Prenatal Steroid Administration Leads to Adult Pericardial and Hepatic Steatosis in Male Baboons

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Abstract

Developmental programming studies indicate that glucocorticoids modify fetal development. We hypothesized that administration of the synthetic glucocorticoid (sGC) betamethasone to pregnant baboons at doses and stages of fetal life equivalent to human obstetric practice to decrease premature offspring morbidity and mortality, programs lipid metabolism. In 10-year-old male baboons (human equivalent 40) exposed in fetal life to betamethasone or saline, we quantified pericardial fat and hepatic lipid content with magnetic resonance imaging and spectroscopy. sGC offspring delivered at term as do most sGC exposed human neonates. Pericardial fat thickness (7.7 ± 3.6 mm vs. 3.1 ± 1.1 mm, M ± SD; p = 0.022; n=5) and hepatic fatty acids (13.3 ± 11.0 % vs. 2.5 ± 2.2 %; p = 0.046; n=5) increased following sGC without birth weight or current body morphometric differences. Our results indicate that antenatal sGC therapy caused abnormal fat deposition and adult body composition in mid-life primate offspring. The concern raised is that this degree of pericardial and hepatic lipid accumulation can lead to harmful local lipotoxicity. In summary, developmental programing by sGC produces a mid-life metabolically obese but normal weight phenotype. Prior studies show sexually dimorphic responses to some programming challenges thus female studies are necessary.

Keywords

Developmental programming; antenatal glucocorticoid; adiposity; baboons

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Introduction

The developmental programming hypothesis states that challenges in critical fetal developmental time windows alter developing structure and function of fetal organs, predisposing to several chronic conditions including obesity and cardiovascular disease. Glucocorticoids play a central role in fetal cardiovascular development (1). Women at risk for premature delivery receive synthetic glucocorticoids (sGC) to accelerate fetal lung maturation, reducing perinatal mortality and morbidity in premature babies. Respiratory distress declines from about 70% to 12% (2). However, more women who receive a complete course of sGC deliver after 34 weeks than before by 4:1 (3). Human studies indicate adverse postnatal effects in offspring exposed to antenatal sGC delivering preterm, including cardiovascular (4) and attention problems (5). Given the well-established therapeutic benefits of sGC, information on long-term side effects need to be assessed in relation to cost-benefit. There is a need for more information. Current human studies are limited by focusing on sGC-exposed babies born prematurely, excluding the majority of exposed babies born at term. Multiple animal studies show that fetal exposure to glucocorticoid levels higher than appropriate for current fetal development can program obesity and cardiovascular function (6).

Little information is available on adverse effects of fetal primate exposure to sGC (7). A single course of maternal sGC alters fetal skeletal muscle metabolism (8). Early clinical studies showed that beneficial fetal effects of sGC only lasted a week (2), leading to some women threatening preterm delivery receiving multiple weekly sGC courses. We hypothesized that antenatal betamethasone administration to pregnant baboons in doses equivalent to those administered clinically results in increased adult offspring adiposity. Using magnetic resonance imaging and spectroscopy, we examined the extent of hepatic and pericardial lipid deposition in 10-year-old baboons (human equivalent 40 years) exposed as fetuses to the equivalent of three weekly sGC courses.

Materials and Methods

All procedures were approved by the Texas Biomedical Research Institute IACUC and conducted in AAALACi approved facilities.

Animal Model

Non-pregnant, female baboons (*Papio species*) of uniform phenotype were randomized into two groups (5 per group) and bred to the same male. Pregnant baboons were given intramuscular betamethasone phosphate (sGC; Celestan; Essex Pharma, Munich, Germany), two injections of 175 μg/kg spaced by 24h) or saline (CTL) at weekly intervals at 0.60, 0.64, and 0.68 gestation (pregnancy day 111/112, 118/119, and 125/126), equivalent to 24, 26, and 28 weeks of human pregnancy. This dose represents the human clinical dose, weight-adjusted for a 70kg woman. Offspring delivered spontaneously at term and were housed with their mothers until weaned and reared in juvenile group cages. Feed was Monkey Diet 5038 (Purina LabDiets, St Louis, MO) containing 13% calories from fat, 18% calories from protein, 69% calories from carbohydrates, mineral and vitamin additives, and metabolizable energy content of 3.22 kcal/g.

Morphometric Measurements, Magnetic Resonance Imaging, and Spectroscopy

Morphometric measurements and MRI were performed on two groups of baboons: sGC (5 males, age = 10.5 ± 0.7 yr; mean $\pm$ SD) and age-matched control baboons (CTL, 5 males, age = 10.4 ± 0.4 yr. Morphometric evaluation included measurement of body length, crown-rump length, chest circumference, waist circumference, and hip circumference. Body mass index was calculated using the standard formula

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

weight (kilograms), and body length (meters). MRI was performed using a 3.0 Tesla MR scanner (TIM Trio, Siemens Healthcare, Malvern, PA). T1 weighted inversion recovery short axis cardiac images were obtained centered at the midventricular level (TE/TR/TI 1.75/934/480 ms). Spatial localized magnetic resonance ^{1}H spectroscopy (MRS) was performed with regions of interest defined in the right hepatic lobe, avoiding visible portal bundles. All studies were conducted between 9 and 11 am after overnight fast. Anesthesia was induced with ketamine hydrochloride (12 mg/kg, i.m.) and maintained with isoflurane (0.8–1.0%).

Image Processing and Analysis

The CMR42 image analysis package (Circle Cardiovascular, Calgary, AB) was used for assessment of pericardial fat. Using short axis images, thickness of pericardial fat was measured at the cardiac mid-ventricular level anteriorly. The MRS data were fitted using jMRUI software with Lorentzian shape and the Advanced Magnetic Resonance (AMARES) algorithm to measure concentration of intrahepatic triglycerides.

Data were analyzed using GraphPad Prism 7. Grubbs' test (extreme Studentized deviate) was used to determine outliers. Normal distribution was assessed by the d'Agostino-Pearson test. Student's t test and Pearson's correlation were performed. Data are presented as mean $\pm$ SD, N = 5 per group; significance at $p < 0.05$.

Results

Birth weight (CTL 1.0 ± 0.07 kg, sGC 1.0 ± 0.05 kg, M $\pm$ SD) and body weight at MRI (34 $\pm$ 3.1 kg, 33 $\pm$ 4.0 kg) were similar between groups. Body mass index (22 ± 2.2, 22 ± 2.2), whole body length (124 ± 5.3 cm, 122 ± 4.3 cm), crown-rump length (74 ± 4.9 cm, 73 ± 3.6 cm), chest circumference (68 ± 6.8 cm, 67 ± 2.8 cm), waist circumference (62 ± 9.4 cm, 62 ± 8.5 cm), and hip circumference (54 ± 3.7 cm, 51 ± 3.6 cm) were similar.

Mid-ventricular pericardial fat thickness was 148% higher in sGC (7.7 ± 3.6 mm, M $\pm$ SD) vs. CTL (3.1 ± 1.1 mm, $p = 0.022$), Figure 1. Hepatic parenchymal lipid content increased 432% in sGC (13.3 ± 11.0 %) vs. CTL (2.5 ± 2.2 %, $p = 0.046$), Figure 2. Anterior pericardial fat and intrahepatic lipid content correlated positively ($r = 0.66$, $p < 0.05$).

Discussion

We demonstrated increased pericardial and hepatic fat deposition in baboons exposed to prenatal sGC without difference in birth weight, current body weight, or other body measurements, indicating programming of adiposity. Our results represent a major concern and indicate long-term alteration in lipid metabolism without discernible change in external phenotype following fetal sGC exposure at a clinical equivalent weight-adjusted dose. Though not NIH recommended, multiple courses are often given to women threatening preterm delivery not delivering within a week or as rescue therapy in late pregnancy.

Our finding of sGC induced pericardial and hepatic steatosis confirmed rodent studies showing metabolic dysregulation. Administration of dexamethasone in late gestation causes glucose intolerance (9) and alters GC receptor expression in hepatic and visceral fat (10) in adult rat offspring. Increased intra-abdominal fat deposition has been documented in prenatal dexamethasone exposed rats with concurrent serum leptin increase (11). Our findings suggest similar abnormal adult primate fat deposition.

Besides implicating metabolic derangement, increased hepatic and pericardial fat deposition raise cardiovascular concerns. The Framingham Heart Study reported association between pericardial adiposity and coronary artery calcification, thought to reflect local toxic pericardial fat effects on adjacent vasculature (12). Likewise, the Multi-Ethnic Study of Atherosclerosis (MESA) concluded that pericardial fat predicts coronary heart disease incidence independently of conventional risk factors (13). Hepatic steatosis is the first sign of nonalcoholic fatty liver disease affecting up to one third of Americans (14). Excess hepatic lipid deposition leads to oxidative stress, mitochondrial dysfunction, and hepatic lipid peroxidation, potentiating nonalcoholic steatohepatitis, presaging end-stage liver disease.

Our finding of unchanged birth weight is noteworthy because it suggests the lack of change in birth weight alone should not be considered evidence for lack of adverse programming. Studies on asthmatic pregnant mothers (15) and fetuses suspected of congenital adrenal hyperplasia (16) revealed no decrease in birth weight with antenatal glucocorticoid treatment, interpreted as an indicator of treatment safety. Further, our findings of unchanged birth weight or current weight indicate a potentially large at-risk population. Our results agree with the Auckland Steroid Trial, which reported insulin resistance but not birth weight and current weight difference in adult offspring who received antenatal betamethasone treatment (17). The concurrent increase in hepatic and pericardial fat deposition and unchanged body weight are consistent with alteration in body composition. We postulate prenatal programming may contribute to the curious "metabolically obese but normal weight" phenotype (18). Our study highlights the need to consider prenatal steroid exposure as part of offspring medical history even if the child is delivered at term, as programming is likely to predispose to lipid metabolic dysfunction and affect life course health. Our study also indicates the need for evaluating pathophysiological levels of maternal GC resulting from maternal stress in pregnancy on offspring obesity phenotype.

In summary, we report a metabolically vulnerable phenotype in adult male baboons exposed to human clinical equivalent antenatal sGC treatment, evidenced by increased hepatic and pericardial fat deposition without changes in body weight. The presence of pericardial and hepatic lipid abnormalities is of concern as they can exert local cytotoxic effects. While the number of subjects is only 5, careful standardization of pre-pregnant maternal phenotype and weight adjustment of the sGC doses allowed demonstration of a highly statistically significant (p =0.022) 150% increase in pericardial fat thickness and 432% increase in liver lipid (p=0.046) in baboons exposed as fetuses to equivalent of three weekly human clinical courses of sGC therapy. Importantly we adjusted the sGC dose for maternal weight a standardization not performed in clinical obstetric practice. Our findings agree with the MACS study that multiple courses of sGC are not recommended (19). Sexually dimorphic responses to antenatal sGC treatment have been documented in rats (20), and studies in females are needed to determine whether sexual dimorphism exists in primates.

Acknowledgments

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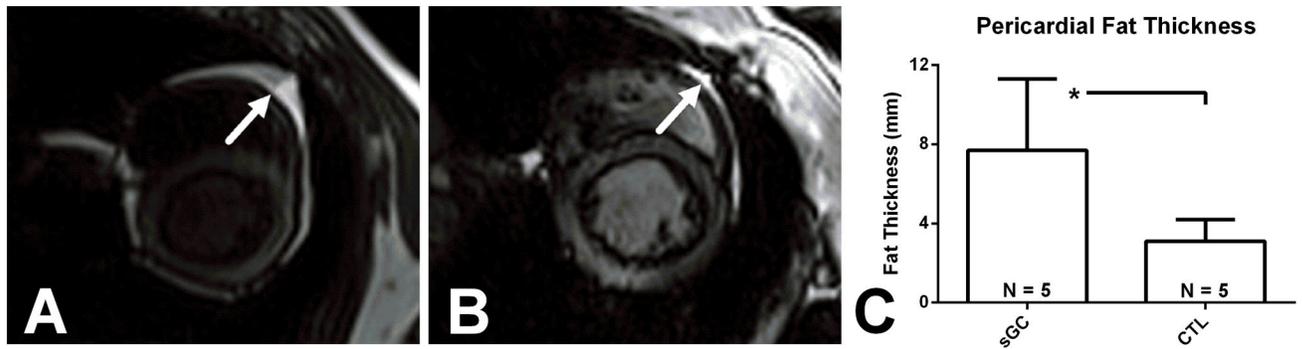
Figure 1. Prenatal betamethasone administration results in increased pericardial fat deposition
Sample short axis images depicting increased amount of pericardial fat in sGC subjects (A, arrow) compared to CTL (B) at the level of mid-ventricle. With quantification, pericardial fat thickness was higher in the sGC group (7.7 ± 3.6 mm, n = 5) compared to CTL (3.1 ± 1.1 mm, n = 5, p = 0.022).
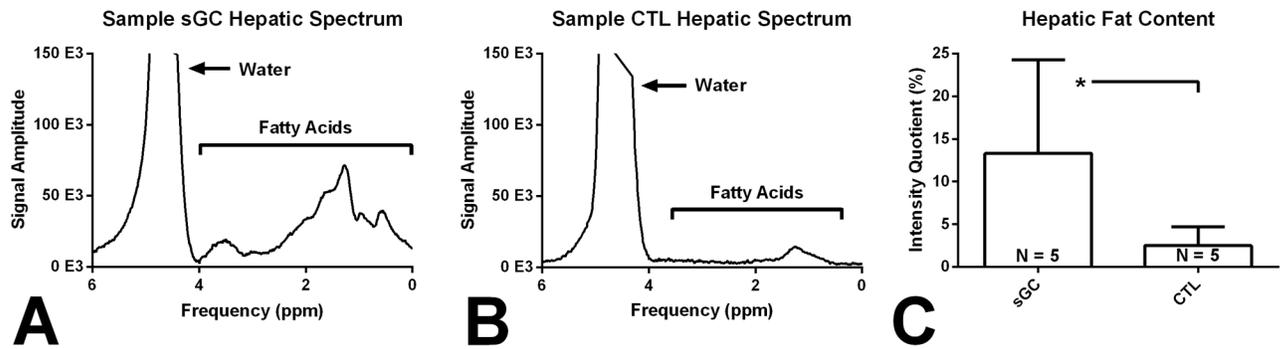
Figure 2. Prenatal betamethasone administration results in increased hepatic steatosis

Sample raw hepatic magnetic resonance spectra depicting increased amount of hepatic fatty acids in sGC subjects (A) compared to CTL (B). Overall hepatic fat content was higher in the sGC animals (13.3 ± 11.0 %, n = 5) compared to CTL (2.5 ± 2.2 %, n = 5, p = 0.046).