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

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Abstract

Growth hormone (GH) exerts its cell division-stimulating (mitogenic) effect not directly on cells but indirectly through the mediation of a mitogen whose synthesis and release are induced by growth hormone. This mitogen is called insulin-like growth factor I (IGF-I). The secretion and activity of IGF-I can be influenced by the nutritional status of the individual and by many hormones other than GH. GH resistance occurs in human Laron type dwarfism due to mutations in the GH receptor (GHR) and a mammalian model for GH resistance has been developed in mice through targeted disruption or "knockout" (KO) of the GHR. In GHR-KO mice, GH resistance leads to quantitative deficits in reproductive development and functions, but does not preclude fertility in either sex (Bartke et al., 1999). Secondary consequences of GH resistance in mice include hyperprolactinemia, hypoinsulinemia, IGF-I insufficiency, mild hypothyroidism, and decreased core body temperature. Many of these phenotypic manifestations are consistent with and suggestive of nutritional stress. Sexual maturation and adult reproductive function are tightly linked to nutritional status. Delayed puberty onset and retarded progression of sexual maturation in GHR-KO mice may be due, at least in part, to nutritional stress exacerbated in rapidly growing young and adolescent individuals. Dietary sucrose supplementation aimed at alleviating nutritional stress in the juvenile-to-adult transition period did not advance puberty onset in GHR-KO mice. The current study attempts to examine the effects of sucrose supplementation on late stages of sexual maturation in GHR-KO mice. Specifically, we tested the hypothesis that dietary sucrose supplementation during the juvenile-to-adult transition would promote more rapid progression of later stages of sexual maturation and advance maternal age at first

conception (MAFC) in female GHR-KO mice. At 65 days post-partum normal and GHR-KO female mice were randomly assigned to one of four treatment groups: normal untreated (N-U, n=15), GHR-KO untreated (KO-U, n=15), normal sucrose-supplemented (N-SS, n=15) and GHR-KO sucrose-supplemented (KO-SS, n=15). Untreated control mice received normal lab chow diets throughout the remainder of the study, while treated females received standard lab chow supplemented with sucrose (2 parts chow: 1 part sucrose). Five days later females were paired with normal adult males and subsequently monitored daily for pregnancy and parturition. Litter size was recorded and pups were removed on the day of delivery. Females were sacrificed 7 days post-partum and body, uterine, and ovarian weights were recorded. Analysis of variance and Fischer PLSD post hoc analysis were used to determine significant effects of genotype, treatment, and genotype x treatment interaction between groups, with significance at $p<0.05$. MAFC was significantly affected by genotype ($p=0.003$), treatment ($p=0.034$), and genotype x treatment interaction ($p=0.024$). Regardless of treatment, normal mice conceived first litters roughly 20 days earlier than untreated GHR-KO females (N-U=83.7 +/- 2.3 days, N-SS=84.2 +/- 3.4 days, KO-U=103.6 +/- 5.8 days). MAFC in sucrose supplemented GHR-KO females (KO-SS=87.0 +/- 2.9 days) did not differ significantly from that of N-SS ($p=0.58$) or from N-U ($p=0.51$) but was advanced significantly ($p=0.008$) compared to KO-U. These results support our hypothesis and warrant further research that studies the mechanism(s) by which sucrose affects the rate of progression of sexual maturation GH resistant states.

Introduction

Growth hormone (GH), secreted by the anterior pituitary, has little or no effect on fetal growth, but is the most important hormone for postnatal growth. Growth hormone exerts its cell division-stimulating (mitogenic) effect not directly on cells but indirectly through the mediation of a mitogen whose synthesis and release are induced by GH. This mitogen is called insulin-like growth factor I (IGF-I). The importance of IGF-I in mediating the major growth-promoting effect of growth hormone is illustrated by the fact that dwarfism can be due not only to decreased secretion of growth hormone, but also to decreased production of IGF-I or failure of the tissues to respond to IGF-I.

In humans, growth hormone insensitivity syndrome, Laron Dwarfism, is due to genetic mutations that cause the growth hormone receptor to fail to respond to growth hormone. The result is failure to produce IGF-I in response to growth hormone. Thus female mice with GH resistance due to targeted disruption of the GH receptor/GH binding protein gene (GHR-KO mice) have suppressed plasma IGF-I levels, reduced growth, and the dwarf phenotype (Danilovich et al., 1999) suggesting that GHR-KO mice mimic the human condition in many ways. The most notable similarities include growth retardation, delayed sexual maturation, severely depressed serum IGF-I levels and a complete inability to utilize GH (List et al., 2001).

In mice and humans GH resistance leads to quantitative deficits in reproductive development and functions, but does not preclude fertility in either sex. (Bartke et al., 1999) The secretion and activity of IGF-I can be influenced by the nutritional status of

the individual and by many hormones other than growth hormone. Estrogen also stimulates the secretion of IGF-I by cells of the uterus and ovaries. Growth hormone (GH), insulin-like growth factor (IGF-I), and prolactin (PRL) can influence various aspects of reproductive functions in both females and males. Bartke et al. (1999) suspect that PRL and the GH-IGF-I axis provide partially overlapping (redundant) regulatory inputs to the hypothalamic-pituitary-gonadal (HPG) axis, and consequently, targeted disruption of either signaling pathway has relatively mild consequences on many functions related to reproduction. Growth hormone and the main mediator of its action, IGF-I, can exert direct and indirect effects on gonadal function and these actions are particularly evident during sexual maturation. The GHR/BP -/- (aka GHR-KO) mice do not tend toward obesity, exhibit normal to low glucose levels, and have severely decreased insulin levels when compared to normal (+/+) animals (List et al., 2001). GH and IGF-I and their receptors are widely distributed throughout the HPG axis, along with numerous binding proteins and proteases known to regulate GH/IGF bioavailability and action (Zazcek et al., 2002). GH antigens are identified in pituitary cells containing FSH and LH messenger RNAs and GnRH receptors, indicating that either GH cells are transitory gonadotrophs, or GH is present in these pituitary cells, possibly to control their function (Chandrashekar et al., 1999). Furthermore, GH binding protein antigens were identified in pituitary cells that contained LH and FSH, indicating a paracrine effect of GH on the function of the gonadotrophs (Chandrashekar et al., 1999). GH and IGF-I have been implicated in the following aspects pertinent to female reproduction: 1) regulation of the timing of puberty onset and the progression of sexual maturation, 2) regulation of GnRH and gonadotropin release from the hypothalamus and pituitary, respectively, and,

3) modulation of ovarian follicular growth and development, steroidogenesis, and apoptosis (Zazcek et al., 2002). In addition, GH and IGF-I influence numerous processes related to pregnancy including oocyte maturation and fertilization, early embryo development, implantation, fetal/placental growth and development, and litter size (Zazcek et al., 2002).

Fulgeshu et al. (1997) evaluated the influence of insulin level on the ovarian response to FSH when inducing ovulation in patients affected by polycystic ovarian syndrome (PCOS) and found that while ovulation rate was similar in normoinsulinemic and hyperinsulinemic subjects, the incidence of ovarian hyperstimulation was significantly higher in the latter group. Yoshimura et al. (1996) conducted a study, which provided proof that addition of IGF-I to the perfusate stimulated follicular growth and the resumption of meiosis in follicular oocytes and increased estradiol production in cultured rabbit ovaries in a dose dependent manner.

Type I diabetes is associated with abnormalities of the GH-IGF-I axis. Such abnormalities include decreased circulating levels of IGF-I (Bartke et al., 1999). Type 1 diabetes has received particular attention because the relative portal insulin deficiency of this condition is thought to be responsible for the reduced circulating levels of IGF-I which in turn, through decreased negative feedback, leads to increased secretion of GH. The metabolic profile of GHR-KO mice suggests these animals may experience some level of nutritional stress, but to date it has not been established how nutritional stress effects sexual maturation and reproductive functions in GHR-KO mice.

Materials and Method

GHR-KO (-/-) and normal (N, +/+ or +/-) control mice were produced by mating +/- or -/- males to +/- females in our breeding colony, which was derived from our original GHR/GHBP KO (Zhou et al., 1997b) crossed with animals derived from C57BL/6 x C3H hybrids and thereafter bred in closed colony at the Southern Illinois University animal facility. Mice were kept in the SIUC vivarium at strict light and temperature conditions, which included a 12L/12D photoperiod at $22+2^0$ C. Once ther animals reached weaning age (21 days) they were segregated by sex but not by genotype and housed with up to five other mice of the same sex per cage. Subsequently ad libitum access to tap water and standard lab chow (Purina Formulab 5008 Rodent; El Mel Inc., Florissant MO) was given. At 65 days post-partum normal and GHR-KO female mice were randomly assigned to one of four treatment groups: normal untreated (N-U, n=15), GHR-KO untreated (KO-U, n=15), normal sucrose-supplemented (N-SS, n=15) and GHR-KO sucrose-supplemented (KO-SS, n=15). Untreated control normal and GHR-KO female mice received normal lab chow diets throughout the remainder of the study, while sucrose supplemented females were fed standard lab chow and sucrose on a ratio of 2 parts chow: 1 part sucrose. Five days after the sucrose supplemented females were started on the new 2 parts chow: 1 part sucrose diet, they were paired with normal adult males and subsequently monitored daily for vaginal plugs (between 0800 and 1030 am) and parturition to determine effects of sucrose supplementation on latency to first mating. All cages contained one GHR-KO female and one normal female to ensure that both GHR-KO and normal females would be similarly exposed to any male effects (e.g. delayed puberty, male sub-fertility/infertility). The mean age of females at pairing did not differ

between groups. There is distinct size differences between normal males and GHR-KO females at 65 days post-partum which, contribute to occasional injuries to the female during mating and consequently a total of 5 GHR-KO females died in the course of the study because of physical injuries caused by the normal males during mating. The date of delivery and litter size was recorded and pups were removed on the day of delivery. Females were sacrificed 7 days post-partum and body, uterine, and ovarian weights were recorded. Analysis of variance and Fisher post hoc analyses were used to determine significant effects of genotype, treatment, and genotype x treatment interaction between groups with significance at $p<0.05$. Experiments were approved by the University Animal Care and Utilization Committee and were conducted in accordance with NIH (national institute of health) animal care guideline.

Results

To determine the effect of sucrose supplementation on the rate at which sexual maturation proceeds and fertility is achieved, we analyzed maternal age at first conception (MAFC) in normal and GHR-KO mice maintained on standard lab chow (untreated control) diets or sucrose supplemented diets during the juvenile-to-adult transition period. MAFC was significantly affected by genotype ($p=0.003$), treatment ($p=0.034$), and genotype x treatment interaction ($p=0.024$) (Fig.1). Regardless of treatment, normal mice conceived first litters roughly 20 days earlier than untreated GHR-KO females (N-U=83.7 +/- 2.3 days, N-SS=84.2+/- 3.4 days, KO-U=103.6 +/- 5.8 days). MAFC in sucrose supplemented GHR-KO females (KO-SS=87.0+/- 2.9 days) did not differ significantly from that of N-SS ($p=0.58$) or from N-U ($p=0.51$) but was advanced significantly ($p=0.008$) compared to MAFC in GHR-KO mice maintained on the control diet. All normal females in the study conceived and delivered first litters. Consistent with previous mating studies in GHR-KO mice, a subset ($n= 5$) of GHR-KO females died (2 KO-U and 3 KO-SS) due to mating related trauma and a subset ($n=10$) of GHR-KO females (6 KO-U and 4 KO-SS) failed to conceive when housed continually with males of proven fertility for 110 or more days (Table 1).

For those females that did conceive we used analysis of variance and Fisher post hoc comparisons to determine significant genotype, treatment, and/or genotype x treatment interaction effects on litter size between groups. Consistent with previous studies, litter size was significantly influenced by genotype ($p=0.001$), but no significant effects of treatment or genotype x treatment interaction was found ($p=0.253$ and $p=0.572$, respectively) (Fig. 2) Regardless of treatment, normal females had larger-sized litter, by

roughly 2 pups per litter, compared to GHR-KO counterparts (N-U=6.1 +/- 0.4 pups/litter, N-SS=5.1 +/- 0.6 pups/litter, KO-U=3.7+/-0.9 pups per litter, and KO-SS=3.4 +/- 0.3 pups/litter).

Because sexual maturation and fertility are intimately tied to nutritional status, which is reflected in fat stores and body weight, we used similar analyses to detect significant differences in body weight [at sacrifice] between groups due to genotype, treatment, and/or genotype x treatment interactions. Genotype, but not treatment or genotype x treatment interaction, significantly affected body weight at sac ($p<0.0001$, $p=0.58$, and $p=0.32$, respectively) (Fig. 3). Body weight at sacrifice was similar in normal females, regardless of treatment (N-U=21.4 +/- 1.0g and N-SS=20.9 +/- 0.5g, $p=0.69$) and was roughly 40% greater than that of untreated or sucrose supplemented GHR-KO females, who did not differ from one another (KO-U=13.3 +/- 0.8g and KO-SS=14.9 +/- 1.4g, $p=0.35$).

Absolute ovarian weight at sacrifice was also significantly affected by genotype ($p<0.0001$) but not by treatment ($p=0.78$) nor by genotype x treatment interactions ($p=0.34$) (fig. 4). Absolute ovarian weights of normal mice were similar between treatment groups (N-U=9.4 +/- 0.6g, N-SS=9.8 +/- 0.4g, $p=0.55$) and roughly double that of ovary weights in GHR-KO females on standard or sucrose supplemented diets (KO-U=5.5 +/- 0.6g, KO-SS= 4.7 +/- 0.5g, $p=0.46$). However, relative ovarian weight (ROW= ovary weight expressed as a percentage of total body weight) was significantly affected by genotype ($p=0.005$) and genotype x treatment interactions ($p=0.04$). Sucrose supplementation differentially influenced ROW in normal and GHR-KO mice, producing slight but not significant increases in ROW in normal mice (N-U=0.044 +/- 0.002% vs.

N-SS=0.047 +/- 0.002%, p=0.28). In contrast, sucrose supplementation resulted in slight decreases in GHR-KO mice that tended toward significance (KO-U=0.041 +/- 0.004% vs. KO-SS= 0.033 +/- 0.004%, p=0.09) and rendered KO-SS females significantly different from N-U females (p=0.008) and N-SS females (p=0.006).

Analysis of variance revealed no significant effects of genotype (p=0.19), treatment (p=0.14), nor genotype x treatment interaction (p=0.21) on absolute uterine weight at sacrifice (fig. 5). However, mean absolute uterine weight at sacrifice in sucrose supplemented GHR-KO mice was 71.4 +/- 5.2g, while in all other treatment groups mean absolute uterine weights were approximately 100g (N-U=105.4 +/- 14.1g, N-SS=102.5 +/- 5.3g, and KO-U=104.9 +/- 12.5g). In Fisher post hoc analysis, two-way comparisons between groups revealed significant differences between KO-SS and N-U groups (p=0.04), nearly significant differences between KO-SS and N-SS groups (p=0.06) and a trend toward significant differences between KO-SS and KO-U groups (p=0.10). Relative uterine weight (uterine weight expressed as a percentage of total body weight) was significantly affected by genotype (p=0.009), treatment (p=0.020) and genotype x treatment interaction (p=0.017). Sucrose supplementation did not affect RUW in normal mice (N-U=0.49 +/- 0.05g, N-SS=0.49 +/- 0.03g, p=0.96). RUW was greater in untreated GHR-KO mice (0.80 +/- 0.11g), with this treatment group being significantly different from both untreated and sucrose-supplemented normal females (p=0.001 and p=0.001, respectively). Sucrose supplementation reduced RUW in GHR-KO mice (0.51 +/- 0.6g) such that KO-SS females were significantly different from untreated GHR-KO mice (p=0.005) but did not differ from untreated or sucrose supplemented normal mice (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

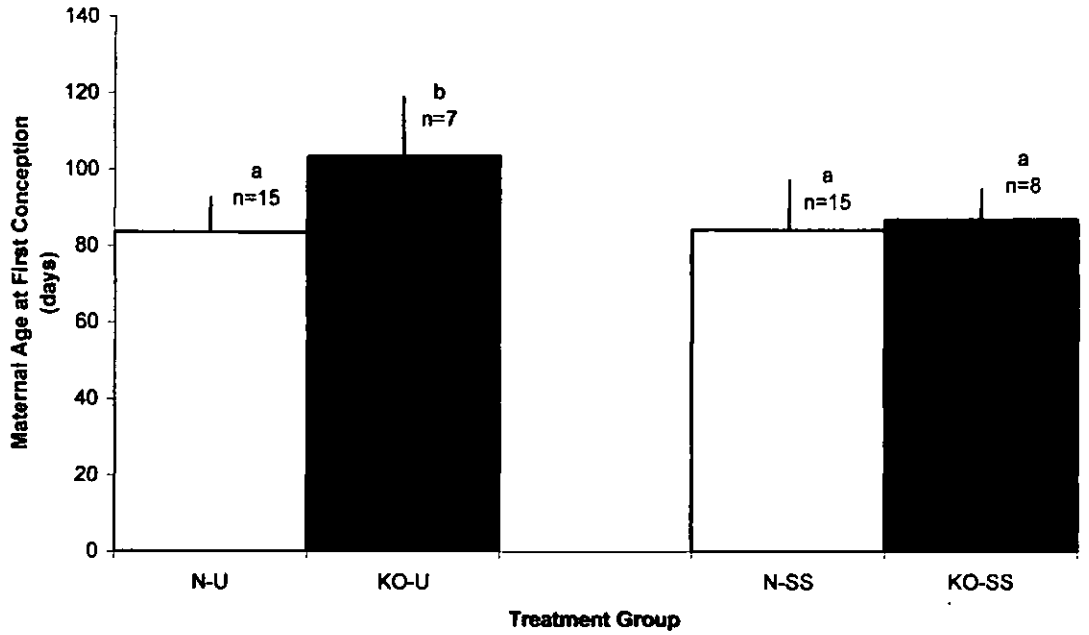

Figure 1: Effect of sucrose supplementation during the juvenile-to-adult transition period (beginning at 65 days post-partum) on the progression of sexual maturation, as assessed by maternal age at first conception, in normal and growth hormone receptor knockout (GHR-KO) mice. Treatment groups= normal 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 $\pm$ 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	0.0082	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

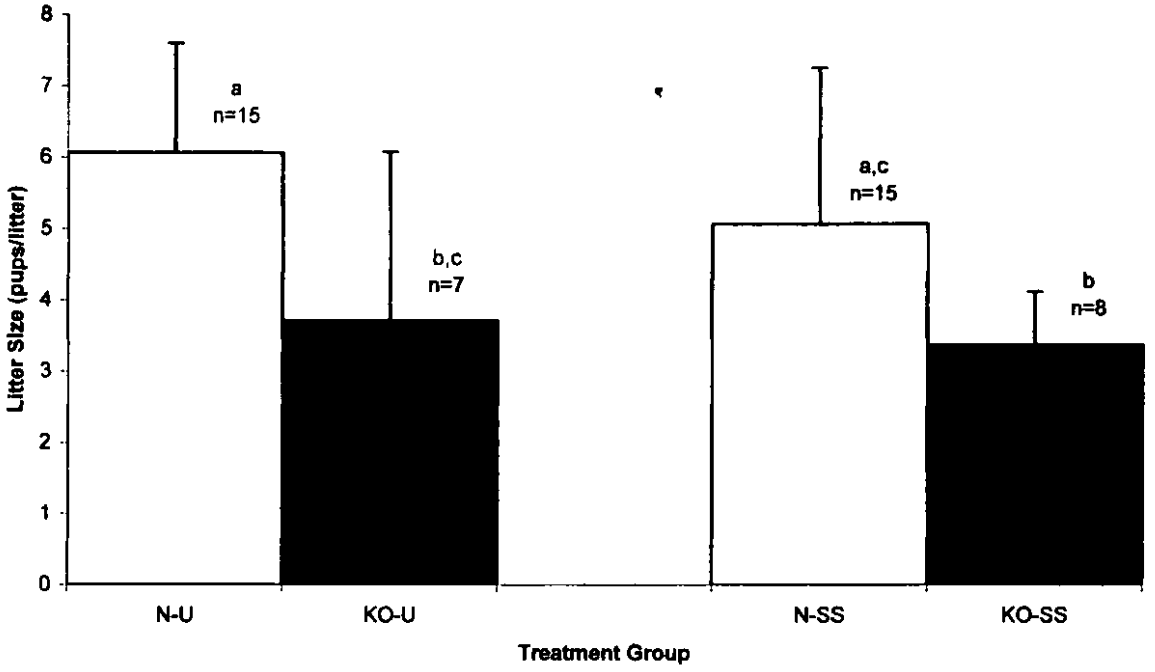

Figure 2: Effect of sucrose supplementation during the juvenile-to-adult transition period (beginning at 65 days post-partum) on litter size in normal and growth hormone receptor knockout (GHR-KO) mice. Treatment groups= normal untreated control (N-U), GHR-KO untreated control (KO-U), normal sucrose supplemented (N-SS), and GHR-KO sucrose supplemented (KO-SS) mice. Bars represent mean ± 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

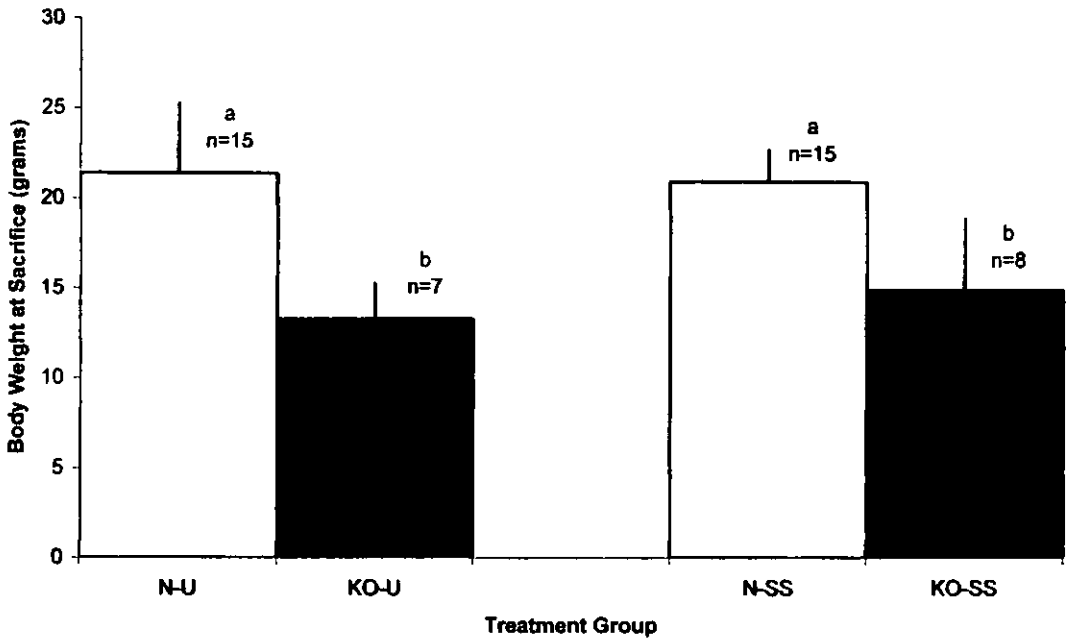

Figure 3: Effect of sucrose supplementation during the juvenile-to-adult transition period (beginning at 65 days post-partum) on body weight in normal and growth hormone receptor knockout (GHR-KO) mice. Treatment groups= normal untreated control (N-U), GHR-KO untreated control (KO-U), normal sucrose 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

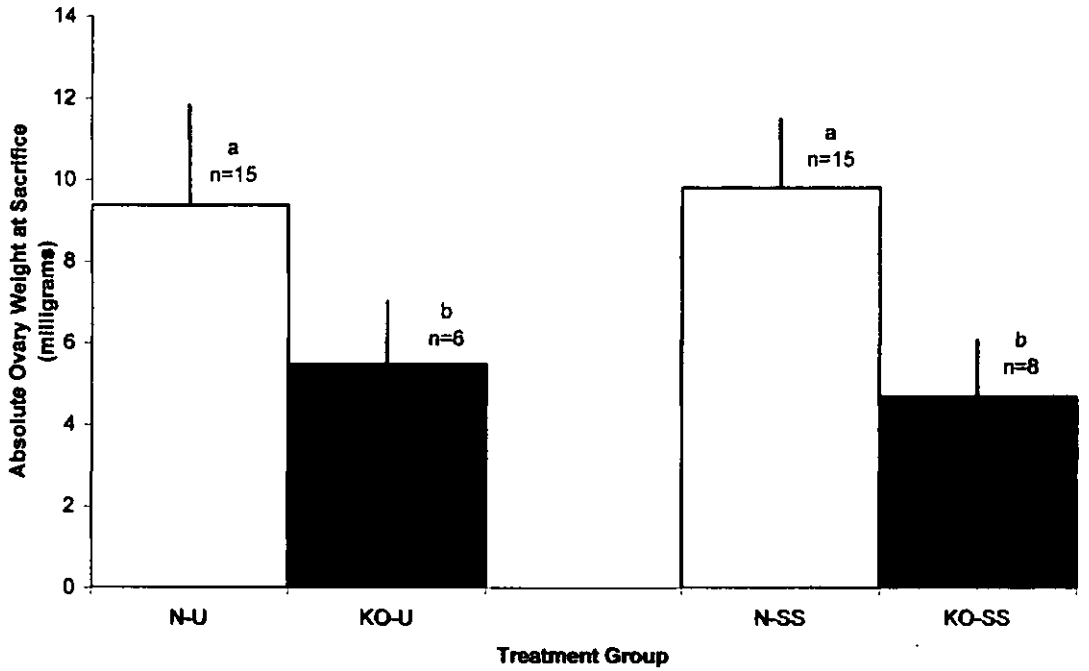

Figure 4: Effect of sucrose supplementation during the juvenile-to-adult transition period (beginning at 65 days post-partum) on absolute ovary weight in normal and growth hormone receptor knockout (GHR-KO) mice. Treatment groups= normal untreated control (N-U), GHR-KO untreated control (KO-U), normal sucrose supplemented (N-SS), and GHR-KO sucrose supplemented (KO-SS) mice. Bars represent mean $\pm$ 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	0.4556	
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

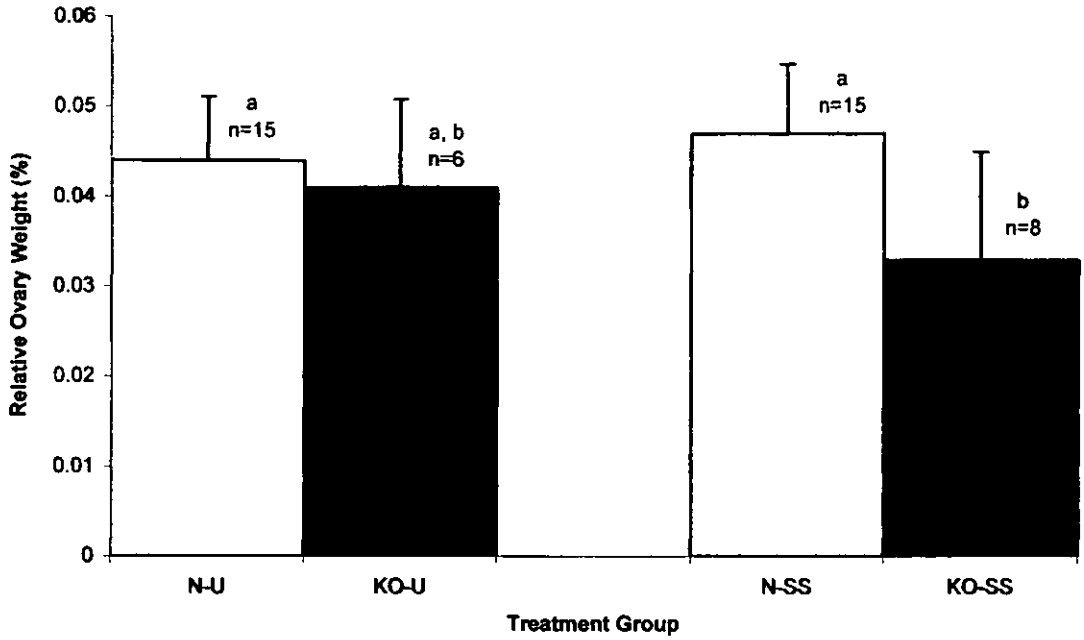

Figure 5: Effect of sucrose supplementation during the juvenile-to-adult transition period (beginning at 65 days post-partum) on relative ovary weight (ROW= ovary weight expressed as a percentage of total body weight) in normal and growth hormone receptor knockout (GHR-KO) mice. Treatment groups= normal 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

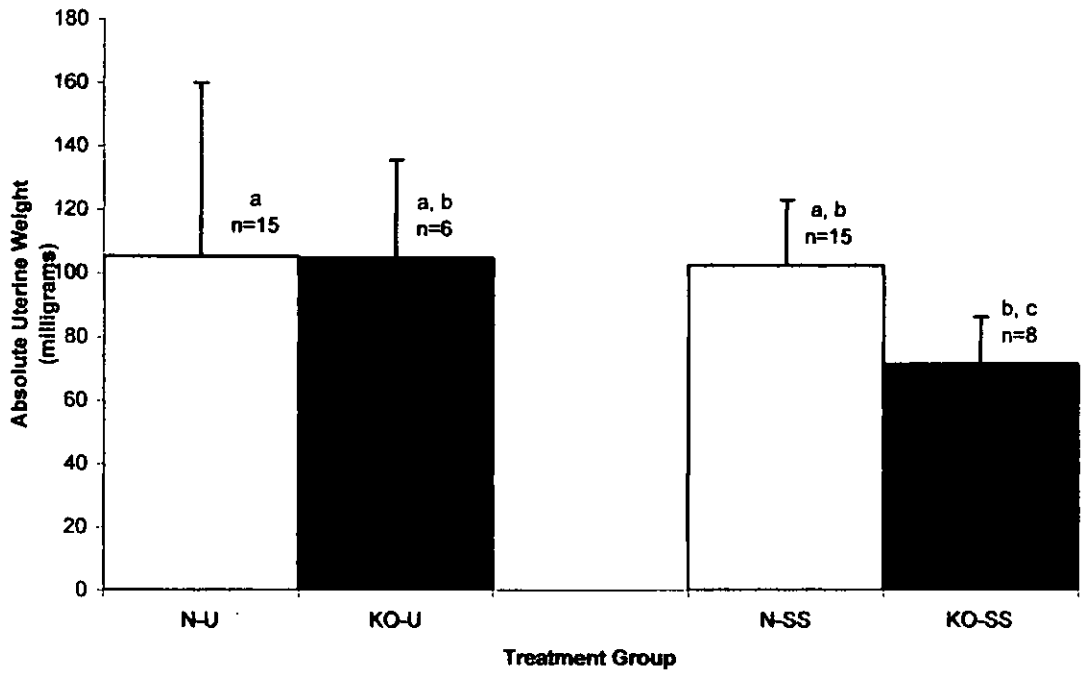

Figure 6: Effect of sucrose supplementation during the juvenile-to-adult transition period (beginning at 65 days post-partum) on absolute uterine weight in normal and growth hormone receptor knockout (GHR-KO) mice. Treatment groups= normal untreated control (N-U), GHR-KO untreated control (KO-U), normal sucrose supplemented (N-SS), and GHR-KO sucrose supplemented (KO-SS) mice. Bars represent mean ± 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

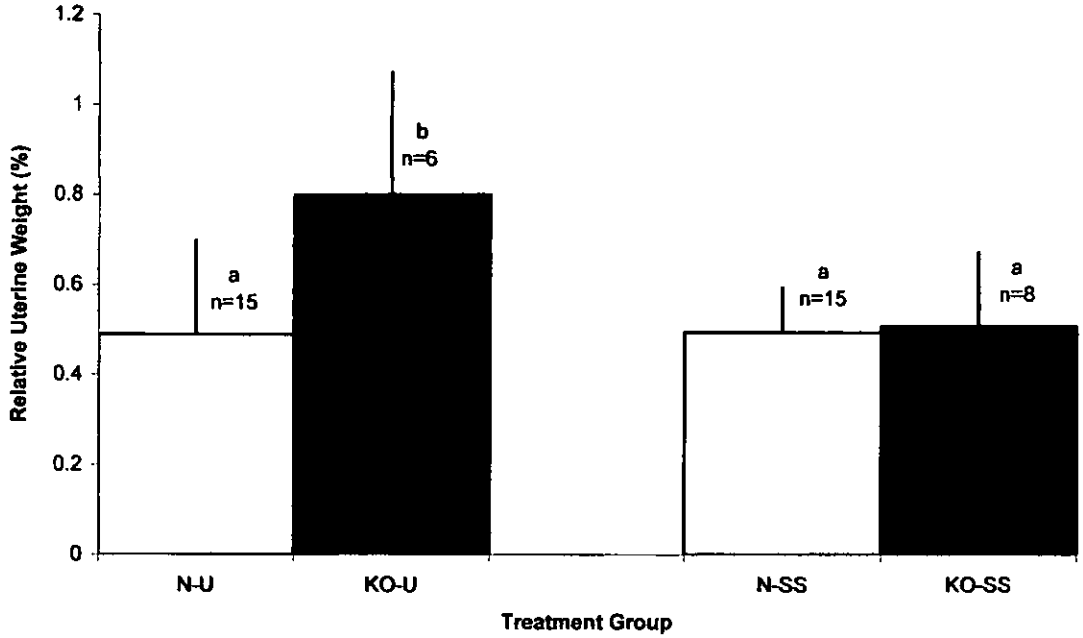

Figure 7: Effect of sucrose supplementation during the juvenile-to-adult transition period (beginning at 65 days post-partum) on relative uterine weight (RUW= uterine weight expressed as a percentage of total body weight) in normal and growth hormone receptor knockout (GHR-KO) mice. Treatment groups= 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 $\pm$ 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	0.0050	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

Growth hormone, secreted by the anterior pituitary, has little or no effect on fetal growth, but is the most important hormone for postnatal growth. Growth hormone induces the release of IGF-I, which in turn exerts its mitogenic effects on cells. GHR-KO female mice have delayed sexual maturation, which has a negative influence on their reproductive functions. To summarize GH and IGF-I have been implicated in the following aspects pertinent to female reproduction: 1) regulation of the timing of puberty onset and the progression of sexual maturation, 2) regulation of GnRH and gonadotropin release from the hypothalamus and pituitary, respectively, and, 3) modulation of ovarian follicular growth and development, steroidogenesis, and apoptosis. (Zazcek et al., 2002) The present study was undertaken in order to examine the effects of diet sucrose supplementation on sexual maturation in GHR-KO mice.

From the results presented previously one generalization can be made in particular: sucrose supplementation speeds up final sexual maturation in GHR-KO female mice. There was no significant difference in MAFC in sucrose supplemented GHR-KO females compared to N-SS ($p=0.58$) or to N-U ($p=0.51$) but it was advanced significantly ($p=0.008$) compared to MAFC in GHR-KO mice maintained on the control diet. Treatment group also significantly affected ROW and RUW. There was a significant difference in ROW between KO-SS compared to N-SS ($p=0.0006$) and to N-C ($p=0.0076$) furthermore there was no significant difference between KO-C compared to N-SS ($p=0.1636$) and N-C ($p=0.5617$) but ROW was not significantly different between KO-SS and KO-C ($p=0.0866$). There was no significant difference in RUW for KO-SS

compared to N-SS ($p=0.8631$) or to N-U ($p=0.8274$) but it RUW was significantly lower in KO-SS compared to KO-U ($p=0.005$).

It is known that in women a drastic reduction in the size of the adipose reserve, as happens in overtrained athletes or in pathological situations is associated with amenorrhea and infertility, and this association persists until the body mass index returns to normal values (Casabiell, 2001). Menstrual dysfunction is common in active women, especially competitive athletes who participate in lean-build sports. Although the mechanism for this menstrual dysfunction has not yet been identified, it is now clear that negative energy balance, due to high-energy expenditure and inadequate energy intake, is a major contributing factor (Manore, 2000). For some athletes, negative energy balance is due to purposeful energy restriction to either maintain or achieve a low bodyweight. Both the restrictive energy intake and the exercise-induced menstrual deficiency in active women that may result can have serious health consequence (Manore, 2002). Eating disorders during pregnancy are associated with miscarriage, low birth weight, obstetrics complications and post-partum depression furthermore amenorrhea due to starvation is a diagnostic criterion for anorexia (James, 2001). Menstrual irregularities are common in women with bulimia. Inadequate body fat results in inadequate estrogen levels because estrogen is stored in body fat (James, 2001). Lowered estrogen levels may lead to infertility in these women with eating disorders. Although anovulation is common, long-term infertility is not. Seventeen percent body fat is required to begin or resume normal menses (James, 2001). In Type II diabetes, tissues of the body such as muscle, liver and fat are resistant to the action of insulin (known as insulin resistance) while the pancreas produces some, but not enough, insulin to overcome this resistance. As a result, blood

sugar (glucose) backs up in the bloodstream. Insulin receptors and insulin signaling proteins throughout the central nervous system play an integral role in regulating metabolism, as well as appetite and fertility (Morris F., 2005). In another study Morris et al. (2005) concluded that when considered with previous studies, this study demonstrates that genetically determined insulin resistance in classical insulin target tissues, such as muscle and fat, may combine with insulin resistance in non-classical target tissues, such as the brain and beta cell. This interaction may occur synergistically, resulting in obesity, insulin resistance, glucose intolerance, and high blood fat levels, leading to the complex metabolic syndrome associated with type II diabetes (Morris F., 2005). Women with insulin-dependent diabetes mellitus (Type I Diabetes) who had been diagnosed prior to menarche had a higher probability of delayed menarche, and were at higher risk for development of menstrual disturbances, including amenorrhea with subsequent fertility disorders (Yeshaya A., 1995).

The mechanism by which dietary sucrose normalizes final sexual maturation time in knockout gene mice is not yet understood. GHR-KO mice are very sensitive to insulin which is due to the low amount of insulin that they produce and to the up-regulation of the insulin receptors in their liver (Kimura et al., 2004). Increased levels of glucose in the blood lead to an increase in levels of insulin, which through some negative feedback mechanism decreases the amount of prolactin (Kimura et al., 2004). In the present study while body weights did not differ significantly in those GHR-KO females maintained on control or sucrose-supplemented diets, the mean body weight of sucrose supplemented females was numerically higher, by about 10%, than the mean weight of non-supplemented GHR-KO mice. Leptin is a 16kDa protein that is secreted almost

exclusively by the adipocytes. Therefore increased body fat would in turn increase leptin secretion. The main action of leptin is to decrease appetite and increase energy expenditure (Messinis, 1999). Mutations in the ob gene (ob/ob) in mice that lead to leptin deficiency result in hyperphagia and profound obesity as well as diabetes, insulin resistance and infertility (Messinis, 1999). Evidence has been provided since the early 1970s that fat mass may directly affect ovulation and fertility. In particular, anorectic women demonstrate a decreased response of luteinizing hormone (LH) to gonadotrophin-releasing hormone (GnRH) and, as in pre-pubertal women; follicle stimulating hormone (FSH) response is greater than that of LH (Messinis, 1999). Furthermore a critical body weight is required for a girl at menarche. All of these observations together with the recent isolation of leptin, support the hypothesis that leptin is probably the missing link between body fat and reproduction (Messinis, 1999). Since insulin is known to increase leptin mRNA in adipocytes, it is possible that insulin may stimulate the secretion of leptin, and, therefore, leptin may participate in certain cases of PCOS (Messinis, 1999). While we did not measure leptin levels in our female mice, this would be an important parameter to assess in subsequent studies.

Insulin, a pancreatic peptide hormone produced in the ß-cells of the islets of Langerhans, plays a major role in the regulation of carbohydrate, fat, and protein metabolism. The classical target organs for insulin action are muscle, adipose tissue, and liver. While the pituitary ovarian regulators, LH and FSH, are of paramount importance to ovarian function, the insulin-related ovarian regulatory system likewise participates in normal follicle development. Its alterations may be important in the ovarian dysfunctions observed in a number of disorders, including diabetes mellitus, obesity, polycystic ovary

syndrome (PCOS), and syndromes of extreme insulin resistance (Poretsky et al., 1999). In vitro, insulin stimulates ovarian steroidogenesis by both granulosa and thecal cells, increasing production of androgens, estrogens, and progesterone (Poretsky et al., 1999). Another group of studies has examined the effects of food intake or oral or intravenous administration of glucose on circulating androgen concentrations. In normal women, Parra et al. (1995) found an increase in free testosterone and no change in androstendione after breakfast, but a decline of free testosterone after an oral glucose load. The role of insulin in the ovary may be summarized as follows: 1) insulin receptors are widely distributed throughout all ovarian compartments, 2) at this time there is no convincing direct in vivo evidence that hyperinsulinemia acutely stimulates ovarian steroid production, but there is direct in vitro evidence and indirect in vivo evidence for a stimulatory effect of insulin on ovarian steroidogenesis, 3) the effects of insulin on ovulation and the ovary are complex (Poretsky et al., 1999). A threshold level of insulin is likely to be required for the normal function of the hypothalamic-pituitary-ovarian axis, either because of the direct stimulatory effects of insulin on this axis or because of the stimulatory effects of insulin on leptin secretion (both direct, with insulin stimulating adipocyte production of leptin, and indirect, because of insulin-stimulated lipogenesis (Poretsky et. al, 1999). On the other hand, excessive circulating insulin, particularly in the setting of insulin resistance, may enhance ovarian androgen production and thus may contribute to the development of anovulation (Poretsky et al., 1999). Insulin may amplify its own effects, the effects of IGFs, and those of gonadotropins by up-regulating type I IGF receptors and gonadotropin receptors, as well as by inhibiting production of IGFBP-1, both in the liver and ovary (Poretsky et al., 1999). In the setting of insulin resistance

and hyperinsulinemia, therefore, a cycle of events that leads to a self-perpetuating amplification of the ovarian effects of insulin and IGFs can develop (Poretsky et al., 1999). While we did not measure plasma insulin in our animals in this study, presumably, sucrose consumption would at least transiently elevate endogenous insulin levels, and sucrose supplementation may increase the rate at which sexual maturation proceeds due to changes in plasma insulin levels that affect any or all of the aforementioned sites in the hypothalamic-pituitary-gonadal axis.

Actions of PRL on luteal function depend on species and the stage of the estrous cycle. In rodents, prolactin can either be luteotrophic after mating or luteolytic in the absence of a mating stimulus (Freeman, 2000). Ameral et al. (2003) studied the effect of prolactin on the insulin signaling pathway in neonatal rat pancreatic islets and they found that PRL significantly potentiates glucose-induced insulin secretion in islets cultured for 7 days moreover, decreased levels of PRL in turn increase levels of Activin A. The study by Tamura et al. (2000) demonstrated that Activin A stimulated basal GH secretion and inhibited basal PRL secretion in GH3 cells via modulation of the transcription of the GH and PRL genes. Therefore maybe PRL and Activin A inhibit each other thus when prolactin levels are low, Activin A levels would be high. Increased Activin levels stimulate secretion of FSH or increase the number of cells secreting FSH (Miyamoto et al., 2003). Miyamoto et al. (2003) results suggest that (1) Activin A has effects on female rat pituitary cells that increase not only the amount of FSH secretion per cell, but also the number of FSH-secreting cells, and (2) Activin A also decreases both the amount of PRL secretion per cell and the number of PRL-secreting cells. Since FSH is important in follicle stimulation, increased amounts of FSH increases stimulation of ovarian follicles

thus leading to faster cycling and ovulation, which could be how sucrose supplementation induces earlier final sexual maturation in GHR-KO female mice. Future studies that measure plasma prolactin and prolactin receptor levels in the hypothalamus, pituitary gland, ovaries and uterus would elucidate further the role of prolactin in reproduction.

Studies that examine the connection between sucrose nutritional supplementation of GHR-KO mice and sexual maturation and reproduction are really important because these findings can help develop new ways of counteracting the negative effects that diseases such as Type I Diabetes have on the reproductive functions of the people affected by the disease. Future research that studies the mechanism by which sucrose affects sexual maturation is warranted.

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