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Differential body weight, blood pressure and placental inflammatory responses to normal versus high-fat diet in melanocortin-4 receptor-deficient pregnant rats

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Abstract

Objectives—Although obesity increases the risk for hypertensive disorders of pregnancy, the mechanisms remain unclear. Neural melanocortin-4 receptor (MC4R) deficiency causes hyperphagia and obesity. Effects of MC4R deficiency on body weight, blood pressure (BP) and placental inflammatory responses to high-fat diet (HFD) are unknown. We tested two hypotheses: MC4R deficiency results in higher body weight, BP and placental inflammation under normal-fat diet (NFD) conditions and HFD exaggerates these responses in MC4R-deficient pregnant rats.

Methods—MC4R^{+/+} and MC4R^{+/-} rats were maintained on NFD (13% kcal fat) or HFD (40% kcal fat) for ~15 weeks, then measurements made on gestational day 19.

Results—MC4R^{+/-} pregnant rats had greater body mass and total body fat and visceral adipose tissue weights along with greater circulating total cholesterol (TC) and leptin levels than MC4R^{+/+} rats regardless of diet. On NFD, circulating adiponectin levels were lower and placental TNF α levels and BP (conscious with carotid catheter) were higher in these heavier rats. Circulating adiponectin levels were lower and placental TNF α levels and BP were higher in MC4R^{+/+} rats compared with NFD controls. These parameters were not affected by HFD in the already heavier and hypertensive MC4R^{+/-} pregnant rats.

Conclusion—Obesity in MC4R deficiency and HFD in MC4R^{+/+} rats result in higher BP and placental inflammation during pregnancy. However, HFD did not exaggerate these responses in already obese MC4R^{+/-} pregnant rats. These data suggest that obesity and HFD are independently related to hypertension and placental inflammation in pregnancy.

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Conflicts of interest

There are no conflicts of interest.

Keywords

adipose; genetics; inflammation; obesity; placenta; preeclampsia

INTRODUCTION

Hypertensive disorders of pregnancy, which include chronic hypertension, gestational hypertension, preeclampsia and preeclampsia superimposed on chronic hypertension, are the most common complications of pregnancy [1]. Such disorders increase pregnancy-related deaths. According to statistics from the Preeclampsia Foundation, this maternal disorder contributes to 76 000 maternal and 500 000 fetal deaths per year worldwide (about preeclampsia, 05 July 2010, retrieved from <http://www.preeclampsia.org/health-information/about-preeclampsia>). Its prevalence is highest in developing countries, but recently, the USA has seen a rise in the rates of preeclampsia in all geographical regions and age groups [2]. Epidemiological studies suggest that a contributor to the rise in rates of hypertension during pregnancy is obesity [3]. Indeed, the risk can be as high as 50% in super-obese pregnant women with a BMI more than 50 [4]. This degree of obesity is not uncommon in the southern USA, where the prevalence of hypertension during pregnancy is ~15% higher compared with northeast, midwest and western regions [2]. Although there is solid evidence supporting an association between obesity and the risk for having hypertension during pregnancy [5], the mechanisms are far less understood.

Animal models of high-fat diet (HFD) feeding have been utilized to try and examine the effects of obesity on blood pressure (BP) regulation during pregnancy. Hayes *et al.* [6] found in Sprague–Dawley rats that 19 weeks of a HFD containing 45% kcal from fat had higher BP, which was measured by tail-cuff plethysmography at gestational day 15. However, maternal body weight and fat mass was not examined during pregnancy. Studies in Japanese macaques found that 4 years of a 32% HFD resulted in greater maternal body weight accompanied by higher placental inflammatory markers compared with control diet-fed monkeys. Maternal BP was not examined [7]. Thus, there are a lack of studies examining BP regulation in obese pregnant experimental animal models.

Genetic mutations account for a significant proportion of the obesity occurring in humans. Heterozygous mutations in the coding region of the melanocortin-4 receptor (*MC4R*) gene are a common cause of monogenetic obesity, accounting for 1–6% of cases [8–16]. Studies have demonstrated, in male rodents, that MC4R deficiency induces hyperphagia and obesity, which are exaggerated by HFD. However, this occurs without concurrent inflammation and hypertension implicating this signaling pathway as crucial for obesity-induced hypertension in males [17–19]. In contrast, female MC4R-deficient rats have elevated BP and obesity compared with their wild-type counterparts, but the effects of heterozygous deficiency of the MC4R on body weight, BP and placental inflammatory responses to HFD during pregnancy are unknown.

The novelty of using MC4R-deficient rats to examine the effects of obesity and HFD on BP regulation during pregnancy lies in their significantly greater amount of body fat and body weight, which has not been possible via HFD feeding alone, as we found in the present

study. Therefore, we set out to test two hypotheses: MC4R deficiency results in higher body weight, BP and placental inflammation under normal-fat diet (NFD) conditions and HFD exaggerates these responses in MC4R-deficient pregnant rats.

MATERIALS AND METHODS

Animals

All animal experiments were conducted in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* with all animal-use protocols approved by The University of Mississippi Medical Center's Institutional Animal Care and Use Committee. MC4R^{+/+} wild-type and MC4R^{+/-} heterozygous female rats were obtained from a colony maintained at UMMC from founders purchased from Charles River Laboratories (Wilmington, Massachusetts, USA) and originally developed by Mul *et al.* [18]. Our rat colony was maintained by nonbrother-sister mating of heterozygous rats, as ad-libitum-fed MC4R^{-/-} female rats do not mate. MC4R^{+/+} rats included littermate controls with additional animals produced by mating wild-type rats. Our experiments only used MC4R^{+/+} and MC4R^{+/-} rats, which were produced from at least three different breeding pairs. Breeding pairs for generation of experimental animals were maintained on standard chow diet from Harlan Laboratories (unautoclaved, Teklad 7013/NIH31; Indianapolis, Indiana, USA) and water, *ad libitum*. All rats were maintained on a 12 : 12 h light : dark cycle at 23 °C.

Genotyping

At weaning (3 weeks), all offspring were placed on NIH31 diet. Tail biopsies were collected for genotyping. This obese rat model was developed by an N-ethyl-N-nitro-sourea-induced mutation in Wistar Hannover rats, which resulted in a truncated form of the receptor with the inability to be expressed in the plasma membrane [18]. Furthermore, this mutation introduced an additional Ddel restriction site (CTNAG) within the *MC4R* gene. Thus, genotyping was performed using a restriction enzyme-based PCR assay utilizing the following primers: Forward: TGATCATGTGTAAACGCTGTCAT, Reverse: AGAGACCTGCAGAATTTGTCA. The PCR product was subjected to Ddel enzyme restriction. Wild-type rats were identified by two bands at 120 and 57 kDa and heterozygous rats having an additional band detected at 106 kDa.

Diets

At ~6 weeks of age, female rats were started on NFD (Teklad, catalog TD.07055, 13% kcal from fat, 0.1% sodium) or HFD (Teklad, catalog TD.07054, 42% kcal from fat, 0.12% sodium) and maintained on respective diets until 15 weeks of age. At this time, females were mated with genotyped-matched males, which had been maintained on NIH31 diet, for the generation of timed-pregnant experimental rats. The observation of sperm in vaginal smears was indicative of gestational day 0. Rats were then housed separately. This resulted in four groups of pregnant rats: NFD MC4R^{+/+}, HFD MC4R^{+/+}, NFD MC4R^{+/-} and HFD MC4R^{+/-}. The average ages at gestational day 0 were, in weeks: NFD MC4R^{+/+}: 17.5 ± 0.3; HFD MC4R^{+/+}: 18.4 ± 0.6; NFD MC4R^{+/-}: 17.9 ± 0.7 and HFD MC4R^{+/-}: 19.3 ± 0.9 (*P* > 0.05). Pregnancy success rate was calculated as (total number of rats that became pregnant/total number of initial rats × 100). Cumulative gestational weight gain was determined from

gestational day 0–19, and gestational caloric intake was examined in a subset of rats from each group from gestational day 0–18. Furthermore, caloric intake was examined at the end of pregnancy over gestational day 17–18. These food intake studies were conducted in their home cages. All rats were fasted overnight on gestational day 18 prior to assessment of BP and tissue harvest on gestational day 19.

Echo-MRI

On gestational day 18, total body fat mass and total lean mass were examined using Echo-MRI-700 equipment (Echo Medical Systems, Houston, Texas, USA). For this, rats were placed in restraint cages and the average of two scans recorded for each rat, a process taking less than 5 min.

Mean arterial blood pressure

On gestational day 18, indwelling catheters were implanted in the left carotid artery and exposed at the nape of the neck under isoflurane anesthesia (Butler Schein Animal Health, Dublin, Ohio, USA) using aseptic techniques. Catheters consisted of V/1 tubing attached to V/3 tubing (Scientific Commodities Inc., Lake Havasu City, Arizona, USA).

Approximately 2.5 cm of the V/3 end of the catheter was inserted into the carotid. Catheters were filled with sterile heparin-0.9% saline solution (300 mg/ml; Pfizer, New York City, New York, USA) and stoppered with a stainless steel catheter plug (SP22/12; Instech Laboratories, Inc., Plymouth Meeting, Pennsylvania, USA) to maintain patency. On gestational day 19, in a quiet room, rats were placed in restraint cages and catheters connected to pressure transducers (MLT0699; ADInstruments, Colorado Springs, Colorado, USA) coupled to a computerized data acquisition system (PowerLab; ADInstruments). BP was then recorded for approximately 1 h. By the end of this time, tracings had stabilized to provide a steady BP reading for at least 20 min. This last 20 min of mean arterial blood pressure (MAP) values were averaged and represent the pressure for each rat.

Tissue harvest

On gestational day 19, under isoflurane anesthesia, a midline incision was made and uterine horns with fetuses were exteriorized. Blood was collected from the abdominal aorta into Vacutainer K₂EDTA tubes (BD, Franklin Lakes, New Jersey, USA). The number of viable and reabsorbed fetuses in each animal was recorded along with individual fetus and placenta weights. Data presented are the combined averages from these measurements for each pregnant rat and total fetal and placental weights. Placental sufficiency was calculated as fetal weight divided by placental weight for each fetal-placental unit and then averaged for each pregnant rat; this is similar to the calculation for this measure in humans [20].

Retroperitoneal adipose tissue (including that fat from around the kidneys and adrenals) was weighed. Total body fat mass, lean body mass (both from EchoMRI) and retroperitoneal fat weight are presented as a percentage of maternal body weight (minus fetal and placental weights). Tibias were isolated and their lengths measured. Tissues were snap-frozen in liquid N₂ until processed for biochemistry, as detailed below.

Tissue processing

One hundred to 150 mg of placental (combined decidua and villi) and adipose tissue was homogenized by a Fast-Prep-24 Instrument for 2 min in 2 ml Lysing Matrix D Tubes (MP Biomedicals, Santa Ana, California, USA). For this purpose, placentas were homogenized in 1 ml of radio-immunoprecipitation assay lysis buffer with a protease inhibitor cocktail as per manufacturer's instructions (sc-24948; Santa Cruz Biotechnology, Santa Cruz, California, USA). Adipose tissue was homogenized in 1 ml lysis buffer consisting of 1 mmol/l EDTA tetrasodium salt dihydrate (Fisher Scientific, Waltham, Massachusetts, USA), 10 mmol/l Tris (pH 7.5) and 0.25 mol/l sucrose along with 0.6 mmol/l phenylmethylsulfonylfluoride, 1 mmol/l sodium orthovanadate, 0.27 U/ml aprotinin, 10 μ l/ml protease inhibitor cocktail for mammalian cells and 10 μ l/ml phosphatase inhibitor cocktail 2 (Sigma, St. Louis, Missouri, USA). Homogenates were quickly centrifuged at room temperature at 13 200 rpm and supernatants stored at -80°C until analyzed. Total protein concentration in each sample was quantified by the bicinchoninic acid method (Thermo Scientific, Rockford, Illinois, USA).

Biochemistry

The soluble fms-like tyrosine kinase-1 (sFlt-1) and TNF α levels in placental and adipose tissue supernatants and plasma were quantified using a mouse Flt-1 or rat TNF α ELISA kit, respectively, per manufacturer's instructions (R&D Systems, Minneapolis, Minnesota, USA). The tissue levels were normalized to total protein. Plasma TC was assessed by colorimetric assay (Wako Diagnostics, Osaka, Japan). Plasma leptin and adiponectin (all R&D Systems) were examined by rat ELISAs.

Statistical analysis

Data were graphed and analyzed using GraphPad Prism 5 (La Jolla, California, USA). All data are expressed as mean $\pm$ SEM. For the body weight measurements prior to starting NFD or HFD, data were analyzed using a Student's *t* test. All other data were analyzed using a two-way analysis of variance, the results of which are detailed in each figure panel, where P^{Strain} represents statistics comparing rat genotypes as a whole, regardless of diet; P^{Diet} represents statistics comparing differences between diets as a whole, regardless of rat genotype; and $P^{\text{S}\times\text{D}}$ represents statistics examining whether the differences found are dependent on both rat strain and diet. When a statistically significant interaction was detected, a Holm-Sidak's multiple comparison test was performed as posthoc, as detailed in the appropriate figure legends. The results of the multiple comparison tests are presented in the figure legends as well and noted in the figure panel with corresponding symbols. Statistical significance was set at *P* less than 0.05.

RESULTS

Body weights

Prior to NFD or HFD diets, at ~ 6 weeks of age, the MC4R $^{+/-}$ female rats weighed more than their wild-type MC4R $^{+/+}$ counterparts (168 ± 5 versus 144 ± 10 g, respectively; $P < 0.0004$). A statistical interaction was detected for absolute body weight at gestational day 0, where this measurement was significantly greater ($P < 0.0001$) in MC4R $^{+/-}$ versus MC4R $^{+/+}$ rats

maintained on NFD (Fig. 1a), whereas this significance was not as strong ($P < 0.01$) between the two genotypes on HFD even though MC4R^{+/-} rats were still heavier (Fig. 1a). At gestational day 19, body mass was greater in MC4R^{+/-} versus MC4R^{+/+} rats independent of diet with no effect of HFD within either rat strain (Fig. 1b).

Cumulative weight gain, since beginning NFD or HFD until gestational day 0 of pregnancy was in NFD MC4R^{+/+} 124 ± 4 g ($N = 9$), HFD MC4R^{+/+} 135 ± 10 g ($N = 9$), NFD MC4R^{+/-} 174 ± 10 g ($N = 8$) and HFD MC4R^{+/-} 143 ± 8 g ($N = 8$). Here, an interaction was detected whereby a significant difference was identified between NFD and HFD MC4R^{+/-} versus NFD MC4R^{+/-} ($P < 0.01$). As for gestational weight gain a significant interaction was detected, but posthoc analysis did not reveal any significant differences between any groups although this value appeared to be greatest in the HFD MC4R^{+/-} pregnant rats (Fig. 1c). To assess longitudinal growth, tibia lengths were similar between all groups: NFD MC4R^{+/+} 3.71 ± 0.04 cm ($N = 13$), HFD MC4R^{+/+} 3.73 ± 0.03 cm ($N = 12$), NFD MC4R^{+/-} 3.74 ± 0.03 cm ($N = 11$) and HFD MC4R^{+/-} 3.71 ± 0.06 cm ($N = 7$).

Adipose tissue mass

Adipose tissue masses were examined at the end of pregnancy. Echoing the body weight data, total body fat mass was greater in MC4R^{+/-} over MC4R^{+/+} pregnant rats irrespective of diet type (Fig. 2a). Similarly, visceral retroperitoneal adipose tissue mass was higher in the MC4R^{+/-} strain overall compared with MC4R^{+/+} pregnant rats (Fig. 2b). Total body lean mass was similar between all four experimental groups (Fig. 2c).

Caloric intake

Cumulative caloric intake from gestational day 0–18 of the NFD was greater in MC4R^{+/-} versus MC4R^{+/+} pregnant rats (Fig. 3a). This was not altered by HFD in MC4R^{+/+} pregnant rats, but intake was significantly less in the HFD group of MC4R^{+/-} pregnant rats (Fig. 3a). However, when caloric intake was examined at the end of pregnancy, from gestational day 17 to 18, there was no significant difference in caloric intake between any of the four groups (Fig. 3b).

Blood pressure

At gestational day 19, there was a significant difference in MAP between obese MC4R^{+/-} versus lean MC4R^{+/+} under NFD conditions (Fig. 4a). Heart rate (HR) was not different between obese MC4R^{+/-} versus lean MC4R^{+/+} pregnant rats on NFD (Fig. 4b).

In response to HFD, MAP (Fig. 4a) was higher by gestational day 19 in MC4R^{+/-} rats, whereas there was only a tendency for a greater HR (Fig. 4b) compared with their NFD genetic counterparts. On the contrary, there was no effect of HFD on hemodynamics in MC4R^{+/-} pregnant rats compared with their NFD genetic counterparts (Fig. 4a and b).

Fetal and placental weights and placental sufficiency

Average fetal weights were similar ($P > 0.05$) between all groups (NFD MC4R^{+/+} 1.83 ± 0.04 g; HFD MC4R^{+/+} 1.78 ± 0.05 g; NFD MC4R^{+/-} 1.78 ± 0.05 g and HFD MC4R^{+/-} 1.75 ± 0.03 g). Average placental weights were heavier ($P < 0.05$) in MC4R^{+/-}

rats regardless of diet (NFD MC4R^{+/+} 0.41 ± 0.02 g; HFD MC4R^{+/+} 0.44 ± 0.02 g; NFD MC4R^{+/-} 0.47 ± 0.02 g and HFD MC4R^{+/-} 0.48 ± 0.02 g). The numbers of live fetuses were NFD MC4R^{+/+} 12 ± 1 , HFD MC4R^{+/+} 11 ± 1 ; NFD MC4R^{+/-} 12 ± 1 and HFD MC4R^{+/-} 10 ± 1 . These values were statistically similar among all groups.

Placental sufficiency, a surrogate measure of the nurturing capability of the placenta, was significantly less only in the obese MC4R^{+/-} pregnant rats, which did not depend on dietary conditions (NFD MC4R^{+/+} 4.43 ± 0.18 ; HFD MC4R^{+/+} 4.12 ± 0.12 ; NFD MC4R^{+/-} 3.81 ± 0.14 and HFD MC4R^{+/-} 3.80 ± 0.12). The pregnancy success rate for each group was NFD MC4R^{+/+} 76%; HFD MC4R^{+/+} 88%; NFD MC4R^{+/-} 76% and HFD MC4R^{+/-} 59%.

Circulating obesity-related metabolic factors

Plasma TC (Fig. 5a) and leptin (Fig. 5b) levels were higher in obese MC4R^{+/-} versus lean MC4R^{+/+} pregnant rats regardless of dietary fat content. In contrast, an interaction was identified in plasma adiponectin levels (Fig. 5c). They were lower under NFD conditions in MC4R^{+/-} compared with MC4R^{+/+} pregnant rats, and HFD feeding elicited a lowering of adiponectin in MC4R^{+/+} pregnant rats. In contrast, HFD did not significantly alter adiponectin levels in MC4R^{+/-} pregnant rats compared with their NFD genetic counterparts. Next examined were placental, adipose and plasma levels of the antiangiogenic factor sFlt-1 and pro-inflammatory factor TNF α .

Levels of soluble fms-like tyrosine kinase-1 and TNF α

There were no differences in placental sFlt-1 levels between any of the four groups (Fig. 6a). Adipose tissue levels of sFlt-1 were significantly lower with HFD regardless of rat genotype (Fig. 6b), whereas plasma levels were similar between all four groups (Fig. 6c).

NFD MC4R^{+/-} pregnant rats had greater placental levels of TNF α over those found in MC4R^{+/+} pregnant rats (Fig. 6d). With HFD, there were greater placental TNF α levels in only MC4R^{+/+} pregnant rats over their NFD controls (Fig. 6d). Adipose tissue levels were higher in the HFD groups with no differences between genotypes (Fig. 6e). Plasma levels were not significantly different between the four groups (Fig. 6f).

DISCUSSION

We found that MC4R^{+/-} pregnant rats, regardless of NFD or HFD, had a higher body weight, fat mass and circulating TC and leptin levels compared with MC4R^{+/+} pregnant rats. These diets had no effect on any of these parameters in either rat strain. In contrast, differences in the effects of the diets between the obese and lean rat strains were detected in circulating adiponectin levels, placental TNF α levels and BP. Plasma adiponectin levels were lower, and placental TNF α was greater in the obese pregnant rats on NFD. Significantly, BP levels were higher in the MC4R^{+/-} versus MC4R^{+/+} pregnant rats maintained on a NFD. Although we initially hypothesized that HFD-induced hypertension would be exaggerated in MC4R^{+/-} obese pregnant rats, HFD resulted in higher BP in MC4R^{+/+} pregnant rats with lower in plasma adiponectin and higher placental TNF α levels, HFD did not significantly alter the adiponectin, TNF or BP levels in MC4R^{+/-} pregnant rats when compared with NFD-fed MC4R^{+/-} pregnant rats.

There is a large body of epidemiological evidence supporting that obesity is a major risk factor for hypertension during pregnancy [3,21–31]. Women with pregnancy-induced hypertension had a higher BMI compared with women with a normotensive pregnancy [32]. However, the mechanisms linking obesity to this maternal hypertension are far from understood and thus require studies in animal models. This has been difficult using HFD feeding alone. Thus, we took advantage of the MC4R heterozygous rat strain, which is obese and fertile in comparison with the other genetic models of obesity [33,34]. Use of MC4R^{+/-} rats is of particular interest and clinically relevant for the purpose of studying the effects of obesity during pregnancy. Indeed, haploinsufficiency of the MC4R is one of the most common causes of monogenetic obesity in humans, and it has been shown that MC4R mutations are present in 1.72 and 0.15% in obese versus nonobese subjects, respectively, and are associated with overeating in adults and children [8,35,36]. It has been shown that HFD exaggerates the development of obesity in nonpregnant MC4R^{+/-} female mice [19,37,38]. We examined the effects NFD and HFD on body weight and BP regulation in pregnancy in lean and obese rats. Our model of obesity during pregnancy was MC4R^{+/-} rats, which were compared with lean MC4R^{+/+} pregnant rats.

When maintained on a NFD, MC4R^{+/-} rats had significantly greater absolute prepregnancy (gestational day 0) body mass as compared their MC4R^{+/+} counterparts. HFD did not promote any dramatic differences in prepregnancy (gestational day 0) body weight between MC4R^{+/+} and MC4R^{+/-} rats. The fact that we did not observe a significant increase in body weight in the wild-type rats in response to HFD feeding as compared with other studies may have resulted from such studies not always utilizing the proper control NFD [39]. This may skew the outcomes for HFD-induced changes in body weight as the macronutrient and micronutrient changes are not necessarily from the same source as the NFD. Furthermore, it is important to utilize rats that are obtained from the same breeding colony so as to control for maternal and paternal dietary conditions, such as in our study. At gestational day 19, absolute body weight and adipose tissue masses were greater in the MC4R^{+/-} over the MC4R^{+/+} rat strain as a whole. HFD did not alter these weights in either strain. The lack of an effect of HFD on body and fat weights, particularly in the MC4R^{+/-} pregnant rats, could be due to the lower caloric intake only in this strain in response to HFD feeding.

Cumulative caloric intake during gestation was higher in MC4R^{+/-} versus MC4R^{+/+} with NFD, which has been found in nonpregnant states as well. The caloric intake of the HFD was not different in MC4R^{+/-} versus MC4R^{+/+} rats and therefore was lower when compared with MC4R^{+/-} pregnant rats on NFD. This lower caloric intake of HFD with MC4R deficiency seems to be specific to pregnancy as Cone's group showed that food intake of a 60% HFD was increased in nonpregnant female MC4R^{+/-} mice and was exaggerated in MC4R^{-/-} along with increases in body weight gain [19]. It has been shown that nonpregnant female MC4R^{-/-} mice have reduced O₂ consumption, as a measure of metabolic activity [40]. However, at the end of pregnancy, as there were no differences in food intake between gestational day 17 and 18 in any of the groups, the BP levels, absolute body weight and adipokine and placental factor levels may be independent of amount of calories consumed.

It is known that adiponectin increases metabolic rate. It has been shown that obesity *per se* is associated with reduced adiponectin levels, which we found in NFD MC4R^{+/-} versus NFD

MC4R^{+/+} pregnant rats. As we also showed that HFD feeding in pregnant MC4R^{+/+} rats was accompanied by similarly lower levels of adiponectin as found in the MC4R^{+/-} pregnant rats on NFD, these data suggest that lower adiponectin levels are not responsible for the increased body weight in the MC4R^{+/-} pregnant rats on NFD. In fact, it suggests that pregnancy is a state of adiponectin resistance. This is supported by the human literature showing that increased gestational body weight gain is accompanied by increased basal metabolic rate but adiponectin levels were not examined. In our rodent study, there was a tendency for greater adiponectin levels and greater gestational weight gain in MC4R^{+/-} rats on HFD. Again, this suggests that, in pregnancy, there is resistance in the link between the effects of adiponectin on metabolic activity and body weight.

In humans, absolute obesity measured as BMI during mid-to-late pregnancy is associated with greater adiposity and hypertensive disorders of pregnancy [41]. Therefore, based on our findings that MC4R^{+/-} obese pregnant rats on NFD have increased body weight and adipose tissue mass at gestational day 19, we hypothesized that BP would be increased in these rats. Indeed, MC4R^{+/-} pregnant rats on NFD had elevated BP compared with MC4R^{+/+} control rats. Subsequently, because body weight was still elevated in MC4R^{+/-} versus MC4R^{+/+} pregnant rats on HFD and was now also accompanied by greater gestational weight gain along with exaggerated visceral adipose tissue mass at gestational day 19, it was expected that BP would be greater in MC4R^{+/-} versus MC4R^{+/+} pregnant rats both on HFD and potentially even greater than NFD-fed MC4R^{+/-} pregnant rats. However, this was not the case.

A potential mechanism mediating the higher BP in MC4R^{+/+} pregnant rats on HFD and MC4R^{+/-} pregnant rats on NFD is the elevated placental levels of TNF α found in these animals compared with MC4R^{+/+} pregnant rats on NFD, as we previously found that infusion of TNF α elicits hypertension in pregnant rats [42]. In addition, circulating leptin levels were doubled in MC4R^{+/-} versus MC4R^{+/+} pregnant rats on NFD. Studies in nonpregnant female MC4R^{+/-} mice have shown a purified NFD having 10% energy from fat have greater body weight and fat mass at 30 weeks of age, but that there is no significant difference in leptin levels compared with wild-type mice [43]. These data suggest that pregnancy exaggerates leptin production in genetic obesity, most likely coming from both placental and adipose tissue sources. Furthermore, it is important to remember that our MC4R^{+/-} pregnant rat model has only one functional MC4R allele. This receptor is necessary for leptin to raise BP as demonstrated by the lack of a BP response to leptin in MC4R global knockout mice [44]. This BP regulatory effect of leptin occurs first by leptin in obesity gaining access through the blood brain barrier by uptake via leptin receptors followed by leptin's action on its receptors located on POMC neurons in the hypothalamus to activate downstream MC4R-expressing second-order neurons [45]. Significantly, it has been shown that pregnant versus nonpregnant rats have reduced satiety responses to α -melanocortin stimulating hormone, which is the molecule linking leptin signaling to MC4R activation, but BP responses were not examined [46]. Future studies should examine if MC4R receptors mediate the increased BP in our NFD MC4R^{+/-} pregnant rats. However, we believe that the BP and food intake effects of MC4R signaling to be disconnected in our model. We have previously demonstrated that nonpregnant female MC4R^{-/-} rats are obese

but unlike males have elevated BP. This suggests that this pathway may not mediate obesity-induced hypertension in females and during pregnancy.

In conclusion, our data suggest that genetic obesity induced by MC4R deficiency and HFD in MC4R^{+/+} rats increase BP and placental inflammation during pregnancy. However, HFD did not exaggerate BP levels over the increased levels already found in NFD MC4R^{+/-} pregnant rats or over the increase it induced in MC4R^{+/+} pregnant rats, even though the former rat strain had greater body weight and fat mass. Thus, obesity and HFD seem to be independently related to hypertension in pregnancy with no synergistic effects on BP. We did not examine which of the four hypertensive disorders of pregnancy is modeled by our obese pregnant rats. We only present that they have increased BP at the end of pregnancy. Future telemetry-based studies will determine whether our obese female rats have increased BP before pregnancy and/or whether pregnancy induces hypertension in the same obese rats. It is important to highlight that placenta dysfunction is thought to drive the pathogenesis of preeclampsia. Human studies support that preeclamptic women have ‘inappropriately heavy placentas’ resulting in reduced placental sufficiency [47], as we found in our obese pregnant rat strain. We propose that our MC4R^{+/-} obese pregnant rats are an excellent model to examine the effects of obesity and obesity-related metabolic factors on implantation and placentation. Moreover, based on our findings, we propose that the hypertensive response to surgically induced placental ischemia and hypoxia utilizing the reduced uterine perfusion pressure model may be exaggerated in the presence of HFD feeding or obesity. This is clinically relevant as placental ischemia is a major hypothetical pathogenic factor for preeclampsia, and increased BP response to this insult under obesogenic conditions may explain why obese women are at increased risk for preeclampsia.

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Abbreviations

BP	blood pressure
GD	gestational day
HFD	high-fat diet
MAP	mean arterial blood pressure
MC4R	melanocortin-4 receptor
NFD	normal-fat diet
sFlt-1	soluble fms-like tyrosine kinase-1
TC	total cholesterol

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FIGURE 1.

Prepregnancy body weights at 15 weeks of age (a), following Holm–Sidak’s multiple comparison test, number of comparisons per family = 6, number of families = 1; * $P < 0.0001$ versus melanocortin-4 receptor^{+/+} on normal-fat diet; ** $P < 0.01$ versus melanocortin-4 receptor^{+/+} on high-fat diet), gestational day 19 body weights (b) and gestational weight gain from gestational day 0 to 19 (c), following Holm–Sidak’s multiple comparison test, number of comparisons per family = 1, number of families = 1; *** $P = 0.04$ versus melanocortin-4 receptor^{+/+} on high-fat diet) in melanocortin-4 receptor^{+/+} ($N = 15$ – 16 /group) and melanocortin-4 receptor^{+/-} ($N = 10$ – 13 /group) pregnant rats on normal-fat diet or high-fat diet, respectively.

FIGURE 2.

Total body fat mass (EchoMRI) (a) retroperitoneal adipose tissue mass (b) and lean mass (EchoMRI) (c) in melanocortin-4 receptor^{+/+} ($N=11/\text{group}$) and melanocortin-4 receptor^{+/-} ($N=7-10/\text{group}$) pregnant rats on normal-fat diet or high-fat diet, respectively, at gestational day 19.

FIGURE 3.

(a) Gestational caloric intake of normal-fat diet or high-fat diet measured in kcal from gestational days 0 to 18 in melanocortin-4 receptor^{+/+} ($N = 5$ or $N = 4$, respectively) and melanocortin-4 receptor^{+/-} ($N = 4$ or $N = 4$, respectively) pregnant rats. Following Holm–Sidak’s multiple comparison test, number of comparisons per family = 6, number of families = 1; * $P < 0.05$ versus melanocortin-4 receptor^{+/+} on normal-fat diet; ** $P < 0.05$ versus melanocortin-4 receptor^{+/-} on normal-fat diet. (b) Caloric intake of normal-fat diet or high-fat diet from gestational day 17 to 18 in melanocortin-4 receptor^{+/+} ($N = 5$ or $N = 6$, respectively) and melanocortin-4 receptor^{+/-} ($N = 6$ or $N = 6$, respectively) pregnant rats.

FIGURE 4.

Mean arterial blood pressure (a) following Holm–Sidak’s multiple comparison test, number of comparisons per family = 6, number of families = 1) and heart rate (b); * $P = 0.049$ versus melanocortin-4 receptor^{+/+} on normal-fat diet; ** $P = 0.031$ versus melanocortin-4 receptor^{+/+} on normal-fat diet) in melanocortin-4 receptor^{+/+} ($N = 10$ – 16 /group) and melanocortin-4 receptor^{+/-} ($N = 6$ – 15 /group), respectively, on normal-fat diet or high-fat diet at gestational day 19.

FIGURE 5.

Plasma total cholesterol (a), leptin (b) and adiponectin (c) following Holm-Sidak's multiple comparisons test, number of comparison per family = 2, number of families = 1; * $P = 0.04$ versus melanocortin-4 receptor^{+/+} on normal-fat diet) in melanocortin-4 receptor^{+/+} ($N = 12-16$ /group) and melanocortin-4 receptor^{+/-} ($N = 10-15$ /group) pregnant rats on normal-fat diet or high-fat diet, respectively, at gestational day 19.

FIGURE 6.

Placental, adipose tissue and plasma soluble fms-like tyrosine kinase-1 (a–c, respectively) and TNFα levels (d–f, respectively) following Holm–Sidak’s multiple comparisons test, number of comparisons per family = 6, number of families = 1; * $P = 0.03$ versus melanocortin-4 receptor^{+/+} on normal-fat diet, ** $P = 0.02$ versus melanocortin-4 receptor^{+/+} on normal-fat diet) in placenta from melanocortin-4 receptor^{+/+} ($N = 12$ –16/group) and melanocortin-4 receptor^{+/-} ($N = 10$ –14/group) pregnant rats on normal-fat diet or high-fat diet, respectively, at gestational day 19.