0013-7227/99/$03.00/0
Endocrinology

Vol. 140, No. 6
Printed in U.S.A.

Deficits in female reproductive function in GH-R-KO mice; role of IGF-I*

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Abstract: Mice homozygous for targeted disruption of the GH receptor/GH binding protein gene (GH-R-KO mice; -/-) exhibit reduced plasma IGF-I levels, elevated plasma GH levels, and dwarf phenotype. Although most GH-R-KO mice are fertile, age at first conception is greatly delayed in -/- x -/- matings. Here we report that the age of vaginal opening is significantly delayed in GH-R-KO vs. normal mice, but it can be advanced by treatment with recombinant human (rh)IGF-I. In pregnant GH-R-KO females, fetal size is reduced and pregnancy is prolonged while placental weight is, unexpectedly, increased. Alterations in fetal and placental weight are related to maternal rather than fetal genotype. Moreover, litter size and body weight of newborn pups are significantly reduced in GH-R-KO vs. normal females. Reduction in litter size reflects both dam and sire effects. We conclude that GH resistance and consequent reduction in peripheral IGF-I levels is associated with delay of female puberty, alterations in fetal and placental growth, delay of parturition, and reduced litter size.

It is well documented that growth hormone (GH) and the main mediator of its action, insulin-like growth factor-I (IGF-I) can exert direct and indirect effects on gonadal function and that these actions are particularly evident during sexual maturation (1). During puberty, the circulating levels of IGF-I increase sharply in mice (2), rats (3), and primates (4). Moreover, it has been demonstrated in vitro that IGF-I is capable to act within the median eminence to induce LHRH release (5). In vivo, IGF-I infused into the third brain ventricle of immature rats induced LH secretion via activation of LHRH release and advanced puberty (6).

Female mice with GH resistance due to targeted disruption of the GH receptor/GH binding protein gene (GH-R-KO mice) have suppressed plasma IGF-I levels, reduced growth, and dwarf phenotype (7). Although most of the homozygous GH-R-KO animals (-/-) are fertile, the age of first conception in the -/- x -/- matings is considerably delayed in comparison to +/+ x +/+ or +/- x +/- matings (7) presumably reflecting a delay of sexual maturation in -/- males. Moreover, litter size of -/- females mated to -/- males was reduced to approximately 40% of litter size in +/- x +/- and +/+ x +/+ matings (7).

The present study was undertaken to determine the age of sexual maturation in GH-R-KO females, to determine whether delay of sexual maturation in these animals can be ascribed to reduction in peripheral IGF-I levels, to evaluate breeding performance of these animals including fetal and placental growth, and to separate the effects of maternal, paternal, and fetal genotype on fetal growth and litter size.

Received 03/01/99.

Materials and Methods

GH-R-KO (-/-) mice and their normal (N; +/+ or +/-) siblings were produced by mating +/- animals or by mating -/- males to +/- females and maintained under controlled conditions of light (12 hL : 12 hD) and temperature (22 ± 2C) with ad libitum access to food (Lab Diet Formulab 5008, PMI Feeds, St. Louis, MO) and tap water. Genotype of phenotypically normal (+/+ vs. +/-) mice was determined by polymerase chain reaction DNA analysis, as described previously (8). Females were examined daily for vaginal opening. To determine the effects of exogenous IGF-I on sexual maturation, 17 GH-R-KO and 16 N mice were used. Eleven GH-R-KO and 4 N animals were injected s.c., b.i.d. with 2 μg of recombinant human IGF-I (Intergen Company, Purchase, NY) per g body weight and 6 GH-R-KO and 12 N mice with a saline vehicle alone. The injections were started one week before the expected age of vaginal opening. Thus, N mice were treated with IGF-I or vehicle starting at 24 days of age, while GH-R-KO animals were treated starting at 31 days of age. The animals were checked daily for establishment of vaginal

opening. On the day of vaginal opening, the IGF-I injections were discontinued and vaginal smears were taken daily. On the day of the first vaginal estrus, the animals were killed and uterine weight was recorded.

For the study of reproductive performance, fetal and placental growth, adult (3-4 months old) normal and GH-R-KO females were mated to normal males, checked daily for vaginal plugs, and sacrificed on days 14, 15, 16, 17, or 18 of gestation. Fetal weight and crown-rump length, placental weight, and number of dead or resorbed fetuses were recorded. Additional GH-R-KO and N females were mated to either GH-R-KO or N males, checked daily for vaginal plugs, and either sacrificed on day 17 for the determination of fetal and placental weight or allowed to go to term. Pregnancy length, litter size, and body weight of pups at birth were recorded. Additional data on litter size and pup weight were derived from animals which were not checked for the dates of matings. All animal protocols were approved by an institutional committee.

Results

Age of puberty and effects of IGF-I. In the first group of animals we examined, vaginal introitus was established at a significantly later age in GH-R-KO than in N females (38.4±0.65 vs. 30.9±0.39 days; P<0.001). In the animals used for the study of the effects of IGF-I, vaginal opening occurred at an earlier age, but a significant difference between GH-R-KO and N mice was again observed (Table 1).

Table 1. Age of vaginal opening in GH-R-KO and normal female mice injected twice daily with rhIGF-I or with saline vehicle (sal) starting one week before the expected age of sexual maturation.

Genotype	Treatment	n	Age at vaginal Opening (days)
normal	saline	12	28.6 ± 0.6*
normal	IGF-I	4	26.5 ± 0.3
GH-R-KO	saline	6	35.7 ± 0.2**
GH-R-KO	IGF-I	11	32.9 ± 0.3

*P<0.01; **P<0.0001; Means ± SEM

The delay of vaginal opening in GH-R-KO as compared to N mice was nearly identical in the two groups of animals, 7.4 and 7.1 days. There were no significant differences in the age of vaginal opening between homozygous wild type (+/+) and heterozygous (+/-) females (data not shown). Administration of recombinant human (rh)IGF-I accelerated vaginal opening by approximately 3 days in GHR-KO mice and by approximately 2 days in N mice (Table 1). At the time of first vaginal estrus, uterine weight was significantly greater (P<0.02) in the GH-R-KO mice treated with IGF-I (19.8±3.72 mg) than in the GH-R-KO animals given saline (7.8±1.47 mg), while body weight was not affected. The interval between the day of vaginal opening and the day of the first vaginal estrus was significantly longer (P<0.02) in the GH-R-KO females given IGF-I (5.2±0.75 days) than in the control GH-R-KO animals (2.8±0.17 days).

Fetal and placental development. The weight and crown-rump length of fetuses in GH-R-KO females on days 14, 15, and 16 of pregnancy were comparable to the values measured in N females, except for a modest but statistically significant reduction in the fetal weight in KO females on day 14 (200 ± 25 vs. 242 ± 26 mg; P<0.01). Between days 16 and 17, the rates of fetal growth began to diverge, and fetuses of GH-R-KO females were significantly smaller than fetuses of N females on days 17 and 18 and weighed significantly less on day 17 (Fig. 1).

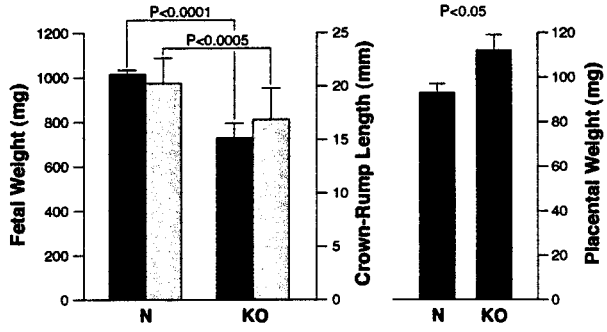

FIG. 1. Fetal and placental weight (solid bars) and crown-rump length (stippled bars) in normal (N) and GH-R-KO (KO) mice mated to N males and examined on day 17 of pregnancy. Means ± SEM.

In contrast to the reduced fetal size, placental weight was significantly greater in GH-R-KO than in N females on days 16 and 17 of pregnancy (Fig. 1), with numerically similar differences on days 14 and 15 and a difference approaching statistical significance on day 18 (0.05<P<<0.10).

To determine whether reduced fetal weight and length and increased placental weight may have been due to fetal rather than maternal factors, additional GH-R-KO females were mated to GH-R-KO or N males and examined on day 17 of

pregnancy. There were no differences in fetal weight, crown-rump length, or in placental weight between litters sired by GH-R-KO or N males (data not shown). Moreover, the coefficients of variations for these values were not greater in litters sired by N males and, therefore, consisting of N, or KO and N fetuses than in those sired by KO males and thus consisting exclusively of KO fetuses.

The number of live fetuses was significantly reduced in GH-R-KO females mated to N males as compared to N females mated to N males, at each of the stages of pregnancy examined (4.83 ± 0.40 vs. 7.19 ± 0.43; $P<0.05$). The number of dead or resorbed fetuses in these two types of matings did not differ (0.93 ± 0.25 vs. 1.06 ± 0.28). At day 17 of pregnancy, the number of fetuses was not significantly influenced by the genotype of the male. The number of live pups at birth was significantly reduced in GH-R-KO as compared to N females (3.17 ± 0.51 vs. 6.09 ± 0.77; $P<0.005$). Comparisons of litter size in GH-R-KO and N females mated to N males (4.1 ± 0.59 vs. 6.3 ± 0.87; $P<0.05$) and in GH-R-KO females mated to GH-R-KO or N males (1.6 ± 0.40 vs. 4.1 ± 0.59; $P<0.05$) indicate that both maternal and paternal genotype influence litter size. Average body weight of live pups at birth was 1.38 ± 0.06 g in GH-R-KO females and 1.65 ± 0.06 g in N females; $P<0.01$. Length of pregnancy was significantly greater in GH-R-KO than in N females (20.0 ± 0.0; n=5 vs. 19.0 ± 0.0 days; n=6; $P<0.05$).

Discussion

The main finding of the present study is that sexual maturation (as evidenced by establishment of the vaginal introitus) is significantly delayed in GH-R-KO female mice and can be advanced by systemic administration of IGF-I. It has been suggested that IGF-I is a potential signal linking growth and puberty in mammals (2-4). Previous studies showed that IGF-I activates the LHRH/LH-releasing system during female puberty in rats (5), that intraventricular administration of IGF-I can advance vaginal opening (6), and that this effect is accompanied by increased synthesis of IGF-I receptors in the median eminence during first proestrus (6). Systemic infusion of recombinant human IGF-I accelerated the age of the first ovulation in juvenile female rhesus monkeys (9), but did not advance the age of vaginal opening in rats (10). In the present study, systemic (s.c.) treatment of immature GH-R-KO or N mice with rhIGF-I significantly advanced the age of vaginal opening. Record of vaginal smears suggests that the age of the first ovulation in these animals was either not advanced or affected only slightly. This may have been due to discontinuing the IGF-I treatment on the day of vaginal opening. Recent study has shown that administration of IGF-I via osmotic mini-pumps can shorten the interval between menarche and first ovulation in rhesus monkeys (9). Differences in the age of vaginal opening in different groups of control (untreated or vehicle injected) animals examined in the present study may have been due to seasonal effects or, more likely, to genetic drift in this genetically heterogeneous line derived from crosses of several unrelated strains.

The uterine weight in GH-R-KO mice treated with IGF-I was increased 2.5-3-fold over the values measured in GH-R-KO animals given saline. This could be due to stimulation of ovarian steroidogenesis by IGF-I (1) or to a direct action of exogenous IGF-I on uterine epithelium. IGF-I is a potent epithelial mitogen in the uterus (10). Moreover, exogenous IGF-I could facilitate mitogenic effects of estradiol-17ß on uterine epithelium (11).

Our findings that the fetal weight and crown-rump length were diminished in GH-R-KO compared to N females indicate that severe IGF-I deficiency or other consequences of GH resistance including reduced levels of serum glucose and insulin (Danilovich and Bartke, unpublished observations) interfere with fetal growth. Moreover, it has been shown that IGF-I may enhance vasodilatation in human term placental explants by inhibiting the release of vasoconstrictors such as thromboxane B2 and prostaglandin F2 α (12). Thus, greatly decreased levels of IGF-I in GH-R-KO mice (7), might diminish the supply of nutrients available for the growth of the fetus resulting in fetus growth retardation.

We do not know whether prolongation of pregnancy in GH-R-KO females is related to inhibition of fetal growth and/or development. It is interesting to note that in spite of significantly longer period of gestation, the weight of newborn pups was lower in GH-R-KO than in N females. We also have no explanation for the significant increase in placental weight in pregnant GH-R-KO females in which fetal weight is significantly reduced. It is tempting to speculate that placental enlargement in GH-R-KO females may represent a compensatory mechanism in these hypoglycemic animals. It could also conceivably be related to suppression of ovarian steroidogenesis in these animals, since estrogen deprivation in pregnant

rats was shown to lead to placental enlargement (13). Comparison of results obtained in GH-R-KO females mated to N or to KO males indicates that increase in placental weight, similarly to the reduction in fetal weight is due to maternal factors rather than to genotype of the embryo.

Major reduction in litter size in GH-R-KO females (7, and the present findings) appears to be due primarily to reduced ovulatory rate because there was no evidence for increased fetal mortality. However, reduced fertilization rate and/or increased pre-implantation losses of embryos may have been involved. In GH-R-KO females mated to GH-R-KO rather than N males, there was a further significant reduction in litter size. Data on the numbers of live vs. dead or resorbed fetuses suggest that this may have been due to reduced fertilization rate in matings involving GH-R-KO males.

Findings in the GH-R-KO animals, including results of the present study, contrast sharply with the findings in IGF-I-KO mice which are characterized by increased perinatal mortality and in the surviving animals, infertility, and infantile reproductive system (14). Apparently the amounts of IGF-I present in GH-R-KO mice and presumably produced independently of GH are sufficient for survival, sexual maturation, and qualitatively normal, although quantitatively severely altered, reproductive functioning. Absence of GH-dependent IGF-I production in these GH resistant animals is presumably responsible for delayed puberty, retarded fetal development, prolonged pregnancy, reduced litter size, and other reproductive abnormalities in these animals. Results of IGF-I replacement clearly implicate GH-dependent IGF-I production in the timing of female puberty in this species.

Acknowledgments

This work was supported by NIH, HD20001. DW participated in this research as member of SIU-C Honors Program. We thank Dr. V. Chandrashekar for help and advice, Mr. Markus Riders and Ms. Rebecca Cerven for technical assistance, and Ms. Mae Hilt for typing.

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