Original Article

MOLECULAR METABOLISM

Sex dependent impact of gestational stress on predisposition to eating disorders and metabolic disease

Mariana Schroeder [1,2,], Mira Jakovcevski [2], Tamar Polacheck [1], Yonat Drori [1], Shifra Ben-Dor [3], Simone Röh [4], Alon Chen [1,2,*]**

ABSTRACT

Objective: Vulnerability to eating disorders (EDs) is broadly assumed to be associated with early life stress. However, a careful examination of the literature shows that susceptibility to EDs may depend on the type, severity and timing of the stressor and the sex of the individual. We aimed at exploring the link between chronic prenatal stress and predisposition to EDs and metabolic disease.

Methods: We used a chronic variable stress protocol during gestation to explore the metabolic response of male and female offspring to food restriction (FR), activity-based anorexia (ABA), binge eating (BE) and exposure to high fat (HF) diet.

Results: Contrary to controls, prenatally stressed (PNS) female offspring showed resistance to ABA and BE and displayed a lower metabolic rate leading to hyperadiposity and obesity on HF diet. Male PNS offspring showed healthy responses to FR and ABA, increased propensity to binge and improved coping with HF compared to controls. We found that long-lasting abnormal responses to metabolic challenge are linked to fetal programming and adult hypothalamic dysregulation in PNS females, resulting from sexually dimorphic adaptations in placental methylation and gene expression.

Conclusions: Our results show that maternal stress may have variable and even opposing effects on ED risk, depending on the ED and the sex of the offspring.

© 2018 The Authors. Published by Elsevier GmbH. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Keywords Activity based anorexia; Binge eating; Obesity; Metabolic syndrome; Early life programming; Stress

1. INTRODUCTION

Eating disorders (ED) are damaging mental and metabolic illnesses that dramatically diminish the quality of life and induce life-threatening side effects [1]. EDs predominantly affect women [2,3] and have an adolescent/young adulthood onset [4]. The main EDs include anorexia nervosa (AN, largely characterized by self-starvation), bulimia nervosa (BN, compulsive/binge eating followed by compensatory behaviors) and binge eating disorder (BED, compulsive/binge eating without compensatory behaviors). While the early origins of these disorders remain largely unknown, recent evidence suggests that epigenetic mechanisms may be involved in initiating and maintaining them [5,6]. Epigenetic changes involved in complex adult psychiatric and neurodegenerative disorders include DNA methylation/hydroxymethylation, histone acetylation/deacetylation, noncoding RNAs and microRNAs. While epigenetic mechanisms are known to modulate behavior and health at various stages during the life cycle, it is in the womb where these processes begin to shape the exposed offspring, with a potentially adverse impact on later physical health, emotional adjustment, and stress reactivity. This maladaptation is particularly frequent in response to intrauterine stress, with the general consensus anticipating that early life stress can dramatically increase the probability of developing an ED later in life [1,7–9]. However, the frequent positive association between early life stress and EDs reported in epidemiological studies may be incomplete. There is an imperative need to make appropriate distinctions between different types of early life stress (e.g., anxiety/depression, trauma, nutrition or obstetric complications) and the different developmental windows at which these stressors occur (e.g., gestation (whole/early/late), lactation, childhood or adolescence)

[1]Department of Neurobiology, Weizmann Institute of Science, Rehovot, 76100, Israel [2]Department of Stress Neurobiology and Neurogenetics, Max Planck Institute of Psychiatry, Munich, 80804, Germany [3]Bioinformatics and Biological Computing Unit, Biological Services, Weizmann Institute of Science, Rehovot, 76100, Israel [4]Department of Translational Research in Psychiatry, Max Planck Institute of Psychiatry, Munich, 80804, Germany

*Corresponding author. Department of Stress Neurobiology and Neurogenetics, Max Planck Institute of Psychiatry, Munich, 80804, Germany. E-mails: alon_chen@psych.mpg.de, Alon.Chen@weizmann.ac.il (A. Chen).

**Corresponding author. Department of Stress Neurobiology and Neurogenetics, Max Planck Institute of Psychiatry, Munich, 80804, Germany. E-mails: marianaschroeder.mail@gmail.com, mariana_schroeder@psych.mpg.de (M. Schroeder).

Abbreviations: ABA, activity based anorexia; BE, binge eating; EDs, eating disorders; FR, food restriction; HF, high fat; PNS, prenatal stress

Received June 19, 2018 • Revision received August 8, 2018 • Accepted August 14, 2018 • Available online 20 August 2018

https://doi.org/10.1016/j.molmet.2018.08.005

 © 2018 The Authors. Published by Elsevier GmbH. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

to truly understand the link between early life factors and subsequent predisposition to disease. It is also necessary to make a clear distinction between the different EDs, which may have very different developmental origins.

This distinction must also be applied to the overweight extreme of the eating disorder spectrum (obesity with metabolic syndrome). *In utero* stress is broadly considered a contributor to subsequent risk of obesity and metabolic dysfunction [10,11]. However, when males are exposed

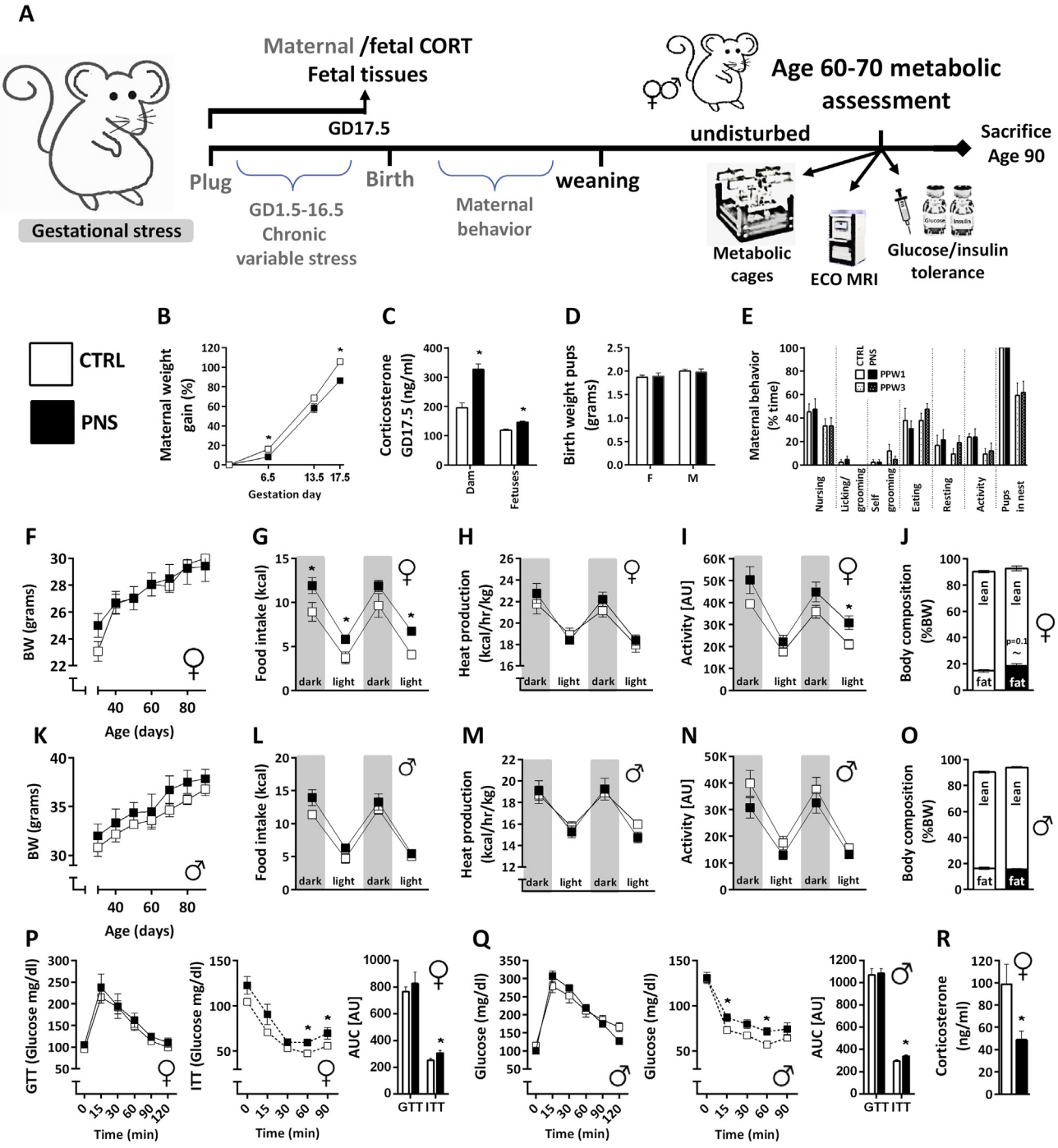

Figure 1: Prenatal stress (PNS) causes basal metabolic abnormalities specifically in female offspring. (A) Experimental design. **(B)** PNS dams weighed less than controls (CTRLs) during gestation ($F_{(1,18)} = 7.01$, $p = 0.016$). **(C)** Corticosterone levels were higher in PNS dams ($t_{(18)} = 5.15$, $p < 0.0001$) and fetuses ($t_{(18)} = 5.79$, $p < 0.0001$) on GD.17.5 compared to CTRLs. **(D)** Pups of both groups had similar body weight (BW) at birth. **(E)** Maternal behavior in the first and third postpartum weeks (PPW) was similar between the groups. **(F–J)** PNS female offspring displayed similar BW **(F)** and heat production **(H)** but tended to high adiposity **(J)** and displayed higher food consumption (($F_{(1,14)} = 6.19$, $p = 0.026$) **(G)**) and activity levels (($F_{(1,14)} = 7.68$, $p = 0.015$) **(I)** than CTRLs**. (K–O)** Male PNS offspring displayed a normal metabolic profile. **(P–Q)** Glucose tolerance was normal but insulin tolerance was affected by PNS in both sexes ($F_{(1,15)} = 5.60$, $p = 0.033$ for females **(P)** and $F_{(1,15)} = 7.39$, $p = 0.017$ for males **(Q).** Glucose tolerance test (GTT) is displayed on the left y-axis and insulin tolerance test (ITT) on the right y-axis. **(R)** Plasma corticosterone levels were lower in PNS than in CTRL females ($t_{(10)} = 2.55$, $p = 0.029$). Data presented as mean and S.E.M. N = 6–10.

 © 2018 The Authors. Published by Elsevier GmbH. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

to prenatal stress in early gestation, they have a reduced body weight and adiposity when exposed to a high fat (HF) diet [12]. Sex-specific differences in the response to ED modeling challenges may be linked to placental expression of O-linked bN-acetyl glucosamine (O-GlcNAc) transferase (OGT). This enzyme, which senses changes in maternal energy homeostasis and regulates epigenetic marks on chromatin, is reduced by early gestational stress in males only and therefore may regulate sex-specific epigenetic modifications of genes important for adult metabolism [13]. In contrast, the impact of late-gestation prenatal stress on adult responsivity and metabolism appears to be more profound in females [14–16].

According to clinical and preclinical studies, intrauterine fetal stress that occurs during sexual differentiation induces sex-dependent effects on fetal brain development. These effects occur within highly sexually dimorphic regions that regulate mood, the stress response, metabolic function, and the autonomic nervous system. Thus, maternal stress has been shown to have a sex-dependent impact that is timing specific during gestation, affecting different developing brain-areas and, consequently, resulting in multiple systemic sex-dependent effects on metabolic functions, ultimately predisposing male and female offspring to different diseases [17–19]. Although fetal programming, mediated by the placenta, may be intended to benefit the health and survival of the offspring, it can lead to a variety of complications when the developmentally adapted organism is exposed to unexpected challenges throughout its life. Furthermore, compromised placental function can have both short- and long-lasting consequences for the developing fetus [17], as is the case for maternal hypoxia [20], pre-eclampsia [21], or placental insufficiency [22].

Here, we explored the effects of chronic variable stress during the whole period of gestation on basal metabolism, the response to food restriction (FR), and susceptibility to develop activity based-anorexia (ABA), BE and metabolic syndrome in male and female offspring. We show that fetal programming by PNS is mediated by global and robust sex-specific alterations in placental DNA/RNA methylation and gene expression. Finally, we focused on hypothalamic programming by PNS specifically in females and detected a variety of abnormalities that underlie the response to the different ED-inducing protocols. Altogether, we report that chronic PNS induces sexually dimorphic effects on placental function, affecting fetal hypothalamic programming and subsequent basal metabolism and the response to a variety of different metabolic challenges.

2. RESULTS

2.1. Prenatal stress induces basal metabolic abnormalities in female offspring

To examine the potential link between gestational stress and a later predisposition to eating and metabolic disorders, we examined the metabolic profile of prenatally stressed males and females under basal conditions. To create these groups, we exposed pregnant ICR/CD1 dams to a chronic variable stress (prenatal stress - PNS) protocol for most of the pregnancy period, beginning a day after the detection of the copulation plug (gestation day (GD)1.5) and finishing on GD16.5 (Figure 1A). PNS dams gained less weight and had higher corticosterone (CORT) levels towards the end of the protocol (Figure 1B,C), which was already reported in a previous study [23]. PNS fetuses also displayed higher CORT levels but showed similar body weight at birth compared to controls (CTRLs; Figure 1C,D). Maternal behavior did not differ between the groups, excluding this environmental factor as a potential confounding variable (Figure 1E, [23]). Body weight follow-up of the offspring showed no differences between the groups in either sex (Figure 1F,K). At around 10 weeks of age, the offspring's' metabolic profile was examined using metabolic cages. PNS females showed higher basal food intake (Figure 1G), similar heat production (Figure 1H), hyperactivity (Figure 1I) and a trend to increased body adiposity (Figure 1J) compared to CTRLs. PNS males showed a similar basal metabolic profile (Figure 1L–N) and adiposity (Figure 1O) compared to CTRLs. Glucose sensitivity was not affected by PNS in either sex, but insulin sensitivity decreased by PNS in both sexes (Figure 1P,Q), a finding previously reported in humans [10,11]. Finally, CORT levels were lower in adult PNS offspring (Figure 1R) [10]. Thus, PNS appears to have stronger basal metabolic effects in female than in male offspring.

2.2. Prenatally stressed animals cope better with short-term food restriction

As a first step to examine the response of prenatally stressed offspring to later life metabolic challenge, we exposed the offspring to 4 days of moderate FR, starting on postnatal day 40 (Figure 2A). While CTRL females reacted to the challenge with moderate weight loss from the first day, PNS females did not show the same weight drop but rather maintained their weight throughout the protocol (Figure 2B). During the challenge, PNS females consumed fewer calories than CTRLs (Figure 2C). Examination of their metabolic profile post-recovery exposed hypophagia during the dark phase, decreased heat production and similar activity compared to CTRLs (Figure 2D). Male offspring showed a similar but more moderate response (Figure 2E–G). The 4-day FR manipulation resulted in increased post-recovery adiposity in females (Figure 2H), but not in males (Figure 2I) and did not affect glucose or insulin tolerance. In fact, the moderate insulin intolerance observed under basal conditions in PNS mice disappeared in both sexes (Figure 1N–O).

2.3. Prenatal stress abolishes the innate predisposition to activity based-anorexia in adolescent females and causes metabolic adaptations in males

To assess the susceptibility and metabolic response of PNS offspring to ABA, we exposed the adolescent offspring (age 30 days) to a modified version of the ABA protocol [23,24]. The ABA model mimics the choice of exercise over eating, relying on limited food intake while allowing unlimited access to running wheels (RW) [25,26]. The limited period of food access allows them to retain the majority of their BW as long as they engage in compensatory food intake when accessible, instead of excessive wheel running. The ABA protocol is characterized by adolescent vulnerability and sex specificity to females [23,27,28], hyperactivity [23,25,29], dramatic weight loss as a consequence of decreased food intake [23,24,30], and circadian disruption in the running pattern [23,28]. Briefly, we habituated the animals to voluntary wheel running for one week with free access to food, followed by four days of free access to wheels combined with gradual FR in the form of 3–4 h of food access a day during the dark cycle (Figure 3A). As expected, CTRL females split into ABA-prone and resilient, with ABA-prone females showing dramatic weight loss, decreased food intake, increased running, and circadian disruption (Figure 3B), all of which was reported in a previous study [23]. ABA-prone females showed increased food intake and activity in the light phase and higher heat production post-recovery compared to CTRL resilient females, highlighting the long-lasting circadian disruption in this group (Figure 3C). As previously reported, none of the PNS females developed ABA [23] and showed a similar profile to resilient CTRL females (Figure 3B,C). In an extended cohort, we found here that the ABA challenge did not affect body adiposity in CTRL females, but did result in higher body

 © 2018 The Authors. Published by Elsevier GmbH. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

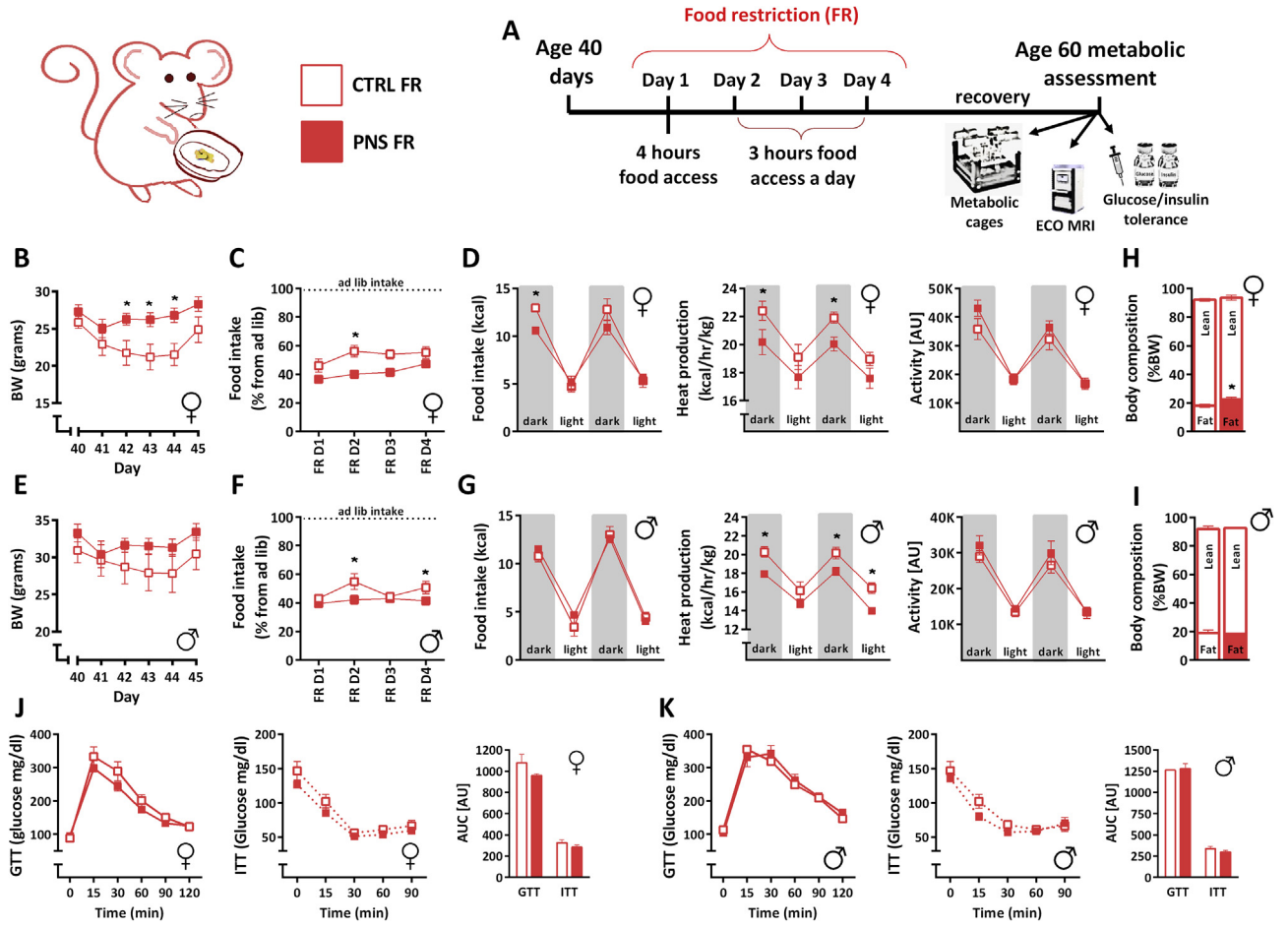

Figure 2: The metabolic response to food restriction differs between control (CTRL) and prenatally stress (PNS) animals. (A) Experimental design. **(B)** The pattern of weight loss was different in PNS females and CTRLs, with PNS animals maintaining their body weight (BW) throughout the protocol ($F_{int(3,8)} = 6.20$, $p = 0.018$). **(C)** The pattern of food intake during food restriction differed between PNS and CTRLs females ($F_{int(4,7)} = 5.59$, $p = 0.024$). **(D)** Food intake and activity levels post-recovery were similar between the groups. Heat production was lower in PNS ($F_{(1,10)} = 5.90$, $p = 0.036$). **(E)** In males, BW change did not differ between the groups. **(F)** PNS males consumed less calories than CTRLs when FR ($F_{(1,10)} = 14.19$, $p = 0.004$). **(G)** Food intake and activity levels post-recovery were similar between the groups. Heat production was lower in PNS FR mice ($F_{(1,10)} = 10.21$, $p = 0.01$). **(H–I)** Body fat was higher in PNS females ($F_{(1,11)} = 4.92$, $p = 0.05$), but not in males compared to CTRLs. **(J–K)** Food restriction (FR) did not affect glucose or insulin sensitivity in the PNS group. Glucose tolerance test (GTT) is displayed on the left y-axis and insulin tolerance test (ITT) on the right y-axis. Data presented as mean and S.E.M. $N = 6$.

adiposity in (ABA resilient) PNS females compared to resilient CTRLs (Figure 3F). As expected, males were largely resilient to the protocol regardless of prenatal treatment and beside one male in the CTRL group, all others coped remarkably well with the ABA manipulations (Figure 3D). However, coping with ABA resulted in decreased heat production and hyperactivity in PNS males (Figure 3E), without affecting adiposity (Figure 3G). Glucose tolerance was normal in all groups and the moderate insulin intolerance observed under basal conditions in PNS animals disappeared in both sexes (Figure 3H,I), as seen after FR (Figure 2J,K). Altogether, the female PNS group showed a remarkable improvement in their capacity to cope with the ABA-inducing protocol compared to CTRL females, an adaptation that may grant them increased survival chances in a food deprived environment.

2.4. Prenatal stress abolished the propensity of females to binge on palatable food, but induced metabolic abnormalities in response to intermittent access to western diet in both sexes

Since PNS females appear to be more efficient than CTRLs at coping with FR and ABA, gaining body fat after both manipulations, we next explored their vulnerability to binge on HF/high palatable food when exposed to a BE-inducing manipulation. Using the intermittent access protocol [23,31,32], we habituated the offspring to Western diet (WD) for 5 days, switched them back to chow, and then allowed them access to WD for 2 h a day, 3 days a week for a period of 4 weeks (a total of 12 BE sessions) (Figure 4A). Contrary to our expectations, PNS females gained similar weight (Figure 4B), consumed less WD during the habituation period (Figure 4C) when compared to CTRL group and overall did not develop BE (Figure 4D). Thus, the PNS females' response appears to reflect anhedonia rather than the expected compulsivity observed in a different model of late gestational stress [15]. From a metabolic perspective, PNS females exposed to this protocol showed decreased food intake post-recovery, lowered metabolic rate, and similar activity in comparison to CTRLs (Figure 4E). In contrast to females, PNS males showed a significantly different pattern of BW progression throughout the BE protocol compared to CTRLs (Figure 4F). While they showed anhedonia (or maybe hypophagia due to increased anxiety/neophobia) to WD during the habituation period similarly to PNS females (Figure 4G), they displayed a moderate and gradual increase in WD consumption compared to CTRL

 © 2018 The Authors. Published by Elsevier GmbH. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

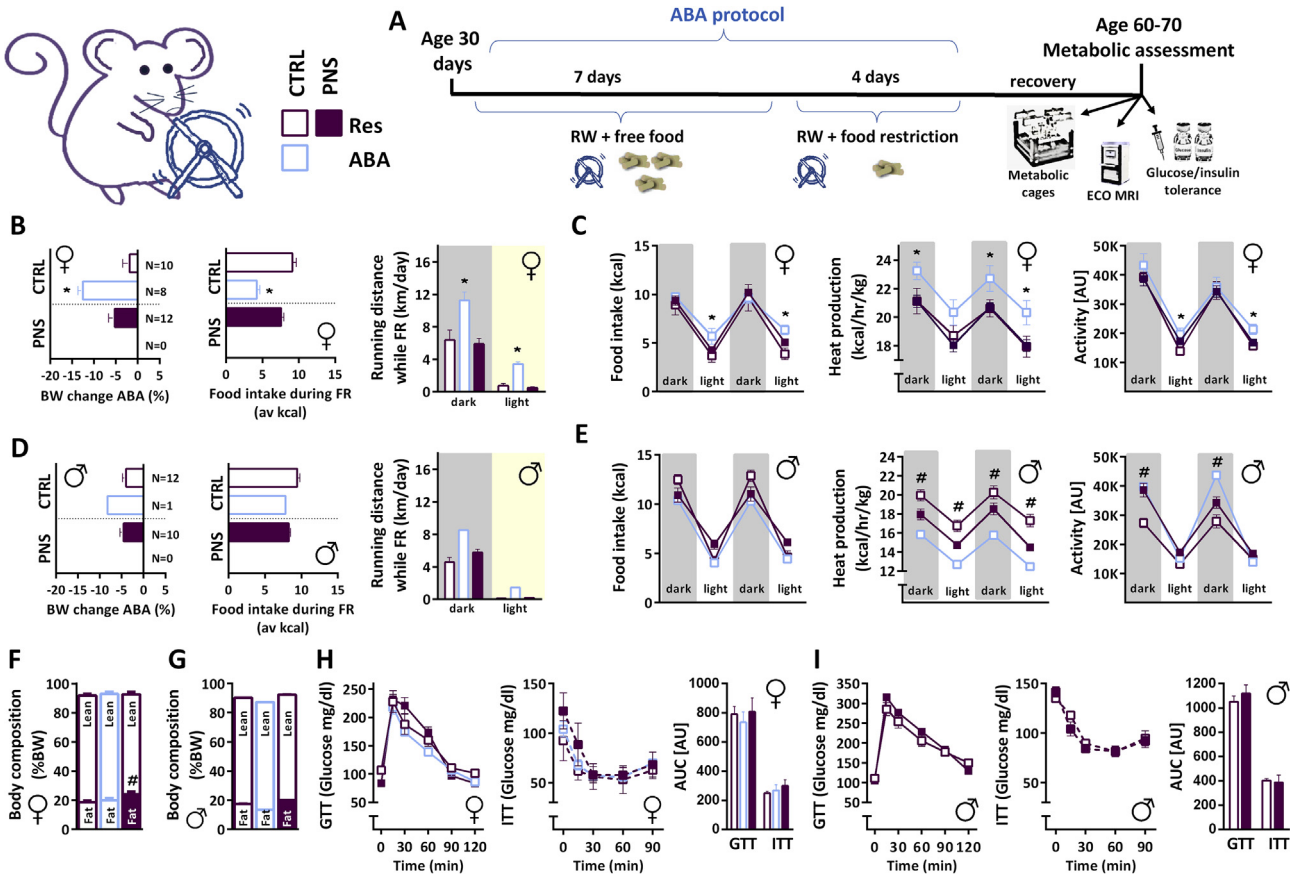

Figure 3: Metabolic and circadian abnormalities resulting from activity-based anorexia (ABA) are specific to females and are abolished by prenatal stress (PNS). (A) ABA timeline. **(B)** Body weight (BW) change and food intake on the last day of the ABA protocol were different only in control (CTRL) ABA females. ABA CTRL females run more in total and in the light phase. **(C)** ABA females (post-recovery) consumed more kcal during the light phase ($F_{(2,27)} = 2.62$, $p = 0.047$), displayed higher heat production ($F_{(2,27)} = 3.90$, $p = 0.033$) and hyperactivity in the light phase compared to resistant CTRL and PNS females. **(D)** Besides one CTRL male, all others were resistant to the ABA protocol, with similar BW and food intake and running distance and pattern. **(E)** All males showed similar food consumption post-recovery, but PNS showed lower heat production ($F_{(2,20)} = 5.73$, $p = 0.011$) and higher activity in the dark phase compared to CTRLs ($F_{(2,20)} = 3.73$, $p = 0.042$). **(F–G)** The ABA protocol increased post-recovery body adiposity in females ($F_{(2,27)} = 4.20$, $p = 0.026$, **G**), but not in males **(G)**. **(H–I)** The ABA protocol had no effects on glucose or insulin tolerance in any of the groups. Glucose tolerance test (GTT) is displayed on the left y-axis and insulin tolerance test (ITT) on the right y-axis. Data presented as mean and S.E.M. Differences between the groups are based on Tukey HSD multiple comparison tests. *$p < 0.05$ for comparisons between CTRL resistant and CTRL ABA groups. $^{\#}p < 0.05$ or comparisons between CTRL resistant and PNS groups. N = 8–12.

males (Figure 4H). In contrast to the PNS females, the BE protocol induced an increase in metabolic rate in males (Figure 4I) without affecting any other metabolic parameters. The BE protocol did not affect body adiposity in males but tended to increase it in females (Figure 4J,K). Glucose tolerance was not affected in either sex (Figure 4L,M). The insulin tolerance test revealed a different pattern of response in PNS females compared to CTRLs (Figure 4L) and a tendency to improvement in PNS males compared to CTRLs (Figure 4M). Taken together, the BE protocol did not induce the expected compulsive consumption of palatable food in PNS females, but exposed metabolic abnormalities leading to decreased heat production, a potential mechanism of exaggerated long-term weight/adiposity gain. In males, the pattern was the opposite, and the increased heat production and improved insulin tolerance may paradoxically hint at a better coping mechanism when exposed to high caloric diets.

2.5. Prenatal stress programs females to diet induced obesity and males to leanness when exposed to high fat diet

In order to explore the possibility that females may be programmed to gain adiposity/weight, while males may be programmed to lose adiposity/weight as a result to PNS, we exposed a further set of PNS offspring to 3 weeks of 60% HF diet (Figure 5A). After a period of recovery, we found that PNS HF females gained significant amounts of weight compared to CTRLs (Figure 5B), while PNS males gained less weight than CTRLs (Figure 5C). Female PNS HF offspring consumed similar amounts of food post-recovery but showed dramatically lower heat production (Figure 5D). Male PNS HF offspring consumed similar amounts of food post-recovery but showed dramatically higher heat production and hyperactivity (Figure 5E). These findings led to higher adiposity in PNS females (Figure 5F) and lower adiposity in PNS males compared to CTRLs (Figure 5G). Exposure to HF diet had no evident effects on glucose and insulin sensitivity in PNS females (Figure 5H), but dramatically affected PNS males. Remarkably, PNS males showed improved glucose tolerance and insulin sensitivity after HF (Figure 5I).

2.6. Prenatal stress programs the female fetuses to abnormal metabolic responses through robust placental adaptations

In order to explore the origins of the metabolic responses of PNS offspring to the different protocols, we next focused on the placenta. We first explored the effects of PNS on global DNA (5-mc) methylation (Figure 6A), DNA (5-hmc) hydroxymethylation (Figure 6B), and RNA (m6A) methylation (Figure 6C) of placentas of both sexes. These first

 © 2018 The Authors. Published by Elsevier GmbH. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

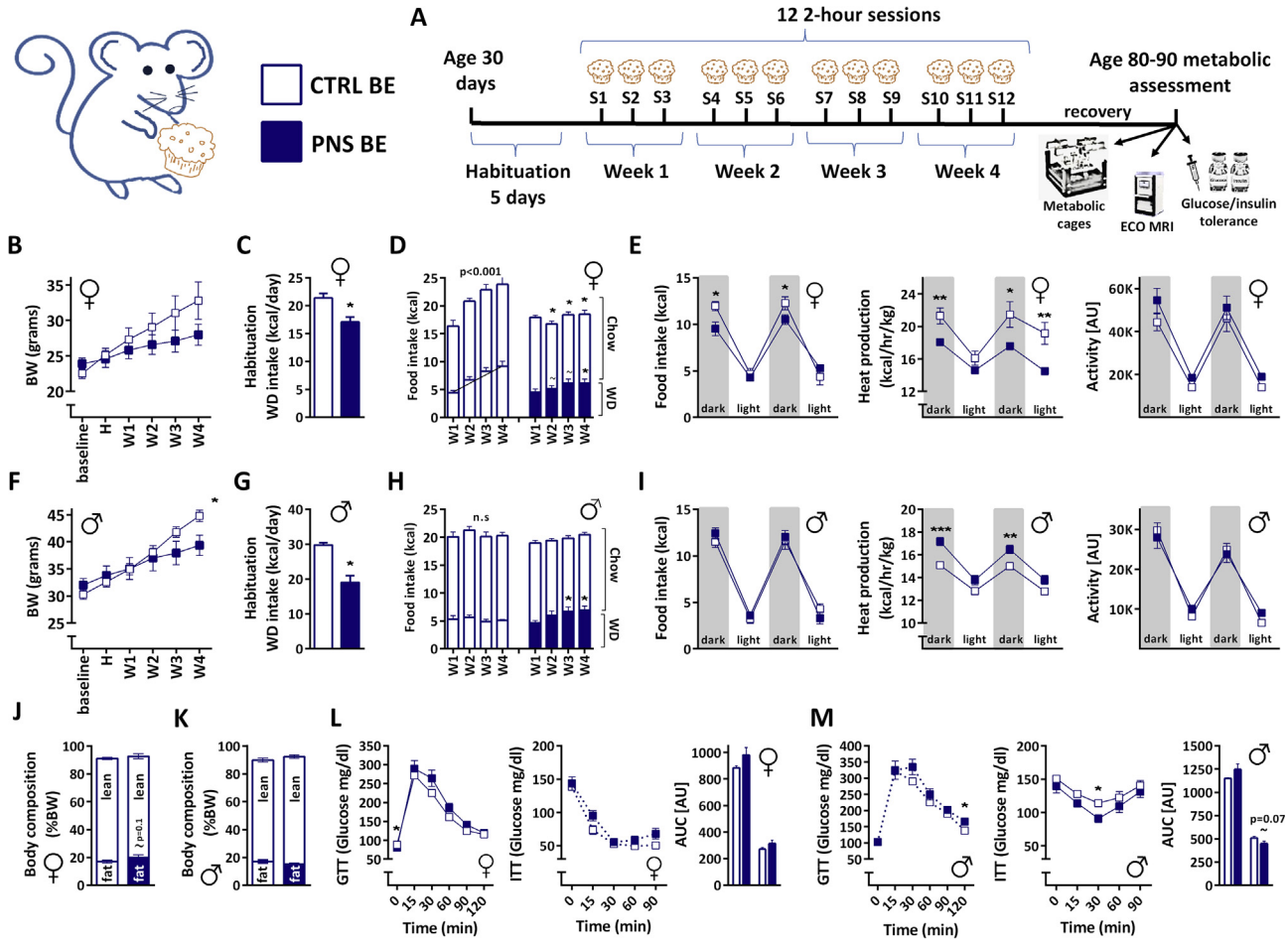

Figure 4: The Intermittent access binge eating (BE) protocol induced BE in control (CTRL) females and overeating in prenatal stress (PNS) males and exposed metabolic abnormalities. (A) BE protocol timeline. **(B)** Body weight (BW) during the BE protocol was similar between the groups in females. **(C)** During the habituation to Western diet (WD), PNS females ($F_{(1,16)} = 13.21$, $p = 0.002$ consumed less kcal than CTRLs. **(D)** Contrary to CTRL females, PNS females did not develop BE ($F_{(1,15)} = 4.68$, $p = 0.047$). **(E)** PNS females consumed less food during the dark phase ($F_{int(3,13)} = 4.76$, $p = 0.019$), displayed lower heat production ($F_{int(3,13)} = 3.67$, $p = 0.041$) and similar activity compared to CTRL females. **(F)** BW was lower in PNS males compared to CTRLs ($F_{int(4,7)} = 17.26$, $p = 0.001$). **(G)** During the habituation to WD, PNS males ($F_{(1,11)} = 28.52$, $p = 0.000$) consumed less kcal than CTRLs. **(H)** The BE protocol induced a different response in PNS males; they consumed less at first and more WD in the last week of the protocol ($F_{int(3,8)} = 4.23$, $p = 0.046$). **(I)** PNS males consumed similar food and showed comparable activity levels post-recovery, but displayed higher heat production ($F_{(1,10)} = 11.86$, $p = 0.006$) than CTRL males. **(J–K)** PNS and BE did not affect body adiposity in males but tended to increase body fat mass in females. **(L)** Glucose tolerance was normal but the response to insulin differed in PNS females ($F_{int(4,12)} = 7.01$, $p = 0.004$). **(M)** In males, glucose tolerance was normal and insulin tolerance tended to be improved by PNS ($F_{(1,10)} = 3.31$, $p = 0.099$). Glucose tolerance test (GTT) is displayed on the left y-axis and insulin tolerance test (ITT) on the right y-axis. Data presented as mean and S.E.M. N = 6–9.

two processes are known to be important regulators of gene expression in the placenta [33,34] and to be affected by stress [35]. N6-Methyladenosine (m6A) RNA methylation, not studied in the placenta until now, is an abundant modification in mRNA preferentially enriched within 3′UTRs and around stop codons, allowing for potential alternative polyadenylation and miRNA binding regulation [36]. We found that PNS increased DNA methylation (Figure 6A) and reduced RNA methylation (Figure 6C), without affecting DNA hydroxymethylation in females (Figure 6B), hinting at major global effects of PNS on placental function. In males, the results were not significant. Accordingly, enzymes responsible for these processes, such as DNA methyltransferase *(Dnmt)3a* (Figure 6D), the Ten-eleven translocation *(Tet)* de-methylation enzymes *2–3* (Figure 6E), the RNA methylation regulatory subunits WT1 associated protein *(Wtap)* and *KIAA1429* and the de-methylases Fat mass and obesity-associated protein *(Fto)* and Alkylated DNA repair protein alkB homolog *(Alkbh)5* (Figure 6F,G) were all increased by PNS specifically in females. Lastly, to gain insight into the potential effects of PNS on gene expression and function of the uterinal environment that the fetuses were exposed to, we performed RNA-Seq comparing CTRLs and PNS female placentas. Pathway enrichment analysis revealed significant enrichment for 15 KEGG signaling pathways (Table S1). Among these, strong changes were observed for the ABC transporters (q-value = 0.073), (Figure 6H, Table S2), circadian entrainment (q-value = 0.060) (Figure 6I, Table S2), cytokine–cytokine receptor interaction (q-value = 0.004) (Figure 6J, Table S2) and the oxytocin pathway (q-value = 0.073) (Figure 6K, Table S2). Finally, we focused on the solute carrier (SLC) group of membrane transport proteins and the insulin-like growth factor (IGF) family, which play a key role in placental structure and function [23,37]. Interestingly, PNS placentas express lower expression levels of the amino acid transporters *Slc6a15*, *Slc1a4*, *Slc6a17*, *and Slc43a1*, the choline transporters *Slc44a5* and *Slc22a4* and the B Vitamin transporters *Slc5a6* (B5, B7) a*nd Slc19a2* (B1) potentially affecting methylation processes in the fetus. In contrast, several other

 © 2018 The Authors. Published by Elsevier GmbH. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

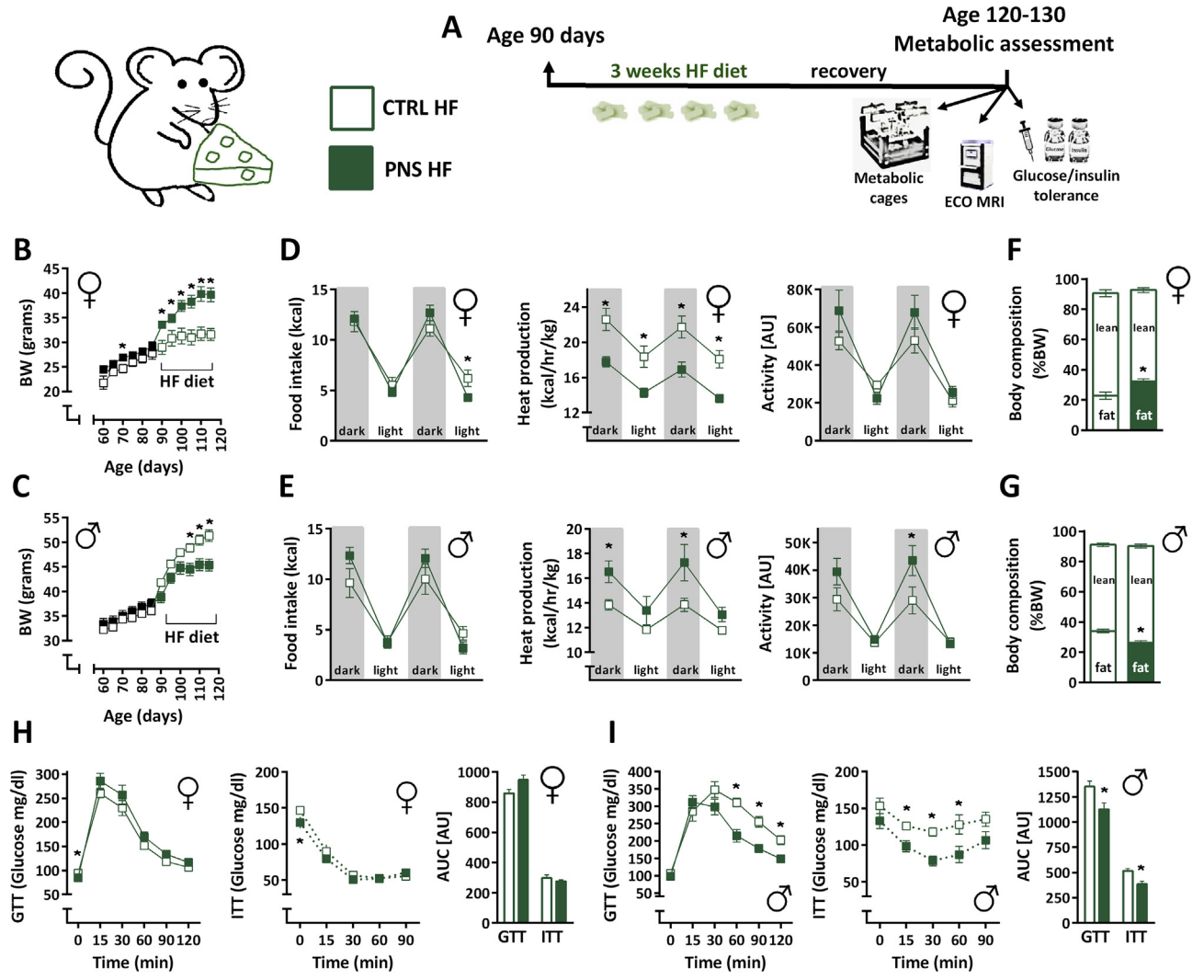

Figure 5: Prenatal stress (PNS) induced opposite metabolic effects in males and females when exposed to high fat (HF) diet. (A) Experimental outline. **(B–C)** Body weight (BW) in the PNS groups exposed to HF was differently affected according to sex ($F_{int:timexgroupxsex(11,18)} = 2.80$, $p = 0.025$). PNS female offspring gained more weight than controls (CTRLs) when exposed to HF diet ($F_{(1,14)} = 12.59$, $p = 0.003$). **(C)** PNS male offspring gained less weight than CTRLs when exposed to HF diet ($F_{int(11,4)} = 110.57$, $p = 0.000$). **(D)** Post recovery PNS females showed similar food consumption, a different profile of activity ($F_{int(3,12)} = 4.35$, $p = 0.027$) and lower heat production than CTRLs ($F_{(1,14)} = 18.10$, $p = 0.001$). **(E)** PNS males displayed similar food consumption, but higher heat production ($F_{int(3,12)} = 3.87$, $p = 0.043$) and a tendency to dark phase hyperactivity ($F_{int(3,12)} = 2.74$, $p = 0.055$) compared to CTRLs. **(F–G)** PNS females had higher body adiposity ($F_{(1,14)} = 11.91$, $p = 0.004$), while PNS males had lower body adiposity ($F_{(1,14)} = 17.09$, $p = 0.001$) compared to same sex CTRLs. **(H–I)** PNS had dramatically different effects on glucose ($F_{int(5,24)} = 5.27$, $p = 0.002$) and insulin ($F_{int(4,25)} = 7.17$, $p = 0.001$) tolerance depending on the sex of the offspring. PNS did not affect glucose or insulin tolerance in females **(H)**, but dramatically improved both the glucose ($F_{(5,10)} = 4.07$, $p = 0.028$) and insulin ($F_{(1,14)} = 7.32$, $p = 0.027$) tolerance in PNS males exposed to HF diet **(I)**. Glucose tolerance test (GTT) is displayed on the left y-axis and insulin tolerance test (ITT) on the right y-axis. Data presented as mean and S.E.M. N = 6–10.

far more abundant amino acid transporters were upregulated, such as *Slc38a6*, *Slc36a1*, *Slc43a2*, and, in particular, the Slc7 family (*Slc7a1* and *Slc7a4 (CATs)* and *Slc7a5*, *Slc7a7*, *Slc7a8* (LATs)). In addition, PNS placentas further displayed higher expression levels of the main glucose transporter *Slc2a1* and *Igf*$_2$ (Figure 6L). All significant genes are shown in Table S3.

2.7. Prenatal stress affects life-long hypothalamic gene expression

To explore the mechanisms underlying our metabolic findings (summarized in Table 1) and the impact of differential placental methylation caused by PNS, we examined gene expression in the adult hypothalamus using a whole-genome gene expression array comparing CTRL and PNS females. Gene ontology analysis revealed abnormalities in a variety of biological processes (Figure S1), including upregulation of the innate immune response and the response to stress (Figure 7A). Long-lasting inflammation in different areas of the brain has previously been reported in the context of PNS [38,39] and likely partially underlies the observed long-term metabolic abnormalities and response to challenge found here and by others [40]. Our findings further include upregulation of 17 long intergenic non-coding RNAs (lincRNAs) (Table S4). LincRNAs functions are largely unclear, but they have recently been implicated in the cellular stress response [41] and in anxiety-like behavior in mice [42]. But more remarkably, the long-term effects of PNS on the adult female hypothalamus are linked to long-lasting alterations in genes linked to neuronal processes, more notably in the vesicle,

 © 2018 The Authors. Published by Elsevier GmbH. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

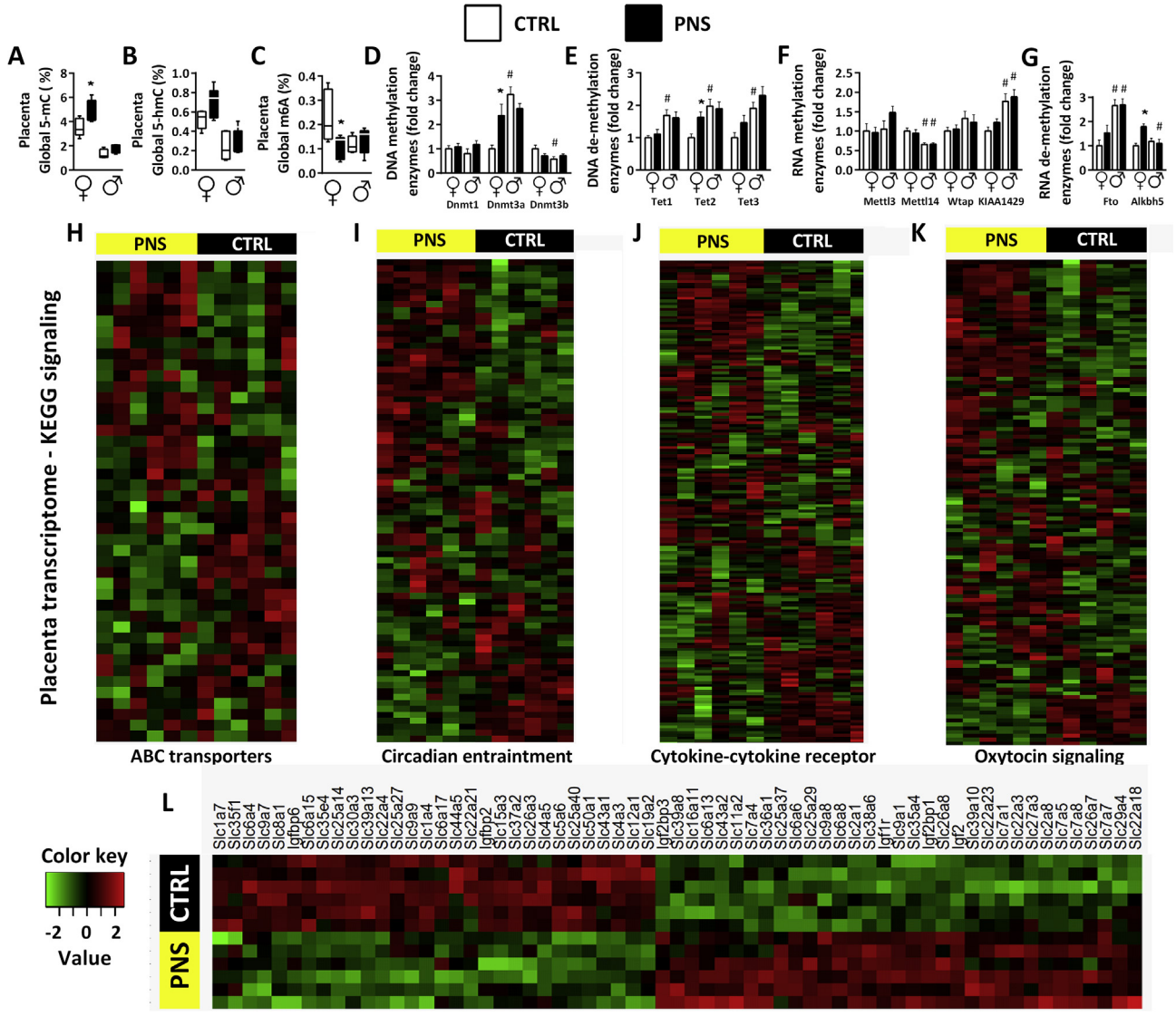

Figure 6: Prenatal stress (PNS) affects global placental DNA/RNA methylation and gene expression in females. (A) Global DNA methylation differed between the groups and was higher in PNS females compared to female controls (CTRLs; Kruskal Wallis H = 19.71, p = 0.000). **(B)** Global DNA hydroxymethylation methylation differed between the groups and was higher in the PNS groups (Kruskal Wallis H = 15.9, p = 0.001). **(C)** Global RNA methylation differed between the groups and was lower in PNS females compared to female CTRLs (Kruskal Wallis H = 10.86, p = 0.013). Data presented as min. to max. with median. Specific group comparisons based on Mann Whitney tests. **(D–G)** Placental gene expression for enzymes involved in DNA methylation and regulation of hydroxy(de)methylation and RNA methylation and demethylation were mainly determined by fetal sex ($F_{(12,17)} = 7.65$, p = 0.000). Dnmt3a ($F_{int(1,31)} = 7.32$, p = 0.011) **(D),** Tet2 ($F_{int\ (1,31)} = 5.60$, p = 0.025) **(E)** and Alkbh5 ($F_{(int(1,31)} = 10.18$, p = 0.003) **(G)** further showed a prenatal treatment × sex interaction and were upregulated by PNS only in females. Significance between the groups was based on post hoc Tukey HSD for multiple comparisons. *p < 0.05 between CTRLs and PNS in the same sex and #p < 0.05 between males and females in the same group. Data presented as mean and S.E.M. N = 8. **(H–K)** Gene expression heatmap of genes contained in 4 significant KEGG pathways from RNA-Seq of CTRL (N = 6) and PNS female placentas (N = 6). **(H)** ABC transporter pathway (q-value = 0.073)**, (I)** circadian entrainment (q-value = 0.060), **(J)** cytokine–cytokine receptor interaction (q-value = 0.004) and **(K)** oxytocin pathway (q-value = 0.073). **(L)** Heatmap showing the significant changes in expression of nutrient transporters resulting from PNS. (N = 6).

synapse, axon, dendrite, presynapse and neuronal cell body (Figures 7A, S1 and S2), suggesting major structural and/or functional remodeling in this group (all predictions based on STRING database [43]).

Finally, we focused on a few individual genes affected by PNS that are heavily involved in the maintenance of energy homeostasis and the response to stress [44–47] (Table S4). Among them, we validated oxytocin *(Oxt)*, arginine vasopressin *(Avp)*, adrenergic receptor beta 3 *(Adrb3)*, neuromedin S *(Nms)*, and neuronatin *(Nnat)*, most of which are involved in many of the processes revealed by GO analysis (Figure 7A,C). *Oxt*, *Avp*, *Adrb3*, and *Nnat* were downregulated, and *Nms* was upregulated by PNS (Figure 7B). Finally, in order to test the fetal origins of the observed phenotypes, we examined the expression levels of these genes in the fetal hypothalamus. We found significant alterations in all five genes in response to PNS, suggesting these abnormalities are programmed *in utero* and remain into adulthood, producing the abnormal responses of PNS females to metabolic challenge.

 © 2018 The Authors. Published by Elsevier GmbH. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Table 1 — Effects of the different ED protocols on metabolic parameters. BW was assessed during the ED protocols and all the rest of the parameters were assessed post recovery. For the ABA group, PNS animals were compared to resilient CTRLs.

effects on metabolic parameters

Experiments — PNS vs. CTRLs ♀	BW change	Food intake	Metabolic rate	Activity	Adipositiy	Glucose tolerance	Insulin tolerance
Basal response to PNS	↔	↗	↔	↗	↗ ~	↔	↘
Food restriction	↗	↔	↘	↔	↗	↔	↔
Activity based anorexia (compared to resilient)	↔	↔	↔	↔	↗	↔	↔
Binge eating	↔	↘	↘	↔	↗ ~	↔	↔
High fat diet	↗	↔	↘	↔	↗	↔	↔

effects on metabolic parameters

Experiments — PNS vs. CTRLs ♂	BW change	Food intake	Metabolic rate	Activity	Adipositiy	Glucose tolerance	Insulin tolerance
Basal response to PNS	↔	↔	↔	↔	↔	↔	↘
Food restriction	↔	↔	↘	↔	↔	↔	↔
Activity based anorexia (compared to resilient)	↔	↔	↘	↗	↔	↔	↔
Binge eating	↘	↔	↗	↔	↔	↔	↗ ~
High fat diet	↘	↔	↗	↗	↘	↗	↗

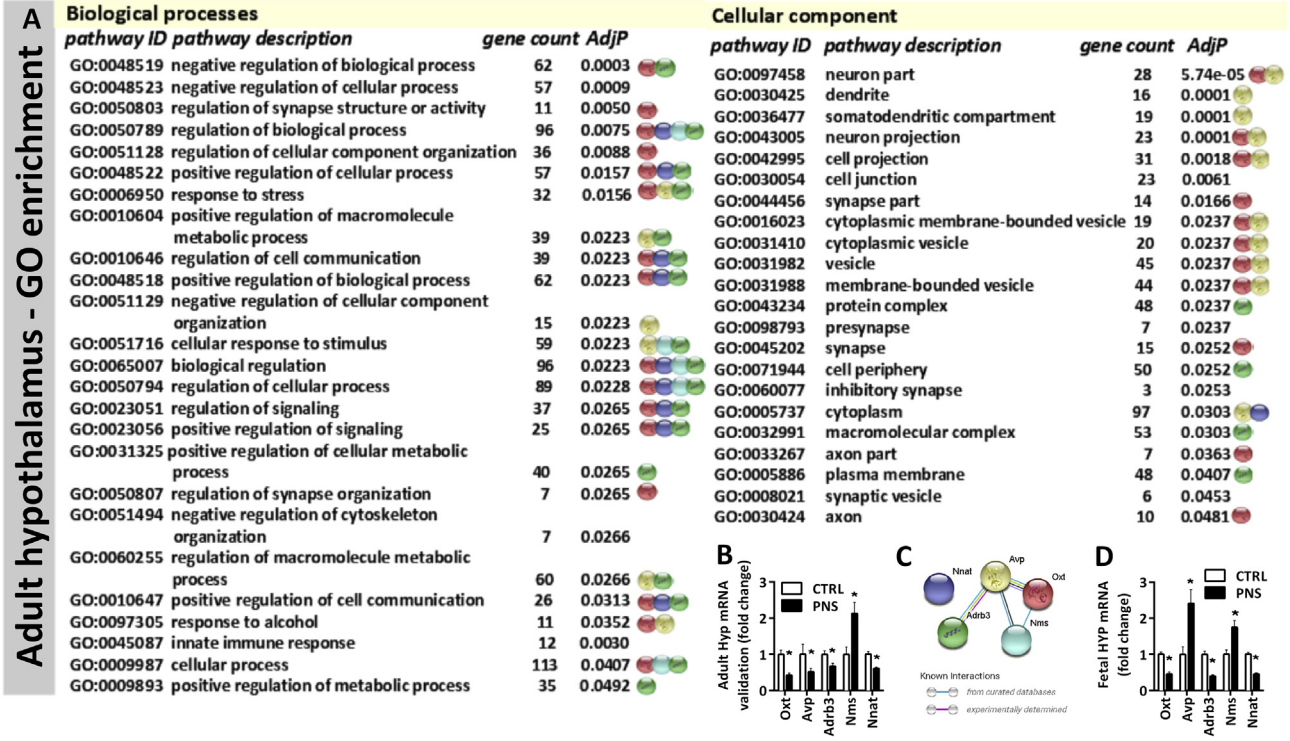

Figure 7: Prenatal stress (PNS) induced abnormalities in hypothalamic gene expression in adult females and fetuses. (A) Gene ontology (GO) enrichment analysis based on a hypothalamic gene expression array of the adult female hypothalamus of control (CTRL) and PNS animals. **(B)** RT-PCR validation shows different gene expression between the groups ($F_{int(4,69)} = 9.48$, $p = 0.001$). **(C)** STRING pathway analysis linking the affected genes (https://string-db.org). **(D)** Relative expression of the selected genes in the female fetal hypothalamus shows abnormalities resulting from PNS ($F_{int(4,80)} = 17.74$, $p = 0.001$). Data presented as mean and S.E.M. N = 6–12.

 © 2018 The Authors. Published by Elsevier GmbH. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

3. DISCUSSION

In the present set of studies, we aimed to address a common confounding variable encountered in studies exploring the effects of early life stress and predisposition to eating and metabolic disorders by using the same stress protocol to test vulnerability to a variety of different EDs and challenges. It is well known that the effect of PNS on the offspring depends on the severity, timing, sex of the offspring and the variables being tested. Thus, early or "first trimester" PNS reduces BW and adiposity in males exposed to HF diet (females not tested) [12], but late gestation stress increases BW, glycemia, and food intake in male mice (females not tested) [48,49] and insulin resistance in rats of both sexes when exposed to HF diet [16,50,51]. Interestingly, late PNS and HF diet increases adiposity in female, but not male rats [52]. Late PNS further increases susceptibility to BE in female mice [15] and to ABA in a subset of passively stress-coping female rats [52]. In contrast, when PNS takes place during the whole gestational period, the females' innate susceptibility to ABA disappears [23]. In girls, prenatal stress during the whole pregnancy period is associated with an increased overall risk of BN but not AN [4], and this is particularly true for late gestation stress [53]. Chronic stress and psychiatric comorbidity are also associated with the onset of EDs when the stressor takes place in the year preceding or generally close to the onset of the ED [54]. Again, this effect was more robust for BN and BED than for AN, which showed mixed results [55]. In addition, stress alone does not always trigger EDs/metabolic disorders, and it is likely that rather a combination of an innate predisposition pooled with the additive effect of early life trauma is more likely to lead to disease [8,56]. Thus, different underlying processes appear to be involved in the different EDs, and these processes may be differentially affected by early life alterations depending on the developmental window at which they occur. The heterogeneity of the protocols and time windows considered, combined with the fact that the majority of the still limited amount of studies in the field examined only one sex or one ED with the same protocol, makes drawing conclusions about PNS-linked predisposition to EDs rather difficult. However, these findings highlight that maternal stress is associated with risk of EDs in the offspring with a different time window for each particular ED and sex.

The stress protocol used here, unlike the ones cited above, included the whole period of pregnancy in an attempt to mimic a real-life situation in which the mother experiences chronic anxiety. The resulting responses to the ED protocols and metabolic challenges according to the sex were remarkable, with females showing clear programming to diet induced obesity through fat accumulation and decreased metabolic rate, regardless of the metabolic challenge. This was especially visible in the FR experiment, in which the sudden food limitation led to a transitory but sharp weight loss in CTRLs but not in PNS animals, suggesting body weight changes are more tightly regulated in this group (Figure 2B). In contrast to the females, the males' metabolic rate was adapted to the type of manipulation (decreased when FR and increased when overfed), so they maintained the appropriate levels of fat and even coped more effectively than CTRLs with HF diet. This was interesting given the usual consensus that PNS combined with HF diet increases insulin resistance, overeating and obesity as suggested in some studies [16,57]. This may be again related to the window of intervention, since other studies focusing on different time-windows reported the opposite effect [12,51,58]. Altogether, while late gestation adversities appear to more profoundly affect females [14–16] and early gestation adversities appear to have more profound effects on males, whole pregnancy adversities appear to have strong and opposite effects for each sex, at least regarding metabolic responses to challenge.

The dramatically different coping style PNS females had compared to CTRLs in response to the different ED protocols is likely mediated by hypothalamic adaptations resulting from PNS. These include abnormal levels of *Avp*, *Oxt*, *Adrb3*, *Nms*, and *Nnat*, all deeply involved in several aspects of metabolic regulation, combined with a lifelong inflammatory state and structural and/or functional remodeling of the hypothalamic neurons. Avp is robustly expressed in parvocellular cells of the paraventricular nucleus of the hypothalamus (PVN) and has been implicated in the regulation of blood glucose, locomotor activity and food intake through melanocortin dependent feeding pathways [44,59–61]. There is also substantial evidence supporting the role of Oxt, a stress-buffering hormone, in energy homeostasis since Oxt neuron ablation makes mice more sensitive to HF diet-induced obesity due solely to reduced energy expenditure [62]. Oxt circulating levels are also decreased in obese diabetic patients, potentially worsening their phenotype [46]. Nnat, an endoplasmic reticulum proteolipid implicated in intracellular signaling, is primarily concerned with antenatal brain growth and development. In addition, Nnat expression in the hypothalamus is responsive to acute nutrient and leptin signaling, is decreased by fasting/increased in obesity [63] and is associated with acute appetite and energy homeostasis and in metabolic-inflammation [64]. Nms, specifically expressed in the suprachiasmatic nucleus of the hypothalamus, is involved in the regulation of circadian rhythms [65] and feeding behavior. Intracerebroventricular administration of Nms induced phase shifts in the circadian rhythm of locomotor activity and had an anorexigenic effect [66] through increases in proopiomelanocortin mRNA expression in the arcuate nucleus and corticotropin releasing factor in the PVN. Nms is also involved in thermoregulation via Adrb3 [47]. In turn, polymorphisms in the adrenergic receptor genes have been extensively studied for association with metabolic syndrome and diabetes [67] and appear to have a sex-specific effect [68]. In addition, beta-adrenergic receptor stimulation can prevent androgenization of the neonatal brain [69]. This is particularly relevant given that PNS can, through prenatal androgen exposure, masculinize the food intake pattern of female offspring [70]. Thus, Adrb3 down-regulation can enable masculinization, leading to the (CTRL) male-like response that PNS females show in response to the different ED protocols. Finally, the adipogenic response of PNS females to energy deficit (Figure 1P) in the FR experiment may be linked to CORT rises, similarly to those found among obese women. FR (and probably BE and HF) stress-related CORT rises can potentially facilitate fat deposition [71]. Mechanisms underlying this process may include direct central nervous system action on peripheral tissues with high glucocorticoid receptor concentration in regions that are sexually dimorphic, producing multiple systemic endocrine and metabolic effects [19]. Altogether, a combination of all these defects can induce lipid accumulation through lower metabolic rate and CORT, selective masculinization and metabolic inflammation despite similar food consumption, in response to any metabolic challenge.

Finally, it is well known that when it comes to gestational programming, the placenta plays a critical role in fetal development, by carrying out the feto-maternal exchange, barrier, and endocrine functions during pregnancy. Placentas exhibit pronounced sexual dimorphism in response to variations in the maternal environment both in humans [72] and mice [12,73–75]. The sexual dimorphism found here in placental DNA and RNA methylation in response to PNS can induce dramatic differences in morphology and functioning and consequently in fetal the environment. Specifically, dysregulation of the expression of the transmembrane ATP-binding cassette (ABC) transporters in PNS

 © 2018 The Authors. Published by Elsevier GmbH. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

females can affect the distribution of nutrients and exchange of waste metabolites across the placenta, consequently playing a critical role in regulating immunological responses and lipid trafficking [76]. Abnormal placental cytokine—cytokine receptor interaction further hints at heightened immunological response resulting from PNS. Next, abnormalities in the placental Oxt signaling pathway, which is thought to play a critical role in placental function [77], may in addition be linked to the low levels in Oxt gene expression observed in the stressed fetal and adult hypothalamus. The observed circadian entrainment in PNS placentas, including abnormally high levels of the Period (Per) canonical clock genes, hints at alterations in placental rhythmicity, which is independent from the mother and the fetus [78]. The fetus in turn may be influenced by dynamic circadian signals originating both in the mother and the placenta, adding a further layer of fetal programming resulting from PNS in females, through mediators of maternal—fetal circadian interactions [79]. Finally, *Igf*$_2$ is heavily involved in nutrient transport and can regulate the thickness of the membrane connecting the maternal and fetal circulation [23,80]. Thus, high expression of placental *Igf*$_2$ can decrease the thickness of the junction, further enabling an increase in transport of glucose and amino acids by the over-expressed glucose transporter *Slc2a1* and the *Slc7* family of amino acid transporters to the stressed fetuses, all of which can further alter fetal metabolic programming.

Thus, while it is very well documented that maternal stress impacts the placenta causing varied negative effects on anxiety, depression, cognition, memory [81,82], metabolism [40], and inflammation [38,39] in the offspring, these effects are largely sex-specific [81] arguably originating in evolutionary adaptations aimed at helping each sex better adapt in challenging conditions. A frequent interpretation of these findings proposes that early adversity may threaten the males' viability, culling the weak and creating a surviving cohort of the fittest. While females may be more successful in adjusting to early adversity with a variety of different strategies, this may come at the high price of increased vulnerability to metabolic disease expressed later in development.

In summary, we found that through global sex-specific placental adaptations, the female hypothalamus is programmed to diet induced obesity and not to develop increased vulnerability to EDs, when exposed to chronic PNS. Moreover, our results highlight the complexity and tortuosity of the interaction between PNS and proneness or resistance to different EDs and the importance of using both sexes and applying comparable manipulations during similar time windows to address the early origin of eating and metabolic disease.

4. MATERIALS AND METHODS

4.1. Animal care

ICR (CD1) mice (Harlan Sprague Dawley Inc., Indianapolis, IN) were maintained in a pathogen-free temperature-controlled (22 $\pm$ 1 °C) mouse facility on a reverse 12 h light-dark cycle at the Weizmann Institute of Science, according to institutional guidelines. Food (Teklad global, Harlan Sprague Dawley Inc., Indianapolis, IN) and water were given ad libitum (apart from during FR). All experimental protocols were approved by the Institutional Animal Care and Use Committee of The Weizmann Institute of Science.

4.2. Breeding

Female ICR mice were mated at 11—13 weeks of age. Two or 3 females were housed with 1 male (minimum age 12 weeks) at the beginning of the dark period and were examined for the presence of a vaginal copulation plug at the end of the dark period. Presence of a copulation plug denoted day 0.5 of gestation. After breeding, the females were individually housed.

4.3. Experimental groups

Females with plugs were randomly assigned to the control or PNS treatment. From day 18.5 of gestation, females were checked twice a day for the presence of a litter (9:00—10:00, 17:00—18:00). Newborn litters found by 18:00 were designated as born on that day-postpartum day 0 (PPD 0). On PPD 1, pups were counted, and litters were culled to 10 pups (with sex distribution kept as equal as possible in each litter). Litters with less than 7 pups were excluded.

4.4. Prenatal stress (PNS) protocol

From gestation day (GD)1.5 until GD16.5, pregnant females were exposed to a chronic variable mild stress protocol, including 2 short manipulations during the dark phase and a further overnight (ON) manipulation during the light phase, every day. The chosen stressors did not induce pain and did not directly influence maternal food intake and were repeated on a weekly basis. The short stressors included multiple cage changes (every 15 min for 2 h), cage tilt (15° for 2 h), white noise (1 h), water in cage (250 ml) or no bedding for 2 periods of 2 h, immobilization in a tube or elevated platform (50 cm high in a bucket of cold water) for 30 min and swimming in warm water for 15 min. Overnight manipulations included illumination during the dark phase, saturated bedding (with water), novel object in the cage (10 glass marbles) and overcrowding (with 14 females in the home cage). Using a randomized complete block design to avoid potential litter effects, the offspring were randomly allocated to the following experimental groups:

1) **Food restricted (FR) group.** Pups were weaned (PPD 21—22) and group housed until PPD30. They were then singled housed for a week and then exposed to FR for 4 consecutive days. The first day, food access was limited to 4 h and the following 3 days, food access was limited to 3 h in the dark phase. The BW and food intake data were published in a previous study [23] as a control for the ABA/resistant groups for CTRLs and PNS separately. Here, we performed a new analysis for a different comparison, i.e. between CTRLs and PNS.
2) **Activity based anorexia (ABA) model.** ABA was performed as previously described [23]. Briefly, pups were weaned (PPD 21—22), and offspring were group housed until PPD30. On PPD30, mice were placed in a running Wheel (RW) system in order to record the voluntary running pattern (diameter 115 mm, TSE-Systems, Bad Homburg, Germany). The protocol lasted for 11 days during which food intake and BW were measured daily. The first week was defined as a "training period", during which food and water were given ad libitum. On the first day of the second week, food access was limited to 4 h, and, on the following days, the food access was limited to 3 h. Animals were defined as anorexic in retrospect by cluster analysis according to: 1) Percentage weight loss; 2) Food intake during FR; 3) Food intake recovery (%) during the FR period; 4) Circadian disruption (km ran during the light phase); 5) Days until collapse (become immobile and hypothermic), and 6) Total running distance (km) when FR. Animals that developed ABA were removed from the running wheel cage once they collapsed and were subsequently given food ad libitum and monitored until fully recovered.
3) **Binge eating protocol.** BE was performed as previously described [15] and consisted of sporadic and limited access (for 2 h a day, 3 times a week during the dark cycle) to Western diet (D12079Bi, Research diets Inc., New Brunswick, NJ, USA) for a period of four weeks, without FR. The protocol was preceded by a 5-day

 © 2018 The Authors. Published by Elsevier GmbH. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

habituation to the new diet and started on PPD30, the pre-pubertal period equivalent to adolescence in humans.

4) **High fat group.** Three-month-old mice were fed ad libitum HFD (60% of calories; D12492 Research Diets Inc., New Brunswick, NJ, USA) for 3 weeks.

4.5. Glucose/insulin tolerance tests

Glucose and insulin tolerance tests (GTT and ITT respectively) were performed in awake animals during the dark phase of the day cycle. For GTT, glucose (2 g/kg of body weight) was injected i.p., and whole venous blood obtained from the tail vein at 0, 15, 30, 60, 90, and 120 min after the injection was measured for glucose using an automatic glucometer (Accu-Check performa, Roche diagnostics GmbH, Mannheim, Germany), following 6 h of fasting. For the Insulin tolerance test (ITT), 0.75 U/kg insulin (Sigma–Aldrich) was injected after 4 h of fasting. Blood was tested with the glucometer at 0, 15, 30, 60, and 90 min after the injection. All animals underwent both tests (GTT first and ITT second) with 2 days recovery in between.

4.6. Body composition

Body composition was assessed using Echo-MRI-100™ (Echo Medical Systems, Houston, TX, USA).

4.7. Metabolic assessment

Indirect calorimetry and food and water intake were measured using the LabMaster system (TSE-Systems, Bad Homburg, Germany). The LabMaster instrument consists of a combination of sensitive feeding and drinking sensors for automated online measurement. The calorimetry system is an open-circuit system that determines O_2 consumption, CO_2 production, and respiratory exchange ratio (RER). Data were collected for five consecutive days after 24 h of adaptation for the apparatus.

4.8. Maternal behavior

On days 6/7 and 17/18 postpartum, patterns of undisturbed nocturnal maternal behavior were observed during 160 min sessions. Each mother was observed every 15 min, for 1–3 s. This allowed the identification of the ongoing maternal behavior at the observation time. Various maternal and non-maternal behaviors were recorded in every observation. The score was ''1'' if the behavior occurred and ''0'' if it did not occur. Maternal Behavior measures were based on existing literature [83] and included both self-(grooming, eating) and pup-directed behaviors (nursing, licking/grooming) and activity measures.

4.9. Adult hypothalamic and blood collection

Adult animals were sacrificed at the age of 3 months. After decapitation, the hypothalamus was dissected and immediately frozen. Trunk blood was collected in the PNS and CTRL mice (without any ED protocol) and immediately centrifuged, and plasma samples were stored at −80 °C.

4.10. Placenta and fetal tissue collection

On GD17.5, pregnant females were anesthetized with an overdose of Ketamine-Xylazine (1:1, 20% in saline) and through cesarean section both the embryos and placentas were excised. Embryos were decapitated; the brain was removed from the skull and placed with the cortex down onto a flat dish to expose the ventral part. Typically, the hypothalamus is easily recognized by its unique density, the entrance of the optic nerve, and its slightly bulb-like anatomy, distinguishing it from the rest of the brain. The entire hypothalamus was then excised using a pointed scalpel and a thin metal spatula and immediately frozen on dry ice and stored at −80 °C. Trunk blood was collected in EDTA coated tubes (MiniCollect, Greiner bio-one, Austria). Placentas were immediately stored at −80 °C. A portion of the embryos' tail was removed for later DNA extraction and sex genotyping. For analyses comparing between groups, only one fetus per litter was used. These were chosen from the central uterinal location, with one male and one female as adjacent fetuses.

4.11. Genotyping

Sex of the embryo was determined by Sry genotyping (forward 5′-TCATGAGACTGCCAACCACAG-3′ and reverse 5′-CATGACCACCAC CACCACCAA-3′) [84]. Amplification product was detected by 2% gel electrophoresis.

4.12. RNA extraction and real-time PCR

For placental tissue, purification of total RNA was done using Tri Reagent (MRC Inc., Cincinnati, OH) according to the manufacturer's recommendations. gDNA was removed using Turbo DNAse digestion in solution, followed by heat deactivation (#AM2238, Thermo Fisher Scientific). Total hypothalamic RNA was extracted using the miRNeasy Mini Kit (#217004, Qiagen) in conjunction with on column digestion of gDNA using Turbo DNAse (see above). RNA preparations were reverse transcribed to generate cDNA using miScriptII Reverse transcription kit for miRNAs/mRNA (QIAGEN, Hilden, Germany). Quantitative mRNA expression was done using a SYBR®Green PCR kit (QIAGEN, Hilden, Germany) (#204057 QIAGEN, Germany) respectively, according to the manufacturer's guidelines and a StepOnePlus® Real time PCR system (Applied Biosystems, Waltham, MA), using specific primers. Tbp was used as internal control for mRNA in placental samples and Hprt was used as internal control for mRNA in hypothalamic samples.

4.13. Global DNA methylation and hydroxy-methylation

DNA was extracted using Tri Reagent (MRC Inc., Cincinnati, OH) according to the manufacturer's recommendations. Global methylation of the placenta was measured using the MethylFlash Methylated DNA Quantification ELISA kit (Epigentek Group Inc., Farmingdale, NY) according to the manufacturer's instructions. Global hydroxyl-methylation was determined using the MethylFlash Hydroxymethylated DNA Quantification ELISA kit (Epigentek) according to the manufacturer's instructions.

4.14. Global RNA N6-methyladenosine (m6A) methylation

Global RNA methylation of the placenta was assessed using the Epi-Quik m6A RNA Methylation Quantification ELISA kit (Epigentek Group Inc., Farmingdale, NY) according to the manufacturer's instructions.

4.15. Hypothalamic whole genome array

Whole genome transcriptome of the hypothalamus was analyzed using Agilent's microarray kit SurePrint G3 (G4858A). The bioinformatic analysis was performed using the Limma (Liniar Models for MicroArray Data) package, which is part of the Bioconductor software. We corrected the background with the Normexp method and used the Loess method for the within arrays normalization and the Aquantile for between arrays normalization. A linear model was applied in order to find the differentially expressed genes (a simple Bayesian model). Gene ontology analysis was performed using the STRING database https://string-db.org [43].

 © 2018 The Authors. Published by Elsevier GmbH. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

4.16. RT-PCR primers

Gene	Forward	Reverse
Dnmt3a	AGTGACCACCAAGTCTCAG	GTTCACTCCGCTTCTCCAA
Dnmt3b	TTCAGTGACCAGTCCTCAGACACGAA	TCAGAAGGCTGGAGACCTCCCTCTT
Dnmt1	CCTAGTTCCGTGGCTACGAGGAGAA	TCTCTCTCCTCTGCAGCCGACTCA
Tet1	ACAAAAAGCGTACCTGCACC	TCTGGGGTTTTCACTCCTCC
Tet2	GAGCTGAGCCAAAAGAGGAAG	CTGGGGCTTGGTGTTCAAAT
Tet3	CCAAGTGGGTGATCCGAAGA	ATCACGGCGTTCTGACAATG
Wtap	AAGCAGCAACAGCAGGAGT	AGGTACTGGATTTGAGTGGTGC
KIAA1428	AGTAACTGGACACCGCTCAAG	AGTACTGCTCAGGAAACCCG
FTO	GCGGGAAGCTAAGAAACTGAG	AGGGTATTTCAGCTGCCACT
Alkbh5	TGTGCTCAGTGGGTATGCT	CGCGGTGCATCTAATCTTGT
Mettl3	GAGCTAGGATGTCGGACACG	GGTTCCTTAAATCCAAGTGCCC
Mettl14	GATGAGATTGCAGCACCTCG	CCCACTTTCGCAAGCATACTC
Tbp	CTCAGTTACAGGTGGCAGCA	ACCAACAATCACCAACAGCA
Avp	TCAACACTACGCTCTCCGCTT	CCTTTGCCGCCCGG
Nms	TCCCGACGGCTTGGATATTG	TCCTGGTTTTCCTTAGGTTGG
Oxt	TCACCTACAGCGGATCTCAGAC	AGACACTTGCGCATATCCAGG
Adrb3	GGCAACCTGCTGGTAATCAT	GCATTACGAGGAGTCCCAC
Nnat	GTCCCCTGTGTTCCCTCGTC	TGTCGGTGCTGCTTTTCTGG
Hprt	GCAGTACAGCCCCAAAATGG	GGTCCTTTTCACCAGCAAGCT

4.17. Libraries and RNA-Seq

Libraries were prepared with the Illumina TruSeq Stranded Total RNA Library Preparation kit with Ribo Zero Gold (Illumina, #RS-122-2301) according to the instructions, using 1000 ng total mouse placenta RNA as starting material. Libraries were quantified on a Qubit fluorometer and by qPCR with a KAPA Library Quantification Kit for Illumina libraries (# KK4828). Size distribution was checked using the Agilent High Sensitivity DNA Assay (#5067-4626) on an Agilent Bioanalyzer. Samples were denatured using 1N NaOH, diluted to a concentration of 3 nM with ExAMP mastermix and loaded onto a HiSeq 4000 machine (Illumina, San Diego, CA; #SY-401-4001) with 1% PhiX control (Illumina, #FC-110-3001) spiked in. HiSeq 3000/4000 flow cells and HiSeq 3000/4000 SBS sequencing chemistry were used for paired-end sequencing with a read length of 100 bp for each direction. Sequencing was performed at the Helmholtz Center (Munich, Germany).

4.18. RNA-seq analysis

The quality of sequencing reads was verified using FastQC 0.11.5 (http://www.bioinformatics.babraham.ac.uk/projects/fastqc). Adapters were trimmed using cutadapt v.1.9.1 [85] in paired-end mode. For quantification of gene expression, kallisto 0.43.1 [86] was employed using the mouse Ensembl annotation v79 (downloaded from http://bio.math.berkeley.edu/kallisto/transcriptomes/). Normalization and differential analysis on gene level was performed using DESeq2 version 1.18.1 [87]. RIN value was corrected for. Only genes with minimum of 5 counts in at least 3 samples were included in the analysis; rRNA and genes located on chrM were filtered out. For pathway analysis, the results from the differential analysis were ranked and inputted into FGSEA [88], using KEGG database [89], 100000 permutations and a q-value cutoff of 0.1 for significance. For visualization purpose, R packages were used (edgeR [90], gage [91].

4.19. Statistical approach

Statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) software, Version 20.0 (SPSS Inc., Chicago, IL) and GraphPad Prism 6 (GraphPad software, Inc., La Jolla, CA). Tests included repeated measures ANOVA, *t* tests or one-way ANOVA when relevant. Differences between the groups were assessed using Tukey's multiple comparisons post hocs. When appropriate, non-parametric tests such as Kruskal—Wallis were used. Specific details of N and tests used are provided in the figure legends.

AUTHOR CONTRIBUTIONS

MS designed the experiments, analyzed the data and wrote the manuscript. AC supervised the project. MS, TP, and YD performed the manipulations, RNA and DNA extractions. MS, TP, and YD sacrificed the animals. MJ constructed the libraries and performed NGS. SR and SB performed the bioinformatic predictions and analysis.

ACKNOWLEDGEMENTS

We thank Mr. Sharon Ovadia for his devoted assistance with animal care. This work was supported by: an FP7 Grant from the European Research Council (260463, A.C.); a research grant from the Israel Science Foundation (1565/15, A.C.); the ERANET Program, supported by the Chief Scientist Office of the Israeli Ministry of Health (3-11389, A.C.); the project was funded by the Federal Ministry of Education and Research under the funding code 01KU1501A (A.C.); research support from Roberto and Renata Ruhman (A.C.); research support from Bruno and Simone Licht; I-CORE Program of the Planning and Budgeting Committee and The Israel Science Foundation (grant no. 1916/12, A.C.); the Nella and Leon Benoziyo Center for Neurological Diseases (A.C.); the Henry Chanoch Krenter Institute for Biomedical Imaging and Genomics (A.C.); the Perlman Family Foundation, founded by Louis L. and Anita M. Perlman (A.C.); the Adelis Foundation (A.C.); the Marc Besen and the Pratt Foundation and the Irving I. Moskowitz Foundation (A.C.). MJ was supported by a NARSAD young investigator (grant no 22809).

 © 2018 The Authors. Published by Elsevier GmbH. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

APPENDIX A. SUPPLEMENTARY DATA

Supplementary data related to this article can be found at https://doi.org/10.1016/j.molmet.2018.08.005.

REFERENCES

[1] Keski-Rahkonen, A., Mustelin, L., 2016. Epidemiology of eating disorders in Europe. Current Opinion in Psychiatry 29(6):340–345. https://doi.org/10.1097/YCO.0000000000000278.

[2] Smink, F.R.E., van Hoeken, D., Hoek, H.W., 2012. Epidemiology of eating disorders: incidence, prevalence and mortality rates. Current Psychiatry Reports 14(4):406–414. https://doi.org/10.1007/s11920-012-0282-y.

[3] Smink, F.R.E., van Hoeken, D., Hoek, H.W., 2013. Epidemiology, course, and outcome of eating disorders. Current Opinion in Psychiatry 26(6):543–548. https://doi.org/10.1097/YCO.0b013e328365a24f.

[4] Su, X., Liang, H., Yuan, W., Olsen, J., Cnattingius, S., Li, J., 2016. Prenatal and early life stress and risk of eating disorders in adolescent girls and young women. European Child and Adolescent Psychiatry 25:1245–1253. https://doi.org/10.1007/s00787-016-0848-z.

[5] Steiger, H., Thaler, L., 2016. Eating disorders, gene-environment interactions and the epigenome: roles of stress exposures and nutritional status. Physiology and Behavior 162:181–185. https://doi.org/10.1016/j.physbeh.2016.01.041.

[6] Strober, M., Peris, T., Steiger, H., 2014. The plasticity of development: how knowledge of epigenetics may advance understanding of eating disorders. The International Journal of Eating Disorders 47(7):696–704. https://doi.org/10.1002/eat.22322.

[7] Rayworth, B.B., Wise, L. a., Harlow, B.L., 2004. Childhood abuse and risk of eating disorders in women. Epidemiology 15(3):271–278. https://doi.org/10.1097/01.ede.0000120047.07140.9d.

[8] Favaro, A., Tenconi, E., Santonastaso, P., 2006. Perinatal factors and the risk of developing anorexia nervosa and bulimia nervosa. Archives of General Psychiatry 63(1):82–88.

[9] Favaro, A., Ferrara, S., Santonastaso, P., 2003. The spectrum of eating disorders in young women: a prevalence study in a general population sample. Psychosomatic Medicine 65(4):701–708.

[10] Entringer, S., Wadhwa, P.D., 2013. Developmental programming of obesity and metabolic dysfunction: role of prenatal stress and stress biology. Nestle Nutrition Institute Workshop Series 74:107–120. https://doi.org/10.1159/000348454.

[11] Entringer, S., Wüst, S., Kumsta, R., Layes, I.M., Nelson, E.L., Hellhammer, D.H., et al., 2008. Prenatal psychosocial stress exposure is associated with insulin resistance in young adults. American Journal of Obstetrics and Gynecology 199(5):498.e1–498.e7. https://doi.org/10.1016/j.ajog.2008.03.006.

[12] Pankevich, D.E., Mueller, B.R., Brockel, B., Bale, T.L., 2009. Prenatal stress programming of offspring feeding behavior and energy balance begins early in pregnancy. Physiology and Behavior 98(1–2):94–102. https://doi.org/10.1016/j.physbeh.2009.04.015.

[13] Nugent, B.M., Bale, T.L., 2015. The omniscient placenta: metabolic and epigenetic regulation of fetal programming. Frontiers in Neuroendocrinology. https://doi.org/10.1016/j.yfrne.2015.09.001.

[14] Sandman, C.A., Glynn, L.M., Davis, E.P., 2013. Is there a viability-vulnerability tradeoff? Sex differences in fetal programming. Journal of Psychosomatic Research 75(4):327–335. https://doi.org/10.1016/j.jpsychores.2013.07.009.

[15] Schroeder, M., Jakovcevski, M., Polacheck, T., Lebow, M., Drori, Y., Engel, M., et al., 2017. A methyl-balanced diet prevents CRF-induced prenatal stress-triggered predisposition to binge eating-like phenotype. Cell Metabolism 25(6):1269–1281.e6. https://doi.org/10.1016/j.cmet.2017.05.001.

[16] Paternain, L., Batlle, M.A., De la Garza, A.L., Milagro, F.I., Martínez, J.A., Campión, J., 2012. Transcriptomic and epigenetic changes in the hypothalamus are involved in an increased susceptibility to a high-fat-sucrose diet in prenatally stressed female rats. Neuroendocrinology 96(3):249–260. https://doi.org/10.1159/000341684.

[17] Bronson, S.L., Bale, T.L., 2015. The placenta as a mediator of stress effects on neurodevelopmental reprogramming. Neuropsychopharmacology Official Publication of the American College of Neuropsychopharmacology 41(1):207–218. https://doi.org/10.1038/npp.2015.231.

[18] Davis, E.P., Pfaff, D., 2014. Sexually dimorphic responses to early adversity: implications for affective problems and autism spectrum disorder. Psychoneuroendocrinology 49:11–25. https://doi.org/10.1016/j.psyneuen.2014.06.014.

[19] Goldstein, J.M., Holsen, L., Huang, G., Hammond, B.D., James-Todd, T., Cherkerzian, S., et al., 2016. Prenatal stress-immune programming of sex differences in comorbidity of depression and obesity/metabolic syndrome. Dialogues in Clinical Neuroscience 18(4):425–436.

[20] Giussani, D.A., Davidge, S.T., 2013. Developmental programming of cardiovascular disease by prenatal hypoxia. Journal of Developmental Origins of Health and Disease 4(5):328–337. https://doi.org/10.1017/S204017441300010X.

[21] Pauli, J.M., Repke, J.T., 2015. Preeclampsia: short-term and long-term implications. Obstetrics and Gynecology Clinics of North America 42(2):299–313. https://doi.org/10.1016/j.ogc.2015.01.007.

[22] Walker, C.K., Krakowiak, P., Baker, A., Hansen, R.L., Ozonoff, S., Hertz-Picciotto, I., 2015. Preeclampsia, placental insufficiency, and autism spectrum disorder or developmental delay. JAMA Pediatrics 169(2):154–162. https://doi.org/10.1001/jamapediatrics.2014.2645.

[23] Schroeder, M., Jakovcevski, M., Polacheck, T., Drori, Y., Luoni, A., Röh, S., et al., 2018. Placental miR-340 mediates vulnerability to activity based anorexia in mice. Nature Communications 9(1):1596. https://doi.org/10.1038/s41467-018-03836-2.

[24] Klenotich, S.J., Dulawa, S.C., 2012. The activity-based anorexia mouse model. Methods in Molecular Biology (Clifton, N.J.) 829:377–393. https://doi.org/10.1007/978-1-61779-458-2_25.

[25] Hall, J.F., Smith, K., Schnitzer, S.B., Hanford, P.V., 1953. Elevation of activity level in the rat following transition from ad libitum to restricted feeding. Journal of Comparative and Physiological Psychology 46(6):429–433.

[26] Routtenberg, A., Kuznesof, A.W., 1967. Self-starvation of rats living in activity wheels on a restricted feeding schedule. Journal of Comparative and Physiological Psychology 64(3):414–421.

[27] Barbarich-Marsteller, N.C., Underwood, M.D., Foltin, R.W., Myers, M.M., Walsh, B.T., Barrett, J.S., et al., 2013. Identifying novel phenotypes of vulnerability and resistance to activity-based anorexia in adolescent female rats. The International Journal of Eating Disorders 46(7):737–746. https://doi.org/10.1002/eat.22149.

[28] Chowdhury, T.G., Wable, G.S., Sabaliauskas, N.A., Aoki, C., 2013. Adolescent female C57BL/6 mice with vulnerability to activity-based anorexia exhibit weak inhibitory input onto hippocampal CA1 pyramidal cells. Neuroscience 241:250–267. https://doi.org/10.1016/j.neuroscience.2013.03.020.

[29] Holtkamp, K., Hebebrand, J., Herpertz-Dahlmann, B., 2004. The contribution of anxiety and food restriction on physical activity levels in acute anorexia nervosa. The International Journal of Eating Disorders 36(2):163–171. https://doi.org/10.1002/eat.20035.

[30] Pjetri, E., de Haas, R., de Jong, S., Gelegen, C., Oppelaar, H., Verhagen, L.A.W., et al., 2012. Identifying predictors of activity based anorexia susceptibility in diverse genetic rodent populations. PLoS One 7(11):e50453. https://doi.org/10.1371/journal.pone.0050453.

 © 2018 The Authors. Published by Elsevier GmbH. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

[31] Corwin, R.L., Buda-Levin, A., 2004. Behavioral models of binge-type eating. Physiology and Behavior 82(1):123—130. https://doi.org/10.1016/j.physbeh.2004.04.036.

[32] Czyzyk, T. a., Sahr, A.E., Statnick, M. a., 2010. A model of binge-like eating behavior in mice that does not require food deprivation or stress. Obesity Silver Spring Md 18(9):1710—1717. https://doi.org/10.1038/oby.2010.46.

[33] Green, B.B., Houseman, E.A., Johnson, K.C., Guerin, D.J., Armstrong, D.A., Christensen, B.C., et al., 2016. Hydroxymethylation is uniquely distributed within term placenta, and is associated with gene expression. The FASEB Journal 30(8):2874—2884. https://doi.org/10.1096/fj.201600310R.

[34] Bianco-Miotto, T., Mayne, B.T., Buckberry, S., Breen, J., Rodriguez Lopez, C.M., Roberts, C.T., 2016. Recent progress towards understanding the role of DNA methylation in human placental development. Reproduction 152(1):R23—R30. https://doi.org/10.1530/REP-16-0014.

[35] Gheorghe, C.P., Goyal, R., Mittal, A., Longo, L.D., 2010. Gene expression in the placenta: maternal stress and epigenetic responses. The International Journal of Developmental Biology 54(2—3):507—523. https://doi.org/10.1387/ijdb.082770cg.

[36] Meyer, K.D., Saletore, Y., Zumbo, P., Elemento, O., Mason, C.E., Jaffrey, S.R., 2012. Comprehensive analysis of mRNA methylation reveals enrichment in 3′ UTRs and near stop codons. Cell 149(7):1635—1646. https://doi.org/10.1016/j.cell.2012.05.003.

[37] Lager, S., Powell, T.L., 2012. Regulation of nutrient transport across the placenta. Journal of Pregnancy 2012:1—14. https://doi.org/10.1155/2012/179827.

[38] Diz-Chaves, Y., Pernía, O., Carrero, P., Garcia-Segura, L.M., 2012. Prenatal stress causes alterations in the morphology of microglia and the inflammatory response of the hippocampus of adult female mice. Journal of Neuroinflammation 9:71. https://doi.org/10.1186/1742-2094-9-71.

[39] Szczesny, E., Basta-Kaim, A., Slusarczyk, J., Trojan, E., Glombik, K., Regulska, M., et al., 2014. The impact of prenatal stress on insulin-like growth factor-1 and pro-inflammatory cytokine expression in the brains of adult male rats: the possible role of suppressors of cytokine signaling proteins. Journal of Neuroimmunology 276(1—2):37—46. https://doi.org/10.1016/j.jneuroim.2014.08.001.

[40] Moisiadis, V.G., Matthews, S.G., 2014. Glucocorticoids and fetal programming part 1: Outcomes. Nature Reviews Endocrinology 10(7):391—402. https://doi.org/10.1038/nrendo.2014.73.

[41] Lakhotia, S.C., 2012. Long non-coding RNAs coordinate cellular responses to stress. Wiley Interdisciplinary Reviews RNA 3(6):779—796. https://doi.org/10.1002/wrna.1135.

[42] Spadaro, P.A., Flavell, C.R., Widagdo, J., Ratnu, V.S., Troup, M., Ragan, C., et al., 2015. Long noncoding RNA-directed epigenetic regulation of gene expression is associated with anxiety-like behavior in mice. Biological Psychiatry. https://doi.org/10.1016/j.biopsych.2015.02.004.

[43] Szklarczyk, D., Morris, J.H., Cook, H., Kuhn, M., Wyder, S., Simonovic, M., et al., 2017. The STRING database in 2017: quality-controlled protein-protein association networks, made broadly accessible. Nucleic Acids Research 45(D1):D362—D368. https://doi.org/10.1093/nar/gkw937.

[44] Pei, H., Sutton, A.K., Burnett, K.H., Fuller, P.M., Olson, D.P., 2014. AVP neurons in the paraventricular nucleus of the hypothalamus regulate feeding. Molecular Metabolism 3(2):209—215. https://doi.org/10.1016/j.molmet.2013.12.006.

[45] Bülbül, M., Sinen, O., Gemici, B., İzgüt-Uysal, V.N., 2017. Opposite effects of central oxytocin and arginine vasopressin on changes in gastric motor function induced by chronic stress. Peptides 87:1—11. https://doi.org/10.1016/J.PEPTIDES.2016.11.001.

[46] Qian, W., Zhu, T., Tang, B., Yu, S., Hu, H., Sun, W., et al., 2014. Decreased circulating levels of oxytocin in obesity and newly diagnosed type 2 diabetic patients. The Journal of Clinical Endocrinology and Metabolism 99(12):4683—4689. https://doi.org/10.1210/jc.2014-2206.

[47] Nakahara, K., Akagi, A., Shimizu, S., Tateno, S., Qattali, A.W., Mori, K., et al., 2016. Involvement of endogenous neuromedin U and neuromedin S in thermoregulation. Biochemical and Biophysical Research Communications 470(4):930—935. https://doi.org/10.1016/j.bbrc.2016.01.155.

[48] Darnaudéry, M., Maccari, S., 2008. Epigenetic programming of the stress response in male and female rats by prenatal restraint stress. Brain Research Reviews 57(2):571—585. https://doi.org/10.1016/J.BRAINRESREV.2007.11.004.

[49] Nyirenda, M.J., Lindsay, R.S., Kenyon, C.J., Burchell, A., Seckl, J.R., 1998. Glucocorticoid exposure in late gestation permanently programs rat hepatic phosphoenolpyruvate carboxykinase and glucocorticoid receptor expression and causes glucose intolerance in adult offspring. Journal of Clinical Investigation 101(10):2174—2181. https://doi.org/10.1172/JCI1567.

[50] Tamashiro, K.L.K., Terrillion, C.E., Hyun, J., Koenig, J.I., Moran, T.H., 2009. Prenatal stress or high-fat diet increases susceptibility to diet-induced obesity in rat offspring. Diabetes 58(5):1116—1125. https://doi.org/10.2337/db08-1129.

[51] Paternain, L., de la Garza, A.L., Batlle, M.A., Milagro, F.I., Martínez, J.A., Campión, J., 2013. Prenatal stress increases the obesogenic effects of a high-fat-sucrose diet in adult rats in a sex-specific manner. Stress 16(2):220—232. https://doi.org/10.3109/10253890.2012.707708.

[52] Boersma, G.J., Liang, N.-C., Lee, R.S., Albertz, J.D., Kastelein, A., Moody, L.A., et al., 2016. Failure to upregulate Agrp and Orexin in response to activity based anorexia in weight loss vulnerable rats characterized by passive stress coping and prenatal stress experience. Psychoneuroendocrinology 67:171—181. https://doi.org/10.1016/j.psyneuen.2016.02.002.

[53] St-Hilaire, A., Steiger, H., Liu, A., Laplante, D.P., Thaler, L., Magill, T., et al., 2015. A prospective study of effects of prenatal maternal stress on later eating-disorder manifestations in affected offspring: preliminary indications based on the Project Ice Storm cohort. The International Journal of Eating Disorders 48(5):512—516. https://doi.org/10.1002/eat.22391.

[54] Rojo, L., Conesa, L., Bermudez, O., Livianos, L., 2006. Influence of stress in the onset of eating disorders: data from a two-stage epidemiologic controlled study. Psychosomatic Medicine 68(4):628—635. https://doi.org/10.1097/01.psy.0000227749.58726.41.

[55] Caslini, M., Bartoli, F., Crocamo, C., Dakanalis, A., Clerici, M., Carrà, G., 2016. Disentangling the association between child abuse and eating disorders. Psychosomatic Medicine 78(1):79—90. https://doi.org/10.1097/PSY.0000000000000233.

[56] Favaro, A., Tenconi, E., Santonastaso, P., 2010. The interaction between perinatal factors and childhood abuse in the risk of developing anorexia nervosa. Psychological Medicine 40(4):657—665. https://doi.org/10.1017/S0033291709990973.

[57] Boersma, G.J., Lee, R.S., Cordner, Z.A., Ewald, E.R., Purcell, R.H., Moghadam, A.A., et al., 2014. Prenatal stress decreases Bdnf expression and increases methylation of Bdnf exon IV in rats. Epigenetics Official Journal of the DNA Methylation Society 9(3):437—447. https://doi.org/10.4161/epi.27558.

[58] Panetta, P., Berry, A., Bellisario, V., Capoccia, S., Raggi, C., Luoni, A., et al., 2017. Long-term sex-dependent vulnerability to metabolic challenges in prenatally stressed rats. Frontiers in Behavioral Neuroscience 11:113. https://doi.org/10.3389/fnbeh.2017.00113.

[59] Spruce, B.A., McCulloch, A.J., Burd, J., Orskov, H., Heaton, A., Baylis, P.H., et al., 1985. The effect of vasopressin infusion on glucose metabolism in man. Clinical Endocrinology 22(4):463—468.

[60] Wideman, C.H., Murphy, H.M., 1993. Modulatory effects of vasopressin on glucose and protein metabolism during food-restriction stress. Peptides 14(2):259—261.

[61] Tsunematsu, T., Fu, L.-Y., Yamanaka, A., Ichiki, K., Tanoue, A., Sakurai, T., et al., 2008. Vasopressin increases locomotion through a V1a receptor in orexin/hypocretin neurons: implications for water homeostasis. Journal of Neuroscience 28(1):228—238. https://doi.org/10.1523/JNEUROSCI.3490-07.2008.

 © 2018 The Authors. Published by Elsevier GmbH. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

[62] Wu, Z., Xu, Y., Zhu, Y., Sutton, A.K., Zhao, R., Lowell, B.B., et al., 2012. An obligate role of oxytocin neurons in diet induced energy expenditure. PLoS One 7(9):e45167. https://doi.org/10.1371/journal.pone.0045167.

[63] Vrang, N., Meyre, D., Froguel, P., Jelsing, J., Tang-Christensen, M., Vatin, V., et al., 2010. The imprinted gene neuronatin is regulated by metabolic status and associated with obesity. Obesity (Silver Spring, Md) 18(7):1289–1296. https://doi.org/10.1038/oby.2009.361.

[64] Ka, H.I., Han, S., Jeong, A.L., Lee, S., Yong, H.J., Boldbaatar, A., et al., 2017. Neuronatin is associated with an anti-inflammatory role in the white adipose tissue. Journal of Microbiology and Biotechnology 27(6):1180–1188. https://doi.org/10.4014/jmb.1702.02049.

[65] Lee, I.T., Chang, A.S., Manandhar, M., Shan, Y., Fan, J., Izumo, M., et al., 2015. Neuromedin S-Producing neurons act as essential pacemakers in the suprachiasmatic nucleus to couple clock neurons and dictate circadian rhythms. Neuron 85(5):1086–1102. https://doi.org/10.1016/j.neuron.2015.02.006.

[66] Miyazato, M., Mori, K., Ida, T., Kojima, M., Murakami, N., Kangawa, K., 2008. Identification and functional analysis of a novel ligand for G protein-coupled receptor, Neuromedin S. Regulatory Peptides 145(1–3):37–41. https://doi.org/10.1016/j.regpep.2007.08.013.

[67] Brondani, L.A., Duarte, G.C.K., Canani, L.H., Crispim, D., 2014. The presence of at least three alleles of the *ADRB3* Trp64Arg (C/T) and *UCP1* −3826A/G polymorphisms is associated with protection to overweight/obesity and with higher high-density lipoprotein cholesterol levels in caucasian-brazilian patients with type 2 diabetes. Metabolic Syndrome and Related Disorders 12(1):16–24. https://doi.org/10.1089/met.2013.0077.

[68] Corella, D., Guillén, M., Portolés, O., Sorlí, J.V., Alonso, V., Folch, J., et al., 2001. Gender specific associations of the Trp64Arg mutation in the beta3-adrenergic receptor gene with obesity-related phenotypes in a Mediterranean population: interaction with a common lipoprotein lipase gene variation. Journal of Internal Medicine 250(4):348–360.

[69] Raum, W.J., Swerdloff, R.S., 1981. The role of hypothalamic adrenergic receptors in preventing testosterone-induced androgenization in the female rat brain. Endocrinology 109(1):273–278. https://doi.org/10.1210/endo-109-1-273.

[70] Bánszegi, O., Altbäcker, V., Dúcs, A., Bilkó, A., 2010. Testosterone treatment of pregnant rabbits affects sexual development of their daughters. Physiology and Behavior 101(4):422–427. https://doi.org/10.1016/j.physbeh.2010.07.020.

[71] Vicennati, V., Ceroni, L., Gagliardi, L., Gambineri, A., Pasquali, R., 2002. Response of the hypothalamic-pituitary-adrenocortical Axis to high-protein/fat and high-carbohydrate meals in women with different obesity phenotypes. The Journal of Clinical Endocrinology and Metabolism 87(8):3984–3988. https://doi.org/10.1210/jcem.87.8.8718.

[72] Sedlmeier, E.-M., Brunner, S., Much, D., Pagel, P., Ulbrich, S.E., Meyer, H.H., et al., 2014. Human placental transcriptome shows sexually dimorphic gene expression and responsiveness to maternal dietary n-3 long-chain polyunsaturated fatty acid intervention during pregnancy. BMC Genomics 15:941. https://doi.org/10.1186/1471-2164-15-941.

[73] Mao, J., Zhang, X., Sieli, P.T., Falduto, M.T., Torres, K.E., Rosenfeld, C.S., 2010. Contrasting effects of different maternal diets on sexually dimorphic gene expression in the murine placenta. Proceedings of the National Academy of Sciences of the United States of America 107(12):5557–5562. https://doi.org/10.1073/pnas.1000440107.

[74] Gabory, A., Ferry, L., Fajardy, I., Jouneau, L., Gothié, J.-D., Vigé, A., et al., 2012. Maternal diets trigger sex-specific divergent trajectories of gene expression and epigenetic systems in mouse placenta. PLoS One 7(11):e47986. https://doi.org/10.1371/journal.pone.0047986.

[75] Gallou-Kabani, C., Gabory, A., Tost, J., Karimi, M., Mayeur, S., Lesage, J., et al., 2010. Sex- and diet-specific changes of imprinted gene expression and DNA methylation in mouse placenta under a high-fat diet. PLoS One 5(12):e14398. https://doi.org/10.1371/journal.pone.0014398.

[76] Bloise, E., Ortiga-Carvalho, T.M., Reis, F.M., Lye, S.J., Gibb, W., Matthews, S.G., 2015. ATP-binding cassette transporters in reproduction: a new frontier. Human Reproduction Update 22(2):dmv049. https://doi.org/10.1093/humupd/dmv049.

[77] Kim, S.-C., Lee, J.-E., Kang, S.S., Yang, H.-S., Kim, S.S., An, B.-S., 2017. The regulation of oxytocin and oxytocin receptor in human placenta according to gestational age. Journal of Molecular Endocrinology 59(3):235–243. https://doi.org/10.1530/JME-16-0223.

[78] Waddell, B.J., Wharfe, M.D., Crew, R.C., Mark, P.J., 2012. A rhythmic placenta? Circadian variation, clock genes and placental function. Placenta 33(7):533–539. https://doi.org/10.1016/j.placenta.2012.03.008.

[79] Mark, P.J., Crew, R.C., Wharfe, M.D., Waddell, B.J., 2017. Rhythmic Three-Part Harmony: the complex interaction of maternal, placental and fetal circadian systems. Journal of Biological Rhythms 32(6):534–549. https://doi.org/10.1177/0748730417728671.

[80] Fowden, A.L., Forhead, A.J., Sferruzzi-Perri, A.N., Burton, G.J., Vaughan, O.R., 2015. Review: endocrine regulation of placental phenotype. Placenta 36:S50–S59. https://doi.org/10.1016/j.placenta.2014.11.018.

[81] Glover, V., Hill, J., 2012. Sex differences in the programming effects of prenatal stress on psychopathology and stress responses: an evolutionary perspective. Physiology and Behavior 106(5):736–740. https://doi.org/10.1016/j.physbeh.2012.02.011.

[82] Davis, E.P., Glynn, L.M., Waffarn, F., Sandman, C.A., 2011. Prenatal maternal stress programs infant stress regulation. Journal of Child Psychology and Psychiatry and Allied Disciplines 52(2):119–129. https://doi.org/10.1111/j.1469-7610.2010.02314.x.

[83] Schroeder, M., Zagoory-Sharon, O., Lavi-Avnon, Y., Moran, T.H., Weller, A., 2006. Weight gain and maternal behavior in CCK1 deficient rats. Physiology and Behavior 89(3):402–409. https://doi.org/10.1016/j.physbeh.2006.07.008.

[84] Petropoulos, S., Gibb, W., Matthews, S.G., 2011. Breast cancer-resistance protein (BCRP1) in the fetal mouse brain: development and glucocorticoid regulation. Biology of Reproduction 84(4):783–789. https://doi.org/10.1095/biolreprod.110.088468.

[85] Martin, M., 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet Journal 17(1):10. https://doi.org/10.14806/ej.17.1.200.

[86] Bray, N.L., Pimentel, H., Melsted, P., Pachter, L., 2016. Near-optimal probabilistic RNA-seq quantification. Nature Biotechnology 34(5):525–527. https://doi.org/10.1038/nbt.3519.

[87] Love, M.I., Huber, W., Anders, S., 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biology 15(12):550. https://doi.org/10.1186/s13059-014-0550-8.

[88] Sergushichev, A., 2016. An algorithm for fast preranked gene set enrichment analysis using cumulative statistic calculation. bioRxiv, 060012. https://doi.org/10.1101/060012.

[89] Kanehisa, M., Goto, S., 2000. KEGG: kyoto encyclopedia of genes and genomes. Nucleic Acids Research 28(1):27–30.

[90] Robinson, M.D., McCarthy, D.J., Smyth, G.K., 2010. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics (Oxford, England) 26(1):139–140. https://doi.org/10.1093/bioinformatics/btp616.

[91] Luo, W., Friedman, M.S., Shedden, K., Hankenson, K.D., Woolf, P.J., 2009. GAGE: generally applicable gene set enrichment for pathway analysis. BMC Bioinformatics 10(1):161. https://doi.org/10.1186/1471-2105-10-161.

 © 2018 The Authors. Published by Elsevier GmbH. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).