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Effects of intrauterine undernutrition on hypothalamic *Kiss1* expression and the timing of puberty in female rats

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Recent studies have suggested that intrauterine undernutrition is closely associated with the pathogenesis of diseases after birth. Perinatal undernutrition is known to disturb the development of reproductive function and delay the onset of puberty in some species. Using a rat model, we determined the effects of prenatal undernutrition on the development of the hypothalamic kisspeptin system and evaluated whether the alteration of the kisspeptin system contributes to the delayed onset of puberty induced by prenatal undernutrition. We also evaluated the effects of prenatal undernutrition on the developmental changes in serum leptin levels because leptin was a putative positive regulator of the hypothalamic kisspeptin system. We compared the timing of vaginal opening (VO) and the developmental changes in body weight, hypothalamic *Kiss1* mRNA levels, and serum leptin concentrations between offspring with prenatal undernutrition (UN offspring) and normal nutrition (NN offspring). After birth, the UN offspring showed rapid growth and had caught up to body weight of the NN offspring by postnatal day 12. After postnatal day 16, the UN offspring showed significantly lower *Kiss1* mRNA levels than the NN offspring, despite their significantly higher serum leptin levels (at days 20 and 28). The timing of VO in the UN offspring was delayed compared with that in the NN offspring, and chronic central injection of kisspeptin normalized the timing of VO in the UN offspring. These results suggest that decreased hypothalamic kisspeptin action contributes to the delayed onset of puberty in prenatally undernourished female rats. Increased leptin resistance in the kisspeptin system might be involved in these alterations.

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Abbreviations GnRH, gonadotropin-releasing hormone; HPG, hypothalamic–pituitary–gonadal; IUGR, intrauterine growth retardation; LH, luteinizing hormone; NN, normal nutrition; UN, undernutrition; VO, vaginal opening.

Introduction

Recently, epidemiological and experimental evidence has suggested that intrauterine undernutrition is closely associated with the pathogenesis of diseases after birth (Ravelli *et al.* 1976; Godfrey & Barker, 2000; Breier *et al.* 2001), giving rise to the concept of developmental origins of health and disease (Breier *et al.* 2001; Gluckman & Hanson, 2004). In humans, intrauterine growth retardation (IUGR) and low birth weight are associated with increased rates of insulin resistance, type 2 diabetes, hypertension, and ischaemic heart disease in adulthood (Barker *et al.* 1993*a*,*b*; Phillips *et al.* 1998*a*,*b*). Perinatal undernutrition is known to disturb the development of reproductive function in the offspring in rats and sheep. The onset of vaginal opening (VO) was delayed in female rats with intrauterine growth retardation (IUGR) induced by ligation of the maternal uterine arteries (Engelbregt *et al.* 2000), and testicular and ovarian growth were drastically retarded, and the onset of puberty was delayed in male and female rats where there had been maternal food restriction during the perinatal period (Leonhardt *et al.* 2003). Pubertal onset was delayed and reproductive ageing was accelerated in female rats housed under a protein restricted diet during the perinatal period (Guzman *et al.* 2006). In addition, the ovulation rate in adulthood was reduced in female sheep that experienced undernutrition during the prenatal period (Rae *et al.* 2002). Furthermore, birth weight was reduced, and pubertal onset was delayed in female rats

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subjected to glucocorticoid overexposure during the fetal period (Smith & Waddell, 2000), which was implicated in the association between restricted fetal growth and the programming of diseases after birth (Edwards *et al.* 1993; Phillips *et al.* 1998*a*,*b*; Lesage *et al.* 2001). In humans, it has been suggested that the risk of anovulation is increased in girls with IUGR and/or low birth weight and that ovarian development is disturbed in growth retarded fetuses (de Bruin *et al.* 1998; Ibanez *et al.* 1999). On the other hand, it has been reported that the onset of puberty in such individuals occurs at a similar time or slightly earlier than in the general population (Prece, 1997). Although some studies have suggested mechanistic hypotheses to explain the relationship between perinatal undernutrition and retardation of reproductive function development using animal models (Deligeorgis *et al.* 1996; Rae *et al.* 2002; Leonhardt *et al.* 2003; Guzman *et al.* 2006), no studies have demonstrated prenatal undernutrition-induced alterations in hypothalamic reproductive function.

Kisspeptin, a *Kiss1* gene product (Kotani *et al.* 2001; Ohtaki *et al.* 2001), stimulates gonadotropin releasing hormone (GnRH) release in rats and mice (Irwig *et al.* 2004; Matsui *et al.* 2004). Kisspeptin is a key factor in pubertal onset in humans, mice, rats and monkeys (Funes *et al.* 2003; de Roux *et al.* 2003; Seminara *et al.* 2003; Navarro *et al.* 2004*a*; Shahab *et al.* 2005). Leptin, an adipose tissue-derived hormone, is also a strong candidate as a link between nutritional status and the hypothalamic–pituitary–gonadal (HPG) axis with regard to puberty onset in female mice and rats (Ahima *et al.* 1997; Cheung *et al.* 1997; Cunningham *et al.* 1999; Zeinoaldini *et al.* 2006). Recently, several studies have suggested that leptin is a putative regulator of the hypothalamic kisspeptin system. Hypothalamic *Kiss1* mRNA levels were decreased in leptin deficient (*ob*/*ob*) mice and diabetic rats, and the decreased *Kiss1* mRNA levels in these animals were augmented by leptin replacement (Smith *et al.* 2006; Castellano *et al.* 2006). In addition, the *Kiss1* mRNA level in a hypothalamic cell line was increased by leptin infusion (Luque *et al.* 2007).

Leptin also acts in the hypothalamus to decrease food intake and stimulate energy expenditure (Gertler, 2006). The actions of leptin are decreased in diet-induced obese mice, and these alterations are called leptin resistance (Van Heek *et al.* 1997; El-Haschimi *et al.* 2000). As hypothalamic infertility is induced in mice with diet-induced obesity, it is assumed that leptin resistance leads to the suppression of reproductive function (Tortoriello *et al.* 2004).

The aim of this study was to determine the effects of prenatal undernutrition on the development of the hypothalamic *Kiss1* system and to evaluate whether alteration of the kisspeptin system contributes to the delayed onset of puberty in prenatally undernourished rats. Serum leptin concentrations were also measured because the leptin resistance might be involved in the alteration of the hypothalamic kisspeptin system.

Methods

Animals

Pregnant Sprague–Dawley rats were purchased (Charles River Japan, Inc., Tokyo, Japan) and housed individually. The animal rooms were maintained under controlled lighting (14 h light, 10 h dark cycle) and temperature (24°C). All animal experiments were conducted in accordance with the ethical standards of the institutional Animal Care and Use Committee of the University of Tokushima and complied with the journal policy and UK regulations on animal experiments (Drummond, 2009). The animals were humanely killed by decapitation at the end of the experiments. Surgical procedures were carried out under anaesthesia with intraperitoneal (I.P.) injection of pentobarbital sodium (40 mg (kg body weight)$^{-1}$).

In total, 16 pregnant rats and their offspring were used in this study. The pregnant rats were divided into two groups. In the normal nutrition (NN) group ($n = 8$), the dams were allowed water and standard rat chow *ad libitum* during the gestation and lactation periods. In the undernutrition (UN) group ($n = 8$), the dams received 50% of the daily food intake of the NN group from embryonic day 14 to delivery and then were allowed to feed *ad libitum* during the lactation period. Maternal body weight change in both the NN and UN groups and the food intake in 24 h in the NN group were measured during pregnancy. Litter size was examined 1 day after delivery. To control litter size to 10–12 per dam, pups were culled or moved to other dams and were fostered until weaning, depending on the total pup numbers in each experimental course. The pups were weighed at various postnatal ages and weaned at 21 days of age. After weaning, the pups were housed one per cage. Both male and female offspring were used in the experiment before weaning, and only female offspring were used in the experiments after weaning. To obviate any litter effects, the animals used for each experiment were randomly chosen from different litters.

Experiment 1. Developmental change in serum leptin concentrations and hypothalamic *Kiss1* mRNA levels

At various postnatal stages, several pups of the NN and UN offspring were killed by decapitation between 09.00 and 11.00 h, and trunk blood and the whole brain were obtained for the measurement of serum leptin concentration and hypothalamic *Kiss1* mRNA levels.

Experiment 2. Assessment of serum hormone concentrations, mRNA levels of hypothalamic factors, and the onset of puberty

During the peripubertal period (postnatal day 28 and 33), several pups from the NN and UN offspring were rapidly weighed and decapitated between 09.00 h and 11.00 h. Ovaries were dissected and weighed. Trunk blood and the whole brain were obtained for the measurement of serum hormone (luteinizing hormone (LH) and leptin) concentrations and the mRNA levels of hypothalamic factors (*Kiss1*, *Kiss1r* (kisspeptin receptor), and *GnRH*). Fifteen pups per group were used to assess the onset of puberty. From postnatal day 30 onward, the pups were inspected daily for vaginal opening (VO). The onset of puberty was defined as the time of VO.

Experiment 3. Effect of continuous intracerebroventricular injection of kisspeptin on the onset of VO

On postnatal day 27, eight pups per group were implanted with Alzet minipumps (model 2002, Durect, Cupertino, CA, USA), which delivered 12 μl day^{-1} for 14 days. These pumps were filled with kisspeptin-10 (Sigma, St Louis, MO, USA) and adjusted to deliver 7.5 nmol daily. In our previous study, intravenous injection of this peptide increased the serum LH concentration in female rats (Iwasa *et al.* 2008). In addition, a previous study from another group showed that continuous intracerebroventricular (I.C.V.) infusion of kisspeptin-10 (7.5 nmol day^{-1}) restored VO without desensitization in subnutrition rats (Roa *et al.* 2008). The pups were also fitted with a brain infusion cannula (Brain infusion kit 2, Durect, Cupertino, CA, USA) under anaesthesia with I.P. injection of pentobarbital sodium (40 mg (kg body weight)$^{-1}$) positioned according to stereotaxic coordinates into the lateral ventricle of the brain via a hole drilled in the skull 1.5 mm lateral and 1 mm posterior to bregma and 4.0 mm below the skull surface. The minipumps were implanted subcutaneously into the neck and connected to the lateral ventricle cannula. After brain cannulation, the pups were inspected daily for vaginal opening (VO). On postnatal day 35, the pups were rapidly weighed and decapitated. Trunk blood and the whole brain were obtained for the measurement of serum hormone (LH and leptin) concentrations and the *Kiss1*, *Kiss1r* and *GnRH* mRNA levels in the hypothalamus.

Hormone assays

The serum leptin concentration was measured using an ^{125}I-radioimmunoassay (RIA) kit (Rat leptin RIA kit, Linco Research Inc., MO, USA). The sensitivity of the assay was 0.5 ng ml^{-1}. The inter- and intra-assay coefficients of variation (CV) were 4.8% and 2.4%, respectively. Serum LH concentrations were measured using an ^{125}I-RIA kit (Rat LH [I-125] RIA Kit, Institute of Isotopes Co., Ltd. Tokyo, Japan). The sensitivity of the assay was 0.2 ng ml^{-1}. The inter- and intra-assay coefficients of variation (CV) were 6.6% and 6.5%, respectively.

Quantitative real time polymerase chain reaction

Hypothalamic explants, including of the median preoptic area, anteroventral periventricular nucleus, and arcuate nucleus were dissected out according to the following methods. A brain section was dissected out using coronal cuts 1 mm anterior from the optic chiasm and the posterior border of the mammillary bodies. The section was cut 2 mm from the bottom of the hypothalamus and then trimmed along the hypothalamic sulci. Total RNA was isolated from the hypothalamus using a TRIzol reagent kit (Invitrogen Co., Carlsbad, CA, USA) and an RNeasy Mini kit (Qiagen Gmgh, Hilden, Germany). cDNA was synthesized with oligo (deoxythymidine) primers at 50°C using the SuperScript III First-Standard Synthesis System for RT-PCR (Invitrogen Co., USA). Real-time PCR analysis was performed using the PCR System 7500 (PE Applied Biosystems, Foster City, CA, USA) with SYBR green. The selected forward and reverse primers were as follows: *Kiss1*: F: 5′-AGC TGC TGC TGC TTC TCC TCT GT-3′, R: 5′-AGG CTT GCT CTC TGC ATA CC-3′; *Kiss1r*, F: 5′-GCA GAC CGT CAC CAA TTT CT-3′, R: 5′-GGG AAC ACA GTC ACG TAC CA-3′; *GnRH*: F: 5′-GCA GAA CCC CAG AAC TTG GA-3′, R: 5′-TGC CCA GCT TCC TCT TCA AT-3′; and *β-actin*: F: 5′-TCA TGA AGT GTG ACG TTG ACA TCC GT-3′, R: 5′-CTT AGA AGC ATT TGC GGT GCA CG-3′. The PCR cycling conditions were as follows: initial denaturation and enzyme activation at 95°C for 10 min, followed by 45 cycles of denaturation at 95°C for 15 s; annealing at 63°C for 30 s (*Kiss-1*, *Kiss1r*), 58°C for 30 s (*GnRH*), and 65°C for 30 s (*β*-actin); and extension at 72°C for 1 min. The copy numbers of the transcripts were normalized against those of *β-actin* transcripts for each sample.

Statistical analysis

All data are presented as means ± S.D. Statistical analyses were performed using two-way analysis of variance (ANOVA) with Fisher's least significant difference test or Student's *t* test. Statistical significance was defined as $P < 0.05$.

Results

Experiment 1. Developmental change in serum leptin concentrations and hypothalamic *Kiss1* mRNA levels

There were no significant differences in body weight between the two maternal groups (NN group and UN group) on day 14. Compared with the NN offspring, the UN offspring showed significantly lower body weight gain during pregnancy (two-way ANOVA; $P < 0.001$, $F_{(3,96)} = 58.4$) (Fig. 1).

Compared with the NN offspring, the UN offspring showed a significant reduction in body weight by postnatal day 8. However, their body weight had caught up to that of the NN offspring by postnatal day 12 (Fig. 2). Compared with the NN offspring, the UN offspring showed significantly lower leptin concentrations on postnatal days 8 and 12. However, they showed significantly higher leptin concentrations than the NN offspring on postnatal day 20 (data not shown).

Both the NN and UN offspring showed increased hypothalamic *Kiss1* mRNA levels on postnatal day 12, 16, and 20. However, compared with the NN offspring, the UN offspring showed significantly lower *Kiss1* mRNA levels after postnatal day 16 (two-way ANOVA; $P < 0.001$, $F_{(4,00)} = 23.6$) (Fig. 3).

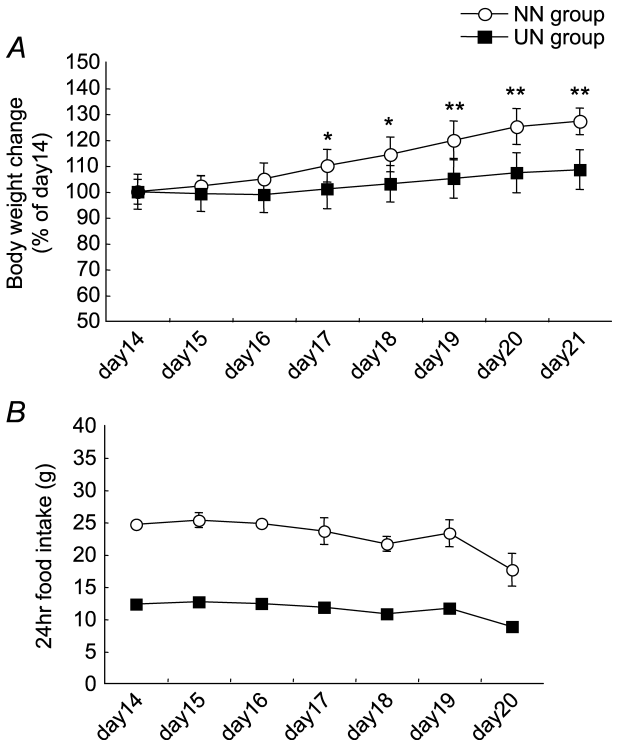

Figure 1. Maternal body weight change (*A*) and food intake in 24 h (*B*) during pregnancy in the normal nutrition (NN) and undernutrition (UN) groups ($n = 8$ per group)
In the UN group, the dams received 50% of the daily food intake of the NN group from day 14. There were no significant differences in body weight between these two groups on day 14. $^*P < 0.05$, $^{**}P < 0.01$ *vs.* UN group.

Experiment 2. Assessment of serum hormone concentrations, mRNA levels of hypothalamic factors, and the onset of puberty

The NN and UN offspring showed similar body weights during the peripubertal period (postnatal days 28 and 33) (Table 1). Compared with the NN offspring, the UN offspring showed significantly lower ovarian weight at postnatal day 28 and significantly lower serum LH concentrations and hypothalamic *GnRH* mRNA levels at postnatal day 33. In addition, the UN offspring showed significantly lower hypothalamic *Kiss1* mRNA levels on postnatal days 28 and 33. On the other hand, the UN offspring showed significantly higher serum leptin concentrations on postnatal day 28.

Compared with the NN offspring, the UN offspring showed a delayed onset of puberty (age at VO: 34.8 ± 0.3 day in the NN offspring *vs.* 36.9 ± 0.3 day in the UN offspring, $P < 0.001$) (Fig. 4), and the UN offspring had a significantly heavier body weight at the time of VO (body weight at VO: 100.4 ± 2.5 g in the NN offspring *vs.* 115.2 ± 2.8 g in the UN offspring, $P = 0.005$).

Experiment 3. Effects of continuous I.C.V. injection of kisspeptin on the onset of VO

To assess whether the delayed VO seen in the UN offspring was caused by decreased hypothalamic expression of *Kiss1* mRNA, continuous infusion of kisspeptin was conducted in peripubertal pups. When kisspeptin was injected continuously, the NN and UN offspring showed similar timing of puberty onset (age at VO: 33.0 ± 0.5 day in the NN offspring *vs.* 33.3 ± 0.4 day in the UN offspring, $P = 0.72$) and body weight at the time of VO (body weight

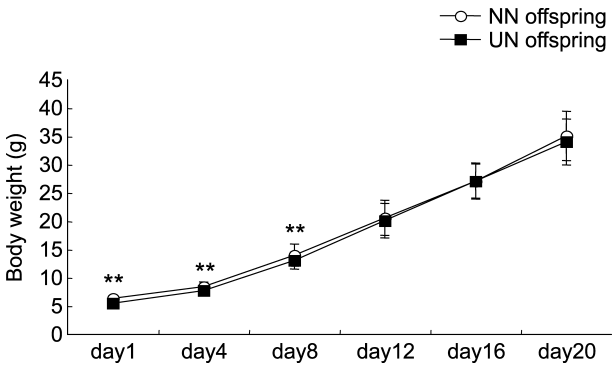

Figure 2. Body weight change during the neonatal development in normal nutrition (NN) offspring and undernutrition (UN) offspring ($n = 49$–94 per group per age)
Pups include both males and females. $^{**}P < 0.01$ *vs.* UN offspring.

Table 1. Body weight, ovarian weight, serum hormone and hypothalamic mRNA profiles of the NN and UN offspring

	Day 28		Day 33	
	NN offspring	UN offspring	NN offspring	UN offspring
Body weight (g)	70.7 ± 3.9	70.9 ± 3.1	96.2 ± 8.5	94.5 ± 3.5
Ovarian weight (mg)	37.9 ± 2.8*	32.5 ± 5.6	59.4 ± 19.7	52.7 ± 8.5
LH (ng ml^{-1})	1.58 ± 0.20	1.59 ± 0.19	1.99 ± 0.23**	1.64 ± 0.14
Leptin (ng ml^{-1})	0.84 ± 0.30*	1.75 ± 0.79	0.91 ± 0.32	1.27 ± 0.44
Kiss1 mRNA	1.27 ± 0.31**	0.88 ± 0.19	1.76 ± 0.23**	1.30 ± 0.25
Kiss1r mRNA	0.95 ± 0.11	0.87 ± 0.17	1.05 ± 0.18	1.00 ± 0.30
GnRH mRNA	1.21 ± 0.16	1.04 ± 0.20	1.28 ± 0.27*	0.98 ± 0.22

The relative expression levels of *Kiss1*, *Kiss1r* and *GnRH* mRNA were calculated by dividing by *β-actin* mRNA expression. NN offspring: normal nutrition offspring; UN offspring: undernutrition offspring. $^{**}P < 0.01$, $^{*}P < 0.05$ *vs.* UN offspring at same age.

at VO: 106.8 ± 5.3 g in the NN offspring *vs.* 105.5 ± 4.5 g in the UN offspring, $P = 0.86$) (Fig. 5).

At the end of kisspeptin infusion (postnatal day 35), the NN and UN offspring showed similar ovarian weight and similar serum LH and leptin concentrations. They also showed similar hypothalamic *GnRH* mRNA levels (Table 2).

Discussion

Several studies have demonstrated mechanistic hypotheses concerning the relationship between perinatal undernutrition and retarded development of reproductive function. It was suggested that the pituitary response to GnRH was decreased in prenatally undernourished lambs (Deligeorgis *et al.* 1996). In addition, a recent study suggested that folliculogenesis is altered by changes in the ovarian expression of gonadotropins and oestrogen receptors in postnatally undernourished rats (Faria *et al.* 2008). Although, the hypothalamus has an important role in the development of reproductive function and the onset of puberty in many species, to the best of our knowledge, there have been no studies focused on the alterations of hypothalamic reproductive functions in perinatally undernourished animals.

Kisspeptin preserves the hypothalamic–pituitary–gonadal axis and promotes the onset of puberty. In humans, mutations in the gene for the kisspeptin receptor, *Kiss1r*, were identified in patients with idiopathic hypogonadotropic hypogonadism (de Roux *et al.* 2003; Seminara *et al.* 2003). These patients did not show spontaneous pubertal development. In addition, VO was advanced by chronic central kisspeptin injection in normal prepubertal female rats (Navarro *et al.* 2004*b*). In addition, retardation of VO was restored by chronic central kisspeptin injection in undernourished female rats (Roa *et al.* 2008). The principal findings of the present study were that hypothalamic *Kiss1* mRNA levels were decreased in prenatally undernourished rats and that replacement of kisspeptin normalized the timing of VO. Our data demonstrated that the decreased function of the kisspeptin system induced by perinatal undernutrition retards the development of reproductive function and the onset of puberty. In humans, it has been reported that the risk of anovulation is increased in girls with IUGR and/or

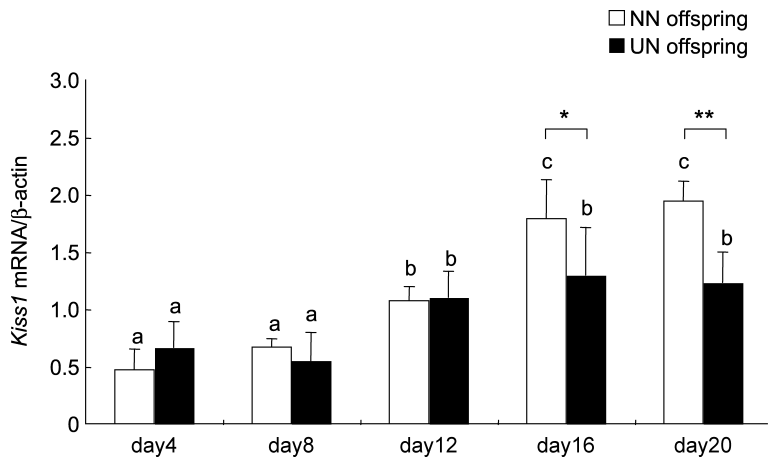

Figure 3. Hypothalamic *Kiss1* mRNA levels during neonatal development in normal nutrition (NN) offspring and undernutrition (UN) offspring (n = 6–10 per group per age)
Pups include both males and females. The relative expression levels of *Kiss-1* mRNA were calculated by dividing by *β*-actin mRNA expression. $^{*}P < 0.05$, $^{**}P < 0.01$ *vs.*each other. Different letters (a–c) show statistically significant differences ($P < 0.05$) within the same group.

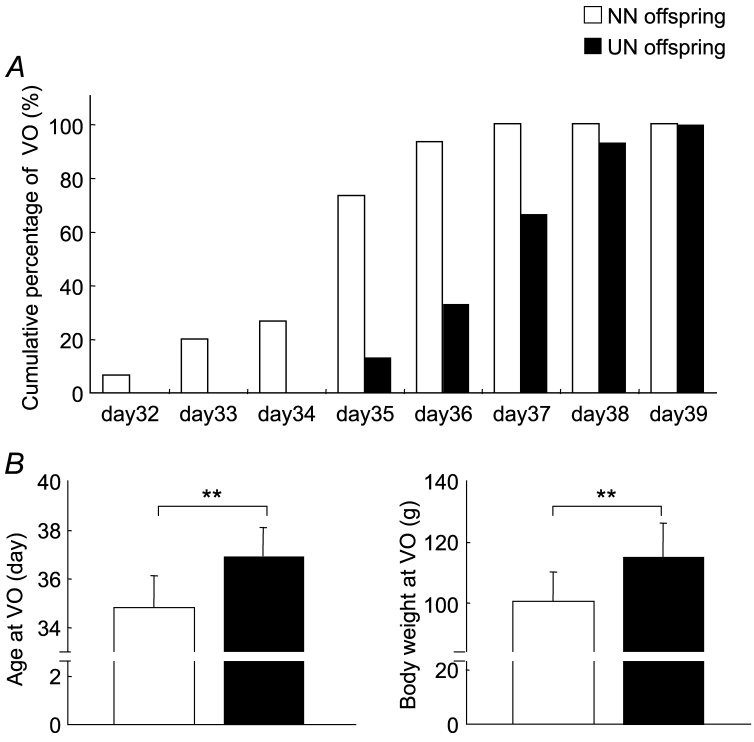

Figure 4. Cumulative percentage of vaginal opening (VO) (*A*) and mean age and body weight at VO (*B*) in normal nutrition (NN) offspring and undernutrition (UN) offspring ($n = 15$ per group)
$^{**}P < 0.01$ *vs*. each other.

low birth weight (Ibanez *et al.* 1999). It is possible that the function of the kisspeptin system is also decreased in humans with IUGR and/or low birth weight and that this alteration disrupts reproductive function. There have been only a few studies in humans on the effects of IUGR on reproductive function, and further studies are needed to clarify the mechanism behind the relationship between IUGR and reproductive function disturbance.

Some studies have suggested that ovarian development was disturbed in perinatally undernourished rats and in growth retarded fetuses (de Bruin *et al.* 1998; Faria *et al.* 2008). In the present study, ovarian weight was significantly lower in normal nutrition offspring than in undernourished offspring at postnatal day 28. We speculate that ovarian development was disturbed in the undernourished offspring in this study and that this

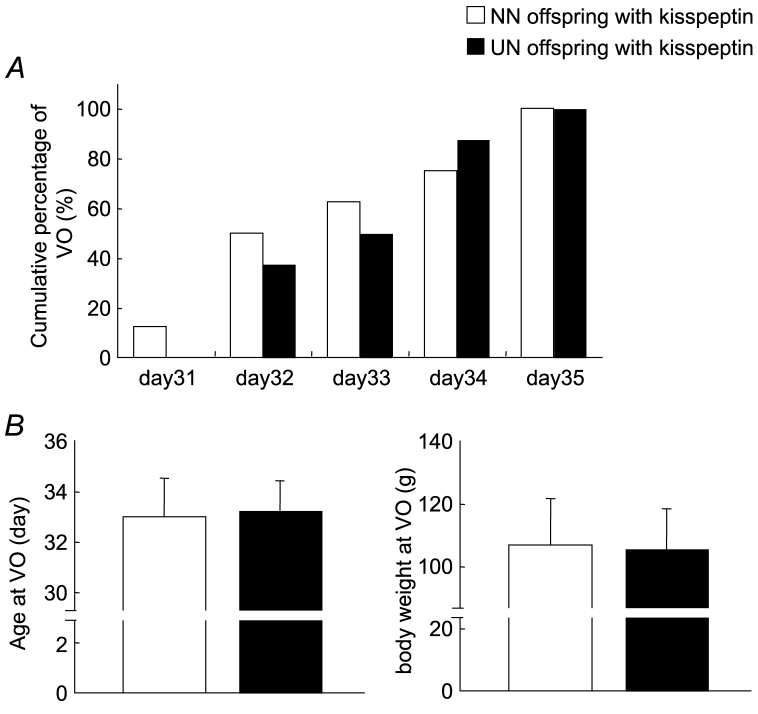

Figure 5. Cumulative percentage of VO (*A*) and mean age and body weight at VO (*B*) in normal nutrition (NN) offspring and undernutrition (UN) offspring under chronic I.C.V. kisspeptin delivery ($n = 8$ per group).

alteration was caused by a decrease in the function of the kisspeptin system, suggesting that chronic injection of kisspeptin would improve ovarian development and normalize the timing of VO in undernourished offspring. Further precise studies including comparisons of ovarian morphological findings at appropriate time points are required to verify this hypothesis.

It has been established that the hypothalamus plays an essential role in the pathology of the 'developmental origins of health and disease'. Undernutrition-induced hypothalamic programming occurs during the prenatal and early postnatal period, and this alteration leads to a long-lasting disturbance in the energy-controlling and appetite regulatory system (Yura *et al.* 2005; Breton *et al.* 2008; Delahaye *et al.* 2008). In particular, the arcuate nucleus (ARC), which plays a pivotal role in the maintenance of energy controlling systems, is highly susceptible to the perinatal nutritional condition. It has been reported that the gene expression of neuropeptide Y (NPY), an orexigenic factor, is increased, and that of proopiomelanocortin (POMC), an anorexigenic factors, is decreased in perinatally undernourished rats compared with normally nourished rats (Plagemann *et al.* 2000; Delahaye *et al.* 2008). As the serum concentration of leptin, which is the regulator of NPY and POMC, is not changed in perinatally undernourished rats, the altered expression of NPY and POMC are caused by the reduction of leptin action on the ARC. These alterations in the energy controlling systems are called leptin resistance and are even seen in the early developmental stage. Leptin resistance is also brought about in animals with diet-induced obesity. In these animals, not only the energy controlling systems but also reproductive functions are disturbed by leptin resistance. For example, hypothalamic infertility, caused by the suppression of the *GnRH* mRNA level occurs in high-fat diet induced obese mice (Tortoriello *et al.* 2004). ARC also plays an important role in the maintenance of reproductive function. Kisspeptin neuronal cell bodies mainly exist in the ARC and anteroventricular nucleus (AVPV) in mice and rats (Smith *et al.* 2005; Adachi *et al.* 2007). It has been established that the *Kiss1* mRNA expression in these two populations is regulated by various peripheral signals, e.g. oestrogen and testosterone, in a region-specific manner (Smith *et al.* 2005; Dungan *et al.* 2006; Adachi *et al.* 2007). Steiner and his colleagues reported that *Kiss1* mRNA expression is inhibited by oestrogen and testosterone in the ARC, whereas it is stimulated in the AVPV (Dungan *et al.* 2006). Therefore, it is possible that prenatal undernutrition may differentially affect *Kiss1* mRNA expression in these two populations. Recently, it has been demonstrated that *Kiss1* mRNA expression in the ARC is also regulated by leptin, a strong candidate as the link between nutritional status and the HPG axis with regard to puberty onset in female mice and rats (Ahima *et al.* 1997; Cheung *et al.* 1997; Cunningham *et al.* 1999; Zeinoaldini *et al.* 2006). Smith *et al.* (2006) showed that a large number of *Kiss1* mRNA expressing cells in the ARC expressed the functional leptin receptor, *ObRb*, and that *Kiss1* mRNA expression in the ARC was decreased in leptin deficient *ob*/*ob* mice. In the present study, we have demonstrated that undernourished offspring showed lower *Kiss1* mRNA levels despite their higher leptin levels compared with normally nourished offspring in the prepubertal period (days 20 (data not shown) and 28). The results of our study and those of others suggest that prenatal undernutrition increases leptin resistance in the hypothalamic kisspeptin system and that this alteration might leads to a decrease in *Kiss1* mRNA expression in the ARC. In the present study, the undernourished offspring showed rapid catch-up growth in the neonatal period. It has been suggested that prenatal undernutrition-induced IUGR rats that demonstrated rapid catch-up growth in the early developmental period showed leptin resistance and increases in body weight and percentage body fat (Desai *et al.* 2005). Therefore, rapid catch-up growth after birth might have accelerated the leptin resistance of the kisspeptin system in the undernourished offspring in this study. However, as whole hypothalami were used to evaluate *Kiss1* mRNA expression in this study, it could not be clarified whether the ARC or AVPV was most sensitive to prenatal undernutrition. Further studies focused on the relationship between perinatal undernutrition and region specific alterations (in the ARC, AVPV, etc.) in *Kiss1* mRNA expression are needed to confirm this hypothesis.

Guzman *et al.* (2006) showed that protein restriction during the perinatal period induced the premature ageing of reproductive function and decreased the fertility rates during adulthood in female rats. These results imply that protein restriction during the fetal and neonatal periods affects reproductive function throughout life. If the decreased function of the kisspeptin system continues after maturation in undernourished offspring, it might bring about infertility and/or premature ageing.

Table 2. Body weight, ovarian weight, serum hormone and hypothalamic mRNA profiles of NN and UN offspring administered kisspeptin

	NN offspring–kisspeptin (day 35)	UN offspring–kisspeptin (day 35)
Body weight (g)	119.5 ± 9.86	117.6 ± 7.4
Ovarian weight (mg)	62.6 ± 13.3	59.1 ± 12.5
LH (ng ml^{-1})	1.99 ± 0.44	1.63 ± 0.39
Leptin (ng ml^{-1})	1.11 ± 0.38	0.92 ± 0.28
GnRH mRNA	0.91 ± 0.27	0.81 ± 0.19

The relative expression levels *GnRH* mRNA were calculated by dividing by *β-actin* mRNA expression. NN offspring: normal nutrition offspring; UN offspring: undernutrition offspring.

In conclusion, these results demonstrate that the action of the hypothalamic kisspeptin system during the developmental period is suppressed in prenatally undernourished female rats. These alterations may contribute to the delayed onset of puberty in these animals because chronic injection of kisspeptin normalized the timing of puberty. Prenatal maternal undernutrition and rapid catch-up growth in the neonatal period might increase the leptin resistance of the kisspeptin system, which might decrease its function.

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Author contributions

T.I. and T.M. have contributed equally to this work. All authors discussed the results and commented on the manuscript. All authors have approved the final version for publication. Where the work was done: Department of Obstetrics and Gynecology, Institute of Health Biosciences, The University of Tokushima Graduate School.