A maternal "junk-food" diet reduces sensitivity to the opioid antagonist naloxone in offspring postweaning

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ABSTRACT **Perinatal exposure to a maternal "junk-food" diet has been demonstrated to increase the preference for palatable diets in adult offspring. We aimed to determine whether this increased preference could be attributed to changes in μ-opioid receptor expression within the mesolimbic reward pathway. We report here that mRNA expression of the μ-opioid receptor in the ventral tegmental area (VTA) at weaning was 1.4-fold (males) and 1.9-fold (females) lower in offspring of junk-food (JF)-fed rat dams than in offspring of dams fed a standard rodent diet (control) ($P<0.05$). Administration of the opioid antagonist naloxone to offspring given a palatable diet postweaning significantly reduced fat intake in control offspring (males: 7.7±0.7 *vs.* 5.4±0.6 g/kg/d; females: 6.9±0.3 *vs.* 3.9±0.5g/kg/d; $P<0.05$), but not in male JF offspring (8.6±0.6 *vs.* 7.1±0.5g/kg/d) and was less effective at reducing fat intake in JF females (42.2±6.0 *vs.* 23.1±4.1% reduction, $P<0.05$). Similar findings were observed for total energy intake. Naloxone treatment did not affect intake of standard rodent feed in control or JF offspring. These findings suggest that exposure to a maternal junk-food diet results in early desensitization of the opioid system which may explain the increased preference for junk food in these offspring.—Gugusheff, J. R., Ong, Z. Y., Muhlhausler, B. S. A maternal "junk-food" diet reduces sensitivity to the opioid antagonist naloxone in offspring postweaning. *FASEB J.* 27, 1275–1284 (2013). www.fasebj.org**

Key Words: fetal programming · high-fat diet · reward

Excessive maternal intake of "junk foods" during pregnancy and lactation has been shown to program an increased preference for fat and sugar in juvenile and adult offspring (1, 2). The term "junk food" can be used to encompass a wide variety of foods that can be high in fat, sugar, or salt as well as energy dense and nutrient poor. The commonality among all junk foods is that they are highly palatable, and since the preference for palatable food is thought to be regulated—at least in part—by activation of the mesolimbic reward pathway, this pathway has become the focus of studies attempting to determine the mechanisms underlying the programming effects of maternal junk food consumption on offspring food preference (3–5).

Within the mesolimbic reward system, opioid signaling plays a central role in eliciting the pleasurable sensation associated with rewarding stimuli (6, 7). The consumption of junk foods is associated with an increased concentration of endogenous opioids within the reward pathway (8, 9), which then bind to opioid receptors in the ventral tegmental area (VTA) to stimulate dopamine release (10). Existing investigations into the mesolimbic reward pathway of adult rats, which have been exposed to a cafeteria diet [a well-established rodent model of junk-food consumption (11), consisting of a variety of foods that are energy dense, nutrient poor, and highly palatable] *in utero* and during the suckling period have highlighted μ-opioid receptor expression within this pathway as being particularly susceptible to alteration. We and others have demonstrated an increased expression of the μ-opioid receptor in the nucleus accumbens (NAc) of adult (3) and juvenile offspring (2) exposed to a cafeteria diet during the perinatal period.

A possible explanation for the effects of a maternal junk-food diet on μ-opioid receptor expression in the offspring is that high levels of endogenous opioids, as would be expected in response to a junk-food diet, may affect opioid receptor ontogeny. Chronic consumption of junk food in adult rodents has been demonstrated to reduce the expression of μ-opioid receptor in the NAc (12), while excessive sugar intake followed by opioid antagonist administration results in symptoms of opiate withdrawal (13). Notably, opioids have been previously demonstrated to readily cross the placenta (14) and into breastmilk (15, 16), which suggests that increases in maternal opioid levels are likely to result in increased concentrations of opioids in fetal and neonatal circulation. However, the effect of a junk food diet and

Abbreviations: C, control diet; C-C, offspring of control diet dams given control saline injections; C-N, offspring of control diet dams given naloxone injections; JF, junk-food diet; JF-C, offspring of junk-food diet dams given saline injections; JF-N, offspring of junk-food diet dams given naloxone injections; NAc, nucleus accumbens; PND, postnatal day; VTA, ventral tegmental area

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subsequent increases in endogenous opioids on the expression of the μ-opioid receptor in the early postnatal period is yet to be explored adequately.

The sensitivity of the developing μ-opioid receptor to the nutritional environment during development, as well as its involvement in the regulation of palatable food intake, has led us to focus on the role of the opioid system in programming of the preference for junk food. Although alterations to μ-opioid receptor expression have been previously observed in adult offspring of dams fed a cafeteria diet during pregnancy and lactation (2, 3), it remains unclear whether these changes in expression are present in the early postnatal period, prior to the increase in palatable food intake. It also remains to be determined whether changes in mRNA expression of the μ-opioid receptor at weaning in these offspring perinatally exposed to a cafeteria diet have functional consequences for the subsequent regulation of food intake in these offspring. Therefore, the current study aimed to determine whether exposure to a maternal junk-food diet during the perinatal period was associated with altered μ-opioid receptor expression in the offspring at weaning and whether these changes affected the efficacy of the opioid antagonist naloxone in reducing the intake of a cafeteria diet in the immediate postweaning period.

MATERIALS AND METHODS

Animals and feeding regime

This study was approved by the Animal Ethics Committee of the University of Adelaide. Albino Wistar rats (17 female and 4 male) were used in these experiments. The animals were allowed to acclimatize to the animal housing facility for 1 wk prior to the commencement of the dietary intervention. During this period, all rats were fed a standard laboratory rodent feed (Specialty Feeds, Glen Forrest, WA, Australia). Following the acclimatization period, rats were assigned into weight-matched groups designated either control (C, $n=8$) or junk food (JF, $n=9$). The C group received a diet consisting of the standard laboratory rodent feed (Specialty Feeds). The JF group was fed a cafeteria diet that included hazelnut spread, peanut butter, chocolate biscuits, savory snacks, sweetened cereal, and a lard and chow mix. Detailed nutritional information on this diet has been previously published (2). Food intake was determined every 2 d by subtracting the amount left uneaten in the cage from the amount initially supplied, and rats were weighed weekly for the duration of the experiment. All animals were individually housed and kept at a room temperature of 25°C in a 12-h light-dark cycle throughout the experiment.

The female rats were provided with their respective diets for 2 wk prior to mating and throughout pregnancy and lactation. Females were mated with 4 proven males (same males used for both C and JF groups), that were maintained on the standard laboratory rodent feed. Vaginal smears were performed to determine the stages of the estrous cycle. On the night of diestrus/proestrus, the 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 considered as confirmation of successful mating and was designated as gestation day 0.

Pups were born on d 21–22 of gestation. On the day after birth [postnatal day (PND) 1], pups were culled to 8 per litter, 4 males and 4 females where possible. Pups were weighed every 2 d during the suckling period and were weaned on PND 21. Pups of C and JF dams are referred to as C offspring and JF offspring, respectively.

Determination of μ-opioid receptor gene expression in the NAc and VTA

At weaning, a subset of both male (C, $n=10$; JF, $n=9$) and female pups (C, $n=8$; JF, $n=8$) were killed, and whole brains were removed. The NAc and VTA were isolated using stereotactic coordinates and microdissection as described previously (2). Total RNA was extracted from these respective brain regions using Trizol reagent (Invitrogen Australia, Mount Waverley, VIC, Australia) and purified with an RNeasy Mini Kit (Qiagen Australia, Doncaster, VIC, Australia). cDNA was synthesized from the purified RNA using Superscript III reverse transcriptase (Invitrogen Australia) and random hexamers. Real-time qRT-PCR was performed on the LightCycler 480 real-time PCR system (Roche Diagnostics, Mannheim, Germany) using the SYBR green system. The primer sequences used for the μ-opioid receptor have been previously published (2); mRNA expression of the reference gene β-actin was measured using the β-actin Quantitect primer assay (Qiagen Australia). The amplification efficiency of the primers was 0.997–0.999, and 2 quality controls were added to each plate to verify interplate consistency. The expression of μ-opioid receptor mRNA relative to β-actin expression was calculated using Q-gene qRT-PCR analysis software (http://www.biotechniques.org).

Naloxone treatment

Pups not used for gene expression analysis were housed with a same-sex littermate and were randomly assigned to receive a daily intraperitoneal injection of either naloxone (5 mg/kg) or an equivalent volume of saline for 10 d postweaning. Naloxone hydrochloride dihydrate (5 mg, purchased from Sigma-Aldrich, St. Louis, MO, USA) was dissolved in 10 ml of sterile saline (a separate aliquot for each animal) and stored away from light at 4°C for the duration of the experiment. Rat pups were weighed prior to each injection to ensure accurate dosing. Injections were given 30 min prior to the onset of the dark cycle (5:30 PM). This generated 4 groups: offspring of C dams given control saline injections (C-C, $n=16$), offspring of C dams given naloxone injections (C-N, $n=16$), offspring of JF dams given control saline injections (JF-C, $n=17$) and offspring of JF dams given naloxone injections (JF-N, $n=17$).

Determination of food preferences

Immediately following the administration of the naloxone/saline injections, the rats were returned to their home cage and provided with free access to both the standard laboratory rodent feed (Specialty Feeds) and the cafeteria diet until the time of the next injection 24 h later. Due to the short half-life of naloxone, food intake of the offspring was measured in the 2-h period immediately following injection (the period during which naloxone has previously been shown to persist in the brain at concentrations that could inhibit food intake; ref. 17), as well as during the entire 24-h period in all offspring.

Food intake was calculated by subtracting the amount left uneaten after the 2- or 24-h time points from the amount supplied at the beginning of each period. The amount of standard laboratory rodent feed and each component of the cafeteria diet consumed within the 2- and 24-h period were recorded, and macronutrient preferences were calculated based on the nutritional composition of each food type. The

amount of food consumed was normalized to offspring body weight. For all statistical analysis, pups in the same cage were considered as one unit. At the conclusion of the 10-d injection period (PND 31), pups were killed and tissues were collected.

Postmortem and tissue collection

Postmortems were performed between 8:00 and 12:00 AM with rats weighed immediately prior to euthanasia. Blood samples were collected in heparinized tubes *via* cardiac puncture and centrifuged at 3500 g at 4°C for 15 min. Body weight, length (nose to tail), and abdominal circumference were determined. All internal organs were weighed, and all visible fat depots, including omental fat (which included the mesenteric depot), retroperitoneal fat, gonadal fat, subcutaneous fat, and interscapular fat, were dissected to determine the fat mass of individual fat depots and total fat mass. The weight of all internal organs and fat were expressed relative to body weight. All tissues and fat depots were frozen in liquid nitrogen and stored at −80°C for future molecular analyses.

Statistical analysis

Analysis of maternal food intake and body weight data as well as birth outcomes was conducted using Student's unpaired *t* tests. The effect of maternal diet on μ-opioid receptor mRNA expression was analyzed by 2-way ANOVA with maternal treatment and sex as factors. The effect of naloxone treatment on the food intake of postweaning offspring was analyzed by 1-way ANOVA in each sex, followed by Duncan's *post hoc* analysis. Where significant differences in food intake (g/kg/2 h) between the saline and naloxone groups were observed for both C and JF offspring, Student's unpaired *t* tests were used to compare the magnitude of the change in food intake caused by naloxone treatment between maternal treatment groups. One- and 2-way ANOVA, as well as the Student's unpaired *t* tests, were performed using SPSS 18.0 statistics software (SPSS Inc., Chicago, IL, USA). Offspring body weight gain was analyzed by 2-way repeated measures ANOVA, which was performed using Stata 11 software (StataCorp, College Station, TX, USA). Male and female offspring were analyzed separately for all measures except where stated. All data are presented as means ± SEM with a value of $P < 0.05$ considered statistically significant.

RESULTS

Dam body weight and nutritional intake during pregnancy and lactation

No difference was found in body weight between the C and JF dams prior to mating (C, 332.8±9.6 g; JF, 324.1±9.4 g) or throughout pregnancy. During pregnancy, the JF dams consumed significantly more fat than the C dams without any differences in protein, carbohydrate, or overall energy intake (**Fig. 1*A***). Throughout lactation, in addition to increased fat intake, the JF dams also consumed less protein and carbohydrate compared to C dams (Fig. 1*B*). The composition of the diet of JF dams during gestation and lactation is shown in Fig. 1*C, D*. All dams ate a variety of foods during both these periods, with the main difference being a higher intake of lard and chow mix during lactation compared to intake during gestation.

Effect of maternal diet on birth outcomes and pup growth

Maternal diet had no effect on litter size (C, 14.6±0.8; JF, 13.2±1.2) or the percentage of males per litter (C, 53.2±4.0%; JF, 60.6±5.1%). Offspring of JF dams had a significantly lower birth weight for both the male (C, 7.0±0.2 g; JF, 6.0±0.1 g; $P<0.01$) and female pups (C, 6.4±0.2 g; JF, 5.7±0.2 g; $P<0.05$). The offspring of JF dams remained lighter than C dams throughout the suckling period and were still significantly lighter than C offspring at weaning (PND 21) in both males and females (male C, 53.7±1.7 g, male JF, 45.0±1.1 g; female C, 52.3±1.6 g, female JF, 43.9±0.8 g; $P<0.01$).

Effect of maternal diet on the expression of the μ-opioid receptor in the NAc and VTA of the offspring at weaning

The mRNA expression of the μ-opioid receptor in the VTA at weaning was lower in offspring of JF dams compared to C dams in both males and females ($P<0.05$; **Fig. 2*A***). In the NAc, μ-opioid receptor mRNA expression at weaning was higher in male offspring of JF dams compared to C dams ($P<0.05$; Fig. 2*B*). No effect of maternal diet on μ-opioid receptor expression in the NAc at weaning in female offspring (Fig. 2*B*) was found.

Effect of maternal diet and naloxone treatment on offspring growth and body composition

Naloxone treatment had no effect on body weight at any time point during the experiment in either the C or JF offspring. At the end of the injection period (10 d after weaning), both male (C-C, 102.6±4.0 g; C-N, 103.8±3.9 g; JF-C, 83.3±2.6 g; JF-N, 88.2±1.8 g; $P<0.01$) and female (C-C, 98.5±3.7 g; C-N, 101.7±4.4 g; JF-C, 82.8±2.3 g; JF-N, 82.5±2.8 g; $P<0.01$) offspring of JF dams were significantly lighter than C dams, independent of whether they were treated with saline or naloxone. In males, offspring of JF dams had a higher subcutaneous fat mass compared to C offspring, independent of whether they received saline or naloxone ($P<0.05$; **Table 1**); no effect of group was observed on the weight of any other fat depots or total fat mass (Table 1). No effect was found of either maternal diet or naloxone treatment on fat deposition in female offspring (Table 1).

Effect maternal diet and naloxone treatment on offspring food intake

Two hours after injection

In C offspring, the intake of fat, carbohydrate, protein, and total energy were all significantly reduced at 2 h after injection in those offspring receiving naloxone injections, compared with those administered saline, in both males and females ($P<0.05$; **Fig. 3**). In the off-

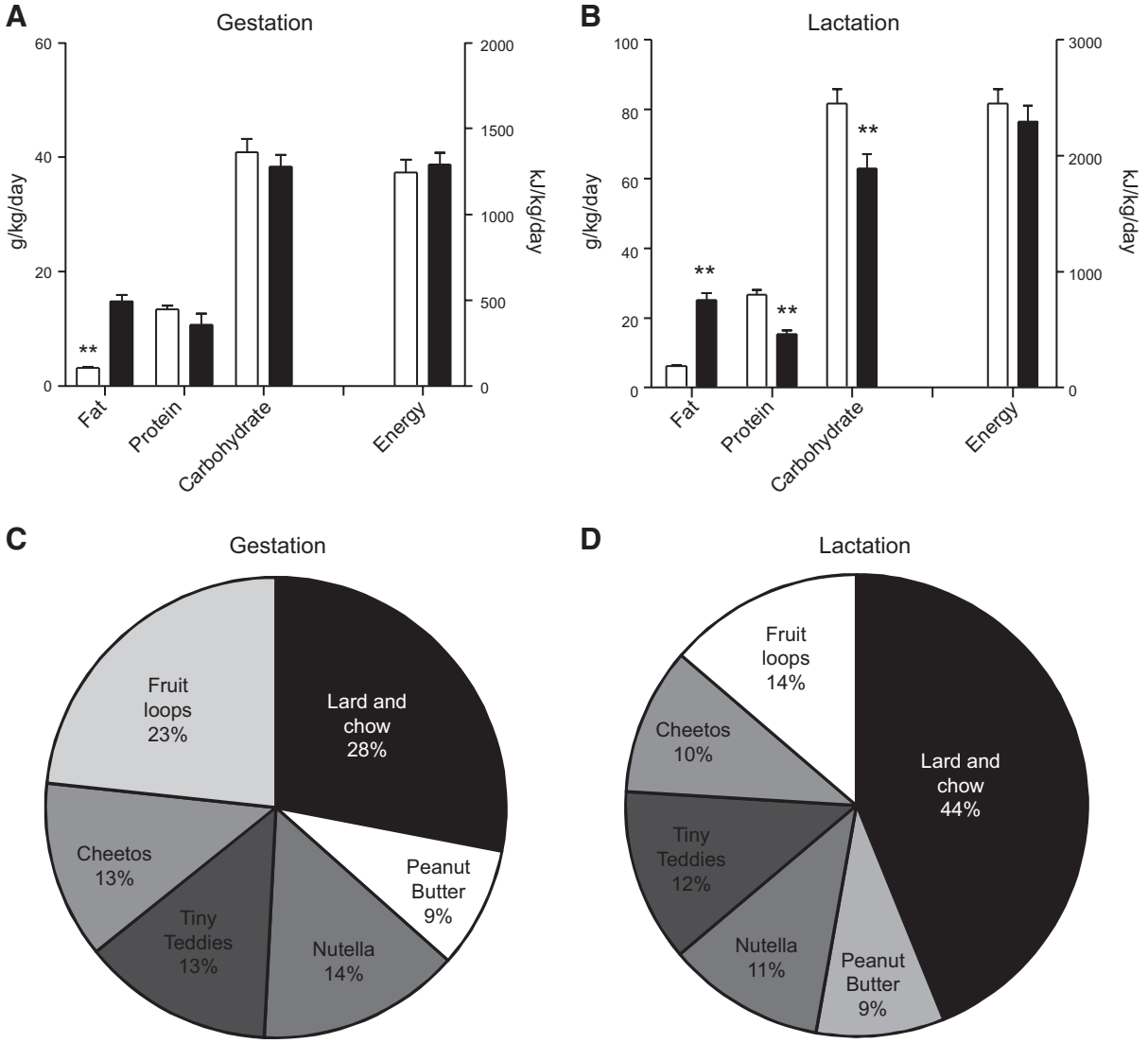

Figure 1. *A*, *B*) Intake of fat, protein, carbohydrate, and total energy of C dams (open bars, $n=8$) and JF dams (solid bars, $n=9$) during gestation (*A*) and lactation (*B*). Results are presented as means ± SEM. $^{**}P < 0.01$. *C*, *D*) Intake of individual components of the cafeteria diet as a percentage of total food intake in the JF dams during gestation (*C*) and lactation (*D*).

spring of JF dams, however, naloxone treatment either had no effect on intake, or it suppressed food intake to a significantly lesser extent when compared to the effects observed in offspring of C dams.

In the male offspring of JF dams, no effect of naloxone treatment on fat intake at 2 h after injection (Fig. 3*A*) was found. In female JF offspring, the decrease in fat intake in naloxone-treated offspring compared to their saline-treated counterparts was significantly less than that observed in the offspring of C dams (C, 42.2±6.0% reduction; JF, 23.1±4.1% reduction; $P<0.05$; Fig. 3*A*).

Total energy intake in both sexes and the intake of carbohydrate in males were significantly reduced in the naloxone-treated offspring of JF dams compared to their saline-treated counterparts at 2 h after injection. However, in all cases, the magnitude of these effects was significantly less than observed in the offspring of C dams (Fig. 3*B*, *D*). Naloxone treatment failed to significantly reduce protein intake in the JF offspring in both males and females, and carbohydrate intake in female JF offspring also did not differ between naloxone and saline-treated animals (Fig. 3*C*, *D*).

Analysis of intake of specific components of the cafeteria diet showed that, in female offspring of both C and JF dams, consumption of hazelnut spread (C-C, 5.7±0.8 g/kg/d; C-N, 2.8±0.5 g/kg/d; JF-C, 7.1±0.7 g/kg/d; JF-N, 4.8±0.7 g/kg/d; $P<0.01$) and peanut butter (C-C, 3.5±0.8 g/kg/d; C-N, 2.1±0.7 g/kg/d; JF-C, 5.9±0.6 g/kg/d; JF-N, 3.6±0.9 g/kg/d; $P<0.05$) was significantly inhibited by naloxone. No other significant effects of naloxone treatment on the intake of other specific junk foods in either male or female offspring were found.

The effects of naloxone on food intake appeared to be specific to the cafeteria diet, since intake of the standard rodent feed offered at the same time was not significantly altered by naloxone treatment in either C or JF offspring in either males (C-C, 7.8±1.3 g/kg; C-N, 5.5±1.2 g/kg; JF-C, 9.45±1.6 g/kg; JF-N, 7.2±1.0 g/kg)

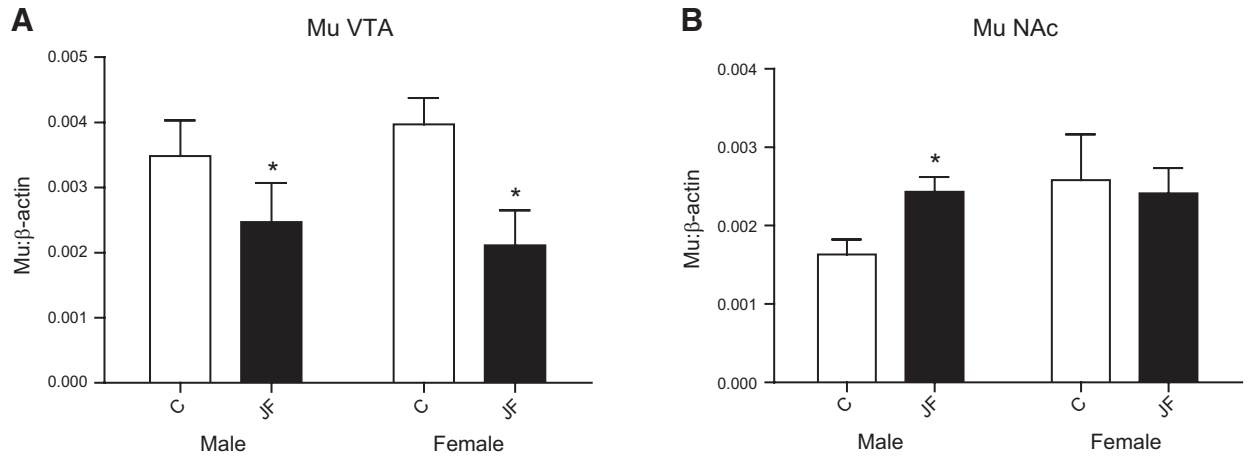

Figure 2. Expression of the μ-opioid receptor in male and female offspring of C dams (open bars, n=18) and JF dams (solid bars, n=17) in the VTA (*A*)and the NAc (*B*)at weaning (PND 21). Results are presented as means ± SEM. *$P < 0.05$.

or females (C-C, 6.0±1.3 g/kg; C-N, 3.4±1.2 g/kg; JF-C, 6.8±1.0 g/kg; JF-N, 8.0±1.8 g/kg).

Twenty-four hours after injection

In females only, offspring of JF dams consumed significantly more fat than their C dam counterparts (**Fig. 4*A***, $P<0.05$). No effects of the naloxone treatment on intake of either total energy or any individual macronutrients in either the C or JF offspring (Fig. 4*B–D*) were found. Also, no difference was found in the intake of either the standard rodent feed or any individual component of the cafeteria diet between saline- and naloxone-treated offspring.

Investigation into the intake of individual foods revealed that, for females only, the offspring of JF dams consumed significantly less sweetened cereal than offspring of C dams, independent of injection treatment (C-C, 32.9±3.9 g/kg/d; C-N, 38.3±7.2 g/kg/d; JF-C, 13.9±2.5 g/kg/d; JF-N, 17.5±6.2 g/kg/d; $P<0.01$). No difference in intake between groups was observed for the other junk foods or in male offspring. No effect of either maternal diet or naloxone treatment on the intake of the standard rodent feed in either male (C-C, 74.6±8.8 g/kg/d; C-N, 89.2±11.4 g/kg/d; JF-C, 82.2±12.4 g/kg/d; JF-N, 104.6±21.0 g/kg/d) or female (C-C, 86.8±8.1 g/kg/d; C-N, 104.1±11.3 g/kg/d; JF-C, 67.6±12.4 g/kg/d; JF-N, 82.5±18.6 g/kg/d) offspring was found.

DISCUSSION

In the present study, we have shown that exposure to a maternal cafeteria diet during pregnancy and lactation is associated with altered expression of the μ-opioid receptor in both the VTA and NAc at weaning in a region- and sex-specific manner, demonstrating for the first time that the effects of perinatal JF exposure on the opioid system are already present immediately following the exposure. We have also demonstrated that the opioid receptor antagonist naloxone was less effective at reducing the intake of the cafeteria diet in offspring exposed to the same diet during the perinatal period, consistent with a reduced sensitivity to opioids in these offspring. This study is the first to demonstrate that the changes in μ-opioid receptor expression previously observed in adult offspring of dams fed a cafeteria diet are already present at weaning and that these changes in expression have functional consequences for the regulation of food intake. This work provides important and novel insights into the pathway linking perinatal exposure to JF consumption with a heightened preference for these foods after birth and

TABLE 1. *Fat depots as a percentage of body weight in male and female offspring of control or JF dams after repeated daily intraperitoneal injections with saline or naloxone for 10 d postweaning (PND 31)*

	Male				Female			
Parameter	C-C	C-N	JF-C	JF-N	C-C	C-N	JF-C	JF-N
Omental fat	0.7 ± 0.05	0.8 ± 0.06	0.9 ± 0.04	0.7 ± 0.04	0.8 ± 0.05	0.8 ± 0.05	0.8 ± 0.04	0.7 ± 0.06
Retroperitoneal fat	1.0 ± 0.13	1.1 ± 0.08	0.9 ± 0.06	1.1 ± 0.04	0.9 ± 0.09	0.9 ± 0.05	0.8 ± 0.02	0.8 ± 0.08
Epigonadal fat	0.7 ± 0.04	0.7 ± 0.03	0.6 ± 0.06	0.6 ± 0.04	0.9 ± 0.05	0.9 ± 0.08	0.7 ± 0.10	0.7 ± 0.06
Interscapular fat	0.6 ± 0.07	0.7 ± 0.08	0.5 ± 0.05	0.6 ± 0.06	0.6 ± 0.04	0.6 ± 0.05	0.5 ± 0.03	0.5 ± 0.04
Subcutaneous fat	6.0 ± 0.41^{a}	6.4 ± 0.27^{a,b}	7.2 ± 0.44^{b,c}	7.8 ± 0.33^{c}	7.8 ± 0.28	6.9 ± 0.48	7.6 ± 0.46	7.9 ± 0.59
Total fat	9.1 ± 0.52	9.8 ± 0.29	10.1 ± 0.58	10.7 ± 0.42	10.8 ± 0.39	10.0 ± 0.62	10.1 ± 0.48	10.6 ± 0.75

Values are expressed as means ± SEM; different superscript letters denote values that are significantly different ($P<0.05$).

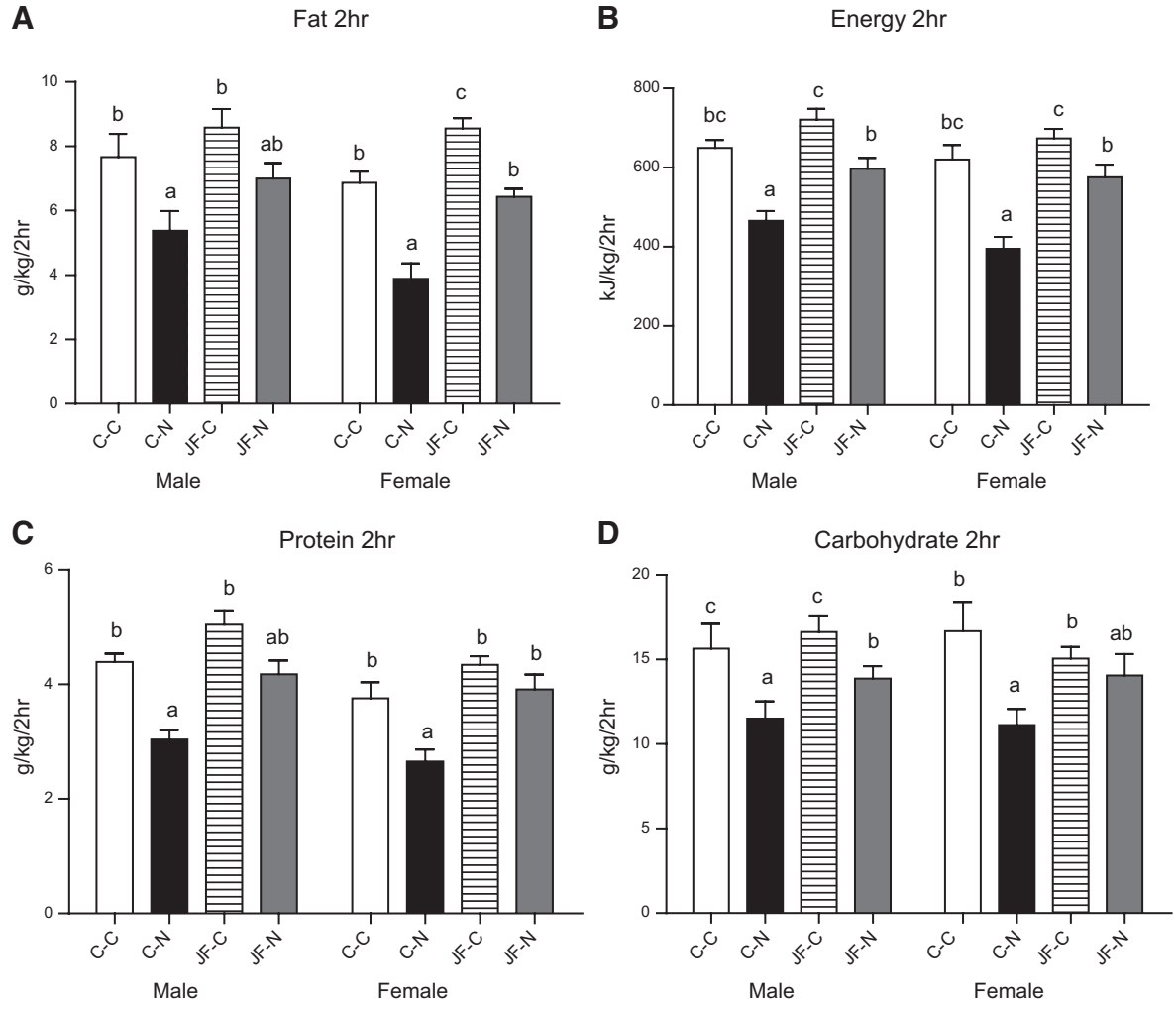

Figure 3. Intake of fat (*A*), total energy (*B*), protein (*C*), and carbohydrate (*D*) 2 h after injection of male and female offspring of C dams given saline (C-C; open bars) or naloxone (C-N; solid bars) and offspring of JF dams given saline (JF-C; striped bars) or naloxone (JF-N; shaded bars). Results are presented as means ± SEM, $n = 8$ pups for all groups except JF-C and JF-N in the male offspring, where $n = 9$. Different letters above the bars denote mean values that differ significantly ($P<0.05$).

adds to the ever growing body of evidence suggesting that maternal JF consumption can alter the development of the reward pathway of the offspring and that these changes affect food choices from weaning into adulthood.

Maternal JF consumption decreases rate of postnatal growth of offspring

Consistent with previous studies (1–2), the offspring of JF dams were born smaller and remained smaller than their C offspring counterparts throughout the suckling period. This reduction in body size has been previously attributed to reductions in protein intake; however, in the current model, protein intake did not differ between maternal groups during gestation. Thus, the decreased birth weight of the offspring of JF dams appears to be driven by a mechanism other than protein deficiency. While the caloric intake of the JF dams was increased relative to C dams, our preliminary analysis of the cafeteria diet provided suggests that it is deficient in a number of key micronutrients, including magnesium and calcium, which have been associated with poor fetal growth outcomes clinically (18–19) and may have contributed to the reduced birthweight of the JF offspring. Whether micronutrient deficiencies also have the potential to affect the development of the reward system has yet to be defined and will be an important question to pursue in future studies. During lactation, the protein intake of JF dams was significantly lower than that of C dams, which is likely to have exacerbated the effect of any other nutrient deficiencies present during gestation and contributed to the reduced body weight of the offspring, since exposure to a low-protein diet during the suckling period has been consistently shown to reduce the body weight of offspring (20, 21).

Maternal JF consumption decreases expression of μ-opioid receptor in VTA

An important finding of the present study was that alterations to the mRNA expression of the μ-opioid receptor in key regions of the mesolimbic reward

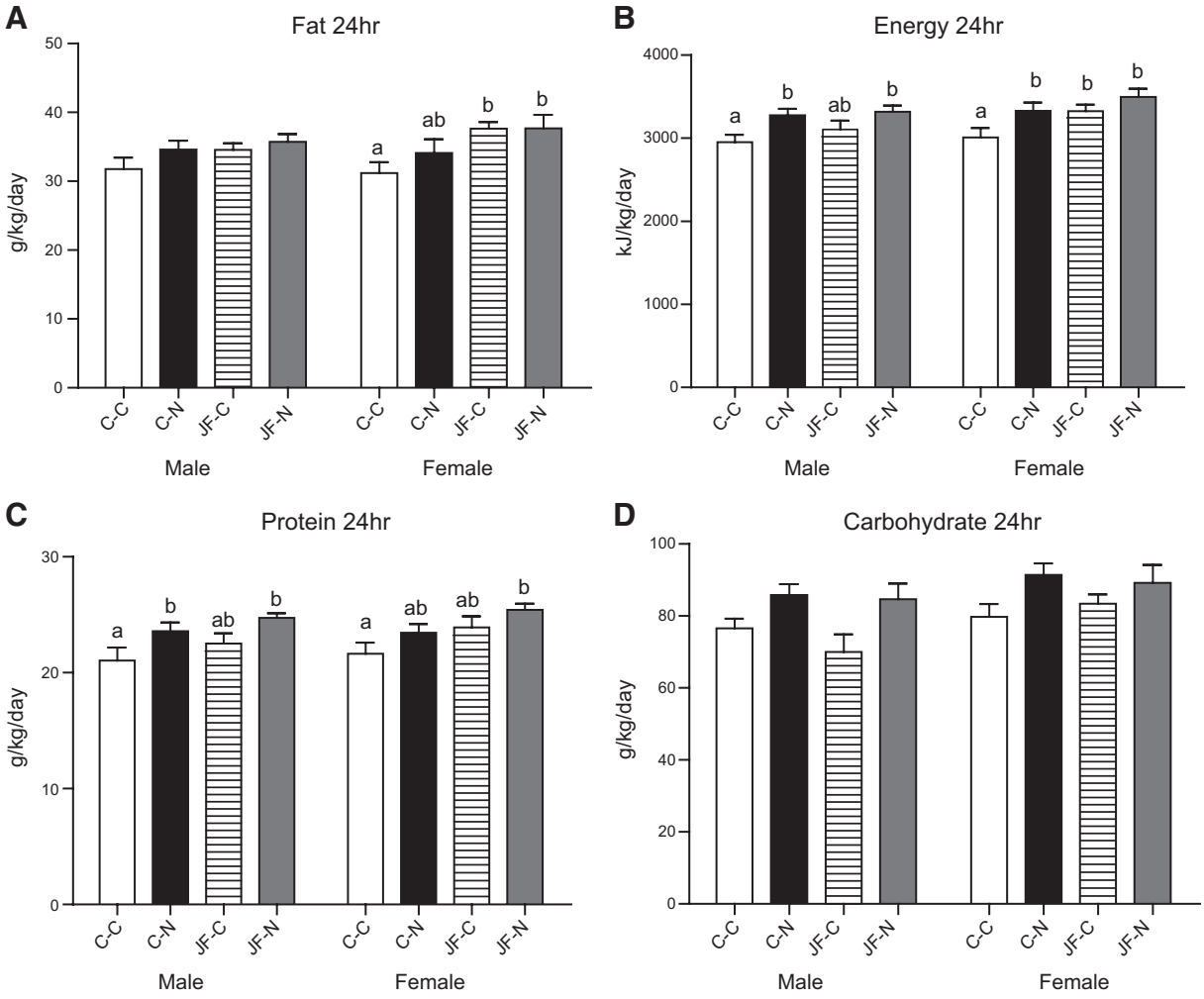

Figure 4. Intake of fat (*A*), total energy (*B*), protein (*C*), and carbohydrate (*D*) 24 h after injection of male and female offspring of C dams given saline (C-C; open bars) or naloxone (C-N; solid bars) and offspring of JF dams given saline (JF-C; striped bars) or naloxone (JF-N; shaded bars). Results are presented as means ± SEM, $n = 8$ pups for all groups except JF-C and JF-N in the male offspring, where $n = 9$. Different letters above the bars denote mean values that differ significantly ($P<0.05$).

system were already present at weaning in the offspring of JF dams. Interestingly, these changes in expression appeared to be dependent on the specific region of the reward pathway examined, with decreased μ-opioid receptor expression observed in the VTA of both sexes and increased expression observed in the NAc of male offspring only. A possible explanation for this disparity of expression between brain areas is their differing rates of development. The appearance and subsequent increase in abundance of the μ-opioid receptor in the brain during development follows a caudal to rostral pattern (22, 23), such that μ-opioid receptor proliferation in the VTA occurs earlier in development than that in the NAc. The effects of opioids on receptor development are greatest at times of rapid proliferation (24), and given our hypothesis that the differences in expression in the offspring of JF-fed dams may be driven by increases in maternal endogenous opioids, it may be that the effect on expression is different depending on the stage of development at which the exposure to increased opioid concentrations occurs.

While the current work is the first to investigate the effect of exposure to a cafeteria diet *in utero* and during suckling on μ-opioid receptor expression at weaning, previous studies have reported changes in mRNA expression of the μ-opioid receptor in adult offspring exposed to similar diets perinatally. Vucetic *et al.* demonstrated that adult offspring of dams fed a palatable high-fat diet and weaned onto a standard rodent feed, had an increased expression of the μ-opioid receptor in the NAc at 18–24 wk of age (3), whereas studies in our own laboratory have shown that offspring of JF dams weaned onto a cafeteria diet exhibited an increased expression of the μ-opioid receptor mRNA in the NAc at 6 wk of age and decreased expression in adulthood after being maintained on a cafeteria for 6 wk postweaning (2). Interestingly, we found no changes in the expression of μ-opioid receptors in the VTA in that same study (2). Viewing these results in light of the current findings suggests that, at least in male offspring, maternal JF consumption increases the expression of the μ-opioid receptor in the NAc at weaning and that this increased expression can persist until adulthood if offspring are weaned onto a standard rodent diet. However, previous work in our own laboratory has revealed that this expression pattern can be reversed by

prolonged exposure to a cafeteria diet during adolescence (2). The studies in the adult offspring of JF dams have focused primarily on changes in gene expression in the NAc rather than the VTA, and it is apparent that further studies are required to better elucidate the effect of a maternal cafeteria diet on the VTA in adulthood.

Maternal JF consumption decreases the effectiveness of naloxone in the offspring

This is the first study to directly demonstrate that changes in μ-opioid receptor expression induced by exposure to maternal JF consumption have functional consequences for the regulation of palatable food intake in the offspring. We found that the offspring of JF dams were significantly less sensitive to the inhibitory effects of the opioid receptor antagonist naloxone on intake of the cafeteria diet than offspring of C dams. We also observed that the offspring of C dams given naloxone consumed significantly more total energy from 2 to 24 h after injection compared to those administered saline, in agreement with studies in adult rodents which have also demonstrated an increase in energy intake after the initial suppression of consumption by naloxone injection (25, 26). That a compensatory increase in energy intake was observed in the offspring of C dams and not in the offspring of JF dams, supports the finding that naloxone was less effective at inhibiting food intake in these animals.

Naloxone was selected as the antagonist, as it only persists at concentrations capable of reducing food intake for 2 h after injection (17, 27); this acute treatment was selected to minimize the effect prolonged suppression of food intake would have on the growth of the pups. In line with this, we did not observe any difference in body weight between those pups given saline and those given naloxone within the same maternal dietary group. Naloxone or saline injections were given at the onset of the dark cycle for all offspring, as it is during this period that rodents have been demonstrated to consume the most food (28), this allowed for the best observation of the effectiveness of naloxone administration on inhibiting food intake.

Previous studies conducted in adult rodents have demonstrated that the effectiveness of naloxone at inhibiting food intake is dependent on the palatability of the food being consumed (9, 29). Consistent with this, we observed no effect of naloxone treatment on the intake of the standard rodent feed in the current study, suggesting that the observed changes in macronutrient intake were specifically due to reduced consumption of the cafeteria diet, rather than an overall decrease in food intake. However, unlike previous studies, which have reported the suppressive effect of naloxone to be fat specific (17, 30), we observed decreases in the intake of all macronutrients (protein, fat, carbohydrate) in the offspring of C mothers where treatment was most effective. This finding can most likely be attributed to the fact that a number of the components of the cafeteria diet provided to the offspring were high in both fat and sugar making it difficult to distinguish between the effects on intake of these different macronutrients. Similarly, the effects on protein intake were likely caused by the inhibitory effect of naloxone on peanut butter intake which contains ~50% fat and 20% protein.

The reduced sensitivity of the offspring of JF dams to the effects of naloxone on the intake of the cafeteria diet suggests that there was reduced μ-opioid receptor binding in the reward pathway of these offspring. Naloxone is a nonspecific opioid receptor antagonist, which binds preferentially to the μ-opioid receptor over κ or δ receptors (31, 32). Naloxone binding to the μ-opioid receptor is thought to inhibit palatable food intake by blocking the binding of endogenous opioids which have been demonstrated to stimulate the intake of junk foods (8, 33). Reduced sensitivity to the effects of naloxone in the JF offspring is in agreement with our finding of reduced μ-opioid receptor expression in the VTA of these offspring at weaning and suggests a reduced μ-opioid receptor binding in the JF offspring. μ-Opioid receptor binding in the VTA regulates the release of dopamine into the NAc, which is the major terminal area of A10 dopamine neurons (34, 35). These results imply that it may be μ-opioid receptor involvement in the control of dopamine release, which is the critical mechanism altered by exposure to a cafeteria diet during the perinatal period. That opioid regulation of dopamine is involved in the programming of food preferences is supported by studies looking at adult offspring of dams fed a cafeteria diet which have demonstrated changes in both the opioid and dopamine systems of these animals (3, 4, 36).

Sex differences in the programming of food preferences by maternal JF consumption

We observed that female, but not male, offspring of JF dams had higher fat intake compared to C dams during the first 10 d postweaning. This was in contrast to previous work in adult offspring which reported a higher preference for fat across both sexes in offspring exposed to a cafeteria diet during the perinatal period (1, 2). However, in a maternal low-protein-diet model, female offspring of the low-protein-fed dams had a higher fat intake when compared to their control counterparts, whereas the same effect was not observed in males (20). Therefore, some evidence suggests that the regulation of fat intake in females may be more susceptible to alteration by maternal diet before birth and early in life than that of male offspring. The differences in fat intake between males and females may be attributed to differences in the rate of development of the opioid system between the sexes, however this is poorly explored in the literature and a need for further investigation remains. Another possible explanation for the sex-specific effect observed is differences in the concentrations of gonadal hormones between the sexes, as estrogen has previously been implicated in the regulation of the endogenous opioid system (37, 38).

Despite our finding that it was only the female offspring JF dams that had an increased preference for

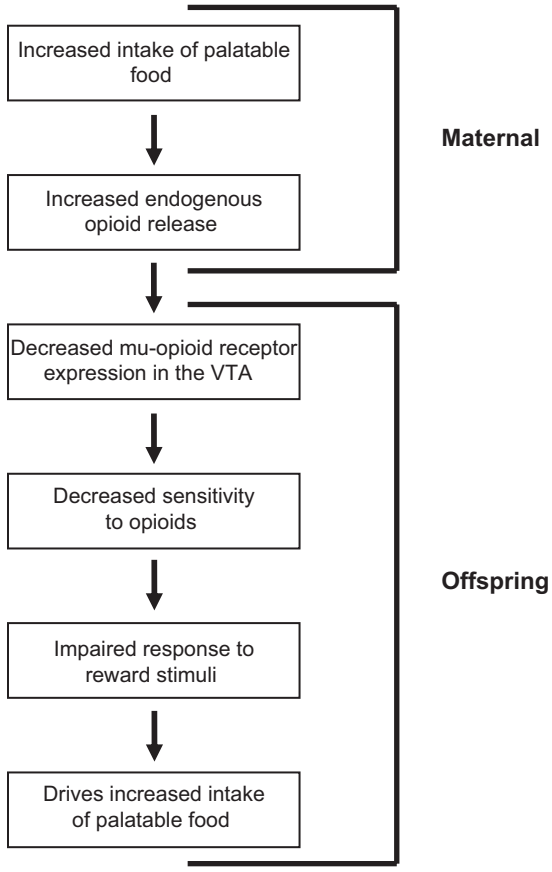

Figure 5. Summary of proposed mechanism through which a maternal JF diet could establish the preference for palatable food in offspring. We speculate that maternal consumption of a JF diet throughout pregnancy and lactation acts to increase maternal endogenous opioids levels. These opioids are then transferred to the offspring *via* the placenta and/or through the breast milk and act on the developing reward pathway to decrease expression of the μ-opioid receptor in the VTA. The resulting densitization to the effect of endogenous opioids in the offspring of JF-fed mothers would drive an increased intake of palatable foods in order to achieve the same level of stimulation in the opioid pathway.

the cafeteria diet in early life; it was the male and not female offspring who had an increase in subcutaneous fat mass. The difference in fat mass in male offspring but not females could be attributed to differences in fat metabolism (39, 40). Nevertheless, the lack of differences in total fat deposition observed between the C and JF offspring in this study has also been reported previously when both the C and JF offspring were weaned on the cafeteria diet and standard chow diet until 6 wk and 3 mo of age (2).

CONCLUSIONS

The present study is the first to demonstrate that maternal JF consumption during pregnancy and lactation has functional consequences on the reward pathway of the offspring immediately postweaning, by reducing the ability of an opioid receptor antagonist to suppress the intake of a cafeteria diet. This study also shows that the alterations in opioid receptor expression are already present at weaning and can affect the regulation of food preferences in the offspring even at this early age. Furthermore, it is likely that decreases in μ-opioid receptor expression and sensitivity present at weaning could have a longer-term effect on the food choices of these offspring, as a need would exist to increase junk-food intake to overcome this early desensitization. We speculate that these changes in the expression and functionality of the opioid system in offspring exposed to a cafeteria diet during the perinatal period may be a result of exposure to high levels of endogenous opioids generated by maternal JF consumption (**Fig. 5**), and it will be important to investigate this hypothesis directly in future studies. This work has provided novel insights into a potential mechanism through which maternal JF consumption increases the preference for junk food in the offspring. A better understanding of this mechanism is crucial if we are to develop possible strategies for intervention and becomes increasingly important in view of the rapidly rising rates of both childhood and adult obesity. FJ

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