The effect of diet control on puberty onset and immunoreactivity of kisspeptin and neurokinin B in female rats

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Abstract. Early onset puberty and irregular estrous cycles occur more frequently in rats which are fed a high-fat diet. Kisspeptin is an essential factor for the regulation of sexual maturation and is co-expressed with neurokinin B in neurons in the hypothalamic arcuate nucleus. However, the effects of a diet change on kisspeptin neuronal signaling are not well-understood. Therefore, in this study, we examined the immunoreactivity pattern of the kisspeptin/kiss1-receptor (KISS1R) and neurokinin B/neurokinin3-receptor (R). Pups born to high-fat diet rats were exposed to a high-fat diet until the onset of puberty. From puberty, the offspring originally exposed to a high-fat diet were fed a normal diet up to 85 postnatal days (PND 85). We examined kisspeptin/Kiss1-receptor and neurokinin B/neurokinin3-receptor immunoreactivity (IR) in the arcuate nucleus of the pups. The onset of puberty in the high-fat group was significantly earlier than the control group. At the onset of puberty, the densities of kisspeptin and neurokinin B IR cells were significantly higher in the high-fat diet group than in the control group; however, the densities of KISS1 and neurokinin 3-receptor IR cells did not differ between the two groups. At PND 85, the density of kisspeptin and neurokinin B IR cells did not differ between control and high fat group. The density of densities of KISS1 and neurokinin 3-receptor IR cells also did not differ between groups at this stage. These data suggest that a high-fat diet can influence puberty onset and the immunoreactivity of kisspeptin and neurokinin B. These effects can be modified by dietary control.

Key words: Diet, High-fat, Kisspeptin, Neurokinin B

WITH CHANGING DIETS, lifestyles and environmental conditions, obesity rates have risen. The prevalence of obesity has been increasing in Korea, along with concerns regarding the prevention of obesity [1]. Obesity can be caused by a variety of factors, including family history and eating habits of children. Being overweight as a child and childhood obesity is linked to obesity in adulthood. Thirty percent of obese adults have a history of being overweight in childhood [2]. Maternal health and nutrition are important factors that can influence the weight of the offspring [3].

Maternal nutrition can affect the onset of puberty in the offspring. Children whose mothers experienced undernutrition during the perinatal period have been observed to have delayed onset of puberty [4]. On the other hand, children whose mothers consumed a high fat diet whilst pregnant showed earlier onset of puberty than children whose mothers consumed a normal diet whilst pregnant [5]. A high-fat maternal diet during lactation has also been seen to lead to an earlier onset of puberty in offspring [6].

Kisspeptin is a neuropeptide derived from the Kiss1 gene. It is an important factor in the modulation of puberty, maturation, and reproduction. Kisspeptin stimulates GnRH and regulates the hypothalamic-pituitary-gonadal axis [7, 8]. In a previous study,
Kisspeptin-immunoreactive cells were observed mainly in the arcuate nucleus (ARC) [9]. Some studies have reported that kisspeptin positive neurons in the hypothalamic arcuate nucleus co-expressed neurokinin B [10, 11]. Neurokinin B expressed in kisspeptin neurons was involved in the process of inducing a rhythmic expression of kisspeptin and the release of GnRH at the median eminence. [12].

Kisspeptin is an essential factor in the regulation of sexual maturation and its expression can be influenced by nutrition [13]. Qunnell et al. showed that mice with luteinizing hormone (LH) deficiency exhibited reduced hypothalamic kisspeptin expression [14]. One study observed increased kiss1 mRNA expression in the offspring of mothers that received a high-fat diet during the lactation period [6]. However, the effects of a diet change on kisspeptin neuronal signaling are not well-understood. Therefore, in this study, we examined the immunoreactivity pattern of the kisspeptin/kiss1-receptor (KISS1R) and neurokinin B/neurokinin3-receptor (R), following diet control.

Materials and Methods

Animals and diets

Animal experiments were performed by the Chosun University Institutional Animal Care and Use Committee (CIACUC2015-A0021). Sprague-Dawley rats were purchased from a certified breeder (Damul Laboratory Animals, Korea). Male and female rats were raised together for three days. Female rats were separated after mating and pregnancy was confirmed on observation of a vaginal plug. After pregnancy was confirmed, rats were divided into two groups based on diet. In the control group, maternal rats \( n = 3 \) were fed a normal diet until parturition. In the other group, defined as the high-fat group, maternal rats \( n = 5 \) were fed a high-fat diet (60% kcal fat, Samtako bio Korea, KOREA) until parturition. Offspring of high-fat diet rats \( n = 22 \) were exposed to a high-fat diet until the onset of puberty. From puberty, the offspring that were originally exposed to a high–fat diet were fed a normal diet up to 85 postnatal days. Offspring of the rats in the normal-diet group \( n = 11 \) were fed a normal diet after birth to 85 postnatal days. Puberty onset was assessed by vaginal opening. From 21 postnatal days (PND), vaginal opening (VO) was observed daily. The body weight of the rats was measured from birth day to 85 postnatal days, including the vaginal opening day.

Tissue preparation and immunohistochemistry

At PND 24–45 and PND 85, transcardial perfusions were performed with 0.9% saline and 4% paraformaldehyde (PFA) solution. Offspring were anaesthetized using Zoletil (10 mg/kg; Vibrac, France) and xylazine (0.15 mg/kg; Bayer, Germany) via intramuscular injection. The brains were removed and fixed for 24 h in 4% PFA. The brains were embedded in paraffin following a gradient ethanol series. Serial coronal sections were obtained (thickness = 12 μm) and mounted to gelatin-coated slides (Fisher Scientific, USA). The sections were then deparaffinized and rehydrated. Antigen retrieval was performed with 0.01 M sodium citrate buffer (pH 6.0) and endogenous peroxidase was blocked with 0.3% hydrogen peroxide solution. Samples were then washed with 0.1 M phosphate buffered saline (PBS; pH 7.4). The sections (8 sections from each rat) were incubated overnight at 4°C with the following primary antibodies: rabbit anti-Kisspeptin (1:50; Bios USA), goat anti-Kiss1 (1:50; Santacruz USA), rabbit anti-KISS1R (1:25; antibodies-online Germany), goat anti-Neurokinin B (1:50 Santa-cruz USA), and rabbit anti-Neurokinin 3R (1:50; Abcam England). On the following day, the sections were incubated with biotinylated secondary antibodies (Vector Laboratories, USA) and counterstained using thionin. Images were captured using a light microscope (Olympus BX41, USA) connected to a digital camera. The density of the immunoreactive cells was analyzed using Image-Pro plus software version 7.0 (Media Cybernetics, USA).

Statistical analysis

All experimental data was analyzed using the Statistical Package for Social Sciences (Information Analysis System, USA). The group data was compared using Student’s \( t \) test and presented as mean ± standard error of the mean (SEM). Statistical significance was considered to be \( p < 0.005 \).

Results

On the vaginal opening day, the mean body weight was significantly higher in the offspring of the high-fat diet group than of the control group. By adulthood (PND
85), the mean body weight of rats originally fed the high-fat diet was similar to that of the control group (Fig. 1). The age of VO in high-fat diet rats was 32.09 ± 5.245 days which was significantly earlier than the age of VO in normal diet rats (39.70 ± 3.093) (Fig. 1).

At adolescence (PND 28–45), the mean body weight of the high-fat diet group (114.36 ± 17.267 g) was significantly increased compared to that of the control group (80.20 ± 7.525 g). By adulthood (PND 85), the mean body weight of rats originally fed the high-fat diet was similar to that of the control group (Fig. 2).

At the onset of puberty, the density of Kisspeptin-IR cells was significantly higher in the high-fat group than in the control group (Fig. 3). However, the densities of KISS1-receptor IR cells did not differ between the two groups (Fig. 3). Simultaneously, the density of neurokininB-IR cells was significantly higher in the high-fat group than in the control group (Fig. 4) and there was no difference in the density of neurokinin 3-receptor IR cells between groups (Fig. 4).

At PND 85, the density of kisspeptin-immunoreactive cells did not differ between groups (Fig. 5). The density of KISS1R-immunoreactive cells also did not differ between groups (Fig. 5). There was no difference in the density of neurokinin B and neurokinin 3R-immunoreactive cells between groups at this stage (Fig. 6).

**Discussion**

In this study, we observed a gain in body weight at the time of puberty onset in rats born to dams fed a high-fat diet during pregnancy, which were then supplied a high-fat diet until puberty. After the onset of puberty, the diet of the high-fat group was altered to a normal diet. At adulthood (PND 85), the mean body weight of the rats originally fed a high-fat diet was similar to the control animals. The onset of puberty in the high-fat group was
Fig. 2  Effects of a high-fat diet on body weight in female rats. At PND 28–45, the mean body weight of the high-fat diet group (114.36 ± 17.267 g) was significantly increased compared to the control group (80.20 ± 7.525 g). At PND 85, the mean body weight of rats originally fed a high-fat diet was similar to that of controls. The data are expressed as mean ± standard error of the mean (SEM, high: black, con: green).

Fig. 3  Kisspeptin (A, B) and KISS1R (C, D) immunoreactivity in the hypothalamus of female rats at PND 30–45. Density of kisspeptin (E) and KISS1R (F)-immunoreactive cells in the control and high-fat diet groups at PND 30–45 (brown colored, arrowed). The data are expressed as mean ± standard error of the mean (SEM). ★, p < 0.005.
significantly earlier than the control group. Nutrition is a critical factor in the onset of puberty [15]. Food restriction in female rats has been shown to lead to delayed puberty [16]. Overnutrition, due to a high-fat diet, can induce early puberty [17]. These studies have indicated that there is a correlation between puberty onset and a high-fat diet.

In our study, at the time of puberty onset, the density of kisspeptin-immunoreactive a cell in the ARC of the high-fat group was higher than that of the control group. The onset of puberty was also earlier. Kisspeptin is an important modulator in the activation of the hypothalamus-pituitary-gonadal axis [18]. Kisspeptin treatment has been shown to induce early onset of puberty in rats [19] and ovulation in seasonally acyclic ewes [20]. Kisspeptin expression is linked to nutritional condition. A prepubertal negative energy balance can result in loss of or delayed onset of puberty [13]. Neonatal undernutrition can also lead to delayed puberty [21]. On the other hand, postnatal overfeeding has been linked to early onset of puberty and an increase in kisspeptin–1R cells [22]. A study observed that during lactation, a maternal diet high in fat led to induction of Kiss1 expression in the ARC of her offspring. A previous study has shown that diet-induced obesity reduced kisspeptin expression [14]. It was also observed that a high-fat diet caused metabolic imbalance, which is related to leptin deficiency in mice. However, some studies showed an increase in leptin and LH pulse frequency in female rats [23, 24]. Interestingly, another study reported that overfeeding with a high-fat diet advanced puberty onset but had no effect on Kiss1 expression [25]. Maria E.K. Lie demonstrated that the absence of an effect on Kiss1 expression was due to the fact that Kiss1 mRNA levels were evaluated on the day of vaginal opening and not on a specific postnatal day [24]. It is possible that the dura-

Fig. 4  Neurokinin B (A, B) and Neurokinin 3R (C, D) immunoreactivity in the hypothalamus of female rats at PND 30–45. Density of Neurokinin B (E) and Neurokinin 3R (F) immunoreactive cells in control and high-fat groups at PND 30–45 (brown colored, arrowed). The data are expressed as mean ± standard error of the mean (SEM). ★, p < 0.005.
tion of high fat diet and variety of species makes some differences about metabolic balance mechanism. At PND 85, the body weight of rats originally fed a high-fat did not differ from that of control animals. At this stage, the density of kisspeptin immunoreactive cells in the hypothalamic arcuate nucleus did not differ between groups. Kisspeptin is a key regulator of reproductive function both at puberty and in adulthood [26, 27]. Here, we suggest that a change in diet is related to kisspeptin immunoreactivity in the ARC.

At the time of puberty onset and adulthood, the density of neurokinin B, co-expression with kisspeptin as mentioned, immunoreactive-cells was the same aspect as the density of kisspeptin. Neurokinin B is a peptide formed from preprotachykinin B in the hypothalamus and is chiefly expressed in the ARC [28, 29]. It is co-localized with kisspeptin and plays a similar function to kisspeptin for example, in its modulation of GnRH secretion [30]. In addition, the expression of neurokinin B is modulated by estradiol (E2) via the estrogen receptor-α [31]. This evidence suggests that neurokinin B is a key regulatory factor for reproduction. The neurokinin-3 receptor (tachykinin receptor 3) binds neurokinin B [32]. We observed no differences in the density of the KISS1R and neurokinin 3R-immunoreactive cells between groups. Some studies have shown similar results to our study in this aspect [33-35]. However, others have been in disagreement [36, 37]. Although it is difficult to understand these diverse findings, several explanations for the contradictory results are possible. Xiao et al. suggest that different experimental methods, including species differences, could lead to contradictory results [23]. NK3R immunoreactivity has been observed in approximately 16% of GnRH-positive neurons in the rat [38]. Other neurotransmitters have also been suggested to regulate reproductive function.

**Fig. 5** Kisspeptin (A, B) and KISS1R (C, D) immunoreactivity (in the hypothalamus of female rats at PND 85. Density of kisspeptin (E) and KISS1R (F) immunoreactive cells in control and high-fat animals at PND 85 (brown colored, arrowed). The data are expressed as mean ± standard error of the mean (SEM). ★, p < 0.005.
In this study, we showed that a high fat diet led to an early onset of puberty and a change in the immunoreactivity of kisspeptin and neurokinin B, but not in KISS1R and neurokinin3R. These data suggest that a high-fat diet can affect the onset of puberty as well as the immunoreactivity of kisspeptin and neurokinin B. These effects can be modulated by dietary control.

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Disclosure Statement

The authors have nothing to disclose.

References


