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The impact of maternal overnutrition and obesity on hypothalamic-pituitary-adrenal axis, response of offspring to stress

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Abstract

We evaluated the effect of maternal obesity before and throughout gestation on offspring hypothalamic pituitary adrenal axis function. Multiparous Rambouillet × Columbia crossbred ewes were fed either 100 % of NRC recommendations (Control, C) or 150 % of NRC (Obese, OB) from 60 d before mating until lambing. Ten lambs born to OB ewes (5 males and 5 females), and 8 lambs born to C ewes (3 male and 5 female) were studied. From delivery to weaning lambs were maintained with their mothers who were all fed 100% NRC recommendations. After weaning all lambs were group housed and fed the same diet to meet NRC requirements. At 19 mo of age lambs were placed in individual pens and fed a pelletized diet to meet maintenance requirements. Jugular vein catheters were placed and 2 d later they received an i.v ACTH challenge followed by an i.v. corticotrophin releasing hormone (CRH)/arginine vasopressin (AVP) challenge 1 d later. Thirty d later offspring were again catheterized and placed into metabolism crates for 2 d before receiving an isolation stress test. ACTH and cortisol responses to the isolation stress test and CRH/AVP challenge and cortisol responses to ACTH challenge were determined. Cortisol was quantified via RIA and ACTH was quantified using an Immulite 1000 and both were analyzed using repeated measures utilizing the MIXED procedure of SAS. Offspring from OB ewes had elevated basal plasma ACTH and cortisol compared to C offspring prior to all three challenges ($P < 0.05$). Offspring from OB mothers tended ($P = 0.06$) to have a greater ACTH response after an intravenous CRH/AVP injection than offspring from C mothers (12340 ± 1430 vs 8170 ± 1570 area under the curve, respectively). Cortisol response to the CRH/AVP and ACTH challenges was not influenced by maternal nutrition ($P < 0.46$), and averaged 4.77 ± 0.2 and $1.94 \pm .01$ $\mu\text{g/dl}$, respectively. The ACTH response following the isolation stress test was also similar ($P = 0.82$) for

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OB and C offspring (147 ± 20 pg/ml). Cortisol response during the isolation stress test was also similar between C and Obese offspring ($P = 0.64$, 5.25 ± 0.3 $\mu\text{g/dl}$). These findings suggest that maternal obesity before and during gestation does not affect stress responses by the offspring, but has an impact on hypothalamo-pituitary-adrenal sensitivity. The lack of differences in cortisol release under the influence of difference concentrations of ACTH during the CRH/AVP challenge could indicate adrenal dysfunction in OB offspring.

Keywords

maternal obesity; offspring; stress response; hypothalamic-pituitary-adrenal axis; sheep

1. Introduction

Obesity and type II diabetes associated with obesity are becoming an epidemic in both developed and underdeveloped countries [1]. In the United States it has been estimated that one third of all women of reproductive age are clinically obese [2]. Epidemiological evidence has demonstrated that children born to obese women have a greater risk of developing obesity, insulin resistance and metabolic syndrome [3,4] than those born to lean or average weight women. We have developed an ovine model of pre-pregnancy maternal obesity and nutritional excess in which fetal weights are increased $\sim 30\%$ by mid-gestation in combination with increased fetal circulating concentrations of glucose, insulin, IGF-1 and cortisol when compared with fetuses from control fed ewes [5,6]. The newborn offspring from obese ewes have increased adiposity at birth along with altered concentration of glucose, insulin and cortisol, [7,8]. In addition offspring from obese ewes have altered postnatal leptin secretion [8] that could result in the altered feed intake and body weight gain observed in this model [9]. In addition to increased feed intake and weight gain adult offspring from obese ewes show insulin and leptin resistance that is either brought about or exacerbated by an *ad libitum* feeding challenge [9].

Nutrient restriction during pregnancy is known to permanently modify hypothalamic-pituitary-adrenal (HPA) development and function in many species including the sheep [10]. Yearling female offspring from ewes that were nutrient restricted during early gestation tend to have an altered response to a CRH/AVP challenge and have a decreased stress response to an isolation stimulus [11]. However, the effects of maternal obesity on HPA activity are unknown in a large animal model. Given the increased fetal cortisol at a critical period of development of the hypothalamo-pituitary-adrenal axis (HPA), we hypothesized that maternal obesity before breeding and throughout gestation would alter HPA function in offspring in adult life.

2. Material and methods

2.1 Animals

All procedures were approved by the University of Wyoming Animal Care and Use Committee. The maternal body weight (BW) and body condition score (BCS) have previously been published along with the maternal and postnatal animal care [9]. Briefly, multiparous whiteface ewes of similar age and body weight were allotted to either a control

(C, 100 % of NRC recommendations [12], n=8) or obese (OB, 150 % of NRC recommendations, n=7) diet based on metabolic body weight ($BW^{0.75}$) from 60 d before mating to a single Columbia sire until the end of gestation. Body weight was determined weekly and diets were adjusted accordingly. Ewe body condition score (BCS; [13]) was assigned to each ewe by two trained individuals at the start of the experiment, at breeding and during mid- and late gestation. Ewes were allowed to lamb unassisted after which all ewes were fed to meet NRC recommendations for a lactating ewe. Birth weight was recorded for all lambs. At 120 ± 3 d of age, lambs were weaned, maintained together thereafter and fed a diet of high quality alfalfa hay and corn to NRC recommendations for growing lambs. At 19.5 ± 0.5 mo of age 8 adult offspring from the C ewes (3 males; 5 females) and 10 adult offspring from OB ewes (5 males; 5 females) were placed in individual pens and adapted over a week period to a pelleted ration meeting 100 % NRC recommendations based on individual metabolic body weight ($BW^{0.75}$). The composition of the ration was previously reported [9] and contained 71.05 % total digestible nutrients, 13.5 % crude protein, and 4.05 % fat. Lambs body composition was determined 24 hours after the initial challenges were conducted and has been previously reported [9] and was not different ($P = 0.86$; C 5.4 ± 0.8 vs OB 5.2 ± 0.7).

2.2 Challenges

To determine the responsiveness of different components of the HPA axis a series of challenges were conducted on C and OB offspring. A catheter (Abbocath, 16ga, Abbott Laboratories, North Chicago, IL) was placed aseptically into the jugular vein 48 hr prior to blood sampling. Catheters were sutured to the skin to secure them and a 124.5 cm extension set (Seneca Medical, Tiffin, OH) was attached to the catheter to allow for infusion and sampling without disturbing the animal. The neck and shoulder area of each animal were covered with a netting material (Surgilast Tubular elastic dressing retainer, Derma Science Inc, Princeton, NJ) to prevent catheter damage. Animals were maintained in neighboring individual pens with free access to water. Bovine CRH, human AVP, and human ACTH were all purchased from Sigma Chemicals (Sigma-Aldridge, St Louis, MO) catalog numbers C2671, V9879, and 02275 respectively. Two d after catheter placement, an ACTH challenge was conducted on all animals on a single day with blood sampling starting at 0800 hr using similar procedures to Turner et al. [14] with slight modifications as previously published [11]. Jugular blood samples were obtained into chilled tubes (heparin; 2.5 mg/mL; Sigma, St. Louis, MO) at -30, -15, -5 and 0 min relative to a $0.2 \mu\text{g/kg}$ intravenous bolus injection of ACTH in saline (2.0 to 3.0 mL) over a 2 sec period. Additional blood samples were collected at 2, 5, 10, 15, 20, 30, 45, 60, 80, 100, 120, and 150 min after ACTH infusion. All blood samples were immediately placed on ice and then centrifuged at $2,500 \times g$ and plasma was collected and stored at -80°C . Four d later, a CRH/AVP challenge was conducted on all animals on a single day starting at 0800 hr as previously reported by Bloomfield et al. [15] with the following modifications previously reported from our laboratory [11]. Jugular blood samples were collected at -30, -15 and 0 min relative to injection of $0.5 \mu\text{g/kg}$ of CRH and $0.1 \mu\text{g/kg}$ of AVP in saline based on each sheep's body weight given as an intravenous bolus (2.0 to 3.0 mL) over a 2 sec period. Then additional blood samples were obtained at 2, 5, 10, 15, 20, 30, 45, 60, 90, 120, 180, and 240 min after CRH/AVP infusion. All blood samples were handled as previously stated for the ACTH challenge.

Thirty d later, a subset of these animals (6 C and 6 OB offspring (3males and 3 females per treatment group) were selected at random were fitted with an indwelling jugular catheter (polyvinyl tubing, 0.5 internal diameter; Fisher Scientific, Pittsburg PA treated with a Tridodecylmethylammonium Chloride heparin complex; Polysciences, Inc, Warrington PA) The catheter was placed in the jugular vein via venipuncture through the overlying skin with a 10 gauge needle. Catheters were sutured in to the skin to secure them and then covered with a netting material (Surgilast Tubular elastic dressing retainer, Derma Science Inc, Princeton NJ), and animals placed in metabolism crates and given *ad libitum* pelleted diet and water. Catheters were flushed every twelve hrs with 3 mls of heparinized saline. Three d after catheter placement, an isolation stress test was conducted on an individual animal with treatment groups alternated to try to prevent time of day from having an effect as previously published [11]. A blood sample was obtained from each offspring in their metabolism crate while they was still housed adjacent to other animals (-45 and 30 min). The offspring was then transported (~ 125 m) to an empty room in their metabolism crate and isolated from the investigators with a curtain. Two additional blood samples were collected from the indwelling catheter which was passed through the curtain, at -15 min and 0 min. Immediately after the 0 min blood sample was collected, the lights in the room in which the animal was placed were turned off and a robot programmed to move randomly, as well as initiate a siren and a strobe light was activated for a one min period. While in the still darkened room, jugular blood samples were collected at 2, 5, 10, 15, 20, 30, 45, 60, 80, 100, 120, and 150 min after the robot was turned on. After collecting the 150 min blood sample the animal was returned to the animal room, and the next animal commenced the protocol.

2.3 Hormone quantification

Plasma concentrations of ACTH were determined as previously described by Gilbert et al. [16] using an Immulite 1000 immunoassay analyzer with a sensitivity of 9 pg/mL (Siemens Medical Solutions, Malvern, PA), which resulted in intra-assay and inter-assay CVs of < 5.5 %. Plasma concentrations of cortisol were determined as previously described by Dong et al. [17] using Coat-A Count Cortisol RIA with a sensitivity of 0.5 µg/dL (Siemens Medical Solutions Diagnostics, Los Angeles, CA) with an intra-assay and inter-assay CV < 4.5 %.

2.4 Statistical analysis

Baseline plasma concentrations of ACTH and cortisol were calculated by averaging the 4 or 3 samples collected prior to infusion during the ACTH and CRH/AVP challenges, respectively, and by averaging the 2 samples collected before the animals were moved during the isolation stress test. Graphpad Prism (GraphPad Software Inc, La Jolla, CA) was used to calculate the area under the curve (AUC) for plasma cortisol and ACTH response curves following CRH/AVP infusion and the isolation stress test, along with plasma cortisol response following ACTH challenge. Plasma cortisol and ACTH response following CRH/AVP infusion and the isolation stress test, along with plasma cortisol response following ACTH challenge 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 statement. Areas under the curves for ACTH and cortisol during CRH/AVP and the isolation stress test and cortisol release during ACTH challenge, along with the baseline concentrations of both cortisol and ACTH were analyzed using the GLM procedure of SAS

with treatment in the model. Offspring sex effects were initially included in all models but were found to be nonsignificant ($P < 0.34$) and therefore removed from the final models. Data are presented as least square means $\pm$ SEM, and differences considered significant at $P < 0.05$, with a tendency at $P < 0.10$.

3. Results

Ewe body weight and BCS along with lamb birthweights and BW have been previously published [9]. Briefly ewes on the OB and C diets had similar BW at the initiation of the study (71.6 ± 3.2 vs 68.3 ± 2.9 kg). Ewes on the OB diet increased their BW ($P < 0.05$) from diet initiation to mating (30 %), d 75 (43 %) and d 135 (52 %) in BW from diet initiation. While C ewes exhibited only modest non-significant ($P > 0.21$) increases in BW from diet initiation to conception (2.9 %), d 75 (8.6 %) or d 135 (15.6 %) of gestation. Body condition scores of C and OB ewes was similar at diet initiation (5.0 ± 0.3 vs 4.8 ± 0.3 , respectively) OB ewes had increased BCS ($P < 0.05$) at mating (7.2 ± 0.2), and further increased ($P < 0.05$) to 8.0 ± 0.2 by d 75, and 8.6 ± 0.2 by d 135. The BCS of control ewes remained relatively constant ($P > 0.38$) from diet initiation to d 135 of gestation averaging 4.9 ± 0.4 . Lamb birthweights of C and OB ewes did not differ, (5.32 ± 0.47 and 6.00 ± 0.30 kg, respectively ($P > 0.28$)). At 19 mo of age offspring BW and BCS remained unaffected by maternal nutrient status (65.9 ± 2.2 and 65.0 ± 1.9 kg and 4.7 ± 0.1 and 4.6 ± 0.1 BCS in C and OB offspring respectively; $P = 0.72$ and 0.61 respectively).

Baseline plasma cortisol was increased ($P < 0.05$) in OB offspring compared to C offspring (9.9 ± 1.4 vs 5.5 ± 1.2 ng/ml) before the ACTH challenge. Baseline plasma cortisol was also increased ($P < 0.05$) in OB offspring before both the CRH/AVP and isolation stress test compared to C offspring (CRH/AVP: 19.3 ± 3.6 vs 11.7 ± 1.9 ; isolation stress test: 15.7 ± 1.3 vs 11.9 ± 1.3 ng/ml respectively). Unlike baseline plasma cortisol, baseline plasma ACTH was unaffected by maternal nutrition during gestation before the CRH/AVP challenge ($P = 0.28$; C 33.15 ± 4.88 vs OB 29.47 ± 3.45) and the isolation stress test ($P = 0.34$; C 21.04 ± 4.51 vs OB 23.54 ± 4.51).

Plasma cortisol response to the ACTH challenge is shown in Figure 1. There was no difference in the cortisol response to ACTH due to maternal treatment ($P = 0.54$) and both groups increase plasma cortisol after infusion of ACTH and then returned back to baseline by 150 min post infusion ($P < 0.0001$). During the CRH/AVP challenge plasma ACTH tended ($P = 0.06$) to be greater, particularly immediately post infusion, in the OB offspring compared to C offspring (Figure 2a). This was confirmed by AUC for plasma ACTH during the CRH/AVP challenge, which tended ($P = 0.06$) to be greater for OB offspring compared to C offspring. However, there was no difference in plasma cortisol during the CRH/AVP challenge (Figure 2b). Plasma ACTH and cortisol response during the isolation stress test are shown the Figure 3. Plasma ACTH and cortisol concentrations were increased in response to the isolation stress test ($P < 0.0001$). However, maternal nutrition had no effect on plasma ACTH or cortisol response during the isolation stress test.

4. Discussion

To the authors' knowledge this is the first demonstration of altered function of the HPA axis in adult offspring born to mothers who were overnourished and obese at conception and throughout pregnancy in an animal model. Of particular interest is the elevated baseline concentration of plasma ACTH and cortisol in the OB offspring. It has been shown that children from mothers that had truncal obesity during gestation have increased fasting plasma cortisol levels compared to children from nonobese mothers [18]. One possible explanation for this increased basal cortisol concentration is increased sympathetic tone. It has been shown that maternal high fat feeding and obesity in the rat leads to increased sympathetic tone that resulted in increased blood pressure before the onset of obesity in offspring from obese mothers [19,20]. The exact mechanism of this altered sympathetic nervous system output is unknown but leptin is thought to be a possible regulator. Leptin has been shown to increase sympathetic nerve activity to brown adipose tissue and other beds including the adrenal gland [21]. Offspring from our obese ewes show leptin resistance, they continue to have increased feed intake even though they have increased circulating leptin concentrations [9]. It has been shown that there is selective leptin resistance in obese rodents. As rodents become obese, increased plasma leptin no longer inhibits feed intake but leptin's ability to increase renal sympathetic activation and arterial pressure is maintained [22].

There has been recent discussion of whether metabolic syndrome is a mild form of Cushing's disease. Cushing's disease and metabolic syndrome share the common manifestations of insulin resistance, hypertension, and abdominal obesity [23]. The elevated ACTH and cortisol observed in this experiment could help explain the insulin resistance and increased adiposity reported in this same group of animals at the end of the *ad libitum* feeding challenge [9]. It has been shown that excess glucocorticoids favors visceral fat deposition [24] and this could be leading to the increased adiposity observed in these animals after ad libitum feeding.

Offspring from obese mothers tended to have increased ACTH release in response to a bolus administration of CRH/AVP compared to offspring from normal fed mothers. In sheep the HPA axis of the fetus has been previously shown to be susceptible to maternal nutrient intake. Specifically, maternal nutrient restriction has been shown to alter HPA axis function during ovine fetal development [25,26,27]. These alterations in the HPA axis of nutrient restricted ovine fetuses persist into the postnatal period, when yearling offspring from nutrient restricted mothers exhibited a greater initial release of ACTH in response to a CRH/AVP challenge in offspring of nutrient restricted vs. control fed mothers [11]. These observations are further supported by the findings of Bloomfield et al. [15] who reported that 30 mo old offspring from ewes exposed to brief bout of undernutrition from d 105-115 of gestation, exhibited an increased ACTH response but no differences in cortisol release when compared with offspring from control fed mothers. In rodent models of maternal obesity and undernutrition, the hypothalamus is a target of programming that results in altered animal physiology in particular energy sensing and feeding behaviors. In rodents maternal obesity is associated with altered leptin signaling in the early postnatal period [28] and this leads to altered feeding behavior in the offspring. We have observed alteration in

postnatal leptin in our model of maternal obesity that could point to this programming of hypothalamic function outside of feeding behavior and peripheral nutrient sensing in this model [8].

The tendency for increased pituitary release of ACTH in OB offspring, but no resultant increase in cortisol secretion when compared to C offspring may indicate comparable adrenal function between the treatment groups. This hypothesis is consistent with the similar cortisol release observed following ACTH infusion in C and obese offspring in this study. However, there could be minor differences in the responsiveness and basal function of the adrenal glands between OB and C offspring. These differences could be due to altered sympathetic tone in the splanchnic nerve as discussed above. The lack of differences in cortisol release during the ACTH challenge in the present study indicates that the direct effects on adrenocortical function via the pituitary is not affected by maternal overnutrition in offspring. This finding is supported by Gardner et al. [29] who found no difference in cortisol release during an ACTH challenge in yearling offspring of ewes undernourished or fed 100 % of requirements from d 0 to 30 of gestation. Together, these findings indicate that adrenal cortical function is not directly affected by maternal malnutrition during pregnancy in the sheep.

Offspring from overnourished obese mothers exhibited a similar plasma ACTH and cortisol response during an isolation stress test. This finding is in contrast to the findings our group has previously reported in maternal nutrient restriction studies in sheep. Yearling female offspring from ewes that were undernourished 50% from d 28 to 78 of gestation had reduced plasma ACTH and cortisol response to the same isolation stress test utilized in this experiment [11]. This finding is supported by Hernandez et al [30] who reported that offspring from undernourished ewes had reduced plasma cortisol and a suppressed behavior reaction to isolation stress. In contrast, maternal high fat feeding in both rodents and primates has been shown to increase hippocampal activity, with increased anxiety during behavioral studies [31,32]. There could be species differences in offspring responsiveness to maternal overnutrition, or differences could result from a global increase in maternal diet as utilized here, and the studies utilizing rodents and primates where maternal overnutrition was accomplished by high fat feeding.

In conclusion, these data clearly indicate that maternal obesity may lead to alterations in HPA function that persist into postnatal life. As the OB offspring approached physical maturity, these alterations could indicate adrenal dysfunction as indicated by the lack of differential cortisol release in response to altered ACTH concentrations. Further studies are needed to investigate how these changes are mediated and if they persist as the offspring age and if changes to the HPA axis either contribute or possibly cause the increase adiposity, appetite and insulin resistance that have been shown in these animals [9].

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Figure 1.

Cortisol response to ACTH challenge in 19 mo offspring of ewes fed 100 % of NRC recommendations (control, ○, n = 8) and ewes fed 150 % of NRC recommendations (obese, OB; •, n = 10). Area under the curve (AUC) is depicted in the inset. Values are means $\pm$ SEM. Treatment $P = 0.54$, time $P < 0.0001$, Treatment by time $P = 0.72$.

Figure 2.

ACTH (a) and cortisol (b) responses to a CRH/AVP challenge in 19 mo old offspring of ewes fed 100 % of NRC recommendations (control, O, n = 8) and ewes fed 150 % of NRC recommendations (obese, OB; •, n = 10). Area under the curve (AUC) is depicted in the inset. Values are means $\pm$ SEM. Treatment $P = 0.06$, Time $P < 0.0001$, Treatment by time $P = 0.64$; and Treatment $P = 0.47$, time $P < 0.0001$, Treatment by Time $P = 0.99$; ACTH and cortisol, respectively.

Figure 3.

ACTH (a) and Cortisol (b) responses to a isolation stress test in 19 mo old offspring of ewes fed 100 % of NRC recommendations (control, ○, n = 8) and ewes fed 150 % of NRC recommendations (obese, OB; •, n = 10). Area under the curve (AUC) is depicted in the inset. Values are means $\pm$ SEM. Treatment $P = 0.82$, time $P < 0.0001$, Treatment by Time $P = 0.52$; and Treatment $P = 0.64$, Time $P < 0.0001$, Treatment by Time $P = 0.79$; ACTH and cortisol, respectively.