J Assist Reprod Genet (2016) 33:535–543
DOI 10.1007/s10815-016-0672-x

REPRODUCTIVE PHYSIOLOGY AND DISEASE

Ovarian kisspeptin expression is related to age and to monocyte chemoattractant protein-1

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Received: 25 November 2015 / Accepted: 26 January 2016 / Published online: 15 February 2016

Abstract
Purpose The objective of this study was to test the hypothesis that ovarian kisspeptin (kiss1) and its receptor (kiss1r) expression are affected by age, obesity, and the age- and obesity-related chemokine monocyte chemoattractant protein-1 (MCP-1).
Methods Ovaries from reproductive-aged and older C57BL/6J mice fed normal chow (NC) or high-fat (HF) diet, ovaries from age-matched young MCP-1 knockout and young control mice on NC, and finally, cumulus and mural granulosa cells (GCs) from women who underwent in vitro fertilization (IVF) were collected. Kiss1, kiss1r, anti-Mullerian hormone (AMH), and AMH receptor (AMHR-II) messenger RNA (mRNA) expression levels were quantified using real-time polymerase chain reaction (RT-PCR).
Results In mouse ovaries, kiss1 and kiss1r mRNA levels were significantly higher in old compared to reproductive-aged mice, and diet-induced obesity did not alter kiss1 or kiss1r mRNA levels. Compared to young control mice, young MCP-1 knockout mice had significantly lower ovarian kiss1 mRNA but significantly higher AMH and AMHR-II mRNA levels. In human cumulus GCs, kiss1r mRNA levels were positively correlated with age but not with BMI. There was no expression of kiss1 mRNA in either cumulus or mural GCs.
Conclusion These data suggest a possible age-related physiologic role for the kisspeptinergic system in ovarian physiology. Additionally, the inflammatory MCP-1 may be associated with kiss1 and AMH genes, which are important in ovulation and folliculogenesis, respectively.

Keywords Kisspeptin · MCP-1 · Ovary · Obesity · Aging

Capsule These data suggest a possible age-related physiologic role for the kisspeptinergic system in ovarian physiology.

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Introduction

The excitatory neuropeptide kisspeptin (kiss1) and its G protein-coupled receptor (kiss1r) are essential for the regulation of gonadotropin-releasing hormone (GnRH) neurons and puberty [1–3]. Additional actions of kiss1 at the level of the ovaries have been suggested [3–6] but remain scarcely studied. Recent data have shown that the intraovarian kiss1 system is required for properly coordinated ovarian function in humans and animals [3–6]. For example, kiss1r hypomorph (i.e., gene with reduced level of activity) mice displayed a premature decline in ovulatory rate, followed by progressive loss of antral follicles, oocyte loss, and a reduction in all categories of pre-antral follicles [3]. Additionally, in vivo administration of the kiss1 antagonist P234 to the ovarian bursa of

22- to 50-day-old rats delayed vaginal opening and disrupted estrous cyclicity [4]. It was also demonstrated that kisspeptin-10 peptide stimulates progesterone secretion in cultured chicken granulosa cells, a phenomenon that was associated with upregulation of the steroidogenic enzymes: steroidogenic acute regulatory protein (StAR), P450 cholesterol side-chain cleavage (P450scc), and 3 beta-hydroxysteroid dehydrogenase (3β-HSD). Altogether, these findings indicate that kiss1 is locally produced inside the ovaries and that it is involved in normal ovulatory process.

Reproductive senescence, in particular, ovarian aging, remains poorly understood in humans. We and others have documented that reproductive aging is characterized by reduced hypothalamic kiss1 expression [7, 8]. On the other hand, women with diminished ovarian reserve have a significant increase in ovarian sympathetic nerve fibers [9] whose stimulation with β-adrenergic agonist induces ovarian kiss1 expression [4]. Given this background, we hypothesized that ovarian kisspeptin expression is upregulated during ovarian aging.

There is mounting evidence that suggests that hypothalamic kiss1 could be affected by adiposity [10]. For instance, rats subjected to food deprivation demonstrated a significant decrease in kiss1 expression in the hypothalamus leading to a reduction in luteinizing hormone (LH) levels [11, 12]. Likewise, diet-induced obese female DBA/2J mice also showed a marked decrease in kiss1 messenger RNA (mRNA) levels in both the arcuate and the anteroventral periventricular nucleus areas of the hypothalamus compared with normal chow (NC)-fed controls [13]. However, given the newly discovered role of the kiss1 system in the ovaries, it remains to be determined whether ovarian kiss1 expression is affected by obesity.

One of the inflammatory molecules that plays a role in the human ovulatory process and relates to both aging and obesity [14–19] is monocyte chemoattractant protein-1 (MCP-1) [20]. MCP-1, a polypeptide composed of 76 amino acids, is produced by various cell types including endothelial cells, fibroblasts, tumor cells, monocytes, and macrophages [20, 21]. It regulates the migration and infiltration of monocytes, basophils, T lymphocytes, and natural killer cells into various tissues, including the ovaries [22]. In humans, physiologic levels of MCP-1 are important for the ovulatory process [20]. As far as aging and obesity, data have shown that systemic MCP-1 levels are increased in aging animals [14–16] and that there is an increased abundance of this pro-inflammatory chemokine in both white adipose tissue and plasma in obese animals [17–19]. Additionally, we have recently demonstrated in women undergoing in vitro fertilization (IVF) that serum and follicular fluid MCP-1 are positively correlated with body mass index (BMI) (Obehi et al, manuscript under review). Taken together, data to date suggest that the pro-inflammatory MCP-1 might play a role in age- and obesity-related ovarian dysfunction hypothetically by attracting large number of monocytes into the ovaries. Despite these findings, data pertaining to the effect of this age- and obesity-related chemokine on ovarian kiss1 expression and other genes important in follicular development (such as anti-Mullerian hormone [AMH] and AMH receptor [AMHR-II]) are lacking. Because kisspeptin and MCP-1 share a similar intracellular pathway, a relationship between these two molecules could potentially exist. At the hypothalamic level, kisspeptin signals via phosphorylation of MAP kinases, such as ERK1/2 and p38 [23, 24]. Similarly, MCP-1 signals via the same MAP kinases, ERK1/2 and p38, in various tissues [25, 26].

Using a high-fat (HF) diet-induced mouse model of obesity and comparing reproductive-aged mice to older mice, we aimed to evaluate the influence of aging and obesity on the expression of ovarian kiss1/kiss1r system. We also used an MCP-1 knockout mouse model in order to determine whether MCP-1 affects kiss1/kiss1r and AMH/AMHR-II. We thus compared ovarian kiss1, kiss1r, AMH, and AMHR-II gene expression between age-matched wild-type and MCP-1 knockout mice.

In order to determine whether alterations in the kisspeptinergic system are occurring at the level of the granulosa cells (GCs), we evaluated in the current studies kiss1/kiss1r expression in human luteinized GCs obtained from women who underwent IVF. We and others have used human luteinized GCs as a long-standing effective model to characterize ovarian physiology in humans [27–31]. Given the new role of kiss1 in ovarian function, it is important to evaluate whether changes in kiss1/kiss1r expression occur in human GCs with aging and obesity.

Materials and methods

Experimental animals and diets

All protocols were conducted in accordance with the National Institutes of Health guide for the care and use of laboratory animals and were approved by the Institutional Animal Care and Use Committee of Albert Einstein College of Medicine and University of Vermont College of Medicine.

Old and reproductive-aged mice on different diets

C57BL/6J wild-type female mice were obtained from Jackson Laboratory (Bar Harbor, ME) at 5 weeks of age. Mice were housed five to a cage and maintained on a 14-h light, 10-h dark cycle with ad libitum access to standard mouse chow and water. After 1 week of acclimation, mice ($n = 12$) were assigned to receive a normal chow diet (PicoLab® Mouse Diet #5058, chemical composition = 9 % fat, 20 % protein, 53 % carbohydrate, 3.59 kcal/g; LabDiet, St. Louis, MO, USA), while another group of mice ($n = 12$) received high-fat (HF) diet (Diet #F3282, chemical composition = 36 % fat, 20.5 % protein, 35.7 % carbohydrate, 5.49 kcal/g; Bioserve,

Frenchtown, NJ, USA). All mice were weighed weekly in order to ensure that mice given HF diet became obese.

Six mice on normal chow and six mice on HF diet were sacrificed at 20 weeks (reproductive-aged group). The other six mice in the normal chow group continued normal chow while the remaining six mice on HF diet were converted to normal chow and both groups were sacrificed at 32 weeks (older group) by cervical dislocation. At the time of sacrifice, oophorectomy was performed and the ovary was snap frozen in liquid nitrogen and stored in −80 °C to be used for gene expression analysis.

Young MCP-1 knockout mice versus young control mice

Young MCP-1 knockout mice (B6.129S4-Ccl2^{tm1Rol}/J) (5–6 weeks old; $n=6$) and young C57BL/6J wild-type control female mice (5–6 weeks old; $n=5$) were housed five to a cage and bred in house under specific pathogen-free conditions and normal light dark cycles (14-h light, 10-h dark cycle) with ad libitum access to standard mouse chow (ProLab® Isopro®, chemical composition = 5 % fat, 22 % protein, 59 % carbohydrate, 3.46 kcal/g; PMI Nutrition International, Brentwood, MO, USA) and water. All mice were sacrificed at 6 weeks of age by cervical dislocation. At the time of sacrifice, oophorectomy was performed and ovaries were snap frozen in liquid nitrogen and stored in −80 °C to be used for gene expression analysis.

Subjects

Fourteen infertile women undergoing fresh IVF and/or intracytoplasmic sperm injection (ICSI) cycles utilizing autologous oocytes at the University of Vermont College of Medicine (UVM COM) in July and August 2013 were prospectively enrolled. Inclusion criteria consisted of women with normal ovarian reserve defined as day 3 follicle-stimulating hormone (FSH) <10 mIU/mL and day 3 estradiol (E2) <80 pg/mL. Reasons for infertility were male, tubal, unexplained, and uterine factors. Women with polycystic ovary syndrome (PCOS) as defined by the Rotterdam criteria [32] were excluded from the study. All patients gave informed consent and the study was approved by the Institutional Review Board of UVM COM (M13-062).

Collection of GCs

Follicular aspirates included mural GCs and oocytes surrounded by cumulus GCs. The follicular fluid from the first aspirated follicle was not used for gene expression evaluation because of possible contamination with vaginal mucosal cells. After removal of the cumulus–oocyte complex by the embryologist, fluid was pooled from each participant in order to isolate mural GCs.

Mural GCs were isolated and concentrated as we previously described [27]. Briefly, mural GCs were added to 40 % PureCeption gradient solution (Cooper Surgical, Trumbull, CT) then centrifuged to remove red and white blood cells. Cells were then washed with phosphate-buffered saline (PBS) and incubated with CD 45+ tagged magnetic beads (Invitrogen, Carlsbad, CA) for 20 min at +4 °C to remove the remaining white blood cells. The beads were then separated, and the remaining fluid was centrifuged for 5 min at 600×*g* to collect the mural GCs.

After identification of the cumulus–oocyte complex in the aspirate, cumulus GCs were collected by mechanically cutting the cumulus cell layer from each oocyte then washed with PBS. Cumulus GCs collected from each participant were also pooled. The collected mural and cumulus GCs were added on the same day to a tube containing TRIzol reagent (Invitrogen, Carlsbad, CA) then stored at −20 °C to be used for gene expression analysis.

RNA extraction, reverse transcription, and real-time polymerase chain reaction from mural and cumulus GCs as well as mice ovaries

Mice ovaries were lysed and homogenized using a homogenizer. For mice ovaries and for GCs, RNA was isolated using TRIzol reagent (Invitrogen, Carlsbad, CA) and chloroform extraction using QIAGEN's RNeasy mini kit according to the manufacturer's instructions. RNA quality analysis was assessed by a Nanodrop spectrophotometer and Agilent Bioanalyzer (Santa Clara, CA). Only samples with a minimum concentration of 10 ng/μL and with an optical density (OD) 260:280 ratio of 1.8 to 2.0 were used for real-time polymerase chain reaction (RT-PCR). The RNA quality was additionally confirmed using RNA electrophoresis. mRNA expression levels were measured by RT-PCR kinetics using the SYBR Green I chemistry (Roche, Indianapolis, IN) as described elsewhere [27]. The primers used (Table 1) were synthesized by Fisher (Pittsburgh, PA). For human GCs, kiss1 and kiss1r were evaluated. For mouse ovaries, kiss1 and kiss1r, as well as AMH and its receptor AMHR-II (these two genes play a role in ovarian folliculogenesis) were evaluated. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) primers were used as a loading control and the levels of mRNA for each gene relative to GAPDH were calculated using the $2^{-\Delta\Delta CT}$ method [33].

Statistics

Data were expressed as mean ± SEM if normally distributed and median (range) if not normally distributed. RT-PCR results were expressed as relative number of copies ± SEM. Mann-Whitney *U* test or *t* test was used as appropriate according to the data distribution. Because the data were not

Table 1 Primers used for RT-PCR in mouse ovaries and human granulosa cells

Gene	Sequence primers (5′–3′)
Mice	
Kiss1	Forward: ATGATCTCAATGGCTTCTTGG Reverse: CCAGGCATTAACGAGTTCCT
Kiss1r	Forward: GCACATGCAGACAGTTACCAA Reverse: CACGCAGCACAGTAGGAAAGT
AMH	Forward: CGTCACCGCAGCCAGCACA Reverse: CCCGCAGAGCACGAACCAAG
AMHR-II	Forward: CCACAGACCACCACCTTTCC Reverse: GTCTGCGTCCCAGCAATCTT
Humans	
Kiss1	Forward: TGGGGAATTCTAGACCCACAGG Reverse: GTAGCAGCTGGCTTCCTCTC
	Forward: GGACCTGCCTCTTCTCACCAA Reverse: TGCTGGCCTGTGGGTCTAGA
Kiss1r	Forward: GGGAACTCGCTGGTCATCTA Reverse: GTTGACGAACTTGCACATGAA

Kiss1 kisspeptin, *Kiss1r* kisspeptin receptor, *AMH* anti-Mullerian hormone, *AMHR-II* AMH receptor

normally distributed, Spearman correlation was used to evaluate the association between kiss1r and clinical parameters in humans. Because data on kiss1r mRNA was not normally distributed, log transformation was performed, followed by multivariate linear regression to test the correlation between age and logkiss1r in cumulus GCs after adjusting for BMI, total days of ovarian stimulation, and total dose of gonadotropins (IUs). All statistical procedures were run on STATA software (StataCorp). $p \leq 0.05$ was considered statistically significant.

Results

Effect of age- and diet-induced obesity on kiss1 and kiss1r mRNA in mice

Mice on HF diet gained (from 15.7 ± 0.2 g at baseline to 25.8 ± 1.4 g) significantly more weight than mice on normal chow diet (from 15.1 ± 0.2 g at baseline to 18.4 ± 0.3 g; $p = 0.0001$) after a 12-week feeding period (Fig. 1). Regardless of diet and weight, mRNA expression levels of kiss1 and kiss1r were significantly higher in older compared to reproductive-aged mice (Fig. 2). There was no difference in kiss1 mRNA expression between reproductive-aged mice on normal chow ($n = 4$) compared with HF diet ($n = 4$) (2.7 ± 0.9 vs. 3.7 ± 0.2, respectively; $p = 0.25$) or between older mice on normal chow ($n = 5$) compared with older mice on HF converted to normal chow diet ($n = 5$) (11.9 ± 3.4 vs. 19.1 ± 8.4, respectively; $p = 0.9$). Similarly, there was no difference in kiss1r mRNA expression between reproductive-aged mice on normal chow compared with HF diet (1.9 ± 0.1 vs. 2.3 ± 0.5; $p = 0.77$) or between older mice on normal chow compared with older mice on HF converted to normal chow diet (4.4 ± 0.5 vs. 7.5 ± 1.5; $p = 0.1$).

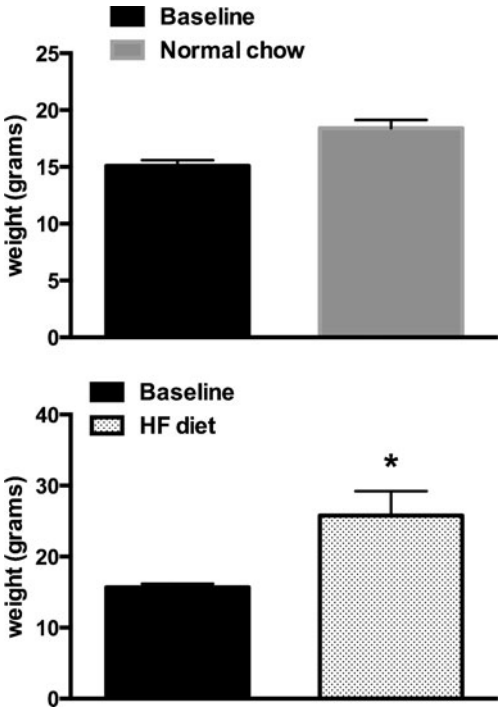

Fig. 1 Weight gain following high-fat (HF) diet. Mice on HF diet ($n = 6$) gained significantly more weight than mice on normal chow diet ($n = 6$) after a 12-week feeding period. Baseline represents 6-week-old mice. *$p < 0.05$

Effect of MCP-1 knockout on kiss, kiss1r, AMH, and AMHR-II mRNA in mice

Young MCP-1 knockout mice ($n = 6$) on normal chow diet had similar weights compared to young wild-type mice controls ($n = 5$) on normal chow diet ($p > 0.05$). Compared to control mice, MCP-1 knockout mice had significantly lower ovarian kiss1 mRNA expression levels ($p = 0.01$) and had a tendency for lower kiss1r mRNA expression levels ($p = 0.1$) (Fig. 3). On the other hand, MCP-1 knockout mice had significantly higher AMH ($p = 0.02$) and AMHR-II ($p = 0.0007$) compared to control mice (Fig. 3).

Humans

Table 2 summarizes the demographics and clinical characteristics of the participants. The age of the participants ranged from 19 to 42. There was no amplification of kiss1 mRNA in our samples despite the use of several types of primers (placental tissue was used as a positive control) and despite previous data indicating its expression in non-luteinized GCs [34]. The kiss1r mRNA levels in cumulus, not mural, GCs were positively correlated with age ($r = 0.71$, $p = 0.01$; $n = 11$

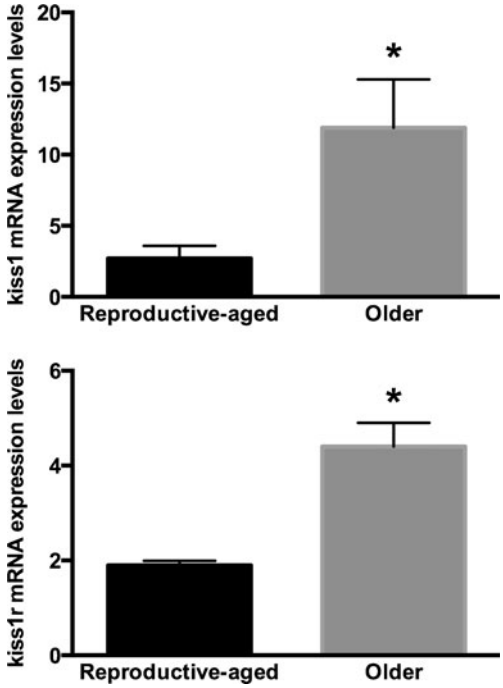

Fig. 2 Ovarian kiss1 and kiss1r expression in relatively reproductive-aged and older mice. Six mice on normal chow or six mice on high-fat diet had oophorectomy at 20 weeks (reproductive-aged group). The remaining six mice in the NC group continued NC while the remaining six on HF diet were converted to NC and both groups had oophorectomy at 32 weeks (older group) of age. Kiss1 and kiss1r mRNA expression levels were significantly higher in older ($n = 10$) compared to reproductive-aged mice ($n = 8$). Diet-induced weight gain did not affect kiss1 or kiss1r mRNA expression levels. *$p < 0.05$

mRNA samples) (Fig. 4). In a multivariate linear regression model adjusting for BMI, total days of ovarian stimulation, and total dose of gonadotropins (IUs), we measured age as a predictor and logkiss1r mRNA in cumulus GCs as the outcome variable of interest. The higher the age, the higher the kiss1r mRNA levels in cumulus GCs mRNA levels ($p = 0.047$). In cumulus GCs, women aged less than 35 ($n = 4$) had significantly lower kiss1r mRNA expression level compared to women aged between 35 and 39 ($n = 6$) who, in turn, had significantly lower kiss1r mRNA expression levels compared to women aged 40 or more ($n = 2$) (4.3 [range = 3.8–4.8], vs. 18 [range = 21–127] vs. 379 [range = 196–562], respectively; $p = 0.004$) (Fig. 5).

In both cumulus and mural GCs, there was no correlation between kiss1r mRNA levels and BMI, baseline day 3 FSH and E2 levels, the total number of days of ovarian stimulation, peak E2 level on the day of HCG maturation trigger, the total dose of gonadotropins administered during the cycle, or the total number of oocytes retrieved (Table 2).

Discussion

The present study evaluated the influence of age and diet-induced obesity on kiss1 and kiss1r mRNA expression levels in mouse ovaries. It also assessed the effect of knocking down MCP-1 gene on ovarian kiss1/kiss1r and AMH/AMHR-II mRNA levels. In order to determine whether alterations in the kisspeptinergic system occur at the level of the GCs, the relationship between age and BMI on kiss1r expression in human cumulus and mural GCs was evaluated. First, the results indicated that kiss1 and kiss1r expression levels in mice were markedly elevated with aging, but were not affected by

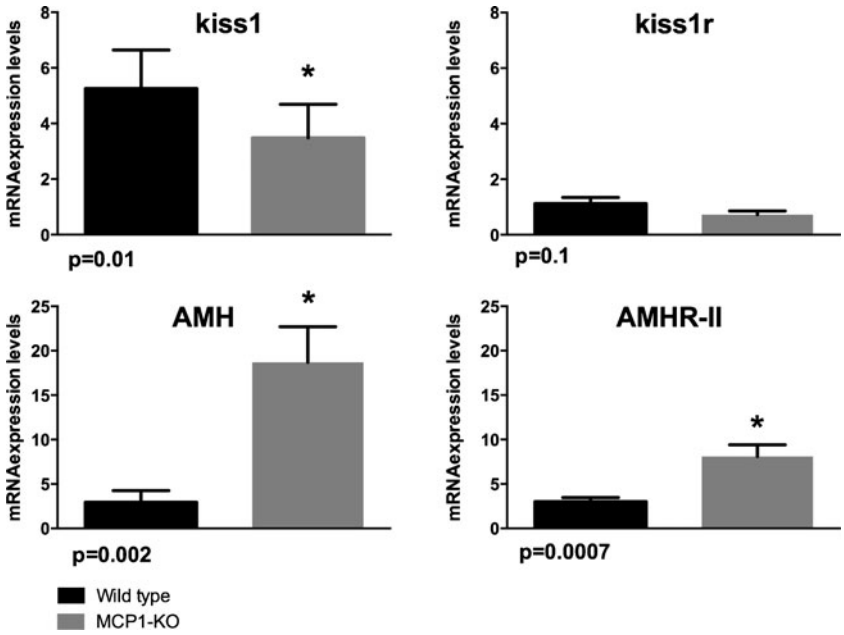

Fig. 3 Ovarian kiss1, kiss1r, AMH, and AMHR-II mRNA expression levels in young MCP-1 knockout and young wild-type mice. Young MCP-1 knockout mice and young C57BL/6J wild-type female mice had oophorectomy at 6 weeks of age. Compared to wild-type mice ($n = 5$), MCP-1 KO mice ($n = 6$) had significantly lower ovarian kiss1 mRNA expression levels ($p = 0.01$) but significantly higher AMH ($p = 0.02$) and AMHR-II mRNA expression levels ($p = 0.0007$). *$p < 0.05$

Table 2 Demographics and clinical characteristics of the participants

All participants ($n = 14$)	Mean ± SEM	Correlation with mural kiss1r	p value	Correlation with cumulus kiss1r	p value
Age (years)	34.6 ± 1.5	−0.4	0.3	0.7	*0.01**
BMI (kg/m^2)	28.5 ± 1.9	−0.3	0.4	−0.1	0.7
Day 3 FSH (mIU/mL)	4.7 ± 0.5	−0.1	0.9	0.1	0.8
Day 3 E2 (pg/mL)	48.2 ± 9.1	0.8	0.1	−0.3	0.6
Total days of ovarian stimulation	11.4 ± 0.5	0.3	0.5	−0.3	0.3
Peak E2 on day of HCG trigger (pg/mL)	1854.0 ± 193.5	0.2	0.7	0.06	0.8
Total gonadotropins dose, IUs	2590.0 ± 304.8	−0.5	0.2	0.3	0.3
Total number of oocytes retrieved	16.0 ± 2.3	−0.4	0.3	−0.1	0.8

BMI body mass index, *E2* estradiol, *FSH* follicle-stimulating hormone, *HCG* human chorionic gonadotropin, *IU* international unit, *Kiss1r* kisspeptin receptor

*$p = 0.047$ in a multivariate linear regression model adjusting for BMI, total days of ovarian stimulation, and total dose of gonadotropins (IUs)

high-fat, diet-induced obesity. Second, kiss1r mRNA expression levels in human cumulus GCs were positively correlated with age, but were not correlated with BMI. These results support the hypothesis that, in addition to its role in the central nervous system, the ovarian kisspeptinergic system may affect ovarian function in older women. Third, MCP-1, a chemokine important for ovulatory function (when present at normal physiologic levels) and abnormally elevated with aging and obesity, is involved in the regulation of ovarian kiss1 and ovarian AMH/AMHR-II.

Cumulus GCs play a vital role in regulating oocyte maturation, and the health of the cumulus cell determines oocyte quality [35, 36]. Although not fully defined yet, cumulus GCs express characteristics distinct from mural GCs, which are steroidogenically more active [37, 38]. Cumulus cells communicate with each other and with the oocyte through specialized gap junctions that allow metabolic exchange and transport of signaling molecules for appropriate follicular development [35, 36, 39]. Interestingly, cumulus and mural GCs have been shown to reflect the characteristics of the oocyte thus providing a noninvasive means to assess oocyte quality [35]. Our current findings suggest that in an infertile population, age-related upregulation in kiss1r expression may alter kisspeptin sensitivity in human cumulus GCs indicating a possible age-related physiologic role, similar to that observed in the hypothalamus [7, 8], for the kisspeptin system in human follicular dynamics. These findings were supported by current data from mice where old mice had significantly higher kiss1 and kiss1r in their ovaries compared to younger reproductive-aged mice. Interestingly, kisspeptin has been shown to decrease the recruitment of follicles by decreasing their sensitivity to FSH (via downregulating FSHR) and thus participates in the reduction of developing follicles observed during aging [40]. As of today, data are still controversial as to which compartment in the ovaries kiss1 is mainly expressed. Although a study by Zhou et al. [41] showed that the major site of kiss1 expression is the theca and interstitial stroma cells, a recent study by Laoharatchatathanin et al. [42] demonstrated that kiss1 is mainly expressed in granulosa cells. Although in our study, kiss1 was not detected in granulosa cells, this could be due to the fact that we have studied luteinized granulosa cells that might behave differently than those that are not luteinized. Taking into account this limitation, further studies are needed to localize the increase in kiss1/kiss1r expression in mammalian ovaries during aging.

We have previously shown in humans that obesity has a negative impact on ovarian function, in particular on genes important in follicular development and ovarian reserve [27, 43–48]. In the current study, we did not find an association between BMI and kisspeptin expression in human GCs and we did not find that diet-induced obesity affected ovarian kiss1/kiss1r expression in mice. This is in contrast to a study by Zhou et al. [41] who demonstrated that HF diet-induced obesity caused a marked suppression of ovarian kiss1 mRNA levels in mice at postnatal days 42 and 70 compared with normal-weight mice on normal chow diet. One explanation for this discrepancy is the fact that we studied mice aged 20 and 32 weeks whose ovaries might be more resistant to insult

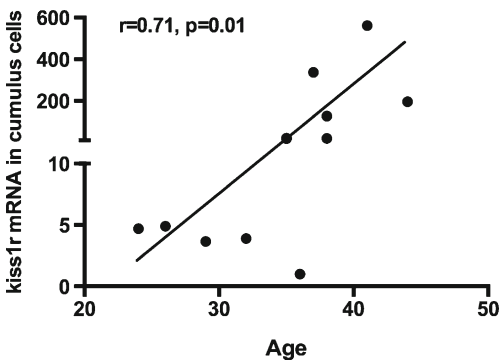

Fig. 4 Correlation between age and kiss1r mRNA expression levels in cumulus granulosa cells (GCs) collected from women who underwent in vitro fertilization. Cumulus GCs were collected by mechanically cutting the cumulus cell layer from each oocyte following oocyte retrieval. Kiss1r mRNA levels were positively correlated with age

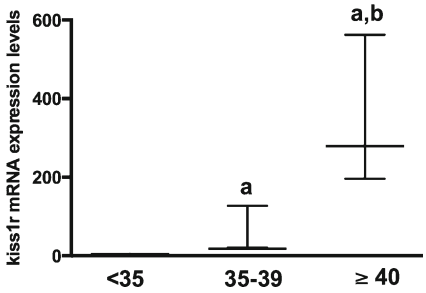

Fig. 5 Stratification by age of kiss1r mRNA expression levels in cumulus granulosa cells (GCs) collected from women who underwent in vitro fertilization. Cumulus GCs were collected by mechanically cutting the cumulus cell layer from each oocyte following oocyte retrieval. Women aged 35 ($n = 4$) had significantly lower kiss1r mRNA expression level compared to women aged between 35 and 39 ($n = 6$) who had significantly lower kiss1r mRNA expression levels compared to women aged ≥ 40 ($n = 2$). Data are described as median (range). *a* $p < 0.05$ compared to <35; *b* $p < 0.05$ compared to 35–39

by HF diet-induced obesity. Interestingly, our findings demonstrated that young MCP-1 knockout mice had suppression in kiss1 mRNA levels, suggesting that MCP-1 gene could be necessary for ovarian kiss1 gene expression which is important in ovulation, further supporting the physiologic role of MCP-1 in the ovulatory process [20].

AMH and AMHR-II genes are important in follicular development and upregulation in these two genes are usually observed in women with ovulatory dysfunction, particularly those with PCOS [30]. Our findings demonstrated that young MCP-1 knockout mice had significantly higher ovarian AMH and AMHR-II mRNA expression levels compared to young wild-type mice. These findings further suggest that MCP-1 may play a role in normal follicular development and ovulation. Understanding the role of MCP-1 in ovarian function may further our understanding of the pathophysiology of ovulatory dysfunction observed in cases where there is upregulation of AMH and/or AMHR-II, such as in PCOS [30].

This study has several limitations. Although our results showed that diet-induced obesity did not affect ovarian kiss 1 or kiss1r mRNA expression levels, the sample size could have been small to detect a difference, if any existed. The luteinized GCs were collected from women who were hyperstimulated with gonadotropins. Although this model may not be the best for studying the ovarian kisspeptinergic system, we [27] and others [30] have shown that GCs could represent a reliable model for studying ovarian physiology and that GCs are responsive to kisspeptin treatment in vitro [34]. Another limitation is that we studied kiss1 and kiss1r expression in the whole mouse ovary, which represents a mixture of different types of cells including granulosa, theca, and stromal cells. In order to bypass this limitation, future experimental work in animals should evaluate separate ovarian compartments. Although MCP-1 knockout mice were used, future experiments should evaluate ovary-specific MCP-1 knockout model to better evaluate the role of this pro-inflammatory marker in ovarian function. Finally, only mRNA expression was evaluated, which might not represent the corresponding protein expression.

In conclusion, alterations in kisspeptin sensitivity in human GCs might provide more insight in understanding the function of ovarian kisspeptin signaling and its implications in ovarian biology. Additionally, the data from the mouse model call for further investigation into the role of the ovarian kisspeptinergic system and ovarian MCP-1 in folliculogenesis, steroidogenesis and ovulation. Given that ovarian kiss1r expression increases with age, future directions should evaluate the response of this receptor to the administration of kisspeptin peptide antagonist (such as peptide 234) in vitro and in vivo and whether this antagonist could reverse some of the features of ovarian aging. It is possible that the rise in adrenergic activity in the ovary that occurs naturally with aging provokes the increase in ovarian kisspeptin [4, 9]. One would expect that the upregulation of kiss1r with age might be due to a decline is systemic plasma kisspeptin levels. However, plasma kisspeptin levels have been shown to be constant in pre- and post-menopausal women [49, 50], thus refuting this hypothesis. Therefore, with age and similar to what happens in the hypothalamus, kiss1r expression changes significantly in the ovary and the sensitivity of the receptors to kisspeptin peptide might unveil some of the mechanisms in reproductive aging. Finally, data pertaining to the role of kisspeptin in assisted reproductive technology are rising. Indeed, the administration of kisspeptin-54 peptide has been used to effectively and safely trigger oocyte maturation in women undergoing IVF treatment [51]. Additionally, kisspeptin and kiss1r might be involved in the fertilization process in the female reproductive tract since the rate of IVF success in animals significantly decreased after treatment of sperm with the kisspeptin antagonist peptide 234 [52]. The pharmacologic manipulation of kisspeptin receptor could represent a potential therapeutic aid in human reproduction.

Compliance with ethical standards

Funding Grant from Ferring Pharmaceuticals Inc. to Z.M. and the American Diabetes Association to M.J.C.

Conflict of interest None

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