5-2004

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Besiana Liti

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Effects of Sucrose Supplementation on Sexual Maturation and Reproductive Functions of Growth Hormone Receptor Knockout Mice

By Besiana Liti

Abstract

1   Growth hormone (GH) exerts its cell division-stimulating (mitogenic) effect not directly

2   on cells but indirectly through the mediation of a mitogen whose synthesis and release are

3   induced by growth hormone. This mitogen is called insulin-like growth factor I (IGF-I).

4   The secretion and activity of IGF-I can be influenced by the nutritional status of the

5   individual and by many hormones other than GH. GH resistance occurs in human Laron

6   type dwarfism due to mutations in the GH receptor (GHR) and a mammalian model for

7   GH resistance has been developed in mice through targeted disruption or "knockout"

8   (KO) of the GHR. In GHR-KO mice, GH resistance leads to quantitative deficits in

9   reproductive development and functions, but does not preclude fertility in either sex

10   (Bartke et al., 1999). Secondary consequences of GH resistance in mice include

11   hyperprolactinemia, hypoinsulinemia, IGF-I insufficiency, mild hypothyroidism, and

12   decreased core body temperature. Many of these phenotypic manifestations are consistent

13   with and suggestive of nutritional stress. Sexual maturation and adult reproductive

14   function are tightly linked to nutritional status. Delayed puberty onset and retarded

15   progression of sexual maturation in GHR-KO mice may be due, at least in part, to

16   nutritional stress exacerbated in rapidly growing young and adolescent individuals.

17   Dietary sucrose supplementation aimed at alleviating nutritional stress in the juvenile-to-

18   adult transition period did not advance puberty onset in GHR-KO mice. The current study

19   attempts to examine the effects of sucrose supplementation on late stages of sexual

20   maturation in GHR-KO mice. Specifically, we tested the hypothesis that dietary sucrose

21   supplementation during the juvenile-to-adult transition would promote more rapid

22   progression of later stages of sexual maturation and advance maternal age at first

1   conception (MAFC) in female GHR-KO mice. At 65 days post-partum normal and GHR-

2   KO female mice were randomly assigned to one of four treatment groups: normal

3   untreated (N-U, n=15), GHR-KO untreated (KO-U, n=15), normal sucrose-supplemented

4   (N-SS, n=15) and GHR-KO sucrose-supplemented (KO-SS, n=15). Untreated control

5   mice received normal lab chow diets throughout the remainder of the study, while treated

6   females received standard lab chow supplemented with sucrose (2 parts chow: 1 part

7   sucrose). Five days later females were paired with normal adult males and subsequently

8   monitored daily for pregnancy and parturition. Litter size was recorded and pups were

9   removed on the day of delivery. Females were sacrificed 7 days post-partum and body,

10   uterine, and ovarian weights were recorded. Analysis of variance and Fischer PLSD post

11   hoc analysis were used to determine significant effects of genotype, treatment, and

12   genotype x treatment interaction between groups, with significance at $p<0.05$. MAFC

13   was significantly affected by genotype (p=0.003), treatment (p=0.034), and genotype x

14   treatment interaction (p=0.024). Regardless of treatment, normal mice conceived first

15   litters roughly 20 days earlier than untreated GHR-KO females (N-U=83.7 +/- 2.3 days,

16   N-SS=84.2 +/- 3.4 days, KO-U=103.6 +/- 5.8 days). MAFC in sucrose supplemented

17   GHR-KO females (KO-SS=87.0 +/- 2.9 days) did not differ significantly from that of N-

18   SS (p=0.58) or from N-U (p=0.51) but was advanced significantly (p=0.008) compared to

19   KO-U. These results support our hypothesis and warrant further research that studies the

20   mechanism(s) by which sucrose affects the rate of progression of sexual maturation GH

21   resistant states.

Introduction

1   Growth hormone (GH), secreted by the anterior pituitary, has little or no effect on fetal

2.   growth, but is the most important hormone for postnatal growth. Growth hormone exerts

3   its cell division-stimulating (mitogenic) effect not directly on cells but indirectly through

4   the mediation of a mitogen whose synthesis and release are induced by GH. This mitogen

5   is called insulin-like growth factor I (IGF-I). The importance of IGF-I in mediating the

6   major growth-promoting effect of growth hormone is illustrated by the fact that dwarfism

7   can be due not only to decreased secretion of growth hormone, but also to decreased

8   production of IGF-I or failure of the tissues to respond to IGF-I.

9   In humans, growth hormone insensitivity syndrome, Laron Dwarfism, is due to

10   genetic mutations that cause the growth hormone receptor to fail to respond to growth

11   hormone. The result is failure to produce IGF-I in response to growth hormone. Thus

12   female mice with GH resistance due to targeted disruption of the GH receptor/GH

13   binding protein gene (GHR-KO mice) have suppressed plasma IGF-I levels, reduced

14   growth, and the dwarf phenotype (Danilovich et al., 1999) suggesting that GHR-KO mice

15   mimic the human condition in many ways. The most notable similarities include growth

16   retardation, delayed sexual maturation, severely depressed serum IGF-I levels and a

17   complete inability to utilize GH (List et al., 2001).

18   In mice and humans GH resistance leads to quantitative deficits in reproductive

19   development and functions, but does not preclude fertility in either sex. (Bartke et al.,

20   1999) The secretion and activity of IGF-I can be influenced by the nutritional status of

21 the individual and by many hormones other than growth hormone. Estrogen also

22 stimulates the secretion of IGF-I by cells of the uterus and ovaries. Growth hormone

23 (GH), insulin-like growth factor (IGF-I), and prolactin (PRL) can influence various

24 aspects of reproductive functions in both females and males. Bartke et al. (1999) suspect

25 that PRL and the GH-IGF-I axis provide partially overlapping (redundant) regulatory

26 inputs to the hypothalamic-pituitary-gonadal (HPG) axis, and consequently, targeted

27 disruption of either signaling pathway has relatively mild consequences on many

28 functions related to reproduction. Growth hormone and the main mediator of its action,

29 IGF-I, can exert direct and indirect effects on gonadal function and these actions are

30 particularly evident during sexual maturation. The GHR/BP -/- (aka GHR-KO) mice do

31 not tend toward obesity, exhibit normal to low glucose levels, and have severely

32 decreased insulin levels when compared to normal (+/+) animals (List et al., 2001). GH

33 and IGF-I and their receptors are widely distributed throughout the HPG axis, along with

34 numerous binding proteins and proteases known to regulate GH/IGF bioavailability and

35 action (Zazcek et al., 2002). GH antigens are identified in pituitary cells containing FSH

36 and LH messenger RNAs and GnRH receptors, indicating that either GH cells are

37 transitory gonadotrophs, or GH is present in these pituitary cells, possibly to control their

38 function (Chandrashekar et al., 1999). Furthermore, GH binding protein antigens were

39 identified in pituitary cells that contained LH and FSH, indicating a paracrine effect of

40 GH on the function of the gonadotrophs (Chandrashekar et al., 1999). GH and IGF-I have

41 been implicated in the following aspects pertinent to female reproduction: 1) regulation

42 of the timing of puberty onset and the progression of sexual maturation, 2) regulation of

43 GnRH and gonadotropin release from the hypothalamus and pituitary, respectively, and,

44   3) modulation of ovarian follicular growth and development, steroidogenesis, and

45   apoptosis (Zazcek et al., 2002). In addition, GH and IGF-I influence numerous processes

46   related to pregnancy including oocyte maturation and fertilization, early embryo

47   development, implantation, fetal/placental growth and development, and litter size

48   (Zazcek et al., 2002).

49   Fulgeshu et al. (1997) evaluated the influence of insulin level on the ovarian

50   response to FSH when inducing ovulation in patients affected by polycystic ovarian

51   syndrome (PCOS) and found that while ovulation rate was similar in normoinsulinemic

52   and hyperinsulinemic subjects, the incidence of ovarian hyperstimulation was

53   significantly higher in the latter group. Yoshimura et al. (1996) conducted a study, which

54   provided proof that addition of IGF-I to the perfusate stimulated follicular growth and the

55   resumption of meiosis in follicular oocytes and increased estradiol production in cultured

56   rabbit ovaries in a dose dependent manner.

57   Type I diabetes is associated with abnormalities of the GH-IGF-I axis. Such

58   abnormalities include decreased circulating levels of IGF-I (Bartke et al., 1999). Type 1

59   diabetes has received particular attention because the relative portal insulin deficiency of

60   this condition is thought to be responsible for the reduced circulating levels of IGF-I

61   which in turn, through decreased negative feedback, leads to increased secretion of GH.

62   The metabolic profile of GHR-KO mice suggests these animals may experience some

63   level of nutritional stress, but to date it has not been established how nutritional stress

64   effects   sexual   maturation   and   reproductive   functions   in   GHR-KO   mice.

1 **Materials and Method**

2 GHR-KO (-/-) and normal (N, +/+ or +/-) control mice were produced by mating +/- or -/-

3 males to +/- females in our breeding colony, which was derived from our original

4 GHR/GHBP KO (Zhou et al., 1997b) crossed with animals derived from C57BL/6 x C3H

5 hybrids and thereafter bred in closed colony at the Southern Illinois University animal

6 facility. Mice were kept in the SIUC vivarium at strict light and temperature conditions,

7 which included a 12L/12D photoperiod at $22+2^0$ C. Once ther animals reached weaning

8 age (21 days) they were segregated by sex but not by genotype and housed with up to

9 five other mice of the same sex per cage. Subsequently ad libitum access to tap water and

10 standard lab chow (Purina Formulab 5008 Rodent; El Mel Inc., Florissant MO) was

11 given. At 65 days post-partum normal and GHR-KO female mice were randomly

12 assigned to one of four treatment groups: normal untreated (N-U, n=15), GHR-KO

13 untreated (KO-U, n=15), normal sucrose-supplemented (N-SS, n=15) and GHR-KO

14 sucrose-supplemented (KO-SS, n=15). Untreated control normal and GHR-KO female

15 mice received normal lab chow diets throughout the remainder of the study, while

16 sucrose supplemented females were fed standard lab chow and sucrose on a ratio of 2

17 parts chow: 1 part sucrose. Five days after the sucrose supplemented females were started

18 on the new 2 parts chow: 1 part sucrose diet, they were paired with normal adult males

19 and subsequently monitored daily for vaginal plugs (between 0800 and 1030 am) and

20 parturition to determine effects of sucrose supplementation on latency to first mating. All

21 cages contained one GHR-KO female and one normal female to ensure that both GHR-

22 KO and normal females would be similarly exposed to any male effects (e.g. delayed

23 puberty, male sub-fertility/infertility). The mean age of females at pairing did not differ

24   between groups. There is distinct size differences between normal males and GHR-KO

25   females at 65 days post-partum which, contribute to occasional injuries to the female

26   during mating and consequently a total of 5 GHR-KO females died in the course of the

27   study because of physical injuries caused by the normal males during mating. The date of

28   delivery and litter size was recorded and pups were removed on the day of delivery.

29   Females were sacrificed 7 days post-partum and body, uterine, and ovarian weights were

30   recorded. Analysis of variance and Fisher post hoc analyses were used to determine

31   significant effects of genotype, treatment, and genotype x treatment interaction between

32   groups with significance at $p<0.05$.   Experiments were approved by the University

33   Animal Care and Utilization Committee and were conducted in accordance with NIH

34   (national institute of health) animal care guideline.

Results

1   To determine the effect of sucrose supplementation on the rate at which sexual

2   maturation proceeds and fertility is achieved, we analyzed maternal age at first

3   conception (MAFC) in normal and GHR-KO mice maintained on standard lab chow

4   (untreated control) diets or sucrose supplemented diets during the juvenile-to-adult

5   transition period. MAFC was significantly affected by genotype ($p=0.003$), treatment

6   ($p=0.034$), and genotype x treatment interaction ($p=0.024$) (Fig.1). Regardless of

7   treatment, normal mice conceived first litters roughly 20 days earlier than untreated

8   GHR-KO females (N-U=83.7 +/- 2.3 days, N-SS=84.2+/- 3.4 days, KO-U=103.6 +/- 5.8

9   days). MAFC in sucrose supplemented GHR-KO females (KO-SS=87.0+/- 2.9 days) did

10   not differ significantly from that of N-SS ($p=0.58$) or from N-U ($p=0.51$) but was

11   advanced significantly ($p=0.008$) compared to MAFC in GHR-KO mice maintained on

12   the control diet. All normal females in the study conceived and delivered first litters.

13   Consistent with previous mating studies in GHR-KO mice, a subset (n= 5) of GHR-KO

14   females died (2 KO-U and 3 KO-SS) due to mating related trauma and a subset (n=10) of

15   GHR-KO females (6 KO-U and 4 KO-SS) failed to conceive when housed continually

16   with males of proven fertility for 110 or more days (Table 1).

17   For those females that did conceive we used analysis of variance and Fisher post hoc

18   comparisons to determine significant genotype, treatment, and/or genotype x treatment

19   interaction effects on litter size between groups. Consistent with previous studies, litter

20   size was significantly influenced by genotype ($p=0.001$), but no significant effects of

21   treatment or genotype x treatment interaction was found ($p=0.253$ and $p=0.572$,

22   respectively) (Fig. 2) Regardless of treatment, normal females had larger-sized litter, by

23 roughly 2 pups per litter, compared to GHR-KO counterparts (N-U=6.1 +/- 0.4

24 pups/litter, N-SS=5.1 +/- 0.6 pups/litter, KO-U=3.7+/-0.9 pups per litter, and KO-SS=3.4

25 +/- 0.3 pups/litter).

26 Because sexual maturation and fertility are intimately tied to nutritional status, which is

27 reflected in fat stores and body weight, we used similar analyses to detect significant

28 differences in body weight [at sacrifice] between groups due to genotype, treatment,

29 and/or genotype x treatment interactions. Genotype, but not treatment or genotype x

30 treatment interaction, significantly affected body weight at sac (p<0.0001, p=0.58, and

31 p=0.32, respectively) (Fig. 3). Body weight at sacrifice was similar in normal females,

32 regardless of treatment (N-U=21.4 +/- 1.0g and N-SS=20.9 +/- 0.5g, p=0.69) and was

33 roughly 40% greater than that of untreated or sucrose supplemented GHR-KO females,

34 who did not differ from one another (KO-U=13.3 +/- 0.8g and KO-SS=14.9 +/- 1.4g,

35 p=0.35).

36 Absolute ovarian weight at sacrifice was also significantly affected by genotype

37 (p<0.0001) but not by treatment (p=0.78) nor by genotype x treatment interactions

38 (p=0.34) (fig. 4). Absolute ovarian weights of normal mice were similar between

39 treatment groups (N-U=9.4 +/- 0.6g, N-SS=9.8 +/- 0.4g, p=0.55) and roughly double that

40 of ovary weights in GHR-KO females on standard or sucrose supplemented diets (KO-

41 U=5.5 +/- 0.6g, KO-SS= 4.7 +/- 0.5g, p=0.46). However, relative ovarian weight (ROW=

42 ovary weight expressed as a percentage of total body weight) was significantly affected

43 by genotype (p=0.005) and genotype x treatment interactions (p=0.04). Sucrose

44 supplementation differentially influenced ROW in normal and GHR-KO mice, producing

45 slight but not significant increases in ROW in normal mice (N-U=0.044 +/- 0.002% vs.

46   N-SS=0.047 +/- 0.002%, p=0.28). In contrast, sucrose supplementation resulted in slight

47   decreases in GHR-KO mice that tended toward significance (KO-U=0.041 +/- 0.004%

48   vs. KO-SS= 0.033 +/- 0.004%, p=0.09) and rendered KO-SS females significantly

49   different from N-U females (p=0.008) and N-SS females (p=0.006).

50   Analysis of variance revealed no significant effects of genotype (p=0.19), treatment

51   (p=0.14), nor genotype x treatment interaction (p=0.21) on absolute uterine weight at

52   sacrifice (fig. 5). However, mean absolute uterine weight at sacrifice in sucrose

53   supplemented GHR-KO mice was 71.4 +/- 5.2g, while in all other treatment groups mean

54   absolute uterine weights were approximately 100g (N-U=105.4 +/- 14.1g, N-SS=102.5

55   +/- 5.3g, and KO-U=104.9 +/- 12.5g). In Fisher post hoc analysis, two-way comparisons

56   between groups revealed significant differences between KO-SS and N-U groups

57   (p=0.04), nearly significant differences between KO-SS and N-SS groups (p=0.06) and a

58   trend toward significant differences between KO-SS and KO-U groups (p=0.10). Relative

59   uterine weight (uterine weight expressed as a percentage of total body weight) was

60   significantly affected by genotype (p=0.009), treatment (p=0.020) and genotype x

61   treatment interaction (p=0.017). Sucrose supplementation did not affect RUW in normal

62   mice (N-U=0.49 +/- 0.05g, N-SS=0.49 +/- 0.03g, p=0.96). RUW was greater in untreated

63   GHR-KO mice (0.80 +/- 0.11g), with this treatment group being significantly different

64   from both untreated and sucrose-supplemented normal females (p=0.001 and p=0.001,

65   respectively). Sucrose supplementation reduced RUW in GHR-KO mice (0.51 +/- 0.6g)

66   such that KO-SS females were significantly different from untreated GHR-KO mice

67   (p=0.005) but did not differ from untreated or sucrose supplemented normal mice

68   (p=0.83 and p=0.86, respectively)

Table summarizing the number of animals that died, conceived, and didn't conceive.

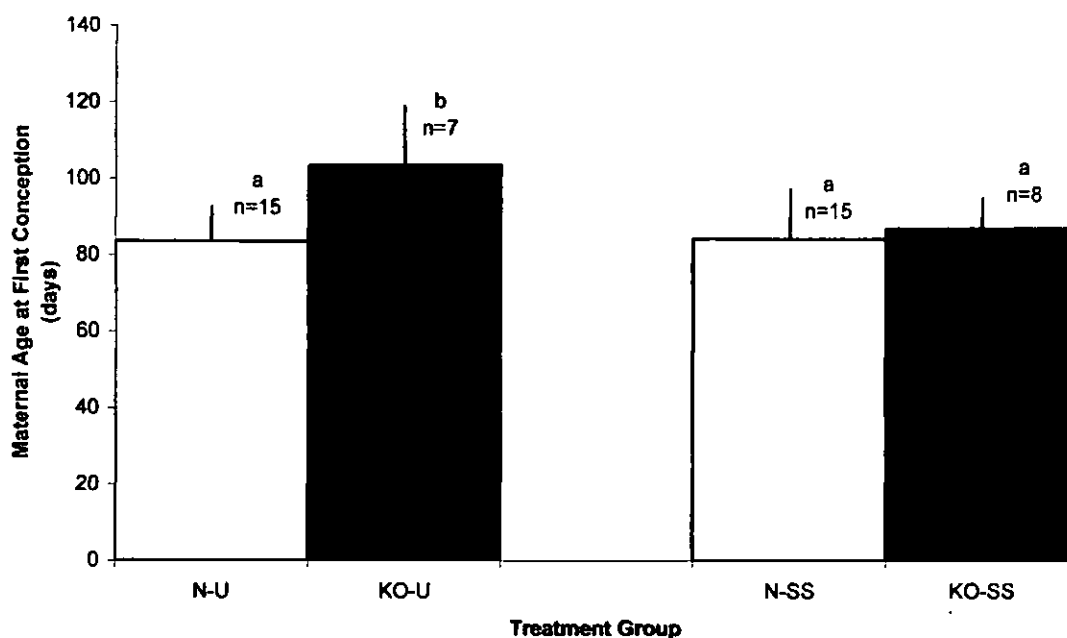| Genotype	Died	Didn't conceive	Conceived
KO-SS	3	4	8
KO-U	2	6	7
N-SS	0	0	15
N-U	0	0	15

Figure 1

Figure 1: Effect of sucrose supplementation during the juvenile-to-adult transition period (beginning at 65 days post-partum) on the progression of sexual maturation, as assessed by maternal age at first conception, in normal and growth hormone receptor knockout (GHR-KO) mice. Treatment groups= normal untreated control (N-U), GHR-KO untreated control (KO-U), normal sucrose supplemented (N-SS), and GHR-KO sucrose supplemented (KO-SS) mice. Bars represent mean ± SEM. Letter designations above bars indicate significant difference (at $p \leq 0.05$) between groups with different letter designations.

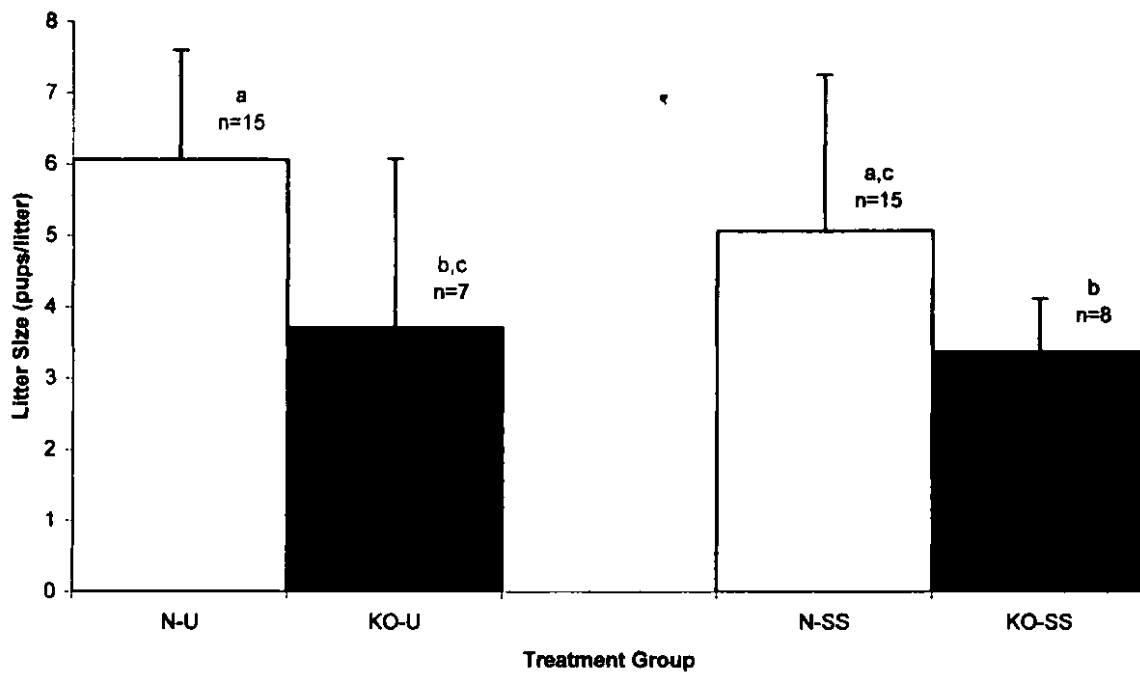| Treatment groups		P value	Significance
ko-ss	ko-u	0.0082	S
ko-ss	n-ss	0.5821	
ko-ss	n-u	0.5128	
ko-u	n-ss	0.0007	S
ko-u	n-u	0.0005	S
n-ss	n-u	0.8998	

Figure 2

Figure 2: Effect of sucrose supplementation during the juvenile-to-adult transition period (beginning at 65 days post-partum) on litter size in normal and growth hormone receptor knockout (GHR-KO) mice. Treatment groups= normal untreated control (N-U), GHR-KO untreated control (KO-U), normal sucrose supplemented (N-SS), and GHR-KO sucrose supplemented (KO-SS) mice. Bars represent mean $\pm$ SEM. Letter designations above bars indicate significant difference (at $p \leq 0.05$) between groups with different letter designations.

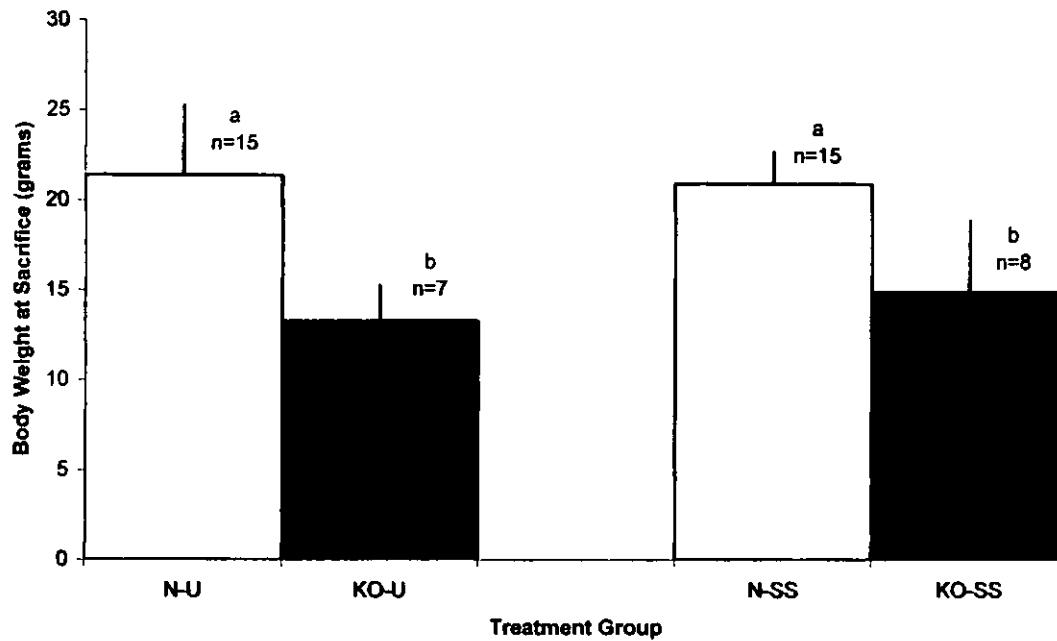| Treatment groups		P value	Significance
ko-ss	ko-u	0.7219	
ko-ss	n-ss	0.0408	S
ko-ss	n-u	0.0017	S
ko-u	n-ss	0.1139	
ko-u	n-u	0.0076	S
n-ss	n-u	0.1420	

Figure 3

Figure 3: Effect of sucrose supplementation during the juvenile-to-adult transition period (beginning at 65 days post-partum) on body weight in normal and growth hormone receptor knockout (GHR-KO) mice. Treatment groups= normal untreated control (N-U), GHR-KO untreated control (KO-U), normal sucrose supplemented (N-SS), and GHR-KO sucrose supplemented (KO-SS) mice. Bars represent mean $\pm$ SEM. Letter designations above bars indicate significant difference (at $p \leq 0.05$) between groups with different letter designations.

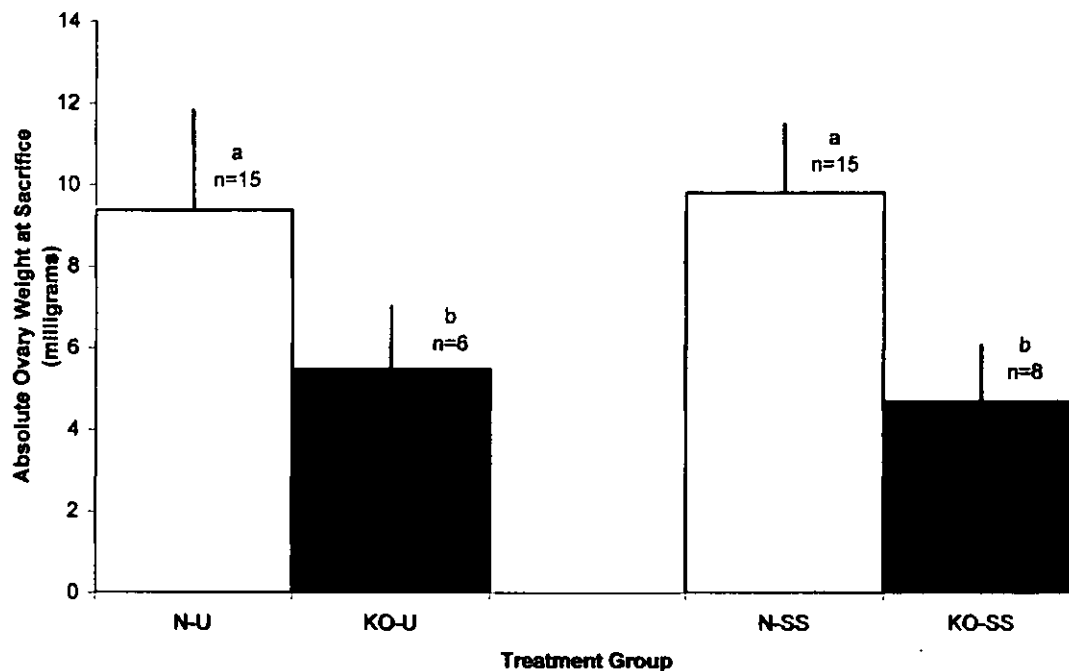| Treatment groups		P value	Significance
ko-ss	ko-u	0.3525	
ko-ss	n-ss	<0.0001	S
ko-ss	n-u	<0.0001	S
ko-u	n-ss	<0.0001	S
ko-u	n-u	<0.0001	S
n-ss	n-u	0.6894	

Figure 4

Figure 4: Effect of sucrose supplementation during the juvenile-to-adult transition period (beginning at 65 days post-partum) on absolute ovary weight in normal and growth hormone receptor knockout (GHR-KO) mice. Treatment groups= normal untreated control (N-U), GHR-KO untreated control (KO-U), normal sucrose supplemented (N-SS), and GHR-KO sucrose supplemented (KO-SS) mice. Bars represent mean ± SEM. Letter designations above bars indicate significant difference (at $p \leq 0.05$) between groups with different letter designations.

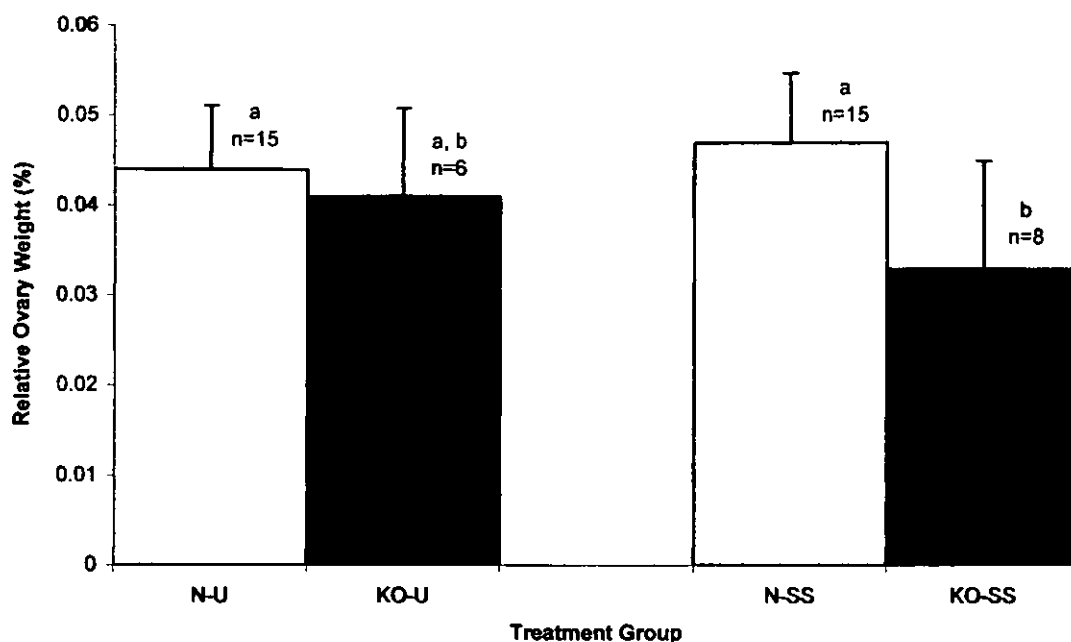| Treatment groups		P value	Significance
ko-ss	ko-u	0.4556	
ko-ss	n-ss	<0.0001	S
ko-ss	n-u	<0.0001	S
ko-u	n-ss	<0.0001	S
ko-u	n-u	0.0002	S
n-ss	n-u	0.5513	

Figure 5

Figure 5: Effect of sucrose supplementation during the juvenile-to-adult transition period (beginning at 65 days post-partum) on relative ovary weight (ROW= ovary weight expressed as a percentage of total body weight) in normal and growth hormone receptor knockout (GHR-KO) mice. Treatment groups= normal untreated control (N-U), GHR-KO untreated control (KO-U), normal sucrose supplemented (N-SS), and GHR-KO sucrose supplemented (KO-SS) mice. Bars represent mean $\pm$ SEM. Letter designations above bars indicate significant difference (at $p \leq 0.05$) between groups with different letter designations.

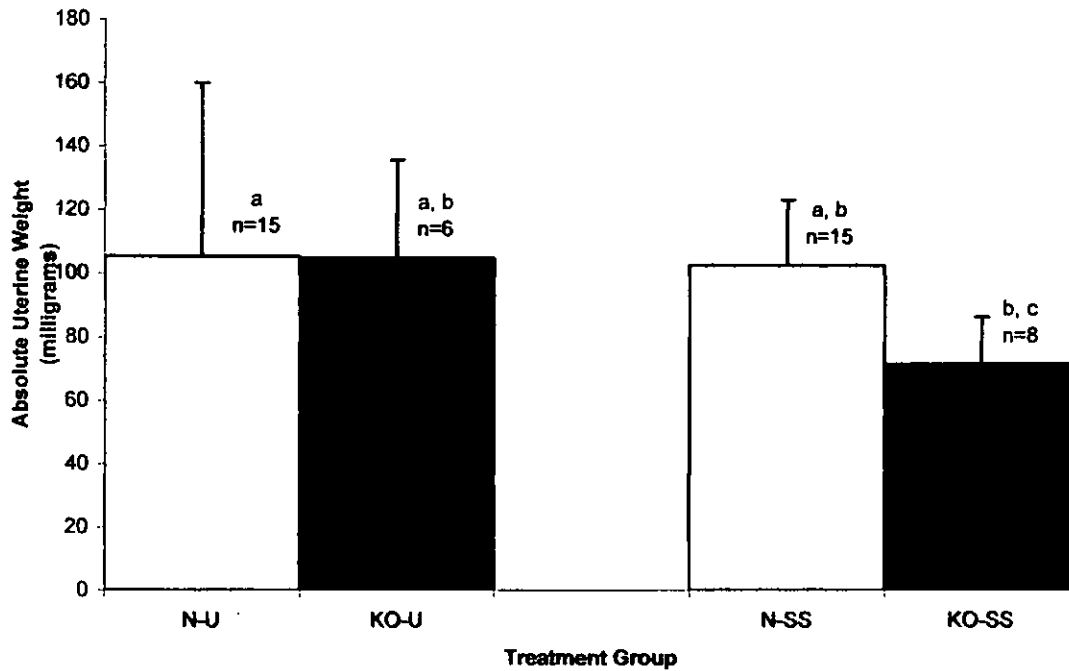| Treatment groups		P value	Significance
ko-ss	ko-u	0.0866	
ko-ss	n-ss	0.0006	S
ko-ss	n-u	0.0076	S
ko-u	n-ss	0.1636	
ko-u	n-u	0.5617	
n-ss	n-u	0.2766	

Figure 6

Figure 6: Effect of sucrose supplementation during the juvenile-to-adult transition period (beginning at 65 days post-partum) on absolute uterine weight in normal and growth hormone receptor knockout (GHR-KO) mice. Treatment groups= normal untreated control (N-U), GHR-KO untreated control (KO-U), normal sucrose supplemented (N-SS), and GHR-KO sucrose supplemented (KO-SS) mice. Bars represent mean $\pm$ SEM. Letter designations above bars indicate significant difference (at $p \leq 0.05$) between groups with different letter designations.

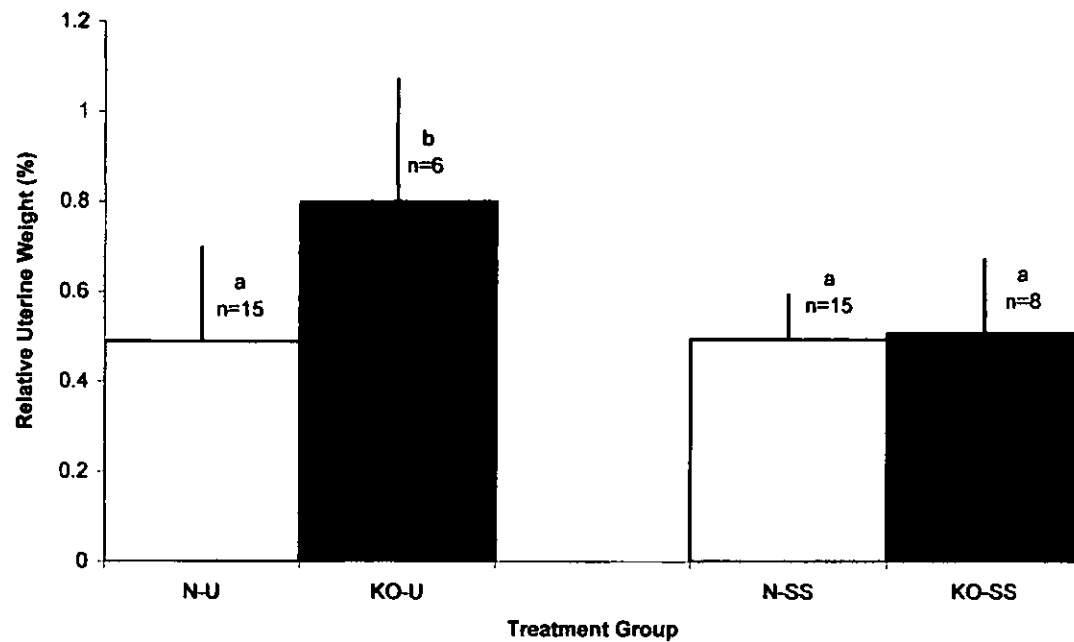| Treatment groups		P value	Significance
ko-ss	ko-u	0.0986	
ko-ss	n-ss	0.0598	
ko-ss	n-u	0.0402	S
ko-u	n-ss	0.8934	
ko-u	n-u	0.9754	
n-ss	n-u	0.8273	

Figure 7

Figure 7: Effect of sucrose supplementation during the juvenile-to-adult transition period (beginning at 65 days post-partum) on relative uterine weight (RUW= uterine weight expressed as a percentage of total body weight) in normal and growth hormone receptor knockout (GHR-KO) mice. Treatment groups= normal untreated control (N-U), GHR-KO untreated control (KO-U), normal sucrose supplemented (N-SS), and GHR-KO sucrose supplemented (KO-SS) mice. Bars represent mean ± SEM. Letter designations above bars indicate significant difference (at p≤0.05) between groups with different letter designations.

Treatment groups		P value	Significance
ko-ss	ko-u	0.0050	S
ko-ss	n-ss	0.8631	
ko-ss	n-u	0.8274	
ko-u	n-ss	0.0012	S
ko-u	n-u	0.0011	S
n-ss	n-u	0.9563	

Discussion and conclusion

1   Growth hormone, secreted by the anterior pituitary, has little or no effect on fetal

2   growth, but is the most important hormone for postnatal growth. Growth hormone

3   induces the release of IGF-I, which in turn exerts its mitogenic effects on cells. GHR-KO

4   female mice have delayed sexual maturation, which has a negative influence on their

5   reproductive functions. To summarize GH and IGF-I have been implicated in the

6   following aspects pertinent to female reproduction: 1) regulation of the timing of puberty

7   onset and the progression of sexual maturation, 2) regulation of GnRH and gonadotropin

8   release from the hypothalamus and pituitary, respectively, and, 3) modulation of ovarian

9   follicular growth and development, steroidogenesis, and apoptosis. (Zazcek et al., 2002)

10   The present study was undertaken in order to examine the effects of diet sucrose

11   supplementation on sexual maturation in GHR-KO mice.

12   From the results presented previously one generalization can be made in

13   particular: sucrose supplementation speeds up final sexual maturation in GHR-KO female

14   mice. There was no significant difference in MAFC in sucrose supplemented GHR-KO

15   females compared to N-SS (p=0.58) or to N-U (p=0.51) but it was advanced significantly

16   (p=0.008) compared to MAFC in GHR-KO mice maintained on the control diet.

17   Treatment group also significantly affected ROW and RUW. There was a significant

18   difference in ROW between KO-SS compared to N-SS (p=0.0006) and to N-C

19   (p=0.0076) furthermore there was no significant difference between KO-C compared to

20   N-SS (p=0.1636) and N-C (p=0.5617) but ROW was not significantly different between

21   KO-SS and KO-C (p=0.0866). There was no significant difference in RUW for KO-SS

22 compared to N-SS (p=0.8631) or to N-U (p=0.8274) but it RUW was significantly lower

23 in KO-SS compared to KO-U (p=0.005).

24   It is known that in women a drastic reduction in the size of the adipose reserve, as

25 happens in overtrained athletes or in pathological situations is associated with

26 amenorrhea and infertility, and this association persists until the body mass index returns

27 to normal values (Casabiell, 2001). Menstrual dysfunction is common in active women,

28 especially competitive athletes who participate in lean-build sports. Although the

29 mechanism for this menstrual dysfunction has not yet been identified, it is now clear that

30 negative energy balance, due to high-energy expenditure and inadequate energy intake, is

31 a major contributing factor (Manore, 2000). For some athletes, negative energy balance is

32 due to purposeful energy restriction to either maintain or achieve a low bodyweight. Both

33 the restrictive energy intake and the exercise-induced menstrual deficiency in active

34 women that may result can have serious health consequence (Manore, 2002). Eating

35 disorders during pregnancy are associated with miscarriage, low birth weight, obstetrics

36 complications and post-partum depression furthermore amenorrhea due to starvation is a

37 diagnostic criterion for anorexia (James, 2001). Menstrual irregularities are common in

38 women with bulimia. Inadequate body fat results in inadequate estrogen levels because

39 estrogen is stored in body fat (James, 2001). Lowered estrogen levels may lead to

40 infertility in these women with eating disorders. Although anovulation is common, long-

41 term infertility is not. Seventeen percent body fat is required to begin or resume normal

42 menses (James, 2001). In Type II diabetes, tissues of the body such as muscle, liver and

43 fat are resistant to the action of insulin (known as insulin resistance) while the pancreas

44 produces some, but not enough, insulin to overcome this resistance. As a result, blood

45   sugar (glucose) backs up in the bloodstream. Insulin receptors and insulin signaling

46   proteins throughout the central nervous system play an integral role in regulating

47   metabolism, as well as appetite and fertility (Morris F., 2005). In another study Morris et

48   al. (2005) concluded that when considered with previous studies, this study demonstrates

49   that genetically determined insulin resistance in classical insulin target tissues, such as

50   muscle and fat, may combine with insulin resistance in non-classical target tissues, such

51   as the brain and beta cell. This interaction may occur synergistically, resulting in obesity,

52   insulin resistance, glucose intolerance, and high blood fat levels, leading to the complex

53   metabolic syndrome associated with type II diabetes (Morris F., 2005). Women with

54   insulin-dependent diabetes mellitus (Type I Diabetes) who had been diagnosed prior to

55   menarche had a higher probability of delayed menarche, and were at higher risk for

56   development of menstrual disturbances, including amenorrhea with subsequent fertility

57   disorders (Yeshaya A., 1995).

58   The mechanism by which dietary sucrose normalizes final sexual maturation time

59   in knockout gene mice is not yet understood. GHR-KO mice are very sensitive to insulin

60   which is due to the low amount of insulin that they produce and to the up-regulation of

61   the insulin receptors in their liver (Kimura et al., 2004). Increased levels of glucose in the

62   blood lead to an increase in levels of insulin, which through some negative feedback

63   mechanism decreases the amount of prolactin (Kimura et al., 2004). In the present study

64   while body weights did not differ significantly in those GHR-KO females maintained on

65   control or sucrose-supplemented diets, the mean body weight of sucrose supplemented

66   females was numerically higher, by about 10%, than the mean weight of non-

67   supplemented GHR-KO mice. Leptin is a 16kDa protein that is secreted almost

68   exclusively by the adipocytes. Therefore increased body fat would in turn increase leptin

69   secretion. The main action of leptin is to decrease appetite and increase energy

70   expenditure (Messinis, 1999). Mutations in the ob gene (ob/ob) in mice that lead to leptin

71   deficiency result in hyperphagia and profound obesity as well as diabetes, insulin

72   resistance and infertility (Messinis, 1999). Evidence has been provided since the early

73   1970s that fat mass may directly affect ovulation and fertility. In particular, anorectic

74   women demonstrate a decreased response of luteinizing hormone (LH) to gonadotrophin-

75   releasing hormone (GnRH) and, as in pre-pubertal women; follicle stimulating hormone

76   (FSH) response is greater than that of LH (Messinis, 1999). Furthermore a critical body

77   weight is required for a girl at menarche. All of these observations together with the

78   recent isolation of leptin, support the hypothesis that leptin is probably the missing link

79   between body fat and reproduction (Messinis, 1999). Since insulin is known to increase

80   leptin mRNA in adipocytes, it is possible that insulin may stimulate the secretion of

81   leptin, and, therefore, leptin may participate in certain cases of PCOS (Messinis, 1999).

82   While we did not measure leptin levels in our female mice, this would be an important

83   parameter to assess in subsequent studies.

84   Insulin, a pancreatic peptide hormone produced in the ß-cells of the islets of

85   Langerhans, plays a major role in the regulation of carbohydrate, fat, and protein

86   metabolism. The classical target organs for insulin action are muscle, adipose tissue, and

87   liver. While the pituitary ovarian regulators, LH and FSH, are of paramount importance

88   to ovarian function, the insulin-related ovarian regulatory system likewise participates in

89   normal follicle development. Its alterations may be important in the ovarian dysfunctions

90   observed in a number of disorders, including diabetes mellitus, obesity, polycystic ovary

91   syndrome (PCOS), and syndromes of extreme insulin resistance (Poretsky et al., 1999). In

92   vitro, insulin stimulates ovarian steroidogenesis by both granulosa and thecal cells,

93   increasing production of androgens, estrogens, and progesterone (Poretsky et al., 1999).

94   Another group of studies has examined the effects of food intake or oral or intravenous

95   administration of glucose on circulating androgen concentrations. In normal women,

96   Parra et al. (1995) found an increase in free testosterone and no change in androstendione

97   after breakfast, but a decline of free testosterone after an oral glucose load. The role of

98   insulin in the ovary may be summarized as follows: 1) insulin receptors are widely

99   distributed throughout all ovarian compartments, 2) at this time there is no convincing

100   direct in vivo evidence that hyperinsulinemia acutely stimulates ovarian steroid

101   production, but there is direct in vitro evidence and indirect in vivo evidence for a

102   stimulatory effect of insulin on ovarian steroidogenesis, 3) the effects of insulin on

103   ovulation and the ovary are complex (Poretsky et al., 1999). A threshold level of insulin

104   is likely to be required for the normal function of the hypothalamic-pituitary-ovarian axis,

105   either because of the direct stimulatory effects of insulin on this axis or because of the

106   stimulatory effects of insulin on leptin secretion (both direct, with insulin stimulating

107   adipocyte production of leptin, and indirect, because of insulin-stimulated lipogenesis

108   (Poretsky et. al, 1999). On the other hand, excessive circulating insulin, particularly in the

109   setting of insulin resistance, may enhance ovarian androgen production and thus may

110   contribute to the development of anovulation (Poretsky et al., 1999). Insulin may amplify

111   its own effects, the effects of IGFs, and those of gonadotropins by up-regulating type I

112   IGF receptors and gonadotropin receptors, as well as by inhibiting production of IGFBP-

113   1, both in the liver and ovary (Poretsky et al., 1999). In the setting of insulin resistance

114   and hyperinsulinemia, therefore, a cycle of events that leads to a self-perpetuating

115   amplification of the ovarian effects of insulin and IGFs can develop (Poretsky et al.,

116   1999). While we did not measure plasma insulin in our animals in this study, presumably,

117   sucrose consumption would at least transiently elevate endogenous insulin levels, and

118   sucrose supplementation may increase the rate at which sexual maturation proceeds due

119   to changes in plasma insulin levels that affect any or all of the aforementioned sites in the

120   hypothalamic-pituitary-gonadal axis.

121   Actions of PRL on luteal function depend on species and the stage of the estrous

122   cycle. In rodents, prolactin can either be luteotrophic after mating or luteolytic in the

123   absence of a mating stimulus (Freeman, 2000). Ameral et al. (2003) studied the effect of

124   prolactin on the insulin signaling pathway in neonatal rat pancreatic islets and they found

125   that PRL significantly potentiates glucose-induced insulin secretion in islets cultured for

126   7 days moreover, decreased levels of PRL in turn increase levels of Activin A. The study

127   by Tamura et al. (2000) demonstrated that Activin A stimulated basal GH secretion and

128   inhibited basal PRL secretion in GH3 cells via modulation of the transcription of the GH

129   and PRL genes. Therefore maybe PRL and Activin A inhibit each other thus when

130   prolactin levels are low, Activin A levels would be high. Increased Activin levels

131   stimulate secretion of FSH or increase the number of cells secreting FSH (Miyamoto et

132   al., 2003). Miyamoto et al. (2003) results suggest that (1) Activin A has effects on female

133   rat pituitary cells that increase not only the amount of FSH secretion per cell, but also the

134   number of FSH-secreting cells, and (2) Activin A also decreases both the amount of PRL

135   secretion per cell and the number of PRL-secreting cells. Since FSH is important in

136   follicle stimulation, increased amounts of FSH increases stimulation of ovarian follicles

137 thus leading to faster cycling and ovulation, which could be how sucrose supplementation

138 induces earlier final sexual maturation in GHR-KO female mice. Future studies that

139 measure plasma prolactin and prolactin receptor levels in the hypothalamus, pituitary

140 gland, ovaries and uterus would elucidate further the role of prolactin in reproduction.

141 Studies that examine the connection between sucrose nutritional supplementation of

142 GHR-KO mice and sexual maturation and reproduction are really important because

143 these findings can help develop new ways of counteracting the negative effects that

144 diseases such as Type I Diabetes have on the reproductive functions of the people

145 affected by the disease. Future research that studies the mechanism by which sucrose

146 affects sexual maturation is warranted.

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