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Elevated Glucocorticoids during Ovine Pregnancy increase Appetite and produce Glucose Dysregulation and Adiposity in Their Granddaughters in response to *ad libitum* feeding at one year of age

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Abstract

Objective—Synthetic glucocorticoids (sGC) are administered to women threatening preterm labor. We have shown multi-generational endocrine and metabolic effects of fetal sGC exposure. We hypothesized that sGC exposure would alter the second filial generation (F2) offspring neonatal leptin peak that controls development of appetitive behavior with metabolic consequences.

Study design—F0 nulliparous ewes were bred to a single ram. Beginning at day 103 of gestation (Term 150), dexamethasone (DEX) ewes received 4 injections of 2 mg DEX i.m, 12 h apart. Control ewes received saline. Ewes lambled naturally. At 22 months of age, F1 offspring were mated to produce F2 offspring. At 10 months of age F2 female offspring were placed on an *ad libitum* feeding challenge for 12 weeks.

Results—DEX F2 female offspring did not show a post-natal leptin peak and their plasma cortisol concentration was elevated in the first days of life. During the feeding challenge DEX F2 offspring consumed 10 % more feed and gained 20 % more weight compared to control F2 offspring. At the end of the feeding challenge DEX F2 offspring had greater adiposity compared to control F2 offspring. F2 sGC offspring showed impaired insulin secretion in response to an IVGTT.

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Conclusions—sGC administration to F0 mothers eliminates the neonatal leptin peak in F2 female offspring potentially by inhibition due to elevated cortisol in the DEX F2 offspring. F2 offspring showed increased appetite, weight gain and adiposity during an *ad libitum* feeding challenge accompanied by decreased insulin response to an IVGTT.

Keywords

synthetic glucocorticoids; fetal; sheep; appetite; leptin; multigenerational

INTRODUCTION

The hormone leptin is produced by adipose tissue and acts on hypothalamic appetitive centers to inhibit food intake.¹ Appropriate regulation of leptin feedback is central to maintenance of normal body weight and composition. Dysregulation of this feedback system can lead to obesity. In neonatal rodents, leptin has a characteristic peak that occurs at postnatal day 8–21.^{1,2,3,4,5,6,7} The precise timing and duration of the leptin peak varies between studies, strains and species. The normal leptin peak is thought to program the balance of competing activity of hypothalamic orexigenic and anorexigenic appetitive neurons and influence leptin sensitivity post-natally.¹ The leptin peak is amplified and prolonged in offspring of obese rats.⁷ Several factors have been implicated in the control of fetal and adult leptin production in rodents including glucocorticoids, glucose, insulin, thyroid hormones and IGF-1.⁸ There is controversy over the timing of the leptin peak in precocial species. We have demonstrated a leptin peak in newborn lambs that occurs between day 6 and 8 of postnatal age and is associated with increased plasma cortisol at birth.⁹

In his classical study, Liggins showed that glucocorticoids (GC) prematurely accelerate fetal lung development in sheep an observation that led to a clinical trial which showed decreased morbidity and mortality in premature babies whose mothers received synthetic GC (sGC).^{10,11} It is now routine clinical practice to treat women threatening preterm delivery before 34 weeks gestation with sGC.¹² While this treatment with sGC confers great benefit by lowering neonatal mortality and morbidity, evidence is accumulating that fetal exposure to GC levels higher than those appropriate for the current stage of fetal maturation produces IUGR in sheep, non-human primates and humans. Synthetic GC administration at 0.75 of gestation results in decreased placenta endothelial nitric oxide synthase function in baboons, which could possible explain the fetal IUGR.¹³ Synthetic CG have also been shown to alter learning and attention in offspring.¹⁴

Developmental programming can be defined as a response to a specific challenge to the mammalian organism during a critical developmental time window that alters the trajectory of development with persistent effects on offspring phenotype and predisposition to future illness. sGC alters the developmental trajectory of many fetal organ systems with potential for adverse developmental programming effects in later life.^{15,16,17,18,19,20} Similarities across species suggest a common underlying mechanism responsible for these adverse outcomes. Animal studies demonstrate that following sGC exposure delayed offspring endocrine, renal, and metabolic effects emerge later in life with the potential to predispose to chronic disease.^{21,22,23,24,25,26,27} Increases in fetal and maternal GC have been demonstrated to occur in response to many challenges that result in developmental programming in both precocial and altricial species.^{28,29} Offspring outcomes resulting from challenges such as poor maternal nutrition can be prevented by inhibiting GC changes occurring in response to the challenge.³⁰ As a result, several investigators consider exposure to excessive endogenous GC levels at critical fetal developmental periods to be a common

causative factor of many, though not necessarily all, fetal programming responses to challenges during development, e.g. maternal stress and poor nutrition.^{31,32}

Most multigenerational studies on effects of fetal exposure to GC have been conducted in rodents – altricial species with a very different profile of fetal and neonatal development to precocial species such as humans and sheep.⁹ However, in sheep we have shown that sGC result in effects on both F1 and F2 female offspring with reduced birth weight and postnatal growth, increased plasma glucose and decreased plasma insulin during intravenous glucose tolerance test and increased basal and reduced stimulated hypothalamic pituitary adrenal axis function.^{21,22} In view of the relationship we have demonstrated of the normal leptin peak in newborn lambs to the plasma cortisol concentration, we hypothesized that exposure of female fetal lambs (F1 generation) to doses of sGC well within the range administered clinically to their mothers (F0 generation) at 27 weeks human gestation equivalent would have effects on F2 female offspring appetite drive, metabolism, endocrine function and weight changes when allowed *ad libitum* food access.⁹ To test this hypothesis, we administered either dexamethasone (DEX) or vehicle to F0 pregnant ewes at 103 and 104 days gestation (Term 150 days), maintained all the F1 female generation (both DEX and control offspring) on a similar diet, and determined F2 female offspring outcomes at approximately one year of age.

MATERIALS AND METHODS

Care and use of animals

All procedures were approved by the University of Wyoming Animal Care and Use Committee. A description of animal generation and housing has previously been published.¹⁹ Briefly, Twenty two founder generation (F0) nulliparous Rambouillet X Columbia crossbred ewes bred to a single ram were used to produce the first filial (F1) and second filial (F2) generation of ewe lambs. After natural mating, F0 ewes were fed in accordance with National Research Council (NRC) maintenance requirements.³³ On day 103 and 104 of gestation, ewes weighed 73.6 ± 4.0 kg (Mean $\pm$ SEM, $n = 22$) body weight and were randomly assigned to one of two treatment groups: DEX ewes ($n = 10$) received four injections of 2 mg of DEX (i.m.; Vedco, St, Joseph, MO) 12 h apart, a dose equal to approx. $60 \text{ ug.kg}^{-1}.\text{day}^{-1}$. Control ewes ($n = 12$) received equivalent volumes of saline i.m. Ewes were allowed to lamb naturally. After lambing, all F0 ewes were given free choice access to alfalfa hay. Prior to two weeks of age, F1 female lambs ($n = 10$ Dex and 12 control F1) were tail-docked as per Federation of Animal Science Societies' recommendations.³⁴ F1 ewe lambs were given free access to a standard commercially available creep feed (Lamb Creep B30 w/Bovatec; Ranch-way Feeds, Ft. Collins, CO) from birth to weaning at four months of age and placed in an outdoor housing facility with shelter and *ad libitum* water. These F1 ewe lambs were maintained in accordance with NRC recommendations requirements for replacement ewes with a diet that consisted of alfalfa and corn with *ad libitum* access to a trace mineral salt block.³³ Diets were adjusted up for weight gain every month.

At maturity nulliparous control and DEX F1 ewes exhibited similar body weights (65.1 ± 3.5 and 64.7 ± 3.7 kg, respectively), and were naturally mated to a single ram of similar breeding. There were 3 F2 control twin (3 male-female twin sets) and 5 F2 control singleton ewe lambs. In the F2 DEX group, there were 4 twin (1 female twin set and 3 male-female twin sets) and 4 singleton ewe lambs. Jugular venous blood was obtained from F2 ewe lambs ($n = 8$, control and 9, DEX; ~ 6 ml of blood) at birth and daily at 06.00 h from postnatal day 1 to 7, and days 9 and 11.

F2 lambs were fed in a similar fashion as their F1 mothers and postnatal body weights were recorded monthly until 10 months of age when lambs were placed on a 12 week feeding challenge using previously published procedures.³⁵ Briefly, F2 lambs were adapted from a hay and grain diet to the experimental ration at maintenance levels over a 10 day acclimation period. The experimental diet was a highly palatable, pelleted diet (containing 71.05 % total digestible nutrients, 1.08 Mcal/ kg Net Energy for gain, 1.64 Mcal/kg Net energy for maintenance, 88.2 % dry matter, 13.5 % crude protein, and 4.05 % fat on an as fed basis (ADM; Alliance Nutrition; Quincy, IL).³⁶ At the end of this acclimation period, lambs were weighed and removed from feed and water for 12 h and evaluated by dual x-ray absorptiometry (DEXA) to determine body composition (fat and lean tissue). The lambs were then returned to the experimental ration, which was fed *ad libitum* for 12 wk period of the feeding challenge

During the feeding challenge, lambs were housed in a single group in an open-front pole barn with free access to water and the pelleted feed available via an automated feeding behavior data acquisition system adapted for use in sheep (GrowSafe Systems Ltd., Airdrie, Alberta, Canada). Feed intake was continuously measured based on the weight difference of the feed bunk (accuracy to .01 kg) at the beginning and end of each feeding event for each individual animal as determined by a unique electronic ear tag worn by each lamb. The feed bunk had an opening which permitted a single lamb's head to enter, allowing only one animal to consume feed at a time and their ear tag to be scanned identifying the lamb consuming feed at each event. Feed was continuously available 24 h per day. Intake measurements were recorded throughout the entire 12 week period. Body weight (BW) was measured every two weeks when jugular blood was collected at 0700 h into a heparinized blood collection tube (BD Vacutainer, Franklin Lakes, NJ; 143 U.S.P. units sodium heparin per 10 mL) prior to weighing. Blood was kept cold and centrifuged within 30 min at 4 °C and 1500 × G for 15 min. Plasma was collected and aliquoted prior to storage at -20 °C for analyses. At the end of the 12 week feeding challenge, a catheter (Abbocath, 16ga, Abbott Laboratories, North Chicago, IL) was placed aseptically into the jugular vein 12 h prior to the start of an intravenous glucose tolerance test (IVGTT). Catheters were sutured to the skin to secure them and an extension set (Seneca Medical, Tiffin, OH) attached for undisturbed infusion and sampling. The neck and shoulder area were covered with netting (Surgilast Tubular elastic dressing retainer, Derma Science Inc, Princeton, NJ) to prevent catheter damage. F2 lambs were maintained in neighboring individual pens with free access to water. No feed was provided for ~ 18 h prior to and during the IVGTT which has been described in detail.³⁶ Jugular blood samples (~ 6 ml) were obtained into chilled tubes (heparin plus NaF; 2.5 mg/mL; Sigma, St. Louis, MO) at -15, and 0 min relative to a 0.25 g/kg intravenous bolus infusion of 50 % dextrose solution (Vedco, St. Joseph, MO) over 5 sec. Blood samples were collected at 2, 5, 10, 15, 20, 30, 45, 60, 90 and 120 min after dextrose infusion. All blood samples were immediately placed on ice, centrifuged at 1,500 × g and plasma was collected and stored at -80° C. Following the IVGTT, F2 lambs were subjected to a DEXA scan to determine body composition at the end of the feeding challenge.

Dual Energy X-ray Absorptiometry (DEXA)

To accurately determine body composition (fat and lean tissue), dual energy x-ray absorptiometry (DEXA, GE Lunar Prodigy™ 8743; Madison, WI) was utilized as previously described and validated for sheep.^{35,36,37} The whole body scan mode was used for all animals and scan times were approximately three minutes depending on the length of the animal. A single, experienced, blinded investigator performed all DEXA scans and quantified % body fat.

Biochemical and Hormone assays

Glucose was measured colorimetrically in triplicate (Liquid Glucose Hexokinase Reagent, Pointe Scientific, Inc., Canton, MI) as previously described.³⁸ Mean intra-assay coefficient of variation (CV) was 1.6 % and inter-assay CV was 2.9 %. Insulin was measured in duplicate by commercial radioimmunoassay (RIA) (Coat-A-Count Insulin RIA, Siemens Medical Solutions Diagnostics, Los Angeles, CA) with intra- and inter-assay CV of 8.4 % and 11.1 %, respectively, and a sensitivity of 2.6 μ IU/ml.³⁸ Plasma leptin was measured by radioimmunoassay (Multispecies leptin RIA, Linco Research, St Charles, MO, USA) as previously described with an intra-assay CV of 4.1 % and inter-assay CV of 5.1 %.³⁹ Concentrations of cortisol were determined as described previously using Coat-A-Count Cortisol RIA with a sensitivity of 5 ng/ml (Siemens Medical Solutions Diagnostics) with an intra-assay CV of 6.1% and inter-assay CV of 9.1%.⁴⁰ Tri-iodothyronine was determined by RIA according to manufacturer's specifications (Coat-a-Count Total T3) with intra- and inter-assay CV of 3.4 % and 4.4 %, respectively, and a sensitivity of 16.2 ng/dl.⁴¹

Statistical analysis

Postnatal hormone and metabolite data were analyzed as a repeated measures analysis using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) with treatment, day and their interaction in the model. Plasma hormone and metabolite concentrations during the feeding challenge were analyzed as repeated measures using the MIXED procedure (SAS Inst. Inc., Cary, NC) with treatment, week and their interaction in the model. The DEXA measurements, feed intakes, and BW gain during the feeding challenge were analyzed using the GLM procedure of SAS, with treatment in the model. Graphpad Prism (GraphPad Software Inc., La Jolla, CA) was used to calculate the area under the curve (AUC) for plasma glucose and insulin response curves during the IVGTT. Baseline concentrations of glucose and insulin in all samples before infusion were averaged to give baseline concentrations. Plasma glucose and insulin during the IVGTT were analyzed as repeated measures using MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) with treatment and time and their interaction in the model. AUC and fasting concentrations of glucose and insulin were analyzed using the GLM procedure of SAS with treatment in the model. Birth type (twin vs. single) was initially included in all models but was found to be non-significant ($P < 0.39$) and was therefore removed. Data are provided as Mean + SEM throughout with $P < 0.05$ was considered significance.

RESULTS

Birth weights and measures together with growth patterns up to 8 months of age of F2 female offspring have been published.²¹ Briefly, birth weight of DEX F2 female lambs was less ($P = 0.03$) than control F2 female lambs (5.72 ± 0.26 vs 6.62 ± 0.29 kg). There was no difference in birth weight between lambs born as singles or twin ($P = 0.39$; single 6.28 ± 0.46 vs twin 5.75 ± 0.55). In control F2 lambs, plasma leptin increased ($P < 0.05$) from postnatal day 2 to 3 and remained higher than values of DEX F2 female lambs on day 4 ($P < 0.05$), returning to levels seen in DEX F2 lambs from day 5 to 11 (Fig. 1A). Plasma cortisol was increased at birth and on day 1, 3, 6 and 7 of age in DEX F2 lambs compared to control F2 lambs (Fig 1B). There was no difference in plasma insulin, glucose, and tri-iodothyronine during the first 11 days of age between DEX and control F2 lambs (Fig. 1 C, D, and E respectively).

At 10 months of age, when F2 ewes were transitioned onto the experimental pelleted ration, there was no difference in control and DEX F2 female offspring body weights (Table 1). There was also no difference in any of the DEXA measurements between control and DEX F2 female lambs before the feeding challenge (Table 1). During the feeding challenge DEX

F2 female offspring put on more weight both in absolute terms and as a percent of initial weight (Figure 2A and B) and consumed more feed (Figure 2C and D) than control F2 female offspring. At the end of the feeding challenge body weight of DEX F2 was 2.5 Kg heavier than controls but due to the fact that controls were 1.5 Kg heavier (not significant) at the beginning of the challenge, the final weights of the two groups were similar between control and DEX F2 offspring (Table 1). However, percent body fat and grams of adipose tissue was increased 28.4 % and 37.6 % of initial measurements respectively in DEX F2 offspring compared to control F2 female offspring (Table 1).

Plasma leptin concentrations were similar at the start of the feeding challenge in the two groups but at day 42, half way through the feeding challenge, and at the end of the feeding challenge, plasma leptin was increased in DEX F2 offspring compared to control F2 offspring (Figure 3A). Plasma cortisol concentrations were similar between F2 offspring at the start and for the first 14 days of the feeding challenge, but from day 28 to the end of the feeding challenge plasma cortisol was higher in DEX F2 female offspring than controls (Figure 3B). Plasma insulin concentrations at the beginning of the feeding challenge were also similar between the two groups but on day 14 to 56 and on day 84 of the feeding challenge plasma insulin was lower in DEX F2 female offspring compared to control F2 female offspring (Figure 3C). Plasma glucose concentrations were elevated in DEX F2 female offspring compared to control F2 female offspring at day 0 to 28 and also days 56 and 84 of the feeding challenge (Figure 3D). During the IVGTT conducted after the feeding challenge, DEX F2 female offspring had elevated plasma glucose in response to a weight adjusted glucose bolus compared to control F2 female offspring (Figure 4A). Plasma glucose AUC during the IVGTT was increased in DEX F2 female offspring compared to control F2 female offspring (Figure 4A insert). Although basal insulin levels were similar in both groups, the increase in plasma insulin in response to the IVGTT glucose load was reduced in DEX F2 offspring compared to control F2 female offspring as was the insulin AUC (Figure 4B).

DISCUSSION

This study is the first to demonstrate that maternal administration of a low dose of sGC two thirds of the way through gestation – a time equivalent to many clinical exposures - alters appetite regulation in the F2 granddaughter generation. Specifically, postnatal changes in the F2 female offspring included a decreased postnatal leptin peak and increased appetite, increased weight gain and adiposity, increased plasma leptin, glucose and cortisol, and decreased plasma insulin in response to an *ad libitum* feeding challenge. These changes are likely to have been programmed in fetal life as we have previously shown that maternal DEX administration leads to a decreased birth weight and reduced morphometrics and reduced growth rates in female F1 and F2 offspring of DEX dams.²¹ We have also observed glucose and insulin regulation changes in these female F2 offspring previous to the age at which we imposed the feeding challenge in the present study.²¹

We hypothesize that the observed changes in the F2 offspring resulted from fetal exposure to levels of GC in excess of those appropriate for the current stage of maturation, just as their F1 mothers experienced in fetal life. This is consistent with the observation that their mothers (F1 offspring) exhibited elevated plasma GC concentrations, and altered GC release during challenges that may result from dysfunction of their pituitary adrenal axis.²² Normal fetal pancreatic growth and development is regulated by physiological levels of GC through regulation of fetal IGF2 secretion.^{42,43,44,45} This concept is supported by the observation that GC concentrations in fetal rodents are negatively correlated with pancreatic insulin content and cell mass⁴⁶ Further, maternal treatment with three courses of sGC in the pregnant ewe results in alterations in fetal cell morphology, and islet insulin content in

concert with decreased expression of pancreatic duodenal homeobox-1 (Pdx-1) protein, a marker of pancreatic development, which plays a major role in the glucose dependent regulation of insulin secretion.^{47,48}

Exposure to high concentrations of glucose, free fatty acid, and other metabolites have been linked to β -cell apoptosis.^{49,50} We have previously shown impaired glucose and insulin regulation in postnatal F1 and F2 DEX offspring maintained on a normal diet without the added food challenge to which ewes were exposed in the present study.⁹ If glucose and insulin dysregulation is maintained in F1 DEX ewes during their pregnancy, as is likely because of the normal insulin resistance of pregnancy, one can hypothesize a similar set of maternal-fetal interactions to those that occur in the setting of maternal obesity in which fetuses are exposed to inappropriate levels of glucose and insulin during gestation constituting a challenge to the developing pancreas.¹⁹

The increased appetite seen in our F2 female offspring does not appear to have been previously observed in any model of prenatal GC administration. Since synthetic GC produces IUGR, we can seek parallels in other models of maternal challenges in pregnancy that result in fetal growth restriction.²¹ Maternal undernutrition in sheep during early gestation results in increased appetite in postnatal offspring, which has been shown to persist as these animals approach old age.^{35,38} The postnatal leptin peak appears to be important in setting the appetitive drive of newborns. In rodents offspring of obese mothers the leptin peak is amplified and prolonged associated with increased post-natal appetite in these offspring.⁷ In lambs born from obese mothers, no leptin peak occurred during the first 11 days of life, and these offspring consumed more feed during the same *ad libitum* feeding challenge used here.⁹ In the setting of maternal obesity in the F0 generation, the lack of the F1 leptin peak persists into the F2 generation, independent of the presence of F1 maternal obesity or differences in maternal nutrient intake.⁵¹ The view that postnatal leptin is important for appetite regulation is supported by the fact that hypothalamic neurons harvested from low birth weight growth restricted newborn rat pups have a reduced ability to proliferate and differentiate when exposed to leptin.⁵²

The increase in adipose tissue mass in our DEX F2 female lambs could have an effect on the observed increased plasma cortisol concentration seen in this feeding study and in basal concentration earlier in the animal's lives.²² 11 β -hydroxysteroid dehydrogenase type 1 (11 β -HSD1) is ubiquitously expressed in adipose tissue and converts inactive cortisone to the active hormone cortisol since 11 β -HSD1 predominately acts as a reductase rather than a dehydrogenase.⁵³ Transgenic mice overexpressing 11 β -HSD1 selectively in adipose tissue have a phenotype showing excess visceral adiposity which is exacerbated by high fat feeding together with diabetes, insulin resistance, hyperlipidemia, and hyperphagia.⁵⁴ Postnatal overfeeding accomplished by reducing litter size in rats results in increased 11 β -HSD1 mRNA and enzyme activity in retroperitoneal adipose tissue of pups by 8 weeks of age.⁵⁵ Maternal undernutrition during gestation results in changes in 11 β -HSD2 but also minor changes in 11 β -HSD1 in adipose tissue after feeding of high fat diets postnatally in rats.⁵⁶ In sheep maternal nutrient restriction during early to mid-gestation results in increased 11 β -HSD1 mRNA in the perirenal adipose tissue of newborn lambs compared to lambs whose mother received adequate nutrition during gestation.⁵⁷ We have recently shown that 11 β -HSD1 activity is increased in a fetal sex dependent manner in perirenal fat and liver in fetuses from nutrient restricted baboons.⁵⁸

The compelling data from numerous laboratories around the world that fetal exposure to GC levels higher than those appropriate for the current stage of gestation raises concerns regarding the short and long-term effects of GC exposures during fetal development when mothers that threaten preterm delivery are given steroids to accelerate fetal lung maturation,

itself an example of an effect of GC at higher levels than would normally be present at this stage of development. Antenatal GC therapy has greatly reduced the burden of morbidity and mortality in prematurely delivered neonates. However, the burden of potential later life chronic disease in individuals who have been exposed to antenatal sGC as fetuses will not be clearly established until enough time has passed in the lives of those exposed. There has never been a clinical trial to evaluate the efficacy of lower doses of sGC than those currently in use. Our past sheep studies support the view that the current dosing regimen of GC is supramaximal and the current study indicates programming effects of one third the clinical dose of GC.⁵⁹ Until further animal studies are conducted it remains unknown at what dose benefits outweigh costs in terms of programming of predisposition to chronic disease. At some stage it may become imperative to conduct a randomized control trial of lower doses compared to the current dose, which as we state above has never been performed. In addition, there should be an active search for therapies that retain the specific effects on maturation of the fetal lung without the unwanted effects on virtually every other fetal organ. Until such time as these issues are resolved, it will be important to monitor the general health of all individuals who were exposed to sGC during development – particularly their cardiovascular, metabolic, and endocrine function.

In conclusion, we have shown that administration of the synthetic GC dexamethasone to sheep in approximately one third the maternal weight adjusted dose given to women threatening to deliver prematurely, results in lasting metabolic and endocrine changes in their female granddaughters.⁶⁰ These F2 offspring exhibited hyperphagia, insulin resistance and increased adiposity when provided unlimited access to feed, as is now generally available in Western societies. Further, these data demonstrate that this impact of fetal exposure to elevated GC is passed multigenerationally even when offspring are fed normally creating a potential self-perpetuating cycle. We hypothesize that the mechanism is the generation of elevated cortisol in the developing F2 generation which produces an altered leptin peak, providing potential targets for intervention.

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Figure 1.

Circulating plasma levels (mean $\pm$ SEM) from birth until postnatal day 11 in F2 female offspring whose grandmothers (F0) received four, injections of 2 mg of dexamethasone 12 hour apart on d 103 and 104 of gestation (DEX; closed symbol, n = 9) and control F2 offspring whose grandmothers (F0) received similar timed injections of saline (open symbol, n = 8). A, plasma leptin (treatment $\times$ day $*P < 0.01$ control vs. DEX F2 lambs within a time point); B, cortisol (treatment $*P < 0.01$ control vs. DEX F2 lambs within a time point); C, insulin (treatment $\times$ day $P = 0.22$); D, glucose (treatment $P = 0.95$); E, tri-iodothyronine (treatment $P = 0.94$).

Figure 2.

A) Body Weight (BW) gain B) BW gain as a percent of initial BW C) feed intake D) Feed intake as a percent of initial BW during feeding challenge in F2 female offspring whose grandmothers (F0) received four, injections of 2 mg of dexamethasone 12 hour apart on d 103 and 104 of gestation (DEX; closed symbol, n = 9) and control F2 offspring whose grandmothers (F0) received similar timed injections of saline (open symbol, n = 8).

Figure 3.

Circulating plasma levels (mean $\pm$ SEM) biweekly during feeding challenge in F2 female offspring whose grandmothers (F0) received four, injections of 2 mg of dexamethasone 12 hour apart on d 103 and 104 of gestation (DEX; closed symbol, $n = 9$) and control F2 offspring whose grandmothers (F0) received similar timed injections of saline (open symbol, $n = 8$). A, plasma leptin (treatment $\times$ day $*P < 0.01$ control vs. DEX F2 lambs within a time point); B, cortisol (treatment $*P < 0.01$ control vs. DEX F2 within a time point); C, insulin (treatment $P < 0.01$ control vs. DEX F2 within a time point); D, glucose (treatment P treatment $*P < 0.01$ control vs. DEX F2 within a time point).

Figure 4a

Figure 4b

Figure 4.

A) Plasma glucose and B) plasma insulin responses to an IVGTT in F2 female offspring whose grandmothers (F0) received four, injections of 2 mg of dexamethasone 12 hour apart on d 103 and 104 of gestation (DEX; closed symbol, n = 9) and control F2 offspring whose grandmothers (F0) received similar timed injections of saline (open symbol, n = 8) at the end of the *ad libitum* feeding challenge. Area under the curve (AUC) is depicted in the inset. Values are means $\pm$ SEM. A) Trt $P < 0.0001$, time $P < 0.0001$, Trt* time $P = 0.8396$; and B) Trt $P = 0.0003$, time $P < 0.0001$, Trt* time $P = 0.1001$. * means differ $P < 0.05$.

Table 1

Pre and post feeding challenge DEXA measurements from C and Dex F2 female offspring

	Control	DEX	P value
	n = 8	n = 9	
Pre feeding challenge			
Body Weight, kg	62.4 ± 3.7	60.9 ± 3.1	0.39
Crown Rump Length, cm	117.4 ± 2.7	115.9 ± 2.4	0.35
Percent Fat	10.1 ± 1.3	11.8 ± 0.9	0.16
Bone Mineral Density	1.12 ± 0.03	1.12 ± 0.02	0.5
Fat Tissue, g	6224 ± 1105	7161 ± 786	0.26
Lean Tissue, g	54046 ± 2624	51055 ± 2587	0.22
Post feeding challenge			
Body Weight, kg	82.5 ± 4.3	85.0 ± 3.3	0.33
Crown Rump Length, cm	125.9 ± 1.8	124.0 ± 1.6	0.24
Percent Fat	17.6 ± 1.6	23.9 ± 1.3	0.003
Bone Mineral Density	1.16 ± 0.02	1.19 ± 0.02	0.22
Fat Tissue, g	13300 ± 1705	17996 ± 1575	0.04
Lean Tissue, g	61273 ± 2492	57565 ± 2261	0.15
Percent change from initial to final			
Body weight, % of initial	32.2 ± 2.9	40.0 ± 2.6	0.05
Percent fat, % of initial	74.1 ± 6.9	102.5 ± 6.1	0.01
Fat tissue, % of initial	113.7 ± 5.6	151.3 ± 4.9	0.02
Lean tissue, % of initial	13.4 ± 1.9	12.8 ± 1.7	0.56