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Age-dependent changes in the reproductive axis responsiveness to kisspeptin-10 administration in healthy men

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Abstract

The present study was designed to assess the responsiveness of hypothalamic–pituitary–gonadal axis to kisspeptin administration with increasing age in men. Human kisspeptin-10 was administered in single iv bolus dose (1 μg/kg BW) to healthy adult, middle and advanced age men. Serial blood samples were collected for 30 min pre- and 120 min post-kisspeptin injection periods at 30-min interval. Analysis of plasma LH by ELISA showed a significant ($p < 0.05$) increase after kisspeptin-10 administration in all groups, whereas plasma testosterone concentration was significantly elevated ($p < 0.05$) after kisspeptin-10 injection only in the adult men group. Present results suggest that in men, central hypothalamic–pituitary axis remains active and shows responsiveness to kisspeptin stimulation across life. However, Leydig cell responsiveness to kisspeptin-induced LH decreases with age in men.

KEYWORDS
hypothalamus, kisspeptin, pituitary, reproduction, testes

1 | INTRODUCTION

Many endocrine alterations related to reproductive functions have been reported to be enhanced in men. A decline in testosterone level and elevation of gonadotropic levels has been reported, previously (Wu et al., 2008). Many functional studies have described progressive androgen decline in males with increasing age (Giusti et al., 1975; Hollander & Hollander, 1958; Morley et al., 1997), particularly from the end of the third or the start of the fourth decade (Baker et al., 1976; Harman, Metter, Tobin, Pearson, & Blackman, 2001). In healthy older men, both the rise in LH level and irregular secretion of LH and testosterone have been reported (Feldman et al., 2002; Liu et al., 2006; Morley et al., 1997; Pincus et al., 1996). Compromised negative feedback to the hypothalamus mediated by the androgen receptors is one of the major causes of ageing-related hypogonadism (Veldhuis et al., 2010). Increasing age stimulates LH secretion, even the suboptimal concentration of GnRH in men. Age determines both testosterone-independent and testosterone-modulated actions of GnRH (Veldhuis, Iranmanesh, & Mulligan, 2005). This notion is further strengthened by the observation that the administration of GnRH and kisspeptin-10 (KP-10) increases serum LH levels in human males (George et al., 2011; Mulligan, Iranmanesh, Kerzner, Demers, & Veldhuis, 1999).

Presently, kisspeptin is believed to be the primary regulator of the hypothalamic–pituitary–gonadal (HPG) axis (Navarro et al., 2004). Kisspeptin receptor 1 (*KISS1R*) expression is found in all the regions of testis except the Leydig cells, suggesting a paracrine/autocrine mode of action (Tariq et al., 2013). In human spermatozoa, kisspeptin and *KISS1R* are mainly expressed in the equatorial, neck and mid-piece regions (Publicover et al., 2008; Suarez, Marquez, Harris, & Schimenti, 2007). An acute rise occurred in serum LH concentration by intravenous bolus KP-10 administration, dose-dependently with 1 μg/kg BW causing the maximal stimulation (George et al., 2011). The HPG-axis is strongly interconnected which shows that all components work together (Liu, Iranmanesh, Nehra, Keenan, & Veldhuis, 2005). After iv administration, the

half-life of KP-10 and KP-54 is about 4 and 27 min respectively (Jayasena et al., 2011).

Kisspeptin is believed to be the primary physiological regulator of the reproductive axis in nonprimate and primate species during different life stages (Okamura, Yamamura, & Wakabayashi, 2013). Exogenous administration of KP-10 intravenous boluses and kisspeptin-54 infusions potently evokes LH secretion in men (Dhillo, Chaudhri, Patterson, Thompson, & Bloom, 2005; George et al., 2011). However, little is known about the involvement of kisspeptin signalling in the cause of senescence-basal modulation of the HPG-axis in any model and particularly in the advanced age men. Therefore, the present study was designed to determine the response of HPG-axis to kisspeptin administration in men at different adult ages, in order to contribute to understand the physiological events at different levels of the HPG-axis on the onset of reproductive senescence.

2 | MATERIALS AND METHODS

2.1 | Ethical approval

Ethical approval was obtained from the Research Ethics Committee of the Quaid-i-Azam University, Islamabad, Pakistan (No. DFBS-2014/July 4, 2014). This trial is also registered in the WHO clinical trial registry under the ID NCT03315325. Prior informed written consents were obtained from all the participants.

2.2 | Study participants

Healthy men (n = 15) were selected as per defined inclusion (age) and exclusion (health problems) criteria from the community of district Lower Dir, Khyber Pakhtunkhwa, Pakistan. These individuals were classified based on their ages in three groups: adult (n = 5), middle age (n = 5) and advanced aged (n = 5). The age ranges were 25–27, 46–50 and 71–75 years in adult, middle and advanced age men, respectively. Individuals with chronic illness or disorder, that is, hepatic and renal complications, epilepsy, pneumonia, asthma, orchitis, hernia, cryptorchidism, cardiovascular diseases, reproductive disorders including mental retardation were excluded from this study. All individuals belonged to a middle-class socio-economic status. Individual anthropometric data of each participant were recorded and are given in Table 1.

2.3 | Experimental design

We used KP-10 because its half-life is 3.8–4.1 min in human after intravenous administration (Jayasena et al., 2011). Several studies published in human regarding to the administration of KP-10 reported no adverse effects (Jayasena et al., 2009; Mead, Maguire, Kuc, & Davenport, 2007; Nabi et al., 2018). The blood sampling was done in the Askari Clinical Laboratory, Talash, Timergara. All the subjects had breakfast at 07:00 a.m. The blood sampling was started at 09:00 a.m. and ended at 12:30 p.m for each group once

TABLE 1 Anthropometric data of study population

Groups and individuals	Age (years)	Body weight (kg)	Height (ft. Inch)	BMI (kg/m^2)
Group	**Adult group**			
1	27	58	5.6	20.64
2	26	62	5.6	22.06
3	25	59	5.6	20.99
4	25	64	5.4	24.22
5	26	63	5.6	22.42
Mean ± *SEM*	25.80 ± 0.37	61.20 ± 1.15	5.54 ± 0.08	22.06 ± 0.63
Group	**Middle age group**			
1	46	58	5.4	21.95
2	46	58	5.6	20.64
3	46	65	5.6	23.13
4	47	52	5.7	17.96
5	50	73	5.7	25.21
Mean ± *SEM*	47.00 ± 0.77	61.20 ± 3.59	5.60 ± 0.05	21.78 ± 1.21
Group	**Advanced age group**			
1	75	55	5.7	18.99
2	75	55	5.5	20.18
3	71	58	5.2	23.39
4	71	59	5.5	21.65
5	74	53	5.1	22.08
Mean ± *SEM*	73.20 ± 0.91	56 ± 1.09	5.40 ± 0.10	21.26 ± 0.70

in total 3 days. Sequential blood samples (2 ml) were obtained for 30 min pre- and 120 min post-kisspeptin injection periods at 30-min intervals (−30, 0, 30, 60, 90, 120). KP-10 (metastin 45–54; Calbiochem, Darmstadt, Germany) was administered (1 μg/kg BW) as an intravenous bolus, immediately after collecting the sample (0 min) to assess the response of GnRH neurons to KP-10 in men by determining plasma level of LH and testosterone. The dose of kisspeptin was selected on the basis of previous studies (George et al., 2011). For the ease of blood sampling and kisspeptin injection, the volunteers were fitted with an infusion cannula (Farcocath; G/Ø/L: 22, 0.9, 25 mm; Farcomake for Advanced Medical Industries SAE Alexandria, Egypt) in the cephalic vein. The blood samples were collected in heparinised syringes (BD 3 ml, Luer-Lok Tip with Precision Glide Needle, 23G×1 TW [0.6 × 25 mm]; Becton Dickinson Pakistan Pvt Ltd). To prevent blood clotting in the cannula, heparin (Heparin; B. Braun Melsungen AG, Melsungen, Germany) was used at the rate of 20 IU/ml in saline as a flushing agent. The sampling was done during a period from August 8, 2014 to August 10, 2014. Human kisspeptin-10 (metastin 45–54) was administered to adult and middle-aged men on one day and the next consecutive day to the advanced age men.

2.4 | Preparation of kisspeptin doses

KP-10 (1 mg vial) was originally purchased in lyophilised form. Stock solution of 1 mg/ml was prepared by adding 1 ml normal saline (0.9% NaCl). Fifteen doses of KP-10 were prepared at the rate of 1 μg/kg BW. The average dose was prepared for adult and advanced age group due to the little differences in individual's BW in each two groups, while for middle age group, individual doses were prepared according to the body weights of middle age men. These doses were prepared in a very hygienic environment. After preparation in the laboratory of Quaid-i-Azam University, Islamabad, Pakistan, these doses were immediately frozen. Shortly before transportations to the Askari Clinical Laboratory, doses were kept on ice. Before kisspeptin administration, 1 ml of sterile saline was mixed with the dose as a vehicle for administration.

2.5 | Blood sampling

After the collection of blood samples in heparinised syringes, 20 IU/ml heparin mixed with sterile normal saline was administered to prevent clotting in the cannula. Samples were then transferred to culture tubes and stored in a refrigerator at 4°C until centrifuged. The process of centrifugation was done just after the completion of blood sampling (within 2 hr) at 3,000 rpm (998 ***g***) for 15 min. Blood plasma was isolated in Eppendorf vials of 1.5 ml capacity and stored at −20°C until hormonal analysis.

2.6 | Hormonal analyses

Enzyme Immune Assay for human testosterone (AMGENIX, San Jose, USA) and LH (Biocheck, Foster City, CA, USA) for measuring plasma testosterone and LH concentration, respectively, were performed according to the manufacturer´s protocol and procedures. The intra-assay coefficient of variation was 8.5% for testosterone and <9% for LH.

2.7 | Statistical analysis

Changes in mean plasma LH and testosterone concentration were assessed by one-way ANOVA followed by post hoc Tukey test in each group. Pre- and post-kisspeptin mean LH and testosterone were compared by paired *t* test. Comparison of plasma LH and testosterone secretion across the ages was done by using one-way ANOVA followed by post hoc test. Both for post-kisspeptin LH and for testosterone, area under the curve (AUC) was measured across the age groups. All data are presented as mean ± *SEM*, and differences were considered significant when $p < 0.05$. Analysis of data was done using the GraphPad Prism, version 5.01 (Graph Pad Software Inc., San Diego, CA, USA).

3 | RESULTS

3.1 | Effect of single iv bolus administration of KP-10 on plasma LH and testosterone levels in adult men

Mean plasma LH concentrations at different time points before and after administration of KP-10 in adult men are shown in Figure 1a. One-way ANOVA with repeated measures on log-transformed data showed that there was a significant effect ($p < 0.05$) observed in mean plasma LH concentration after administration of KP-10. Further post hoc analysis indicated that mean LH level observed at 30 min post-kisspeptin-10 injection was significantly ($p < 0.05$) increased, as compared to the basal (−30 min) value. Overall mean plasma LH concentrations observed during pre- (−30, 0 min) and post (30–120 min)-KP-10 administration periods in adult men are shown in Figure 2a. A paired *t* test on log-transformed data showed that administration of KP-10 significantly increased ($p < 0.05$) mean plasma LH levels in adult men.

Mean plasma testosterone concentrations at different time points before and after administration of KP-10 in adult men are shown in Figure 3a. One-way ANOVA with repeated measures on log-transformed data showed that there was a significant effect ($p < 0.05$) observed in mean plasma testosterone concentration after administration of KP-10. Further post hoc analysis indicated that mean testosterone levels observed at 60 and 90 min post-KP-10 injection was significantly ($p < 0.05$) increased, as compared to the basal (0 min) value. Overall mean plasma testosterone concentrations observed during pre- (−30, 0 min) and post (30–120 min)-KP-10 administration periods in adult men are shown in Figure 2a. A paired *t* test on log-transformed data showed that administration of KP-10 significantly increased ($p < 0.05$) mean plasma testosterone levels in adult men.

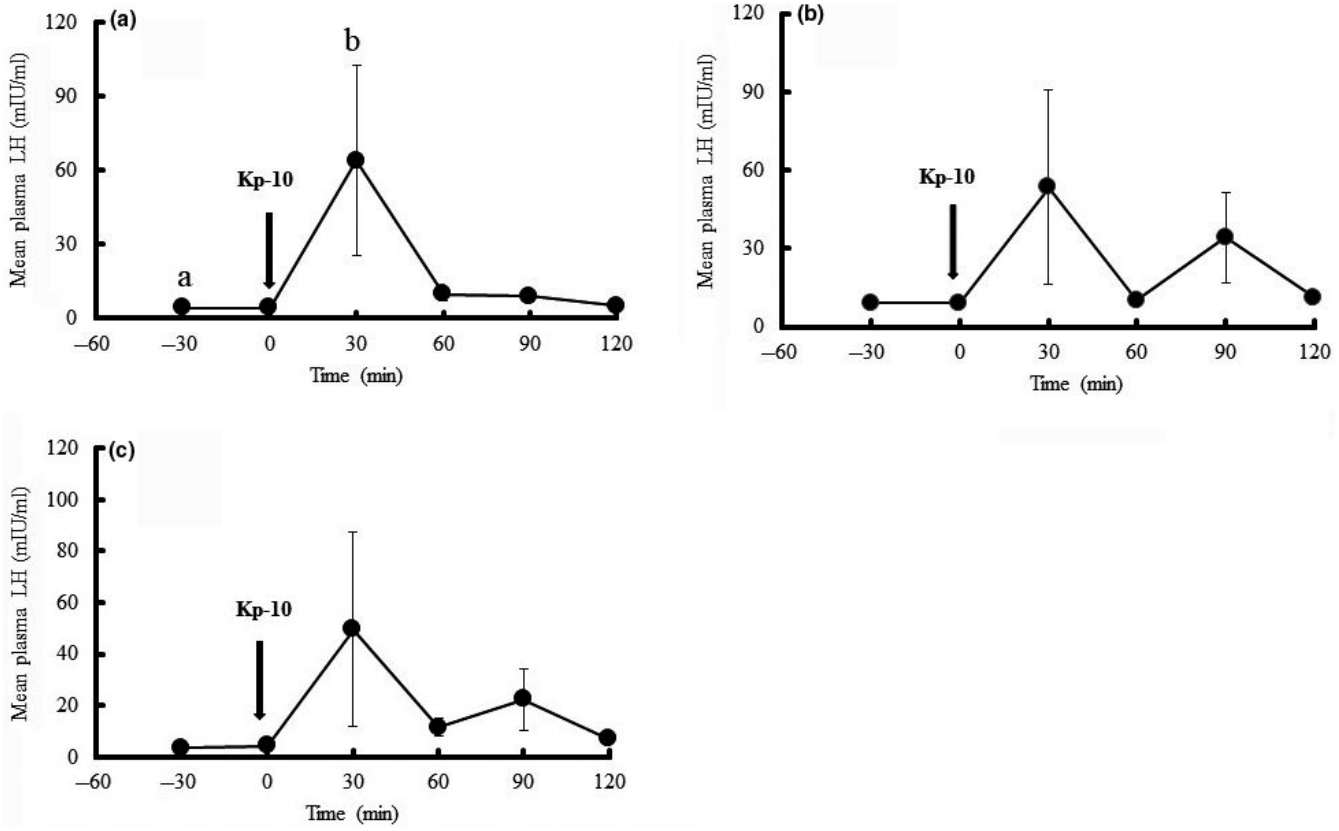

FIGURE 1 Comparison of pre- and post-KP-10 LH values across the different time points in adult (a), middle (b) and advanced age (c) groups. The significant difference in each figure was indicated by the alternate letter at $p < 0.05$

3.2 | Effect of single iv bolus administration of kisspeptin-10 on plasma LH and testosterone levels in middle-aged men

One-way ANOVA with repeated measures showed that there was a nonsignificant increase ($p > 0.05$) observed in mean plasma LH concentration after administration of KP-10, as compared to the basal values (Figure 1b). Overall mean plasma LH concentrations observed during pre- (−30, 0 min) and post (30–120 min)-KP-10 administration periods in middle-aged men are shown in Figure 2b. A paired *t* test on log-transformed data showed that administration of KP-10 significantly increased ($p < 0.05$) mean plasma LH levels in middle-aged men.

One-way ANOVA with repeated measures showed that there was a nonsignificant effect ($p > 0.05$) observed in mean plasma testosterone concentration after administration of KP-10 (Figure 3b). A paired *t* test showed that pre-kisspeptin plasma testosterone level was quite equal to post-treatment mean plasma testosterone level in middle-aged men (Figure 2b).

3.3 | Effect of single iv bolus administration of KP-10 on plasma LH and testosterone levels in advanced age men

One-way ANOVA with repeated measures showed that there was nonsignificant variation ($p > 0.05$) observed in mean plasma LH concentrations after administration of KP-10 (Figure 1c). Overall mean plasma LH concentrations observed during pre- (−30, 0 min) and post (30–120 min)-KP-10 administration periods in advanced aged men are shown in Figure 2c. A paired *t* test on log-transformed data showed that administration of KP-10 significantly increased ($p < 0.05$) mean plasma LH levels in advanced age men.

One-way ANOVA with repeated measures showed that there was nonsignificant effect ($p > 0.05$) observed on mean plasma testosterone concentrations after administration of KP-10 (Figure 3c). Paired *t* test showed that pre kisspeptin plasma testosterone level was quite equal to post-treatment mean plasma testosterone level in advanced age men (Figure 2c).

3.4 | Comparison of KP-10 affected LH and testosterone secretion across ages

Mean post-kisspeptin LH concentration did not vary across the age groups. Although mean LH response to KP-10 injection showed variation at different ages, statistical analysis indicated no significant differences. No significant differences were shown in LH AUC across the groups. Data showed that there was a decreasing trend in mean post-kisspeptin plasma testosterone concentration across the age groups. However, no significant differences were observed. Although mean testosterone response to KP-10 injection showed variation at different ages, statistical analysis

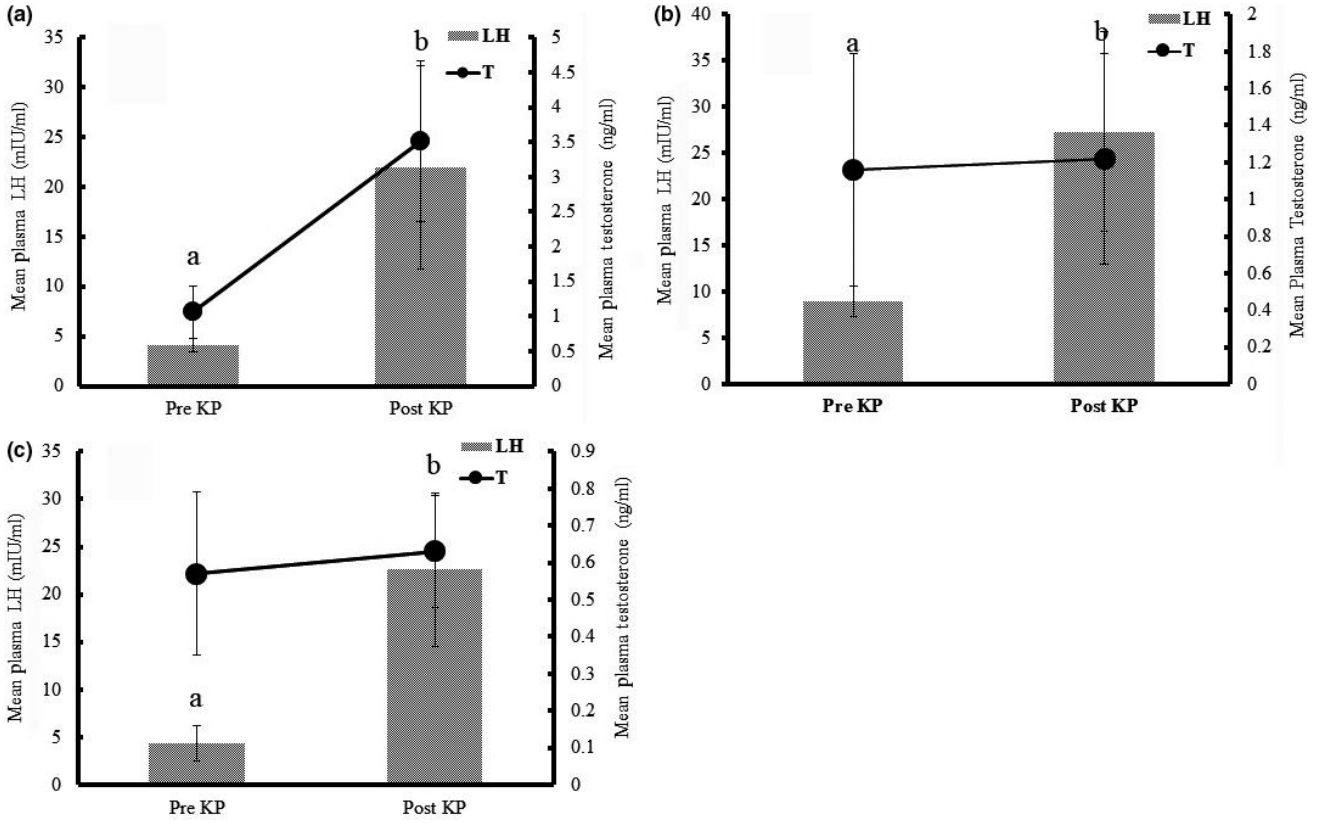

FIGURE 2 Comparison of overall mean ± *SEM* plasma LH and testosterone concentration before and after KP-10 administration in adult (a), middle (b) and advanced (c) age groups. The significant difference in each figure was indicated by the alternate letter at $p < 0.05$

indicated no significant differences. Data showed that there was a decreasing trend in testosterone AUC across the age groups. However, no significant differences were observed.

3.5 | Comparison of basal plasma LH and testosterone concentrations in different age groups

Mean basal LH levels showed some apparent variation across the ages. However, no specific significant difference was observed. A slight decreasing trend with age was evident in these basal testosterone levels with the concentration in adult group was greater than the middle age and advanced age men while middle age basal plasma testosterone concentration was lower than the adult age group but greater than the advanced age group. However, differences in basal plasma testosterone concentrations across the ages were nonsignificant.

4 | DISCUSSION

The administration of KP-10 provoked a peak of plasma LH concentration in a similar way across the ages studied. The effect was acute and similar in all the age groups. In case of testosterone, administration of KP-10 increased plasma testosterone level of adult men while no effect was observed in circulating testosterone levels of middle- and advanced age men. These results suggest that the excitatory influence of KP-10 on LH release was not affected by the age. However, the ability of kisspeptin induced LH to stimulate testosterone secretion decreased with age. Our results suggest that the testosterone level decreases during senescence not due to changes at the hypothalamic–pituitary levels, because no difference of kisspeptin stimulated plasma LH level was found among the age groups. This shows that there may be some problems which occurred at testicular level, which impair testosterone secretion during senescence.

In the present study, the rise of plasma LH levels stimulated by the administration of KP-10 was similar in all groups, which is in line with the foregoing studies (Dhillo et al., 2005; George et al., 2011). In our study, KP-10-stimulated plasma LH levels were similar in all the age groups observed. Our results coincide with the observation of a previous study conducted by Molnar et al. (2012), which demonstrated that in men there is an ageing-related acute increment in hypothalamic kisspeptin signalling and a moderate increase in central NKB signalling. These changes have been related to ageing associated reduced testosterone negative feedback to kisspeptin and NKB neurons. However, in the present study, a preservation of responsiveness to kisspeptin stimulation was noted with age, but enhancement was not evident. In aged men, heightened hypothalamic stimulation of the reproductive axis is attributed to increased kisspeptin and NKB inputs to GnRH neurons, as compared to younger men. A few of the hormonal changes result from

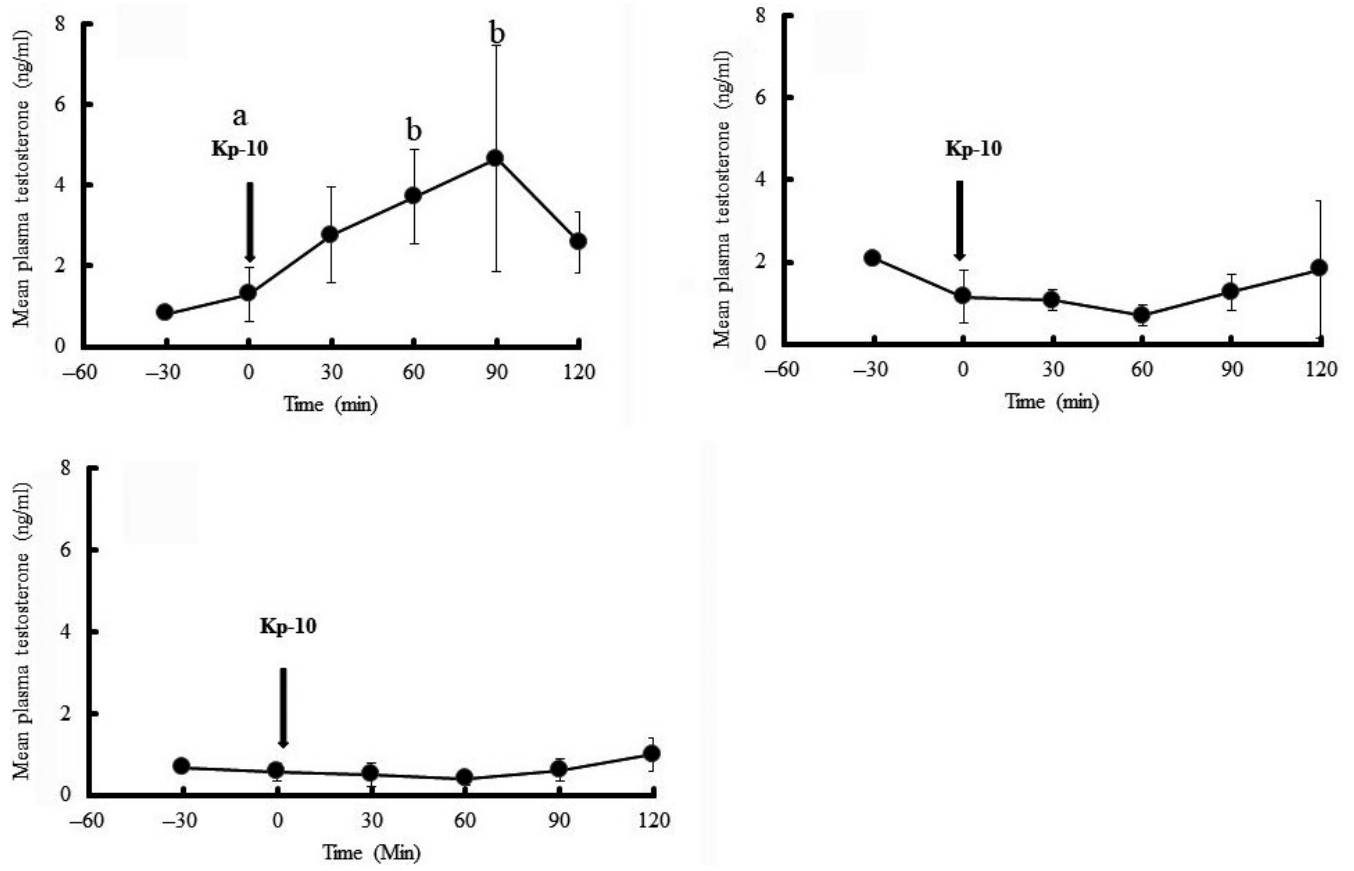

FIGURE 3 Comparison of pre- and post-KP-10 testosterone values across the different time points in adult (a), middle (b) and advanced age (c) groups. The significant difference in each figure was indicated by the alternate letter at $p < 0.05$

a decreased negative feedback to the hypothalamus mediated by androgen receptors (Veldhuis et al., 2010), which probably is due to the kisspeptin and NKB neurons. It is previously known that the hypothalamic mechanisms of negative feedback can influence GnRH/LH secretory frequency, pulsatility and total LH secretion (Veldhuis et al., 2010). In the present study, such a fine characterisation of LH secretion was not done. Nevertheless, our finding of maintained responsiveness to kisspeptin across ages would suggest that foregoing impairment of LH secretion is not related to modulation in kisspeptin responsiveness. Keeping in mind the possible regulation of the kisspeptin/NKB neurons in testosterone negative feedback to hypothalamic neurons (Navarro et al., 2004; Shibata, Friedman, Ramaswamy, & Plant, 2007; Smith, Cunningham, Rissman, Clifton, & Steiner, 2005), the present study also shares some similarity with data which showed an elevated Kiss1 expression in the hypothalamus after menopause (Rometo, Krajewski, Voytko, & Rance, 2007), which might relate to the increase in GnRH/LH secretion (Kermath & Gore, 2012). Kisspeptin mRNA expression was not influenced by increasing age in the pituitary, numbers of kisspeptin immunopositive neurons in the arcuate nucleus, or estradiol-dependent reductions in the kisspeptin mRNA expression of posterior hypothalamus (Lederman et al., 2010). Present results further highlight and specify that GnRH neuronal responsiveness to kisspeptin stimulation is maintained during ageing in men.

The elevated levels of plasma testosterone in adult men with kisspeptin administration, observed in the present study are in line with the findings of a previously reported study (Jayasena et al., 2011). A number of pharmacological studies have highlighted elevated testosterone concentrations (Dhillo et al., 2005; George, Anderson, & Millar, 2012). Chan et al. (2011) on the other hand observed a non-significant increase in plasma testosterone after kisspeptin 112–121 administration. An important finding of the present study was that iv-bolus administration of KP-10 produced no effect on plasma testosterone level of middle and advanced age men. Apparent insensitivity of the testis to LH stimulation by kisspeptin during senescence might be the result of changes in the Leydig cell. Indeed, Harman and Tsitouras (1980) demonstrated the testosterone response to LH was reduced during senescence in men. There can be a number of factors that may be involved in decreased responsiveness of testis to LH mediated stimulation during senescence. Previous studies showed that there is an age-related shift in body composition towards more body fat mass and the loss of muscle mass (Borkan, Hults, Gerzof, Robbins, & Silbert, 1983; Depres et al., 1990; Visser et al., 2005). Increased body fat increases leptin production and its elevated levels are associated with lower testosterone levels in old age attained either by direct suppression of the LH drive to testis or is due to the reduction of the Leydig cells sensitivity to LH (Isidori et al., 1999). Further, expression of leptin receptor is also reported in the testis (Cioffi et al.,

1996) and rat in vitro have exhibited the negative actions of leptin on testicular physiology (Tena-Sempere et al., 1999). Another possible explanation of the decreased testosterone response to kisspeptin in ageing groups can be a potential direct action of kisspeptin on the testis. Kisspeptin has been postulated to play a direct stimulatory effect on testicular tissue in term of testosterone production in mouse (Anjum, Krishna, Sridaran, & Tsutsui, 2012) and monkey (Irfan, Ehmcke, Wahab, Shahab, & Schlatt, 2013). As reported by Anjum et al. (2012), kisspeptin expression in mice testes increases from pre-pubertal to pubertal period and has been suggested to be responsible for increased circulating testosterone level and testicular weight. During this transition in the adult rhesus monkey, kisspeptin in high doses markedly suppresses LH but amplify testosterone production, suggesting the direct and stimulatory action of kisspeptin on Leydig cells (Ramaswamy et al., 2007). Kisspeptin also exerted an intratesticular action in adult primate testes. This intratesticular kisspeptin action accelerated the steroid synthesis after LH or hCG administration (Irfan et al., 2013). In contrast to the above-mentioned stimulatory direct effect of kisspeptin on testosterone in adult testis, kisspeptin may have a possible inhibitory role in testicular tissue during senescence. Such a role is supported by observations in mouse in which immunoreactivity of kisspeptin increased, but testosterone decreased significantly during senescence (Anjum et al., 2012). Similar mechanism may be operational in human also as our data showed that in senescence LH level increased, but testosterone level did not increase after KP-10 administration in the middle age and advanced age men. Further studies are needed to investigate if kisspeptin have a local direct inhibitory effect on testosterone production which is masking the effect of kisspeptin induced LH rise on Leydig cells during senescence in the testes.

5 | CONCLUSION

Conclusively, present study suggests that KP-10 raises plasma LH secretion in adult, middle and advanced age men equally. Plasma testosterone concentration of adult age men is increased by iv bolus administration of KP-10 while no increase occurs in circulating testosterone concentration of middle-aged and advanced age men. Our findings suggest that in men central hypothalamic–pituitary axis remains active and show responsiveness to KP-10 across the life, but Leydig cell responsiveness to gonadotropin decreases with age. These results will provide a great insight into the understanding of kisspeptin physiology in the reproductive system.

CONFLICTS OF INTEREST

The authors have no conflict of interest in relation to this work.

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REFERENCES

Anjum, S., Krishna, A., Sridaran, R., & Tsutsui, K. (2012). Localization of gonadotropin-releasing hormone (GnRH), gonadotropin-inhibitory hormone (GnIH), kisspeptin and GnRH receptor and their possible roles in testicular activities from birth to senescence in mice. *Journal of Experimental Zoology Part A: Ecological Genetics and Physiology*, *317*(10), 630–644. https://doi.org/10.1002/jez.1765

Baker, H. W., Burger, H. G., De Kretser, D. M., Hudson, B., Connor, S., Wang, C., … Rennie, G. C. (1976). Changes in the pituitary-testicular system with age. *Clinical Endocrinology*, *5*(4), 349–372. https://doi.org/10.1111/j.1365-2265.1976.tb01964.x

Borkan, G. A., Hults, D. E., Gerzof, S. G., Robbins, A. H., & Silbert, C. K. (1983). Age changes in body composition revealed by computed tomography. *Journal of Gerontology*, *38*(6), 673–677. https://doi.org/10.1093/geronj/38.6.673

Chan, Y. M., Butler, J. P., Pinnell, N. E., Pralong, F. P., Chan, K. K., & Seminara, S. B. (2011). Kisspeptin resets the hypothalamic GnRH clock in men. *Journal of Clinical Endocrinology and Metabolism*, *96*(6), 908–915. https://doi.org/10.1210/jc.2010-3046

Cioffi, J. A., Shafer, A. W., Zupancic, T. J., Smith-Gbur, J., Mikhail, A., Platika, D., & Snodqrass, H. R. (1996). Novel B219/OB receptor isoforms: Possible role of leptin in hematopoiesis and reproduction. *Nature Medicine*, *2*(5), 585–589. https://doi.org/10.1038/nm0596-585

Depres, J. P., Moorjani, S., Lupien, P. J., Tremblay, A., Nadeau, A., & Bouchard, C. (1990). Regional distribution of body fat, plasma lipoproteins, and cardiovascular disease. *Arteriosclerosis*, *10*(4), 497–511. https://doi.org/10.1161/01.ATV.10.4.497

Dhillo, W. S., Chaudhri, O. B., Patterson, M., Thompson, E. L., & Bloom, S. R. (2005). Kisspeptin-54 stimulates the hypothalamic-pituitary gonadal axis in human males. *Clinical Endocrinology and Metabolism*, *90*(12), 6609–6615. https://doi.org/10.1210/jc.2005-1468

Feldman, H. A., Longcope, C., Derby, C. A., Johannes, C. B., Araujo, A. B., Coviello, A. D., … McKinlay, J. B. (2002). Age trends in the level of serum testosterone and other hormones in middle aged men: Longitudinal results from the Massachusetts male aging study. *Journal of Clinical Endocrinology and Metabolism*, *87*(2), 589–598. https://doi.org/10.1210/jcem.87.2.8201

George, J. T., Anderson, R. A., & Millar, R. P. (2012). Kisspeptin-10 stimulation of gonadotrophin secretion in women is modulated by sex steroid feedback. *Human Reproduction*, *27*(12), 3552–3559. https://doi.org/10.1093/humrep/des326

George, J. T., Veldhuis, J. D., Roseweir, A. K., Newton, C. L., Faccenda, E., Millar, R. P., & Anderson, R. A. (2011). Kisspeptin-10 is a potent stimulator of LH and increases pulse frequency in men. *Journal of Clinical Endocrinology and Metabolism*, *96*(8), 1228–1236. https://doi.org/10.1210/jc.2011-0089

Giusti, G., Gonnelli, P., Borrelli, D., Fiorelli, G., Forti, G., Pazzagli, M., & Serio, M. (1975). Age related secretion of androstenedione, testosterone and dihydrotestosterone by the human testis. *Experimental Gerontology*, *10*(5), 241–245. https://doi.org/10.1016/0531-5565(75)90001-7

Harman, S. M., Metter, E. J., Tobin, J. D., Pearson, J., & Blackman, M. R. (2001). Longitudinal effects of aging on serum total and free testosterone levels in healthy men. Baltimore longitudinal study of aging. *Journal of Clinical Endocrinology and Metabolism*, *86*(2), 724–731. https://doi.org/10.1210/jcem.86.2.7219

Harman, S. M., & Tsitouras, P. D. (1980). Reproductive hormones in aging men. I. Measurement of sex steroids, basal luteinizing hormone, and Leydig cell response to human chorionic gonadotropin. *Journal of Clinical Endocrinology and Metabolism*, *51*(1), 35–40. https://doi.org/10.1210/jcem-51-1-35

Hollander, N., & Hollander, V. P. (1958). The micro determination of testosterone in human spermatic vein blood. *Journal of Clinical*

Endocrinology and Metabolism, *18*(9), 966–971. https://doi.org/10.1210/jcem-18-9-966

Irfan, S., Ehmcke, J., Wahab, F., Shahab, M., & Schlatt, S. (2013). Intratesticular action of kisspeptin in rhesus monkey (*Macaca mulatta*). *Andrologia*, *46*(6), 610–617. https://doi.org/10.1111/and.12121

Isidori, A. M., Caprio, M., Strollo, F., Moretti, C., Frajese, G., Isisori, A., & Fabbri, A. (1999). Leptin and androgen in male obesity: Evidence for leptin contribution to reduced androgen levels. *Journal of Clinical Endocrinology and Metabolism*, *84*(10), 3673–3680. https://doi.org/10.1210/jcem.84.10.6082

Jayasena, C. N., Nijher, G. M., Chaudhri, O. B., Murphy, K. G., Ranger, A., Lim, A., & Dhillo, W. S. (2009). Subcutaneous injection of kisspeptin-54 acutely stimulates gonadotropin secretion in women with hypothalamic amenorrhea, but chronic administration causes tachyphylaxis. *Journal of Clinical Endocrinology and Metabolism*, *94*(11), 4315–4323. https://doi.org/10.1210/jc.2009-0406

Jayasena, C. N., Nijher, G. M., Comninos, A. N., Abbara, A., Januszewki, A., Vaal, M. L., … Dhillo, W. S. (2011). The effects of kisspeptin-10 on reproductive hormone release show sexual dimorphism in humans. *Journal of Clinical Endocrinology and Metabolism*, *96*(12), 1963–1972. https://doi.org/10.1210/jc.2011-1408

Kermath, B. A., & Gore, A. C. (2012). Neuroendocrine control of the transition to reproductive senescence: Lessons learned from the female rodent model. *Neuroendocrinology*, *96*(1), 1–12. https://doi.org/10.1159/000335994

Lederman, M. A., Lebesgue, D., Gonza-lez, V. V., Shu, J., Merhi, Z. O., & Etgen, A. M. (2010). Age-related LH surge dysfunction correlates with reduced responsiveness of hypo-thalamic anteroventral periventricular nucleus kisspeptin neurons to oestradiol positive feedback in middle-aged rats. *Neuropharmacology*, *58*(1), 314–320. https://doi.org/10.1016/j.neuropharm.2009.06.015

Liu, P. Y., Iranmanesh, A., Nehra, A. X., Keenan, D. M., & Veldhuis, J. D. (2005). Mechanisms of hypoandrogenemia in healthy aging men. *Endocrinology and Metabolism Clinics of North America*, *34*(4), 935–955. https://doi.org/10.1016/j.ecl.2005.07.008

Liu, P. Y., Pincus, S. M., Takahashi, P. Y., Roebuck, P. D., Iranmanesh, A., Keenan, D. M., & Veldhuis, J. D. (2006). Aging attenuates both the regularity and joint synchrony of LH and testosterone secretion in normal men: Analyses via a model of graded GnRH receptor blockade. *American Journal of Physiology Endocrinology and Metabolism*, *290*(1), 34–41. https://doi.org/10.1152/ajpendo.00227.2005

Mead, J., Maguire, J., Kuc, R. E., & Davenport, A. P. (2007). Kisspeptins are novel potent vasoconstrictors in humans, with a discrete localization of their receptor, G protein-coupled receptor 54, to atherosclerosis-prone vessels. *Endocrinology*, *148*, 140–147. https://doi.org/10.1210/en.2006-0818

Molnar, C. S., Vida, B., Sipos, M. T., Ciofi, P., Borsay, B. A., Racz, K., … Hrabovszky, E. (2012). Morphological evidence for enhanced kisspeptin and neurokinin b signaling in the infundibular nucleus of the aging man. *Endocrinology*, *153*(11), 5428–5439. https://doi.org/10.1210/en.2012-1739

Morley, J. E., Kaiser, F. E., Perry, H. M., Patrick, P., Morley, P. M., Stauber, P. M., … Garry, P. J. (1997). Longitudinal changes in testosterone, luteinizing hormone, and follicle stimulating hormone in healthy older men. *Metabolism*, *46*(6), 410–413. https://doi.org/10.1016/S0026-0495(97)90057-3

Mulligan, T., Iranmanesh, A., Kerzner, R., Demers, L. W., & Veldhuis, J. D. (1999). Two-week pulsatile gonadotropin releasing hormone infusion unmasks dual (hypothalamic and Leydig-cell) defects in the healthy aging male gonadotropic axis. *European Journal of Endocrinology*, *141*(3), 257–266. https://doi.org/10.1530/eje.0.1410257

Nabi, G., Ullah, H., Khan, S., Wahab, F., Duan, F., Ullah, R., … Shahab, M. (2018). Changes in the responsiveness of the hypothalamic-pituitary-gonadal axis to kisspeptin-10 administration during pubertal transition in boys. *International Journal of Endocrinology*, *2018*, 1–10. https://doi.org/10.1155/2018/1475967

Navarro, V. M., Castellano, J. M., Fernandez-Fernandez, R., Barreiro, M. L., Roa, J., Sanchez-Criado, J. E., … Tena-Sempere, M. (2004). Developmental and hormonally regulated messenger ribonucleic acid expression of KiSS-1 and its putative receptor, GPR54, in rat hypothalamus and potent luteinizing hormone-releasing activity of KiSS-1 peptide. *Endocrinology*, *145*(10), 4565–4574. https://doi.org/10.1210/en.2004-0413

Okamura, H., Yamamura, T., & Wakabayashi, Y. (2013). Kisspeptin as a master player in the central control of reproduction in mammals: An overview of kisspeptin research in domestic animals. *Animal Science Journal*, *84*(5), 369–381. https://doi.org/10.1111/asj.12056

Pincus, S. M., Mulligan, T., Iranmanesh, A., Gheorghiu, S., Godschalk, M., & Veldhuis, J. D. (1996). Older males secrete luteinizing hormone and testosterone more irregularly, and jointly more asynchronously, than younger males. *Proceeding of the Natural Academy of Sciences*, *USA*, *93*(24), 14100–14105. https://doi.org/10.1073/pnas.93.24.14100

Publicover, S. J., Giojalas, L. C., Teves, M. E., de Oliveria, G. S., Garcia, A. A., Barratt, C. L., & Harper, C. V. (2008). Ca^{2+} signaling in the control of motility and guidance in mammalian sperm. *Frontier in Bioscience*, *13*(1), 5623–5637. https://doi.org/10.2741/3105

Ramaswamy, S., Seminara, S. B., Pohl, C. R., DiPietro, M. J., Crowley, W. F. Jr, & Plant, T. M. (2007). Effect of continuous intravenous administration of human metastin 45–54 on the neuroendocrine activity of the hypothalamic- pituitary-testicular axis in the adult male rhesus monkey (*Macaca mulatta*). *Endocrinology*, *148*(7), 3364–3370. https://doi.org/10.1210/en.2007-0207

Rometo, A. M., Krajewski, S. J., Voytko, M. L., & Rance, N. E. (2007). Hypertrophy and increased kisspeptin gene expression in the hypothalamic infundibular nucleus of postmenopausal women and ovariectomized monkeys. *Journal of Clinical Endocrinology and Metabolism*, *92*(7), 2744–2750. https://doi.org/10.1210/jc.2007-0553

Shibata, M., Friedman, R. L., Ramaswamy, S., & Plant, T. M. (2007). Evidence that down regulation of hypothalamic KiSS-1 expression is involved in the negative feedback action of testosterone to regulate luteinising hormone secretion in the adult male rhesus monkey (*Macaca mulatta*). *Journal of Neuroendocrinology*, *19*(6), 432–438. https://doi.org/10.1111/j.1365-2826.2007.01549.x

Smith, J. T., Cunningham, M. J., Rissman, E. F., Clifton, D. K., & Steiner, R. A. (2005). Regulation of Kiss1 gene expression in the brain of the female mouse. *Endocrinology*, *146*(9), 3686–3692. https://doi.org/10.1210/en.2005-0488

Suarez, S. S., Marquez, B., Harris, T. P., & Schimenti, J. C. (2007). Different regulatory systems operate in the mid piece and principal piece of the mammalian sperm flagellum. *Society for Reproduction and Fertility Suppliment*, *65*, 331–334.

Tariq, A. R., Shahab, M., Clarke, I. J., Pereira, A., Smith, J. T., Khan, S. H., … Anwar, T. (2013). Kiss1 and kiss1 receptor expression in the rhesus monkey testis: A possible local regulator of testicular function. *Central European Journal of Biology*, *8*(10), 968–974. https://doi.org/10.2478/s11535-013-0219-4

Tena-Sempere, M., Pinilla, L., Gonzalez, L. C., Dieguez, C., Casanueva, F. F., & Aguilar, E. (1999). Leptin inhibits testosterone secretion from adult rat testis in vitro. *Journal of Endocrinology*, *161*(2), 211–218. https://doi.org/10.1677/joe.0.1610211

Veldhuis, J. D., Iranmanesh, A., & Mulligan, T. (2005). Age and testosterone feedback jointly control the dose-dependent actions of gonadotropin-releasing hormone in healthy men. *Journal of Clinical Endocrinology and Metabolism*, *90*(1), 302–309. https://doi.org/10.1210/jc.2004-1313

Veldhuis, J. D., Takahashi, P. Y., Keenan, D. M., Liu, P. Y., Mielke, K. L., & Weist, S. M. (2010). Age disrupts androgen receptor-modulated negative feed- back in the gonadal axis in healthy men. *American Journal of Physiology Endocrinology and Metabolism*, *299*(4), 675–682. https://doi.org/10.1152/ajpendo.00300.2010

Visser, M., Goodpaster, B. H., Kritchevsky, S. B., Newman, A. B., Nevitt, M., Rubin, S. M., … Harris, T. B. (2005). Muscle mass, muscle strength,

and muscle fat infiltration as predictors of incident mobility limitations in well-functioning older persons. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences*, *60*(3), 324–333. https://doi.org/10.1093/gerona/60.3.324

Wu, F. C., Tajar, A., Pye, S. R., Silman, A. J., Finn, J. D., O'Neill, T. W., … Vanderschueren, D. (2008). Hypothalamic-pituitary-testicular axis disruptions in older men are differentially linked to age and modifiable risk factors: The European male aging study. *Journal of Clinical Endocrinology and Metabolism*, *93*(7), 2737–2745. https://doi.org/10.1210/jc.2007-1972

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