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Sex and age-dependent effects of a maternal junk food diet on the mu-opioid receptor in rat offspring

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Running title: maternal diet and mu-opioid receptor development

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Abstract

Perinatal junk food exposure increases the preference for palatable diets in juvenile and adult rat offspring. Previous studies have implicated reduced sensitivity of the opioid pathway in the programming of food preferences; however it is not known when during development these changes in opioid signalling first emerge. This study aimed to determine the impact of a maternal junk food (JF) diet on mu-opioid receptor (MuR) expression and ligand binding in two key regions of the reward pathway, the nucleus accumbens (NAc) and the ventral tegmental area (VTA) in rats during the early suckling (postnatal day (PND) 1 and 7) and late suckling/early post-weaning (PND 21 and 28) periods. Female rats were fed either a JF or a control diet for two weeks prior to mating and throughout pregnancy and lactation. MuR expression in the VTA was significantly reduced in female JF offspring on PND 21 and 28 (by 32% and 57% respectively, $P<0.05$), but not at earlier time points (PND 1 and 7). MuR ligand binding was also reduced (by 22%, $P<0.05$) in the VTA of female JF offspring on PND 28. No effects of perinatal junk food exposure on MuR mRNA expression or binding were detected at these time points in male offspring. These findings provide evidence that the opioid signalling system is a target of developmental programming by the end of the third postnatal week in females, but not in males.

Key Words: programming, reward, mu-opioid receptor, high-fat diet

Abbreviations

ANOVA, analysis of variance; C, control diet; JF, junk food diet; MuR, mu-opioid receptor; NAc, nucleus accumbens; PND, postnatal day; qRT-PCR, quantitative real-time PCR; SEM, standard error of the mean; VTA, ventral tegmental area.

1. Introduction

Human and animal studies have provided compelling evidence that food preferences are programmed in response to specific nutritional exposures before birth and/or in early infancy. A number of these studies have demonstrated that excessive maternal intake of 'junk foods' (palatable foods that are high in fat and sugar) during pregnancy and lactation results in an increased preference for fat and sugar in the juvenile and adult offspring [1-3]. In adults, palatable food intake is controlled primarily by the mesolimbic reward pathway. Consumption of palatable foods activates this reward pathway by stimulating the release of endogenous opioids. These opioids then bind to opioid receptors in the ventral tegmental area (VTA), activating dopaminergic neurons in this brain region, and in the nucleus accumbens (NAc), the site of dopamine release, to potentiate dopamine signalling [4-6]. Involvement of the opioid pathway in the interactions between the VTA and NAc, and in stimulating dopamine signalling in the reward pathway, is thought to be critical in mediating the acute pleasurable sensation associated with the consumption of palatable food [7, 8].

Altered development of the mesolimbic reward system, in particular the opioid signalling system, has been implicated in the early programming of food preferences [9-12]. A number of studies have reported changes in the expression of a key component of the opioid signalling pathway, the mu-opioid receptor (MuR), in both the VTA and NAc of adult offspring of dams fed a junk food or high-fat diet [1, 3, 13]. We have previously reported that a reduction in MuR expression in the offspring of dams maintained on a junk food diet during pregnancy and lactation is present in the VTA at weaning (3 weeks after birth) [14]. Importantly, we have also shown that this decrease in MuR expression has functional consequences for the regulation of palatable food intake in these animals [14]. However, to date no studies have looked directly at how exposure to a junk food diet may affect the postnatal developmental trajectory of MuR expression before 3 weeks of postnatal age. Identifying when the changes to MuR expression first occur would provide a valuable insight into the mechanisms that underlie the programming of food preferences.

Studies of the ontogeny of MuR in the human fetus and infant are understandably limited by the scarcity of appropriate tissue samples, for this reason there has been a reliance on animal models, particularly rodents, to provide information on their development. The available data show that MuR in the forebrain is expressed as early as embryonic day 13 of the rat [15]. Furthermore, MuR expression in the NAc has been shown to be present at its highest levels during the first postnatal week, decreasing to adult levels over the following two weeks [16].

However, these prior experiments were undertaken only in males and did not evaluate MuR expression in key regions of the mesolimbic reward pathway outside the forebrain, including the VTA. Thus, in addition to investigating the impact of altered nutrition on the development of the reward pathway, further studies are needed to delineate the ontogeny of MuR in both male and female rats during early postnatal life.

Therefore the aim of the present study was to determine the effect of exposure to a junk food diet before birth, during the suckling period and immediately post-weaning on MuR expression and ligand binding in male and female rat offspring. This was achieved by performing *in situ* hybridisation and autoradiographic ligand binding procedures on brain tissue collected from offspring during the early suckling (postnatal day (PND) 1 and PND 7) and late suckling/early post-weaning (PND 21 and PND 28) periods. A secondary aim of the study was to investigate the normal ontogeny of MuR mRNA expression in male and female rats across this period.

2. Methods

2.1 Animals and feeding

The study was approved in advance by the Animal Ethics committee of the University of Adelaide. 10 female Albino Wistar rats were allowed to acclimatise to the animal housing facility for 1 week before the start of the dietary intervention. During this period, all rats were fed a standard laboratory rodent feed (Specialty Feeds, Glen Forrest, WA, Australia). Following the acclimatisation period, female rats were placed into one of two weight matched groups, the control group (C, n=5) or junk food group (JF, n=5). The control group received a diet of standard laboratory rodent feed (Specialty Feeds, Glen Forrest, WA, Australia). The JF group were fed a junk food diet which included hazelnut spread, peanut butter, chocolate biscuits, savoury snacks, sweetened cereal and a lard and chow mix. Detailed nutritional information on this diet has been provided previously [1]. Food intake (g) was determined every 2 days by weight difference (subtracting the amount left uneaten in the cage from that supplied at the start of the 2 day period) and rats were weighed weekly throughout the experiment. The animals were housed individually and kept at a room temperature of 25°C in a 12:12 light-dark cycle.

The experimental diets were provided for 2 weeks prior to mating and throughout pregnancy and lactation. Females were mated with 4 proven males that were maintained on the standard laboratory rodent feed. The same males were used for mating with both the C and JF groups. In the female rats, vaginal smears were performed daily to chart the stages of the estrous cycle.

On the night of pro-estrus, each female rat was placed with a male overnight and vaginal smears were conducted the following morning. The presence of sperm in the vaginal smears was taken as confirmation of successful mating and was designated as gestation day 0. Pups were born on day 21-22 of gestation. On the day after birth (postnatal day (PND) 1), brain tissue was collected from 1 male and 1 female pup per litter and the remaining pups were culled to 10, 5 males and 5 females where possible. Pups were weighed every 2 days during the suckling period and were weaned on PND 21. Weaned pups were housed with a same sex littermate and fed the same diet as their mothers until the conclusion of the experiment on PND 28.

2.2 Post mortem and tissue collection

1 male and 1 female pup from each litter was humanely killed at PND 1, PND 7 and PND 21. The remaining pups in each litter (2 male and 2 female) were killed at the end of week 4 (PND 28). Post mortems were conducted between 0800h and 1200h with rats weighed immediately prior to euthanasia. Blood samples were collected via cardiac puncture into heparinised tubes and centrifuged at 3,500 g at 4ºC for 15 minutes. Body weight, length (nose to tail) and abdominal circumference were recorded. Brain and liver samples were collected from offspring on PND 1 and 7. At PND 21 and 28, all internal organs were weighed and all visible fat depots, including omental fat, retroperitoneal fat, gonadal fat, subcutaneous fat and interscapular fat were collected. The weights of all individual fat depots were added together to determine total body fat mass. All tissues and fat depots were frozen in liquid nitrogen and stored at -80°C.

2.3 Brain tissue sectioning

At post-mortem, brain tissue for *in situ* hybridisation was snap frozen and placed into a cryostat mould to facilitate the cutting of 15µm sagittal sections. Sagittal sections (n=120/ animal) were cut on a cryostat (Leica Cryostat; Leica, Wetzler, Germany) and mounted on Menzel-Glaser Super frost plus slides (25x75x1.0 mm, Menzel-Glaser, Braunschweig, Germany) then stored at -80˚C in sealed containers. Sections were cut in series of 8, meaning the first 8 sections cut from each block were mounted on 8 separate slides, the second set of 8 sections were mounted consecutively below the first and the third set of 8 sections below this. One slide from each series of 8, was stained with haemotoxylin and eosin using standard methods and the anatomical location of the key regions of interest (VTA and NAc) were identified using specific brain landmarks and a neonatal rat brain atlas [17]. Brain tissue for ligand binding autoradiography was cut into serial 14µm coronal sections. Two adjacent sections were thaw-mounted onto Superfrost Plus slides (Sigma-Aldrich, Poole, UK) in four rostrocaudal series for each animal. Slides were allowed to air-dry at room temperature and stored at -80°C.

2.4 Probe synthesis and in situ hybridisation

An antisense probe specific for the MuR was synthesised from PCR primer DNA (5' CAG AGA TGC AAT TAA CCC TCA CTA AAG GGA GAA CTG GGA GAA CCT GCT CAA A; 3' CCA AGC CTT CTA ATA CGA CTC ACT ATA GGG AGA TGT GGT TTC TGG AAT CGT GA) (Applied Biosystems, Foster City, CA, USA) before being purified using QIAquick Gel extraction kit (Qiagen, Limburg, Netherlands) and sequenced to confirm its specificity for the gene of interest. A sense probe was also synthesised to act as a negative control. The probes were labelled using radioactive ^{35}S UTP and the activity quantified by scintillation counting. Successful probe synthesis yielded approximately 2.5 million counts per minute per µl.

In situ hybridisation experiments were performed as previously described [18]. Briefly sections were fixed in 4% paraformaldehyde, acetylated, dehydrated through an ethanol gradient and hybridised overnight at 55°C using [^{35}S]-labelled cDNA probe. The sections were then treated with RNaseA (Ambion, Texas, USA) to remove unhybridised probe and desalted with a high stringency wash (30 min) in 0.1% saline-sodium citrate (SSC) at 65°C. Following dehydration through an ethanol gradient, slides were air-dried before being exposed under photographic emulsion at 4°C for 6 weeks. Under emulsion, silver grain formation was carefully monitored with a series of test slides to ensure that overexposure did not occur and that single cells were distinct. After development, the slides were counterstained with cresyl violet to allow the regions of interest (VTA and NAc) to be accurately located. The number of labelled cells (those containing silver granules) within each of these brain regions was counted at X200 magnification using Image Proplus 5.1 as detailed in [19]. In older animals (PND 21 and 28) two fields of view were counted for the VTA and three for the NAc, whilst in younger animals (PND 1 and PND 7) one field of view was counted for the VTA and two for the NAc, given the much smaller overall brain size.

2.5 Ligand binding autoradiography

Ligand binding autoradiography for MuR was carried out on brain tissue collected from 1 male and 1 female pup per litter at PND 28 (separate pups from those used for *in situ* hybridisation analysis). Details of the procedure have been described previously [20]. Briefly, frozen sections were fixed with 0.1% paraformaldehyde for 2.5 minutes before incubation for 120 minutes at room temperature in Tris-HCl buffer (50mM Tris-HCl, 120mM NaCl, 5mM KCl, 2mM $CaCl_2$, 1mM $MgCl_2$, 0.1% BSA, pH 7.4; Sigma-Aldrich) containing 4nM DAMGO [tyrosyl-3,5-^{3}H(N)] (NET 902; Perkin Elmer, Boston, MA), a ligand that binds to MuR with high affinity. Unbound ligand was removed by washes in Tris-HCl buffer and deionised water.

Sections were then air-dried before exposure for 12 weeks to BioMax MR film (Kodak, Rochester, NY) along with ^{3}H autoradiographic microscale standards (American Radiolabeled Chemicals, Royston, UK). In an adjacent series of sections, competition of DAMGO [tyrosyl-3,5-^{3}H(N)] binding with 1 μM non-radioactive naloxone (N7758; Sigma-Aldrich) resulted in elimination of autoradiographic signals. After film development, sections were counterstained with cresyl violet for microscopic examination. Images of the film autoradiographs and corresponding stained sections were overlayed and quantification of autoradiographic signal was carried out in the appropriate anatomical areas using MCID Core Software (InterFocus Imaging, Cambridge, UK).

2.6 Statistical analysis

Analysis of maternal food intake and body weight data as well as birth outcomes was conducted using Student's unpaired t-tests. Two-way ANOVA with sex and maternal diet as factors was used to analyse MuR expression at each time point as well as body fat mass and ligand binding on PND 28. When a significant interaction between sex and maternal diet was identified, data were separated by sex and the effect of maternal diet analysed using Student's unpaired t-tests. Two-way ANOVA, and Student's unpaired t-tests, were performed using SPSS statistics 18.0 software (SPSS Inc., Chicago, IL, USA). Offspring body weight gain and MuR expression data over time were analysed by two-way repeated measures ANOVA, which was performed using Stata 11 software (StataCorp., TX, USA). All data are presented as mean ±SEM and a *P* value of <0.05 was considered statistically significant.

3. Results

3.1 Dam body weight and nutritional intake during pregnancy and lactation

There was no significant difference in body weight between the control and JF dams prior to mating (control 224.1±6.8g, JF 210.2±10.4g), immediately post-partum (control 391.6±17.6g, JF 446.8±19.8g) or when the pups were weaned (control 338.5±7.6g, JF 366.4g±9.4g). There was also no difference in gestational weight gain (as a percentage of pre-pregnancy bodyweight) between groups (control 33.9±3.8%, JF 39.4±2.3%). During pregnancy, the JF dams consumed significantly more fat and less protein than the controls with no differences in carbohydrate or overall energy intake between groups (Table 1). Throughout lactation, in addition to the increased fat and decreased protein intake, the JF dams also consumed less carbohydrate and had a lower overall energy intake compared to control dams (Table 1).

3.2 Effect of maternal diet on birth outcomes, body composition and growth

Maternal diet had no effect on litter size (control 15.1±1.0, JF 14.3±1.1) or the percentage of males per litter (control 53.5±3.1%, JF 53.2±5.0%). Offspring of JF dams had a significantly lower birth weight for both the male and female pups (Table 2). On PND 28, both male and female control offspring were heavier than their JF counterparts, however, the JF offspring of both sexes had a significantly higher total fat mass relative to bodyweight than the controls (Table 2).

3.3 Effect of maternal diet on MuR expression on PND 1 and PND 7

On PND 1, there was a significant interaction between maternal diet and sex for MuR mRNA expression in the NAc. When this interaction was explored using Student's unpaired t-tests, the number of cells expressing MuR in this brain region tended to be lower in male JF offspring (P=0.08), but higher in female JF offspring (P=0.07) compared to their control counterparts (Fig. 1A). There was no effect of either maternal diet or sex on MuR expression in the VTA on PND 1 (Fig. 1B) or in the VTA or NAc on PND 7 (Fig. 1C, D).

3.4 Effect of maternal diet on MuR expression on PND 21 and PND 28

There was no significant effect of maternal diet on the number of cells expressing MuR in the NAc in offspring of either sex on PND 21 or 28, or in the VTA in male offspring on PND 21 or 28 (Fig. 2A, 3A). However, in the VTA on PND 21 and 28 the number of cells expressing MuR was significantly lower in female offspring of JF dams compared to female offspring of control dams (Fig. 2B, 3B).

3.5 Effect of maternal diet on MuR ligand binding in late postnatal development

On PND 28, MuR binding in the NAc or VTA was not affected by maternal diet in the males (Fig. 4A, B). However, female offspring of JF dams showed a higher level of binding in the NAc than female offspring of control dams; the level in the latter group was also significantly lower than in the two male groups (Fig. 4A). In the VTA, MuR binding in the female offspring of JF dams was significantly lower than in the female offspring of control dams and in the two male groups (Fig.4B).

3.6 MuR expression across postnatal development

Independent of maternal diet, the number of cells expressing the MuR in the NAc was higher on PND 1 and 7 than on PND 21 and 28 in both male and female offspring (Fig. 5A). A similar pattern was observed in the VTA, with a higher number of MuR expressing cells on PND 1 and 7 than on PND 21 in males and females; however, by PND 28 the number of cells

expressing MuR in the VTA had increased and was no longer significantly different from that seen on PND 1 or 7 (Fig. 5B).

4. Discussion

The current study provides novel insights into the ontogenic profile of MuR expression in two key regions of the rat mesolimbic reward pathway, the VTA and NAc. It demonstrates that this developmental profile can, at least in female offspring, be altered by exposure to a junk food diet. We show that the VTA in female offspring exposed to the junk food diet before birth, via the dam's milk and immediately post-weaning has reduced MuR mRNA expression at PND 21 and 28 and reduced MuR ligand binding at PND 28. This, together with the present finding of a sex difference in the response to a maternal junk food diet, provides new information about the programming of food preferences.

4.1 Maternal junk food decreases MuR expression and ligand binding in the VTA in the late suckling/early post-weaning period

In the VTA of JF females we observed significant reductions in the number of cells expressing MuR mRNA at PND 21 and 28 and in the intensity of MuR ligand binding at PND 28; such findings seem consistent with those obtained in our previous study which utilised qRT-PCR to measure MuR expression [14]. However, in contrast to those earlier results, we did not detect a reduction in MuR expression in male offspring. This may be due to differences in the sensitivity of the methods used in the respective studies, as the reduction in MuR expression in the VTA in the previous study was less pronounced in male offspring (a 1.4 fold decrease) than in females (a 1.9 fold decrease) [14]. It is therefore possible that the *in situ* hybridisation method used in the current study did not provide sufficient sensitivity to detect the quantitatively smaller changes in gene expression present in male JF offspring at this age. Furthermore, in addition to reduced MuR expression in their VTA, JF females in our previous study had an increased preference for fat at 4 weeks after birth, an effect not observed in males [14]. This may indicate that female offspring are more susceptible to the effects of a maternal JF diet on food preferences/reward signalling and that there is a threshold for reduction in MuR expression to induce changes in food preference that was not reached in male offspring.

The decreases in MuR expression observed in the VTA in female JF offspring at PND 21 and 28 were not present on PND 1 or 7, suggesting that maternal JF diet exposure has a greater effect on this opioid system in the later period of postnatal brain development than before or immediately after birth. Alternatively, it may be that continual exposure to the junk food

stimulus from before birth to the end of the suckling period is necessary to induce significant changes in MuR expression. Previous studies have reported that exposure to a junk food/high-fat diet during the suckling period or first week post-weaning is associated with a heightened preference for fat and susceptibility to obesity in the adult offspring equivalent to that observed in offspring exposed to the junk food diet before birth as well as through the breastmilk [21-23]. There are currently no studies, however, which have determined the impact of exposure to a junk food diet in the early postnatal period alone on MuR expression; these will be required to definitively establish the importance of the timing of exposure for the programming of opioid signalling and food preferences.

4.*2 Maternal junk food diet exposure has sex-specific effects on MuR expression and ligand binding in the NAc*

No significant changes in MuR mRNA expression were identified in the NAc up to PND 28 in either male or female offspring. However, the MuR binding at this site on PND 28 was lower in control females than control males, but exposure to the junk food diet increased the binding in the females to the level found in their male counterparts. It should be noted that we have previously demonstrated an increase in MuR mRNA in the NAc of male offspring exposed to a maternal junk food diet at 3 and 5 weeks postnatally, an effect which was not seen in females [14, 24]. Nevertheless, in the present study the JF-related rise in MuR binding in the NAc was limited to female offspring; this implies post-translational influences on the MuR, a possibility that requires further investigation.

Despite no significant changes in MuR mRNA expression in the NAc being observed in response to the maternal junk food diet, we did identify an interaction between maternal diet and sex on PND 1. Such that junk food exposure tended to decrease the number of cells expressing MuR in male offspring and increase the number of cells expressing MuR in females. We have previously shown a sex-specific increase in MuR expression in the NAc after cafeteria diet exposure [23]. However, in that study we saw an increase in MuR expression in male but not female JF offspring at 5 weeks of age, highlighting the importance of the age of the offspring at the time that MuR expression is measured on the results [25]. Estrogen has been shown in previous studies to play an important role in opioid pathway regulation in brain areas outside the VTA and NAc [26, 27] and sex differences in response to maternal morphine administration MuR have also been reported [28, 29]. Thus, the sex differences observed in response to a maternal junk food diet could potentially be the result of an interaction between sex-hormones, including estrogen, and increased exposure to endogenous opioids. However, it

is important to note that the animals in this study were all pre-pubertal, and further studies are needed to test this directly.

4.3 MuR expression in NAc is higher in the early suckling period than in the late suckling/ early post-weaning period

Consistent with previous *in situ* hybridisation findings[16], MuR mRNA expression in the NAc was higher at birth and at PND 7 than at PND 21 or 28, independent of maternal diet or sex. The higher level of MuR expression at birth and during early postnatal development, together with evidence suggesting that MuRs from PND 5 to 21 show very low G protein coupling in the NAc, indicate that MuR may have reduced functionality in the early postnatal period, compared to PND 30 and adulthood [30]. Unlike the development of the MuR in the NAc [16], there has been no previous quantification of the ontogeny of MuR in the VTA. In the current study, MuR expression in this region declined from the first week after birth to PND 21, before increasing at PND 28.

The current study is the first to compare the developmental ontogeny of MuR mRNA expression between male and female offspring. Sex differences in the prenatal developmental profile of dopaminergic fibres in the striatum have been previously reported [31]. However, we found no significant differences between male and female control or JF offspring in MuR mRNA expression at any of the time points we observed. Further studies are required to determine whether the similar developmental trajectory between males and females at the mRNA level extends to the receptor's function.

4.4 Conclusions and speculation

The current study is the first to demonstrate that exposure to a maternal junk food diet before birth and during the early postnatal period results in a reduction in MuR expression in the VTA during the third and fourth postnatal week in female offspring. That this reduction in MuR expression was not observed at birth or at the end of the first postnatal week but was present by the time of weaning suggests that the late suckling period is a critical window during which exposure to a maternal junk food diet may have a pronounced effect on opioid signalling. These findings raise the possibility that interventions applied during the late suckling period could potentially counteract the deleterious effects of exposure to a junk food earlier in development. Furthermore, the sex-specific response to maternal junk food diet exposure identified in this study emphasises the need to consider both sexes when investigating reward pathway development and the programming of food preferences. The current work provides novel

insights into the impact of maternal junk food diet exposure on postnatal MuR development and thus provides valuable information on a potential mechanism behind the programming of food preferences.

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Figure legends

Figure 1 Number of labelled cells expressing the mu-opioid receptor per field of view in the NAc (A, C) and VTA (B, D) of the male and female offspring of control dams and junk food (JF) fed dams on PND 1 (A, B) and PND 7 (C, D). An interaction between maternal diet and sex was present in the NAc at birth ($P<0.05$). Results presented as mean±SEM. n=4-5 pups for all groups.

Figure 2 Number of labelled cells expressing the mu-opioid receptor per field of view in the NAc (A) and VTA (B) of the male and female offspring of control dams and junk food (JF) fed dams on PND 21. * indicates significantly different mean ($P<0.05$). Examples of mu-opioid receptor expression demonstrated through silver grain formation, over cells which have been counterstained with cresyl violet in the VTA of control (C) and JF (D) female offspring (x400 magnification). Results presented as mean±SEM. n=5 pups for all groups.

Figure 3 Number of labelled cells expressing the mu-opioid receptor per field of view in the NAc (A) and VTA (B) of the male and female offspring of control dams and junk food (JF) fed dams on PND 28. * indicates significantly different mean ($P<0.05$). Examples of mu-opioid receptor expression demonstrated through silver grain formation, over cells which have been counterstained with cresyl violet in the VTA of control (C) and JF (D) female offspring (x400 magnification). Results presented as mean±SEM. n=5 pups for all groups.

Figure 4 Mu-opioid receptor ligand binding in the NAc (A) and VTA (B) of the male and female offspring of control dams or junk food (JF) fed dams on PND 28. * indicates significantly different mean ($P<0.05$). Results presented as mean±SEM. n=5 pups for all groups. Representative film autoradiographs showing mu-opioid receptor ligand binding at rostrocaudal levels incorporating (C) the nucleus accumbens (NAc) or (D) the ventral tegmental area (VTA) in a female offspring of a junk food (JF) fed dam on PND 28. Scale bars = 2 mm.

Figure 5 Number of labelled cells expressing the mu-opioid receptor per field of view in the NAc (A) and VTA (B) in the offspring of control dams and junk food (JF) fed dams across postnatal development: control males, open bars; JF males, closed bars; control females, striped bars; JF females, grey bars. # indicates significant effect of time ($P<0.05$). Results presented as mean±SEM. n=4-5 pups for all groups.

Table 1 Maternal macronutrient intake during pregnancy and lactation in control and JF dams

	Control	JF
Pregnancy		
Fat (g/kg/d)	3.5±0.1	17.2±1.2**
Protein (g/kg/d)	14.8±0.6	9.1±0.4*
Carbohydrate (g/kg/d)	45.4±1.8	43.1±2.3
Energy (kJ/kg/d)	1362.7±55.4	1461.3±72.7
Lactation		
Fat (g/kg/d)	6.6±0.2	23.3±0.8**
Protein (g/kg/d)	28.3±0.6	14.7±0.7*
Carbohydrate (g/kg/d)	86.6±1.9	60.6±2.7*
Energy (kJ/kg/d)	2598.6±57.6	2130.7±80.3*

alues between

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415 **Table 2** Birth weight, weight at PND 28 and fat mass as a % of body weight at PND 28 from male and female offspring of control and JF dams

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	Male		***Female***	
Parameter	**C**	**JF**	**C**	**JF**
Birth weight (g)	7.1±0.3	6.1±0.2**	6.6±0.3	5.8±0.2*
Body weight PND 28 (g)	98.9 ±4.2	75.4 ±4.0*	90.2 ±2.7	78.1 ±7.6*
% fat mass PND 28	7.5±0.8	12.5±1.8*	8.3±1.2	12.9±30.7*

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Data presented as mean ± SEM, n=5 litters for both groups. Significantly different values between groups within each sex are marked as *P<0.05, ** P<0.01.

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