (F2: age 6 months) have reported impaired glucose metabolism with reduced body weight²⁰ as well as increased weight for length and fat mass in females but not males.²¹ Interestingly, one study of the juvenile grandchildren of ewes receiving glucocorticoids during pregnancy showed increased appetite, greater adiposity, impaired insulin secretion, and altered fat metabolism in adipose tissue.²² A nonhuman primate study in vervet monkeys, studied at age 8 to 12 months, found that despite glucocorticoid-exposed juveniles having reduced body weight, they had greater subcutaneous fat, abnormal glucose tolerance, and reduced number of β cells in the pancreas.²³ At age 30 years, human offspring whose mothers had received a single course of betamethasone in pregnancy showed no differences in body weight, blood lipids, or other cardiovascular risk factors; however, they did show signs of insulin resistance.²⁴

Because prevalence of obesity increases with age,^{25,26} peaking in middle age,²⁷ these data from juveniles and young adults suggest the differences between CTR and sGC-exposed groups will increase with age. The increased adiposity and altered metabolism observed in young adults and juveniles imply greater risk for obesity and its comorbidities in middle age. Combined with metabolic data from other animal models, our MRI and MRS male baboon data⁴ point to predisposition to obesity as a consequence of prenatal exposure to sGC. In this study, we examined 10- to 12.5-year-old baboons (human equivalent approximately 40 years; baboon life span in captivity is estimated at an average of 21 years)²⁸ to determine

whether the combination of prenatal programming by sGC administration and aging results in adverse metabolic outcomes. We note that skeletal growth of captive male baboons is complete at 8 years of age.²⁹⁻³¹

We hypothesized that sGC male offspring would demonstrate dysfunctional lipid metabolism compared to CTR. We are also interested in any effects that programming of lipid dysfunction by sGC has on the normal trajectory of development of age-related lipid dysfunction. We measured their external body morphometry in a variety of dimensions and ran a full blood lipid panel to determine whether adult sGC males exhibit signs of increased adiposity and metabolic dysregulation compared to CTR. We also compared the findings with published data from MRI of the heart and MRS⁴ of the liver to identify valuable external biomarkers of adiposity. To compare biological and chronological age in order to estimate whether sGC males age faster than CTR, we calculated age-based regressions for serum lipid concentrations in a cohort of normal life-course control (NLC) male baboons spanning most of the adult life span (7-23 years).

Methods

Ethics

All procedures were approved by the Texas Biomedical Research Institute Institutional Animal Care and Use Committee and conducted in Association for Assessment and Accreditation of Laboratory Animal Care approved facilities. Animals were treated in accordance with the US Animal Welfare Act.

Maternal sGC Treatment

Pregnant baboons received 3 courses of intramuscular (IM) betamethasone phosphate (sGC group, 175 μ g/kg day $\times$ 2 days) or saline (CTR group) at 0.60, 0.64, and 0.68 gestation (pregnancy days 111-112, 118-119, and 125-126; term 180 days). The timing was equivalent to 24, 26, and 28 weeks of human pregnancy. Offspring delivered spontaneously at term. Maternal body weight and offspring body weight were measured at birth on a digital scale. Synthetic glucocorticoid and CTR mothers were similar in age at delivery (CTR: 11.9 $\pm$ 0.45 years, sGC 10.7 $\pm$ 1.35 years, $P = .31$), gestation length (CTR: 180.1 $\pm$ 1.63 days, sGC 183.0 $\pm$ 2.65 days, $P = .35$), and weight at delivery (CTR: 13.5 $\pm$ 0.26 kg, sGC 14.9 $\pm$ 1.12 kg, $P = .20$); offspring birth weights were similar (CTR: 1.02 $\pm$ 0.06 kg, sGC 0.99 $\pm$ 0.02, $P = .72$). Mothers and their offspring were housed together in social groups until weaning at 9 months of age, at which point offspring were housed in age- and sex-matched groups.

Animal Care and Housing

At the time of the full morphometric panel and blood samples, the sGC ($n = 5$) and age-matched CTR ($n = 10$) male baboon offspring were 10 to 12.5 years of age. Body weight data were

Table 1. Definitions of Morphometric Measurements Taken With (A) Tape Measure or (B) Skinfold Calipers.

Measurement (cm)	Method
A	
Body length	Measured from the back of the head at the intersection of the parietal and lambdoidal sutures to the heel of the right foot
Hip circumference	Measured all the way around the hips at the level of the greatest protrusion of the innominate/pelvic bone
Waist circumference	Measured all the way around the waist at the level of the umbilicus
Measurement (mm)	Method
B	
Abdominal skinfold	Measured 1 cm below umbilicus
Bicep skinfold	Measured halfway between elbow and armpit
Chest skinfold	Measured 6 cm above right nipple
Neck skinfold	Measured 2 cm above transition of cervical to thoracic vertebrae on dorsal side
Subscapular skinfold	Measured 1 cm below inferior angle of scapula
Suprailiac skinfold	Measured 1 cm above iliac crest in midaxillary line
Thigh skinfold	Measured halfway between patella and pubis
Triceps skinfold	Measured halfway between elbow and tip of acromion

available for all animals every year from 1 to 12 years of age. We studied an additional 15 NLC male adults at younger and older ages to cover most of the adult life span, ranging in age from 7 to 23 years (roughly equivalent to human ages 22-80 years).²⁸ All animals were housed in single-sex outdoor social groups of 9 to 16 animals. They ate ad libitum Purina Monkey Diet 5LEO (Purina, St Louis, Missouri), containing 14% calories from fat, 19% calories from protein, 66% calories from carbohydrates, mineral, and vitamin additives, and a metabolizable energy content of 2.98 kcal/g.

Blood Sampling

Blood samples were taken in the group cage area within 10 minutes of isolation and tranquilization with 10 mg/kg ketamine IM. We measured serum total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglycerides, insulin, and glucose. We measured whole blood hemoglobin A_{1c} (HgA_{1c}). All assays were performed on an ACE Chemistry Analyzer, except insulin, which was measured on an Immulite 1000 analyzer with kits from Siemens Healthcare Diagnostics (Flanders, New Jersey).

Morphometrics

We measured total body weight with a digital scale. Trained and experienced personnel used a tape measure to assess body length, hip circumference, and waist circumference (Table 1, A). The same personnel used skinfold calipers to measure

skinfolts of the abdomen, biceps, chest, neck, subscapular, suprailiac, thigh, and triceps (Table 1, B). Triplicate measurements were taken and averaged for use as final values. Body mass index (BMI) was calculated using the formula:

$$\text{BMI} = \frac{\text{Weight}}{(\text{Total Body Length})^2},$$

where weight is measured in kilograms and body length in meters. All measurements were taken under sedation by ketamine at the same time blood samples were drawn. Technicians were blinded to the details of the groups from which samples were obtained.

Magnetic Resonance Imaging

We used MRI cardiac and hepatic data from a published study ($n = 5$ sGC, 5 CTR)⁴ to investigate correlations between adiposity measured by MRI and measured by external morphometrics. Briefly, the MRI study was performed with a 3.0 Tesla MR scanner (TIM Trio; Siemens Healthcare, Malvern, Pennsylvania). T1-weighted inversion recovery short axis cardiac images were obtained centered at the midventricular level (echo time (TE/TR/TI: 1.75/934/480 milliseconds). Spatial localized magnetic resonance ¹H spectroscopy (MRS) was performed with regions of interest defined in the right hepatic lobe. Imaging was performed under anesthesia, induced with ketamine hydrochloride (12 mg/kg IM) and maintained with isoflurane (0.8%-1.0%). The CMR42 image analysis package (Circle Cardiovascular, Calgary, Canada) was used for assessment of pericardial fat. The MRS data were fitted using jMRUI software, version 5.2 with Lorentzian shape and the Advanced Magnetic Resonance algorithm to measure concentration of intrahepatic triglycerides.⁴

Statistics

One-tailed *t* tests were performed in SPSS 23 to compare each measurement in CTR versus sGC. A 1-tailed approach was chosen due to the clear, 1-directional nature of the obesity predisposition hypothesis, with sGC expected to exhibit higher values for all measures. Body weight growth from 1 to 7 years of age was studied with quadratic regression, and adult weight gain from 8 to 12 years of age was studied with linear regression (captive male baboons complete skeletal growth at 8 years of age²⁹⁻³¹). Regressions were calculated separately for each individual then compared between sGC and CTR. Pearson correlation determined whether morphometric parameters were associated with serum lipid levels as well as whether they could predict amount of pericardial fat, a known marker of metabolic cardiac health. Linear regression was used to determine whether lipids changed with age and to develop formulas describing the relationships in the NLC baboons. Low-density lipoprotein was the only variable that changed with age and was also different between age-matched CTR and sGC. We therefore used the LDL normal life-course regression formula to calculate

Figure 1. Body weight in controls (CTR; black, $n = 10$) and sGC (gray $n = 5$). A, Top: Mean body weight (kg) growth from ages 1 to 7 years (before completion of skeletal growth) was similar between groups: $\text{Weight} = 1.28 + 1.91 (\text{Age}) + 0.32 (\text{Age}^2)$. B, Bottom: Adult weight gain from ages 8 to 12 years leveled off in CTR: $\text{Weight} = 25.58 + 0.42 (\text{Age})$, while sGC continued to gain weight, $\text{Weight} = 12.61 + 2.03 (\text{Age})$; $P = .03$.

biological ages of the sGC animals and the age-matched CTR using mean LDL of each group to determine whether sGC and CTR differed in biological age. Data are presented as mean $\pm$ standard error of the mean with significance at $P < .05$.

Results

Life-Course Body Weight

Growth in weight from ages 1 to 7 years was similar between groups (Figure 1A), with growth in both groups captured by the formula: $\text{Weight} = 1.28 + 1.91 (\text{Age}) + 0.32 (\text{Age}^2)$. In adulthood from ages 8 to 12 years, sGC continued to gain weight, while CTR body weight leveled off (Figure 1B; $P = .03$). The CTR equation was $\text{Weight} = 25.58 + 0.42 (\text{Age})$, while that for the GC group was $\text{Weight} = 12.61 + 2.03 (\text{Age})$.

Table 2. Adult Morphometric Measurements in CTR ($n = 10$) and sGC ($n = 5$) Males at Mean Age 11 Years.^a

	BMI (kg/m ²)	Weight (kg)	Body Length (cm)	Waist Circumference (cm)	Hip Circumference (cm)	Chest Skinfold (mm)	Bicep Skinfold (mm)	Abdomen Skinfold (mm)	Thigh Skinfold (mm)	Subscapular Skinfold (mm)	Triceps Skinfold (mm)	Neck Skinfold (mm)	Suprailiac Skinfold (mm)
CTR	19.85	29.35	121.53	59.58	51.87	3.57	2.42	2.90	2.08	5.87	2.02	8.08	3.48
SEM	0.86	1.41	1.34	3.27	1.46	0.41	0.15	0.29	0.12	0.46	0.08	0.67	0.45
sGC	21.98	34.81	125.55	72.49	57.81	4.63	2.53	2.70	2.23	7.17	2.33	11.33	4.30
SEM	0.88	2.45	2.47	4.98	3.55	0.43	0.22	0.19	0.16	0.55	0.19	0.78	0.57
P value	.07	.03	.07	.02	.04	.08	.33	.32	.24	.06	.05	.01	.15

Abbreviations: BMI, body mass index; CTR, controls; sGC, synthetic glucocorticoid; SEM, standard error of the mean.
^aData presented as mean $\pm$ SEM.

Table 3. Lipid Measurements in CTR (n = 10) and sGC (n = 5, Except for Insulin and Insulin: Glucose where n = 3).^a

	%H _g A _{1c}	Cholesterol (mg/dL)	LDL (mg/dL)	HDL (mg/dL)	HDL:LDL	Triglycerides (mg/dL)	Glucose (mg/dL)	Insulin (μIU/mL)	Insulin: Glucose
CTR	4.46	80.70	21.40	50.00	2.39	49.00	112.50	32.11	0.28
SEM	0.08	5.37	1.49	4.21	0.20	6.49	5.10	11.12	0.08
sGC	4.66	96.60	28.20	56.40	2.17	61.20	101.60	29.27	0.28
SEM	0.13	4.06	3.31	4.13	0.40	6.48	5.46	6.58	0.06
P value	.10	.04	.02	.18	.29	.13	.10	.45	.49

Abbreviations: CTR, controls; HDL, high-density lipoprotein; LDL, low-density lipoprotein; sGC, synthetic glucocorticoid; SEM, standard error of the mean.

^aMean ± SEM.

Table 4. Life-Course Regressions of Lipid Variables in CTR Males From 7 to 22 Years of Age.

	%H _g A _{1c}	Cholesterol (mg/dL)	LDL (mg/dL)	HDL (mg/dL)	HDL: LDL	Triglycerides (mg/dL)	Glucose (mg/dL)	Insulin (μIU/mL)	Insulin: Glucose
N	0	25	25	25	25	25	25	23	23
Slope	—	0.39	2.47	−0.27	−0.09	−1.51	−0.11	−1.72	−0.01
R	—	0.10	0.65	0.12	0.56	0.39	0.02	0.23	0.23
P	—	.63	.0004	.59	.004	.05	.91	.30	.29

Abbreviations: CTR, controls; HDL, high-density lipoprotein; LDL, low-density lipoprotein; sGC, synthetic glucocorticoid; SEM, standard error of the mean.

Adult Morphometrics

Full adult morphometric data are presented in Table 2. Synthetic glucocorticoid males weighed more than CTR ($P = .03$) at the time the full panel of measurements was taken (mean age 11 years). Higher BMI ($P = .07$) and body length ($P = .07$) values in sGC approached significance. Synthetic glucocorticoid had greater waist circumference ($P = .02$) and hip circumference ($P = .04$) than did CTR. Skinfolts of the neck ($P = .01$) and triceps ($P = .05$) were also larger in sGC, while skinfolts of the chest ($P = .08$), bicep ($P = .33$), abdomen ($P = .32$), thigh ($P = .24$), subscapular ($P = .06$), and suprailiac ($P = .15$) were similar.

Serum Measurements

Compared to CTR, sGC had greater total serum cholesterol ($P = .04$) and LDL ($P = .02$), but the 2 groups were similar in H_gA_{1c} ($P = .10$), glucose ($P = .10$), insulin ($P = .45$), insulin: glucose ratio ($P = .49$), HDL ($P = .18$), HDL:LDL ratio ($P = .29$), and triglycerides ($P = .13$; Table 3).

Life-Course Lipid Changes and Biological Age of Lipid Measures in sGC-Exposed Baboons

In CTR baboons, LDL increased with age ($P = .0004$), while HDL:LDL ratio ($P = .004$) and triglycerides ($P = .05$) decreased with age; total cholesterol, HDL, glucose, insulin, and insulin:glucose ratio did not change across the life course (Table 4).

Since LDL was found to increase with age (Table 4) and was also different between sGC and CTR (Table 3), we used the LDL age-based regression formula ($y = 1.68 + 2.47x$) to

calculate the biological age of the sGC and age-matched CTR males from their mean LDL values. When corrected for the normal regression, sGC males demonstrated to be 2.75 years advanced in biological age compared to age-matched CTR males.

Correlations of Morphometric Variables With Serum Lipids and Cardiac Adiposity

Pearson correlations between morphometric parameters that were different between sGC and CTR are shown in Table 5. Waist ($P = .05$) and hip ($P = .02$) circumferences were associated with insulin levels, and hip circumference was also associated with insulin:glucose ratio ($P = .04$). Hemoglobin A_{1c} and triceps skinfold approached significance ($P = .08$), as did H_gA_{1c} and neck skinfold ($P = .09$), triglycerides and waist circumference ($P = .06$), and insulin: glucose ratio and waist circumference ($P = .10$).

Triceps skinfold correlated with both apical ($P = .002$) and midventricular ($P = .03$) pericardial fat thickness. Hip circumference correlated with amount of apical fat ($P = .02$) better than with midventricular fat ($P = .07$). Neck skinfold correlated better with midventricular pericardial fat thickness ($P = .05$) than apical fat ($P = .14$). There was no association between weight or waist circumference with either apical fat or midventricular fat.

Discussion

Exposure to 3 courses of sGC prenatally results in predisposition to obesity-related complications. Both obesity and high cholesterol are strong risk factors for cardiovascular disease

Table 5. Pearson's Correlation Associations Between Morphometric Measurements That Were Elevated in sGC Over CTR With Serum Lipids and Cardiac Adiposity as Measured by MRI.^a

Variables Measured	n	Weight		Waist		Hips		Triceps Skinfold		Neck Skinfold	
		r	Direction	r	Direction	r	Direction	r	Direction	r	Direction
%H _A I _c	7 CTR, 5 sGC	0.40	NS	0.40	NS	0.31	NS	0.52 [#]	+	0.52 ^d	+
Cholesterol	10 CTR, 5 sGC	0.21	NS	0.29	NS	0.15	NS	0.27	NS	0.22	NS
HDL	10 CTR, 5 sGC	0.17	NS	0.14	NS	0.12	NS	0.28	NS	0.09	NS
LDL	10 CTR, 5 sGC	0.04	NS	0.18	NS	-0.01	NS	-0.06	NS	0.17	NS
HDL: LDL	10 CTR, 5 sGC	0.07	NS	-0.07	NS	0.07	NS	0.33	NS	-0.03	NS
Triglycerides	10 CTR, 5 sGC	0.25	NS	0.49 ^d	+	0.23	NS	0.36	NS	0.35	NS
Glucose	10 CTR, 5 sGC	0.19	NS	0.33	NS	0.20	NS	0.26	NS	0.04	NS
Insulin	9 CTR, 3 sGC	0.44	NS	0.59 ^b	-	0.65 ^b	-	0.10	NS	0.18	NS
Insulin: glucose	9 CTR, 3 sGC	0.35	NS	0.5 ^d	-	0.61 ^b	-	0.05	NS	0.14	NS
Apical fat thickness	5 CTR, 5 sGC	0.48	NS	0.53	NS	0.73 ^b	+	0.84 ^c	+	0.51	NS
Midventricular fat thickness	5 CTR, 5 sGC	0.43	NS	0.55 ^d	+	0.59 ^d	+	0.7 ^b	+	0.64 ^b	+

Abbreviations: CTR, controls; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NS, nonsignificant; +, positive; -, negative; sGC, synthetic glucocorticoid.

^aDirection refers to direction of association between the 2 variables.

^b $P \leq .05$.

^c $P \leq .01$.

^d $P \leq .10$.

and diabetes, among other morbidities.^{32,33} The sGC-exposed males have increased total and LDL cholesterol, body weight, waist and hip circumferences, and triceps and neck skinfold thickness.⁴ The findings of larger external body size and higher blood lipid content in sGC-exposed males confirm earlier studies indicating prenatal sGC treatment at human clinical levels is associated with metabolic dysregulation and obesity in adulthood in rodents,^{18,19} sheep,⁶ and nonhuman primates.^{4,14} Although 3 courses of sGC are not currently recommended by the National Institute of Health,¹ multiple courses of sGC are still common in both the developed and the developing world.

Body weight was increased in sGC males at the time of the study, age 11 years, at the same time as full panels of morphometric measurements and lipids were obtained. Using weight data available every year up to 12 years, quadratic regression showed that body weight growth was similar between groups from ages 1 to 7 years. However, from ages 8 to 12 years (captive male baboons complete skeletal growth at 8 years²⁹⁻³¹), linear regression showed CTR body weight leveled off, while sGC continued to gain weight (Figure 1). Average baboon life span in captivity is estimated at about 21 years.²⁸ Although a linear correlation between baboon and human ages cannot be made, baboons aged 11 years are roughly equivalent to humans aged 45 years.²⁸ Thus, the difference in body size between the 2 groups emerges in middle age. Follow-up at age 30 years in 1 human randomized controlled trial on offspring exposed to sGC showed no evidence of change in body size, blood lipids, blood pressure, prevalence of diabetes, or history of cardiovascular disease compared to placebo, although they did find evidence of insulin resistance.²⁴ The authors of this study recognized that incidence of cardiovascular disease peaks after 30 years of age, so the finding of increased insulin resistance,

even without other differences, suggests an elevated risk of cardiovascular disease and diabetes with further aging in the exposed cohort.²⁴ Our data showing increased body weight, waist and hip circumferences, skinfolds, and blood lipids in early middle age provide support for this hypothesis. The initial hypothesis we proposed of lipid dysregulation in sGC is also strengthened by our prior report of 150% increase in pericardial fat thickness and 432% increase in liver lipids in adult baboons exposed to sGC as fetuses compared to CTR.⁴ Few other human or nonhuman primate studies have conducted follow-up into and beyond middle age. We further hypothesize that the increase in sGC body size compared to CTR is due to increased body fat mass; more studies are needed.

One goal of this study was to identify external biomarkers of pericardial fat deposition and metabolic dysregulation, which can be used to institute interventions directed toward protecting sGC-exposed offspring before major pathophysiology occurs. We found that triceps skinfold serves this function particularly well for cardiac adiposity since it is correlated with both apical and midventricular fat as measured by sophisticated MRI approaches. The other external measures that were different between sGC and CTR correlate with either apical or midventricular fat but not both. Hip and waist circumferences were positively associated with insulin levels. It is valuable to identify simple measurements that can identify individuals at risk of metabolic dysfunction and obesity. Triceps skinfold, waist circumference, and hip circumference are straightforward measurements with the potential for repetitive, noninvasive risk stratification that are easier, quicker, and less expensive than some other alternatives. Triceps skinfold has long been recognized an effective measure of adiposity^{34,35} and is an excellent predictor of percentage body fat in humans, sometimes even outperforming BMI.³⁶⁻³⁸ Waist and hip circumferences are also

known as strong tools for assessing adiposity and cardiovascular disease risk.^{36,39,40} These simple external morphometric measurements are valuable biomarkers for studying development and aging of sGC-exposed individuals.

An important finding of this study is that birth weight alone is not a good indicator of developmental programming. Change in birth weight is sometimes used as a parameter to assess presence of developmental programming, with lack of change in birth weight from control taken as indicating safety of a treatment.^{41,42} Many investigators now acknowledge birth weight as a measurement does not always represent body composition. Our findings support the principle that birth weight alone is a poor predictor of future phenotype and does not clearly indicate the presence or absence of developmental programming.

We have proposed the concept that developmental programming accelerates aging processes.⁴³⁻⁴⁵ Our regression analysis of NLC enabled us to calculate the biological age of sGC-exposed baboons in relation to the regression that represents overall chronological aging of the control cohort. Low-density lipoprotein cholesterol increased with age in NLC. Low-density lipoprotein was also higher in sGC than age-matched CTR. We found preliminary evidence that the LDL values of the sGC-exposed males were indicative of an accelerated aging phenotype by approximately 3 years, approximating somewhat more than a decade in human equivalent adult life.

A limitation of this study is that it only includes males. Female data are also needed. Differences in developmental programming setting of the level of activity of the hypothalamic–pituitary–adrenal axis by exposure to sGC have been shown in male and female offspring^{46,47} as well as intergenerational effects.⁴⁸ Female morphometric and lipid data are best treated separately from males for several reasons. First, baboons exhibit significant body size sexual dimorphism; male baboons are twice the size of females.⁴⁹ Second, female baboons experience reproductive cycling that may contribute to findings and makes normalization of data difficult. In particular, the pronounced perineal swelling exhibited by females around the time of ovulation, among the largest of primates, changes the dimensions and body composition.⁵⁰ Moreover, hormonal changes and pregnancy histories of females make analysis of female data more complex than that of the males. However, research is needed to investigate predisposition to obesity in female sGC-exposed baboons.

Our study used multiple doses of sGC administration. There is currently much discussion regarding the necessity for, and effects of, repeat sGC administration to pregnant women.^{51,52} One leading study of 5-year-old children compared effects of single and multiple courses. It stated, “There was no significant difference between the groups in the risk of death or neurodevelopmental disability.”⁵¹ Although these represent reassuring conclusions based on a relatively large number of infants, there is a need for more controlled animal studies on a comprehensive set of outcomes including assessment of a wide variety of organ systems and using sensitive

approaches that reflect function, such as RNA sequencing, metabolomics, and proteomics.

One of the fundamental principles of programming is absence of outcomes with a clear phenotype early in life followed by emergence of programming outcomes throughout the life course. Thus, studies well into the life course, such as conducted here, are needed. There may also be a need for exposure to “second hits” (such as aging) for abnormal sGC-dependent phenotypes to manifest themselves, resulting in emergence of altered physiology.^{51,52} Another major life-course event that has the potential to change outcomes is puberty, which studies focusing on young children may not consider. The animals in this report were all postpubertal when morphometrics were measured.

Finally, the baboons in our study delivered at term. Prematurity has lifelong consequences on offspring health later in life. The interaction of prematurity and sGC treatment remains to be studied. Additionally, concern has been expressed about the lack of studies of exposed human fetuses who remain in utero.⁵³ Because of the many causes, difficulties in diagnosis, and unpredictable progression of preterm labor, not all women who receive sGC will deliver prematurely. A minority of pregnant women receiving sGC deliver prematurely. One study indicates that as many as over 70% of pregnancies in which sGC are administered do not deliver before 36 weeks and hence the offspring do not benefit from the sGC exposure.⁵² Our findings suggest there is a need to better understand the effects of exposure to sGC during development on the long-term health of these infants.

In summary, while maternal treatment with sGC is successful in reducing neonatal morbidity and mortality following preterm birth,^{1,2} fetal exposure to multiple doses of glucocorticoids has consequences that may emerge and persist in later life. Studies in experimental animals are needed to identify risks and mechanisms at the tissue, cellular, and molecular levels to continue to safely use the treatment while developing preventive treatments and therapies for unwanted outcomes. This study and others demonstrate that prenatal sGC exposure is associated with predisposition to obesity and metabolic dysregulation in nonhuman primates.⁴ Increases in external body size and blood lipids emerge in middle age, potentially raising risk of cardiovascular disease and diabetes. Establishing biomarkers of cardiac health, such as triceps skinfold, will aid in the identification of at-risk individuals.

Authors' Note

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