

Serum kisspeptin levels in normal and overweight Saudi females and its relation with anthropometric indices

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BACKGROUND AND OBJECTIVES: Animal studies suggest that undernutrition and low body weight are associated with low kisspeptin expression and vice versa. This led us to hypothesize that obese and overweight individuals might have significant differences in kisspeptin levels compared with normal-weight individuals.

DESIGN AND SETTINGS: A prospective cohort study of 1-year duration involving young Saudi female students in the University of Dammam.

SUBJECTS AND METHODS: Young Saudi females studying in a Saudi university were divided into 2 groups: normal weight with a body mass index (BMI)=18.5-24.99 and overweight/obese with a BMI \geq 25. Serum kisspeptin levels were measured by enzyme-linked immunosorbent assay in early follicular, pre-ovulatory, and luteal phases, and anthropometric parameters were recorded.

RESULTS: Mean serum kisspeptin levels in the overweight/obese group (follicular phase: 266.88 [45.53], pre-ovulatory phase: 477.12 [21.18], and luteal phase: 735.13 [52.59]) did not differ significantly compared with the normal-weight group (follicular phase: 259.82 [20.50], pre-ovulatory phase: 448.36 [24.58], and luteal phase 708.18 [49.47]) in any of the menstrual phase. In any of the menstrual phase, kisspeptin levels were not found to be correlated with any of the anthropometric variable in any group.

CONCLUSION: Serum kisspeptin levels in overweight and obese young females may not differ significantly compared with normal-weight females, and there may not be any correlation between serum kisspeptin and anthropometric indices.

Kisspeptin has emerged as a key player in regulation of reproduction. Currently, it is accepted worldwide that kisspeptins are the most powerful gonadotropin-releasing hormone (GnRH) secretagogue.¹⁻³

Kisspeptin has an essential role in the control of puberty onset, as evident by impaired puberty progression and sexual immaturity^{4,5} in humans and mice with the genetic inactivation of kisspeptin receptor. Kisspeptin neurons within the hypothalamus serve as a relay station for transmitting negative-feedback effects of sex steroids on GnRH neurons.⁶ Compelling evidence suggests that there is another population of kisspeptin neurons, which is essential for mediating positive-feedback effects of estrogen on generating pre-ovulatory luteinizing hormone (LH) surge. This was confirmed by the absence of pre-ovulatory LH surge in female mice infused with kisspeptin antagonist.⁷

Kisspeptin gene (KiSS-1) expression has also been reported in adipose tissues of both humans and rodents,^{8,9} suggesting that kisspeptin might play a role in regulating body weight. However, the pathophysiological significance of kisspeptin in processes other than reproduction remains debatable, as KiSS-1 knockout mouse do not show any additional phenotype aside from hypogonadism.¹

Few studies have addressed kisspeptin signaling in relation to body weight and energy metabolism. Undernutrition and low body weight have been reported to be associated with low kisspeptin expression in hypothalamus,¹⁰ and obese females have been shown to have higher kisspeptin levels compared with normal weight.¹¹ In line with this, Li et al¹² determined KiSS-1 and kisspeptin receptor (Kiss1r) mRNA levels in brains of female rats from weaning onward. An increased expression of KiSS-1 was reported in the brains

with high-fat diet. These data suggest that obesogenic diets might be associated with the upregulation of kisspeptin in the hypothalamus of female rats.

A recent study¹³ has reported obesity in Kiss1r knockout female mice. This obesity might be caused by impaired kisspeptin signaling in the brain because kisspeptin neurons communicate with some anorexigenic proopiomelanocortin and orexigenic neuropeptide Y neurons.^{14,15}

Brown et al. quantified KiSS-1 mRNA in adipose tissues of rats and found an increased expression of KiSS-1 by 18-hour food restriction. In contrast, obesity or high-fat diet reduced KiSS-mRNA expression, suggesting that KiSS-1 expression is regulated by food intake and obesity.¹⁶ Similar to this, another study revealed significantly low-plasma kisspeptin levels in obese pregnant females compared with controls.¹⁷

Collectively, all these studies suggest a possible link between body weight and kisspeptin. This led us to hypothesize that alterations in kisspeptin signaling/serum kisspeptin levels may contribute, directly or indirectly, to some aspects of human obesity. In other words, overweight and obese females might have significant differences in kisspeptin levels compared with normal-weight females. To investigate a possible involvement of kisspeptin in the pathogenesis of human obesity, we measured endogenous kisspeptin concentrations in normal-weight and overweight and/or obese females and correlated endogenous kisspeptin levels with various anthropometric indices.

SUBJECTS AND METHODS

The informed consent was obtained from each subject after approval of the experimental protocol by the Deanship of Scientific Research in our university. Twenty-eight female students (14 normal weight and 14 overweight/obese) from various colleges within our university were recruited. The study participants met the following inclusion criteria: 18- to 25-year-old Saudi females, with regular menstrual cycle (cycle length varying between 25 and 35 days with no more than 5 days' variability), and no use of prescription medications (including hormonal contraception) for at least 2 months before the study. Exclusion criteria were the presence of a chronic medical condition, irregular menstruation, and Inaccessibility to follow-up.

Anthropometric measurements were obtained using standard procedures (light clothing without shoes after voiding, following a minimum of a 4-hour fast).¹⁸ All measurements were conducted in duplicate, and the average of the 2 measures was recorded. Weight in kilograms was assessed to the nearest 0.5 kg using

digital or balance beam scales. Height was measured to the nearest centimeter with the participants looking straight ahead; standing upright on the floor, with heels together using a vertical metal centimeter rule was mounted. Waist circumference was assessed to the nearest 1 mm at the level of the iliac crest using a measuring tape. Body mass index (BMI) was calculated and categorized as normal (BMI 18.5–24.9), overweight (BMI 25–29.9), and obese (BMI \geq 30). Waist-hip ratio and waist-stature ratio were calculated by waist circumference/hip circumference and waist circumference/height, respectively.

Blood samples were obtained by venipuncture, after an overnight fast between 8 and 10 AM (to minimize the effect of circadian rhythm on kisspeptin levels). Three blood samples per volunteer were collected at 3 different times.¹⁹

- (1) Early follicular phase: During 2 to 5 days from the onset of menstrual cycle
- (2) Pre-ovulatory Phase: During 11 to 16 days before the start of the next menstrual cycle.
- (3) Luteal phase: Three to 5 days before the onset of the next menstrual cycle.

The verification of the menstrual cycle phase was done using basal body temperature (BBT) chart (high estrogens during follicular phase lowers BBT; high progesterone after ovulation raises BBT)¹⁹ and serum estradiol levels.²⁰

All blood samples were allowed to clot and were centrifuged within 30 minutes after vein puncture. The obtained serum was frozen at -80°C till further analysis using a kisspeptin ELISA Kit.

The statistical analysis was done by SPSS, version 20.0 (IBM SPSS Statistics, Armonk, NY, USA). Mean, maximum, minimum, and standard errors of mean were calculated by descriptive statistics. The comparison between normal-weight and overweight subjects was done by independent sample *t* test. The relationships between serum kisspeptin and various anthropometric variables were examined by Pearson correlation coefficient analyses. The level of significance was set at $P < .05$.

RESULTS

Mean anthropometric variables and serum kisspeptin levels in normal and overweight subjects are shown in **Tables 1 and 2**, respectively. A statistically insignificant difference was observed in serum kisspeptin levels in overweight subjects compared with normal-weight subjects in all 3 phases of menstrual cycle (**Table 2**).

Pearson correlation also failed to reveal any statistically significant relation between serum kisspeptin

levels with any of the anthropometric variables among the 2 groups (Tables 3 and 4; data shown for early follicular phase only).

DISCUSSION

To our knowledge, the findings of this study provide a novel report on plasma kisspeptin levels and their potential relationship with anthropometric data to be carried out in healthy females. Despite clear scientific evidence from animal experiments showing that kisspeptin expression changes with changes in body weight,¹⁰⁻¹³ we found no significant difference in kisspeptin levels in overweight females compared with normal-weight females. Also, no correlation was observed between any of the anthropometric indices and kisspeptin. Since our study subjects were humans, it renders more authenticity to our results.

Our results are in agreement with those of Pita et al²¹ who did not find any correlation between kisspeptin and anthropometric data in neonates or adults. Our study results are also in agreement with the studies that have reported no effect of obesogenic diet such as high-fat diet on adult KiSS1 expression.²²⁻²⁴ There is a strong possibility that it is metabolic dysfunctions associated with obesity, such as hypertriglycemia, and not the degree of obesity per se that modulates kisspeptin expression in the brain. In line with this, Overgaard²⁵ found that the number of kisspeptin neurons was inversely correlated with plasma triglyceride levels, and there was no correlation between body weight and kisspeptin expression. The suggested mechanism underpinning this is the hypertriglycemia-induced lipotoxic inflammation in the hypothalamus, thereby decreasing the number of kisspeptin neurons. Further studies localizing inflammatory markers in kisspeptin neurons are needed to confirm this hypothesis.

However, our results are contrary to the studies

that have found statistically significant difference in kisspeptin in overweight and obese. Pita et al²¹ reported increased kisspeptin in prepubertal obese girls compared with healthy prepubertal girls ($P < .01$). Moreover, kisspeptin levels were related to BMI in healthy as well as obese girls, suggesting a probable role for adipose tissue in the regulation of kisspeptin synthesis. The cause of this discrepancy in results could be different methodological approaches used. Pita et al measured kisspeptin levels by radioimmunoassay (RIA), whereas we used ELISA. The RIA technique is more specific and sensitive than ELISA. Moreover

Table 1. Anthropometric variables in 2 groups.

Variables	Normal weight group (Mean [SEM])	Overweight/obese group (Mean [SEM])
Weight (kg)	50.88 (1.61)	76.40 (14.84)
Height (cm)	153.82 (1.32)	157.88 (1.59)
BMI (kg/m ²)	21.50 (0.58)	30.43 (0.92)
Waist circumference (cm)	72.27 (1.59)	85.00 (3.85)
Hip circumference (cm)	94.64 (1.10)	111.53 (4.76)
Waist-hip ratio	0.76 (0.01)	0.76 (0.01)
Waist-stature ratio	1.43 (0.03)	1.13 (0.05)

BMI: Body mass index, SEM: standard error of mean.

Table 2. Serum Kisspeptin levels in normal and over-weight subjects.

Serum kisspeptin (nmol/L)	Normal weight (Mean [SEM])	Overweight (Mean [SEM])	P value
Early follicular phase	259.8 (20.5)	266.88 (45.5)	.91
Pre-ovulatory phase	448.4 (24.6)	477.12 (21.2)	.39
Luteal phase	708.2 (49.5)	735.13 (52.6)	.72

SEM, Standard error of mean. P value determined by independent sample t test.

Table 3. Pearson correlation of kisspeptin with anthropometric indices in normal-weight subjects.

Serum kisspeptin Levels	Weight	BMI	WC	HC	WHR	WSR
Pearson correlation	0.01	-0.27	.39	.03	.61	.39
Significance (2 tailed)	0.98	0.42	.24	.93	.07	.23

BMI, Body mass index; WC, waist circumference; HC, hip circumference; WHR, waist-hip ratio; WSR, waist-stature ratio.

Table 4. Pearson correlation of Kisspeptin with anthropometric indices in overweight subjects.

Serum kisspeptin levels	Weight	BMI	WC	HC	WHR	WSR
Pearson correlation	.13	.16	.07	.01	.22	.21
Significance (two-tailed)	.62	.55	.79	.98	.41	.42

BMI, Body mass index; WC, waist circumference; HC, hip circumference; WHR, waist-hip ratio; WSR, waist-stature ratio.

their study subjects were prepubertal girls, whereas ours were pubertal girls. The justification for the variations in results of prepubertal and adult females could be that kisspeptin behaves differently throughout development in females. Serum kisspeptin levels are significantly higher in adult females than in prepubertal females.²¹ This finding seems to indicate that in the early stages of life, the nutritional status or body weight influence plasma kisspeptin levels and, once puberty has begun, other factors may regulate kisspeptin.

Briefly, we conclude that in our limited number of subjects, no significant difference was found between

serum kisspeptin levels in obese and normal-weight females. Also no correlation was observed between serum kisspeptin and anthropometric indices.

Limitations and Recommendations

Several limiting factors probably affected the results of the study: small sample size, exclusion of prepuberty subjects, and use of ELISA to determine KP levels.

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