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The Effects of Intra-Uterine Growth Retardation and Postnatal Undernutrition on Onset of Puberty in Male and Female Rats

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ABSTRACT

The nutritional status, prenatally and early postnatally, plays a critical role in postnatal growth and development. Early malnutrition may change the original programming of organs, especially those in developmental phases, which can result in long-term changes in metabolism. The association between a low birth weight and the increased risk on type 2 diabetes, hypertension and cardiovascular disease is well known. In the present study we investigated whether intrauterine malnutrition or direct postnatal food restriction affects the onset of puberty in male and female rats. Intrauterine growth retardation (IUGR) was induced by uterine artery ligation on day 17 of gestation and postnatal food restriction (FR) by litter-enlargement to 20 pups per mother from day 2 after birth until weaning (24 d). Both models of malnutrition resulted in a persistent growth failure postnatally. The parameter of the onset of puberty was balano-preputial-separation (BPS) in the male rat and vaginal opening (VO) in the female rat. In both male IUGR ($n = 26$) and FR ($n = 20$) rats, the age at BPS was significantly delayed, with 48.1 ± 1.9 d ($p < 0.0001$) and 50.4 ± 2.9 d ($p < 0.0001$), respectively, compared with controls ($n = 30$) with 45.8 ± 1.4 d. In female IUGR rats (n = 37) the age at VO was significantly delayed, with 37.4 ± 2.7 d ($p < 0.04$) compared with 36.1 ± 1.5 d in controls ($n = 23$), but not in female FR rats ($n = 18$) with 36.5 ± 2.2 d. Weight at onset of puberty did not differ between male IUGR and control rats, 194.5 ± 20.0 g and 201.7 ± 16.8 g, respectively, but was significantly lower in male FR rats with a weight of 175.6 ± 17.5 g ($p < 0.0001$). In female IUGR as well as in female FR rats, weight at onset of puberty was significantly lower compared with controls: weight in IUGR 106.1 ± 13.1 g ($p < 0.001$), weight in FR 85.3 ± 7.6 g ($p < 0.0001$) and weight in controls 116.9 ± 9.3 g. We conclude that early malnutrition, during late gestation or direct postnatally, results in a delayed onset of puberty in IUGR and FR male rats and in IUGR female rats, but not in FR female rats. The onset of puberty in these growth retarded rats as well as in controls does not depend on the achievement of a certain, crucial weight. The perinatal period appears to be a "critical time period" for the maturational process of pubertal development. **(*Pediatr Res* 48: 803–807, 2000)**

Abbreviations:
IUGR, intra, uterine growth retardation
FR, food restriction
VO, vaginal opening
BPS, balano preputial separation

In rapidly growing organisms malnutrition in early life is a serious challenge to which the system will try to adjust to survive. The quantity or quality of nutrition at these critical periods has permanent consequences for later life. One of the mechanisms to adapt to inadequate supply of nutrients is slowing down the rate of cell division in tissues and organs, which may lead to an altered "programming" of the structure and function of the system (1–3). In the human, malnutrition induced in early life is associated with an increased risk to develop type 2 diabetes, hypertension and cardiovascular disease at long term (1–6).

The prevalence of type 2 diabetes is much higher in adults with a low weight at birth (2, 7) Both insulin deficiency and insulin resistance are thought to be important in the pathogenesis of type 2 diabetes. With respect to the insulin deficiency we know that intrauterine growth failure as a result of malnutrition is associated with a decreased number of islet cells in the pancreas.

Low birth weight is also associated with an increased activity of the adrenal as is shown in adults, whereby persons with a low birth weight had higher cortisol levels (8, 9) Furthermore premature adrenarche more often occurs in girls with a low birthweight (10). Concerning growth and development, in gen-

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eral children will have a catch up growth postnatally, although in 30% to 40% growth will be compromised (11, 12). There are only a few studies on the effects of perinatal undernutrition on puberty and fertility. It seems that the onset of puberty in boys and girls born with a low birth weight starts at an age comparable to that in the general population or slightly earlier (13)

For a long time it is hypothesized that weight plays an important role in the central regulation of puberty. Frisch and Revelle suggested that in girls a critical weight is needed for menarche to occur (14). The question arises whether intrauterine or perinatal weight is also involved in the "programming" of the developmental process of puberty.

In rats experimentally induced IUGR leads to impaired growth in males and females and in altered glucose tolerance in females (15, 16). The relation between intra-uterine growth retardation and onset of puberty in rats has not been studied before, nor other possible mechanisms, like a critical body weight, either at birth and early postnatally or at onset of puberty. In female rats postweaning undernutrition leads to a delayed puberty (17). However, undernutrition before weaning showed various results, which may be explained by the use of different strains of rats as well as by different diets, especially with respect to protein concentrations. Overall, in the female rat undernourished before weaning, onset of puberty seems to be dependent on the severeness of the undernutrition (18). In addition, since puberty did not occur at a same body weight in control and malnourished rats, a critical body weight does not seem to be essential (18).

The aim of this study was to investigate whether perinatal malnutrition, either during late gestation or direct postnatally, modulates pubertal development in male and female rats, and to study the role of body weight in this process.

MATERIALS AND METHODS

Animals. Timed pregnant Wistar rats with an average weight of 200 g were obtained from Harlan CPB (Horst, The Netherlands) and housed under a constant light-dark cycle with lights on from 0600–1800 h and controlled temperature (21–23°C).

Intra-uterine growth retardation (IUGR) was induced by ligation of the uterine artery on day 17 of gestation according to the method of Wigglesworth (19). The pups were born spontaneously at day 21–22 and defined as IUGR, when their weight on day 2 after birth was below 5.3 g, corresponding with minus two standard deviations of the mean of the weight of control pups, born from SHAM-operated dams (mean weight 6.3 g).

Postnatal undernutrition was achieved by litter-enlargement to 20 pups per mother from day 2 after birth until weaning (24 d). During lactation and postweaning pups of all groups received a normal diet (20% protein). After weaning they were kept in group cages, two males or three females per cage, with *ad libitum* access to food and water. From day 30 onwards, the females were inspected for vaginal opening (VO) daily and from day 40 the males for balano-preputial separation (BPS). The onset of puberty was defined as the age (in days) at which VO or BPS occurred. For all experiments, approval was obtained from the Animal Welfare Committee (DEC) of the "Vrije Universiteit" at Amsterdam.

Statistical analysis. Data are expressed as mean ± SD. Statistical analyses were performed using the *t* test. Differences were considered statistically significant when $p \leq 0.05$.

RESULTS

Onset of puberty in male IUGR and FR rats. We obtained 30 control, 26 IUGR, and 20 FR male rats. Figure 1 shows the growth curves of the control, IUGR, and the FR group from day 2 after birth until BPS. Over the whole period weight in IUGR rats was significantly lower ($p < 0.0001$) compared with the controls, and from day 4 on in FR rats compared with the controls ($p < 0.0001$) and the IUGR rats ($p < 0.0001$).

Intrauterine malnutrition resulted in a significantly delayed time of BPS compared with controls (BPS in IUGR at day 48.1 ± 1.9 *versus* in controls at day 45.8 ± 1.4, $p < 0.0001$). Weight at BPS did not differ between the IUGR group and controls (weight 194.5 ± 20.0 g *versus* 201.7 ± 16.8 g, respectively).

In the FR rats BPS occurred at a significantly later age (BPS in FR at day 50.4 ± 2.9 *versus* in controls at day 45.8 ± 1.4, $p < 0.0001$). In the FR group weight at BPS (175.6 ± 17.5 g) was significantly lower compared with controls (201.7 ± 16.8 g, $p < 0.0001$), and even significantly lower compared with IUGRs (194.5 ± 20.0 g, $p < 0.002$).

Figure 2 shows the mean weights of the rats in the three groups from day 40 until BPS and the individual weights at time of BPS. There is a relation between body weight and age, but no "critical" weight at BPS in all three groups.

Onset of puberty in female IUGR and FR rats. We obtained 23 control, 37 IUGR and 18 FR female rats. The growth curves of the control, IUGR and the FR group from day 2 after birth until VO are shown in Figure 3. Just as in the male rats, over this period weight was significantly lower in IUGR rats ($p <$

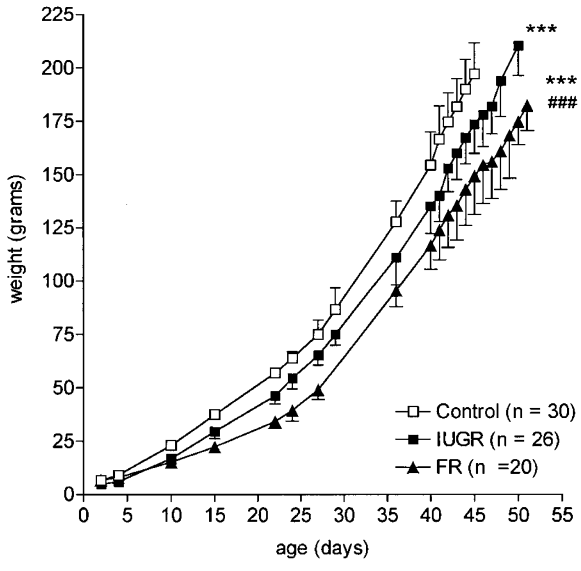

Figure 1. Weight curves of male rats from day 2 after birth until onset of puberty. Values are expressed as mean ± SD. *** IUGR and FR *vs* control, $p < 0.0001$, ###FR *vs* IUGR, $p < 0.0001$.

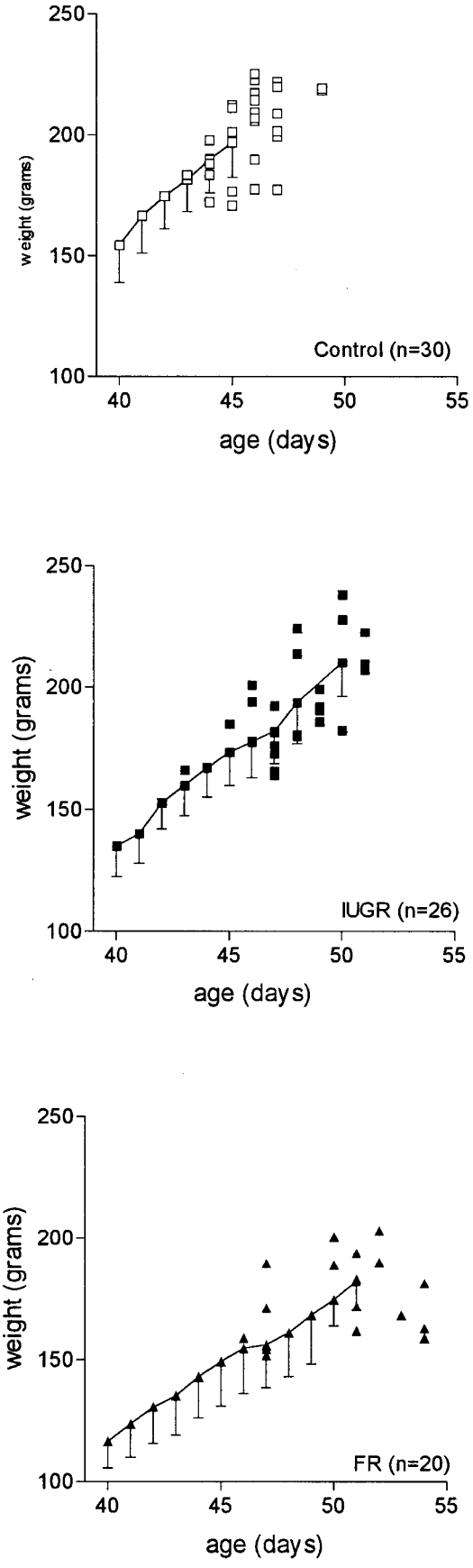

Figure 2. Mean weight from day 40 until BPS and individual weight at BPS of male control ($n = 30$), IUGR ($n = 26$) and FR rats ($n = 20$).

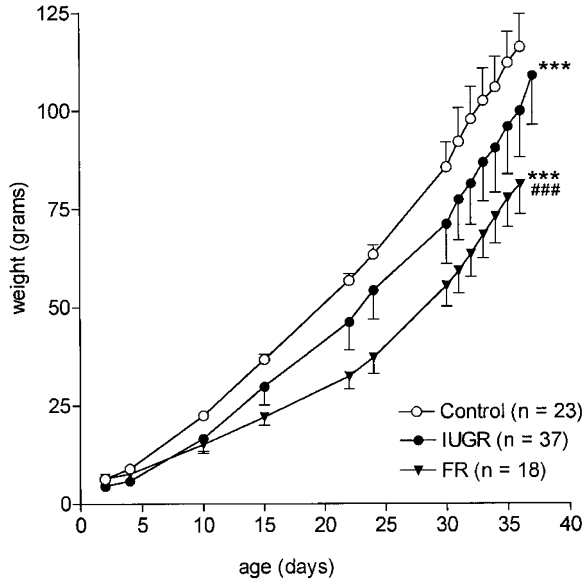

Figure 3. Weight curves of female rats from day 2 after birth until onset of puberty. Values are given as mean ± SD. *** IUGR and FR *vs* control, $p < 0.0001$, ### FR *vs* IUGR, $p < 0.0001$.

0.0001) compared with controls, and from day 4 on in FR rats compared with controls ($p < 0.0001$) and to IUGRs ($p < 0.0001$). In IUGR rats compared with controls onset of puberty was significantly delayed (VO in IUGR at day 37.4 ± 2.7 *versus* in controls at day 36.1 ± 1.5, $p < 0.04$). In the FR rats the age at VO was not significantly different from the control group (VO in FR at day 36.5 ± 2.2 *versus* in controls at day 36.1 ± 1.5). In both IUGR and FR rats weight at VO was significantly lower compared with controls (weight of IUGR 106.1 ± 13.1 g, $p < 0.001$, weight of FR 85.3 ± 7.6 g, $p < 0.0001$, weight of controls 116.9 ± 9.3 g). In FR rats weight was even significantly lower ($p < 0.0001$) compared with IUGR rats.

Figure 4 shows the mean weights of the rats in the control and the two growth retarded groups from day 30 until VO and the individual weights at time of onset of puberty. Just as in the male rats there is a relation between body weight and age, but no "critical" weight at onset of puberty.

DISCUSSION

For human females a hypothesis of a direct relation between a critical weight and menarche was proposed by Frisch and Revelle in 1971 (14). This hypothesis may explain the delaying effect of acute malnutrition on menarche and, *vice versa*, the trend to an earlier menarche by abundant food supplies in the last decades. This "critical weight" theory was rejected by Scott and Johnston (20), who suggested that maturational processes especially of the CNS in association with the neuroendocrine activity provided better explanations for the onset and maintenance of normal reproductive cycles in human females. Already in 1963 it was described that food intake and body weight could be initiating factors for puberty in the rat (21). Underfeeding after weaning resulted in growth retardation and a delayed vaginal opening in female rats. VO in these

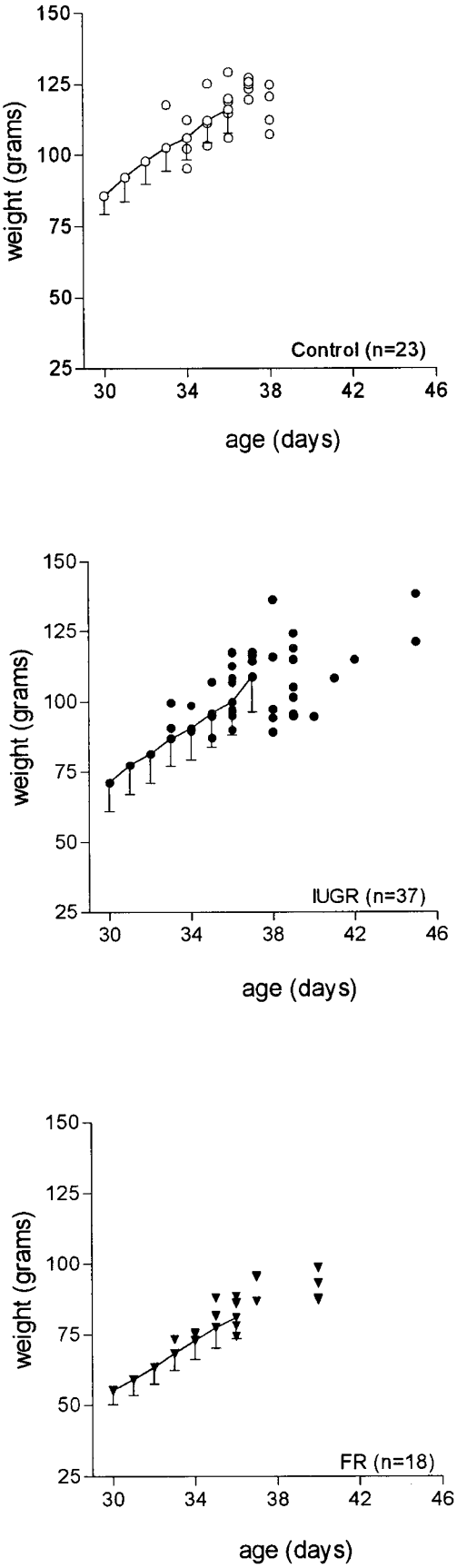

Figure 4. Mean weight from day 30 until VO and individual weight at VO of female control (n = 23), IUGR (n = 37) and FR rats (n = 18).

retarded rats occurred when a specific weight was obtained comparable with weight at VO in optimally grown rats. These observations were disputed by Glass and Swerdloff (18), who could not find a critical body weight at vaginal opening in the female rat. Most of these studies, in the human as well as in the rat, were focused on the effects of acute malnutrition or actual body weight and the onset of puberty.

Barker and Clark *et al.* described the association between early malnutrition and diseases in later life (2). Early malnutrition appears to induce a changed "programming" of development and function of different organs. Critical time windows play an essential role in this.

We addressed the question whether malnutrition during late gestation or during the perinatal period may induce a changed programming of the developmental process of puberty and whether these different time periods have their own characteristic consequences. We defined the clinical signs as balanopreputial separation and vaginal opening as parameter of the onset of puberty in the male and female rats, respectively.

In our study malnutrition during late gestation, which resulted in intrauterine growth retardation, led to a delayed onset of puberty in both male and female rats. Early postnatal food restriction showed different results in males and females. The postnatal food restricted male rats showed a more retarded balano-preputial separation than the intrauterine growth retarded rats with an even lower body weight. In the female rats, early postnatal undernutrition, in contrast to intrauterine malnutrition, resulted in a normal timing of the onset of puberty. Vaginal opening occurred at a similar age as seen in the control group, although weight at time of vaginal opening was significantly lower in the food restricted rats compared with controls. Weight in the FR group with a normal timing of VO was even lower than weight of the IUGR group with a delayed VO.

In both male rats and female rats BPS or VO occurred at body weights showing a large variation. These weights are more related with age than with age at onset of puberty. There is no evidence for a critical weight at the time of either BPS or VO. In case of a critical weight mechanism, we expect in a weight to age diagram, that weights at BPS or VO would vary around a horizontal line indicating the weight, essential to initiate BPS or VO.

In 1987 Frisch hypothesized (22, 23) that, more than weight, an appropriate body composition or a certain percentage of fat is needed to start puberty in rats. It might be of interest to examine the body composition and percentage of fat in these intrauterine growth retarded rats at the onset of puberty. It might be sensible that a low percentage of fat will delay an important event such as puberty and the start of the fertile period in life until the body is "ready" for it.

Regarding gonadal development, De Bruin *et al.* (24) observed a significantly lower percentage of primordial follicles in the ovaries of growth retarded human fetuses compared with age matched controls. This indicates that ovarian development is disturbed in case of intrauterine growth failure and that the ovaries undergo less growth in case of fetal malnutrition. This could imply an increased risk of fertility problems for females born with a low birth weight. Since the gonadotropin releasing hormone (GnRH) and gonadotropin axis is fully active during

the fetal period (25), the question arises whether this inappropriate ovarian development occurs under a normal central regulatory control. In addition to gonadal development, the central regulating factors of puberty are modulated by early food restriction as well. It is well known that the hypothalamic neuropeptide Y and the peripheral peptide leptin, both food dependent, play a role in the developmental process of the control of puberty. Early malnutrition may change the endocrine programming and therefore the timing of the onset and the following sequela of pubertal development.

Our data show that early malnutrition related to low weight is associated with either a normal or a delayed VO in female rats. In male rats early malnutrition with or without a normal weight is associated with delayed onset of puberty. We suggest that, except for a central dysregulation, gonadal failure may responsible for this pubertal disorder as well.

We conclude that early malnutrition affects the onset of puberty in both male and female rats independent of their weight. The perinatal period appears to be a "critical time period" for the maturational process of puberty. Weight does not seem to play a critical role in the process of sexual maturation.

REFERENCES

1. Lucas A 1990 Does early diet program future outcome? Acta Paediatr Scand Suppl 365:58–67
2. Barker DJP, Clark PM 1997 Fetal undernutrition and disease in later life. Rev Reprod 2:105–112
3. Dennison E, Fall C, Cooper C, Barker D 1997 Prenatal factors influencing long-term outcome. Horm Res 48(Suppl 1):25–29
4. Gluckman PD, Breier BH, Oliver M, Harding J, Bassett N 1990 Fetal growth in late gestation - a constrained pattern of growth. Acta Paediatr Scand Suppl 367:105–110
5. Gluckman PD, Harding JE 1997 Fetal growth retardation: underlying endocrine mechanisms and postnatal consequences. Acta Paediatr (Suppl) 422:69–72
6. Gluckman PD, Harding JE 1997 The physiology and pathophysiology of intrauterine growth retardation. Horm Res 48(Suppl 1):11–16
7. Hales CN, Barker DJP, Clark PMS, Cox LJ, Fall C, Osmond C, Winter PD 1991 Fetal and infant growth and impaired glucose tolerance at age 64. BMJ 303:1019–1022
8. Phillips DIW, Barker DJP, Fall CHD, Seckl JR, Whorwood CB, Wood PJ, Walker BR 1998 Elevated plasma cortisol concentrations: a link between low birth weight and the insulin resistance syndrome? J Clin Endocrinol Metab 83:757–760
9. Clark PM 1998 Programming of the hypothalamo-pituitary-adrenal axis and the fetal origins of adult disease hypothesis. Eur J Pediatr 157(Suppl 1):S7–S10
10. Ibanez L, Potau N, Francois I, de Zegher F 1998 Precocious pubarche, hyperinsulinism and ovarian hyperandrogenism in girls: relation to reduced fetal growth. J Clin Endocrinol Metab 83:3558–3562
11. Fitzhardinge PM, Inwood S 1989 Long-term growth in small-for-date children. Acta Paediatr Scand Suppl 349:27–33
12. Westwood M, Kramer MS, Munz D, Lovett JM, Watters GV 1983 Growth development of full-term nonasphyxiated small-for-gestational-age newborns: follow-up through adolescence. Pediatrics 71:376–382
13. Preece MA 1997 Puberty in children with intrauterine growth retardation. Horm Res 48(Suppl 1):30–32
14. Frisch ER, Revelle R 1971 Height and weight at menarche and a hypothesis of menarche. Arch Dis Child 46:695–701
15. Engelbregt MJT, Houdijk ECAM, Popp-Snijders C, Delemarre-van de Waal HA 1997 Persistent growth impairment despite normal growth hormone secretion and normal IGF-1 in male and female intra-uterine growth retarded rats. Horm Res 48(Suppl. 2):277
16. Houdijk ECAM, Engelbregt MJT, Popp-Snijders C, Delemarre van de Waal HA 1997 Sexual dimorphism in long-term alterations in insulin secretion after intra-uterine growth retardation in the rat. Horm Res 48(Suppl 2):222
17. Holehan AM, Merry BJ 1985 The control of puberty in the dietary restricted female rat. Mech Ageing Dev 32:179–191
18. Glass AR, Swerdloff RS 1980 Nutritional influences on sexual maturation in the rat. Fed Proc 39:2360–2364
19. Wigglesworth JS 1964 Experimental growth retardation in the foetal rat. J Pathol Bacteriol 88:1–13
20. Scott EC, Johnston FE 1982 Critical fat, menarche, and the maintenance of menstrual cycles: a critical review. J Adolesc Health Care 2:249–260
21. Kennedy GC, Mitra J 1963 Body weight and food intake as initiating factors for puberty in the rat. J Physiol 166:408–418
22. Frisch RE 1980 Pubertal adipose tissue: is it necessary for normal sexual maturation? Evidence from the rat and human female Fed Proc 39:2395–2400
23. Frisch RE 1987 Body fat, menarche, fitness and fertility. Hum Reprod 2:521–533
24. De Bruin JP, Dorland M, Bruinse HW, Spliet W, Nikkels PGJ, Te Velde ER 1998 Fetal growth retardation as a cause of impaired ovarian development. Early Hum Dev 51:39–46
25. Kaplan SL, Grumbach MM, Aubert ML 1976 The ontogenesis of pituitary hormones and hypothalamic factors in the human fetus: maturation of central nervous system, regulation of anterior pituitary function. Recent Prog Horm Res 32:161–243