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The Effects of Ramadan Fasting on the Level of Sex Hormones in Pre-menarche Girls in Mashhad, Iran

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ABSTRACT

The present study aimed to evaluate the effects of Ramadan fasting on the level of sex hormones in girls between 9-13 years before age at menarche.

This study was conducted on a sample of 58 subjects (age range: 9-13 years), who were divided into two groups of fasting (N=31) and non-fasting (N=27). The levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), progesterone and estradiol were measured in all the subjects before and after Ramadan. Measurements were carried out three days before the start of Ramadan, and one day afterwards.

In this study, FSH levels significantly increased in the non-fasting group (P=0.01), and the level of Dehydroepiandrostenedione (DHEA) had a significant decrease during the time of study in both groups (P=0.001, P=0.006). In addition, serum levels of LH significantly increased in the non-fasting group after Ramadan (P=0.006), and estradiol significantly increased in both groups (P=0.008, P=0.004).

Given the similar changes in the levels of DHEA, progesterone and estradiol in both study groups, it could be concluded that fasting has no effects on these parameters, and the changes in LH and FSH levels could be due to other contributing factors.

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Introduction

Ramadan is a holy time of year in the Islamic culture, which lasts for one month and may coincide with different seasons (1, 2). Therefore, length of fasting varies depending on the season of the year and geographical features. Daily duration of fasting in Ramadan ranges between 12-19 hours (3).

In the month of Ramadan, healthy adults refrain from eating, drinking, smoking and sexual intercourse during daylight hours, starting from dawn to sunset (1, 4, 5). During this time, changes occur in the quality of food, and the nutritional patterns mainly consist of two large meals before dawn (Sahur) and another one after sunset (Iftar) (6, 7).

Alterations in eating habits and lifestyle during Ramadan will lead to decreased frequency of food and fluid intake, reduced nighttime sleep, and increased daytime sleep (8-10). Furthermore, lifestyle changes during Ramadan could affect the metabolism, as well as the endocrine and neuroendocrine systems (11-13).

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Hormonal factors involved in growth consist of the growth hormone (GH), insulin-like growth factor 1 (IGF-1), and sex hormones such as estradiol, progesterone, dehydroepiandestrone (DHEA), follicle-stimulating hormone (FSH), luteinizing hormone (LH), thyroid hormones, insulin, leptin and ghrelin (14, 15).

LH and FSH are among glycoprotein hormones, which are secreted from the anterior pituitary gland, also known as thyroid-stimulating hormones (16). The presence of Gonadotropin-releasing hormone (GnRH), as well as the reduction in serum levels of estrogen and testosterone, stimulate the secretion of FSH and LH from the anterior pituitary gland (16, 17). These glycoprotein hormones have a considerable influence on endocrine glands (i.e. thyroid, ovaries and testicles) (18).

Ovaries are responsible for the secretion of two main sex hormones: estrogen and progesterone. During childhood, estrogen is secreted in small proportions whereas in puberty, the level of this hormone, which is secreted under the influence of gonadotropic hormones of the anterior pituitary, increases by 20 times (18). Both estrogen and progesterone could diminish the secretion of FSH and LH (16, 17).

Hormone fluctuations occur concomitantly with puberty (19). Puberty is a complicated process, which involves the activation and maturation of hypothalamic-pituitary-gonadal (HPG) axis (20, 21). Normal puberty is a predictable process, and girls reach puberty earlier than boys; however, the age of puberty onset may vary under different circumstances (19-21).

On average, girls reach puberty at 10 years old (20). According to the commands of Islam, fasting is obligatory for girls at the age of 9 (22); however, there are some concerns among parents regarding the possible adverse effects of prolonged food deprivation on the growth and health condition of their daughters (23).

Given the importance of growth and puberty in girls during Ramadan, this study aimed to evaluate the effects of Ramadan fasting on the concentration of sex hormones in pre-menarche girls between 9-13 years of age.

Materials and Method

Study Design

This prospective, cohort study was conducted during the Ramadan of 1433 A.H (July-August 2012) in Mashhad, Iran. Participants were selected from the families affiliated to Imam Khomeini Relief Foundation, and based on the willingness of the subjects, they were divided into two groups of fasting and non-fasting. Discernment of pubertal stages (1, 2 and 3) was evaluated by a pediatrician, and determination of menarche was done based on the history and onset of the menstrual cycle of the participants.

Study Population

Sample Size

In this pilot study, the sample size in each group consisted of 30 subjects, and the participants were divided into two main groups. Eventually, 58 subjects, who were residents of Mashhad (located in the north-east of Iran), completed the study (fasting=31 subjects, non-fasting=27 subjects).

Inclusion and Exclusion Criteria

In this study, STROBE 2007 (v4) checklist was used, and the inclusion criteria were as follows: 1) age range between 9-13 years; 2) residence in Mashhad; 3) informed consent for participation; 4) absence of menarche (determined based on the clinical history) and 5) compliance with the study requirements (i.e. fasting for at least 20 days in the fasting group, in addition to 8 hours of sleep, moderate physical activity, and isocaloric diets).

Exclusion criteria of this study were the presence of autoimmune disorder, cardiac or renal diseases, chronic inflammation, thyroid disease, diabetes mellitus, and adherence to special diets.

Questionnaires

Demographic data of the subjects including age, education status, length of fasting and medical history were collected via interviews with the participants and their mothers, and the obtained data were recorded in separate questionnaires.

Anthropometric Measurements

In this study, the height of the participants was measured using a standard clinical stadiometer,

with ±.01 cm accuracy. In addition, body weight was measured with the participants in light clothes and without shoes, using Bioimpedance Analyzer (BIA) (BC-418 MA model).

Blood Sampling and Laboratory Tests

After 12 hours of fasting, blood samples (5 CC) were obtained from the participants three days before the start of Ramadan, and one day afterwards. The collected samples were sent to laboratory for the evaluation of the serum concentrations of sex hormones, such as estradiol, progesterone, DHEA, FSH and LH. Approximately 30 minutes after sampling, the collected sera were centrifuged (3-30K, Sigma, Germany) at 3000 rpm and 4°C for 10 minutes. Afterwards, the sera were placed into sterile micro tubes.

Serum concentrations of estradiol and progesterone were measured using a gamma counter device and Biosecure Radioimmunoassay (RIA) Kits (KIP 0629:96). In addition, serum levels of zinc, iron and ferritin were determined in the laboratory, and serum concentration of zinc was evaluated by atomic absorption spectroscopy instrument.

Ethical Considerations

The objectives and methods of the study were explained to the subjects, and informed consent was obtained prior to participation. The study protocol was approved by the Ethics Committee of the Research Center of Mashhad University of Medical Sciences, Mashhad, Iran.

Statistical Analysis

Statistical analysis of the collected data was performed using SPSS V.11 (SPSS Inc., Chicago, IL, USA), and Kolmogorov-Smirnov test was IL, USA), and Kolmogorov-Smirnov test was used to evaluate the distribution of variables. Moreover, parametric statistics were used for the assessment of the data with normal distribution, and Chi-square test was applied to verify the homogeneity of qualitative variables in the study groups.

Pre-test and post-test comparisons between the two groups were performed via paired t-test, and independent t-test was used to evaluate the Tanner Intervention program in both study groups. Variables were controlled by generalized linear models intervening test, and the obtained results were expressed as Mean ± Standard Deviation (SD). A P value of <0.05 was considered as significant.

Results

In total, 65 healthy girls were enrolled in this study (age range: 9-13 years), who were divided into two groups of fasting (N=31) and non-fasting (N=34). Out of these participants, 58 subjects were able to complete the study, and 3 subjects were excluded from the fasting group (one subject due to menarche issues, and two subjects due to fasting for less than 20 days).

On the other hand, 4 subjects were excluded from the non-fasting group (one due to menarche issues, two cases due to departure from the city of study, and one subject left the study). The 58 remaining participants were followed-up during the study period until the end of Ramadan month.

The mean age of the participants in the fasting and non-fasting groups was 11.06 ± 1.15 and 10.88 ± 1.31 years, respectively (age range: 9-13 years). The baseline characteristics of the studied subjects are shown in Table 1.

The distribution of Tanner stages at the baseline in the two groups is shown in Table 2.

Table 1. Baseline Characteristics of Participants

Quantitative Variable	Fasting Group	Non-fasting Group	Total Number	Independent t-test Results
	Mean ± SD	Mean ± SD	Mean ± SD	
Age (year)	11.06 ± 1.15	10.88 ± 1.31	10.97 ± 1.22	P = 0.58
Primary Height (cm)	143.16 ± 7.44	147.0 ± 8.06	142.01 ± 7.76	P = 0.23
Primary Weight (kg)	35.57 ± 7.99	32.61 ± 7.76	34.20 ± 7.96	P = 0.16
Primary BMI (kg/m^2)*	17.20 ± 2.85	16.26 ± 2.48	16.76 ± 2.70	P = 0.19
Iron (mg/dL)	90.22 ± 30.24	84.37 ± 40.94	87.50 ± 35.42	P = 0.54
Zinc (mg/dL)	86.85 ± 15.46	82.90 ± 13.36	85.01 ± 14.53	P = 0.30
Ferritin (mg/dL)	27.14 ± 15.55	29.45 ± 11.68	28.21 ± 13.81	P = 0.53

*BMI: Body Mass Index

In this study, the variables were assessed between the two groups for normal distribution. According to the results of Kolmogorov-Smirnov test, all the variables were normally distributed in the groups before the month of Ramadan.

According to the information in Table 3, no significant increase was observed in the serum levels of FSH during the time of study in the fasting group (P=0.56), while these levels rose significantly in the non-fasting group (P=0.01). However, this difference was not considered to be statistically significant (P=0.43). Moreover, after controlling the confounding effect of Tanner stages, the FSH levels were not observed to be significantly different between the two study groups (P=0.45, F=0.57), and there was no correlation between the Tanner stage and fasting (P=0.94, F=0.05).

As shown in Table 3, DHEA levels had a statistically significant decrease during the time of study in both groups (P=0.001, P=0.006); however, the changes in the levels of DHEA before and after fasting were not considered to be significantly different between the groups (P=0.58). After controlling the confounding effect of the Tanner stage, the level of DHEA was not observed to be significantly different between the two study groups (P=0.58, F=0.29), and a correlation was found between the Tanner stage and fasting (P=0.05, F=3.18).

According to the information in Table 3, serum levels of LH in the non-fasting group increased significantly after Ramadan (P=0.006). As for the fasting group, the increase in the serum levels of LH was not considered to be significant (P=0.059).

Table 2. Distribution of Tanner Stages at Baseline in the Study Groups

Tanner Stages	Fasting Group		Non-fasting Group		Total Number	
	Number	%	Number	%	Number	%
Stage 1	5	16.1	9	33.3	14	24.1
Stage 2	12	38.7	10	37.0	22	37.9
Stage 3	14	45.2	8	29.6	22	37.9
Total	31	100	27	100	58	100
	Chi-square Results		P=0.25		x^2=2.69	

Stage 1: First stage of breast development (before breast growth)
Stage 2: Second stage of breast development
Stage 3: Third stage of breast development

Table 3. Mean and SD of FSH (mIU/ml), DHEA (ng/ml), LH (mIU/ml), Progesterone (pg/ml) and Estradiol (pg/ml) in the Study Groups (before and after Ramadan)

Variables/ Groups	Before Ramadan	After Ramadan	Level of Changes before and after Ramadan	Paired T-test Results	
				P	T
FSH (mIU/ml): Fasting	5.38±3.07	5.71±4.03	0.33±3.16	0.56	-0.58
Non-fasting	3.73±3.76	4.70±3.09	0.96±2.96	*0.01	-1.68
T-test	P 0.07 t 1.83	P 0.29 t 1.06	P 0.43 t -0.78		
DHEA (ng/ml): Fasting	802.75±463.81	547.30±385.88	-255.45±274.28	*0.001	5.18
Non-fasting	862.07±800.91	544.77±405.92	-317.29±548.24	*0.006	3.00
T-test	P 0.72 t -0.35	P 0.98 t 0.02	P 0.58 t 0.55		
LH (mIU/ml): Fasting	2.58±2.34	3.23±2.63	0.65±1.84	0.059	-1.96
Non-fasting	1.05±0.91	1.64±1.55	0.40±0.80	*0.006	-2.97
T-test	*P 0.002 t 3.35	*P 0.006 t 2.84	P 0.50 t 0.67		
Progesterone (pg/ml): Fasting	0.98±0.96	1.21±0.63	0.23±0.84	0.13	-1.53
Non-fasting	1.05±1.20	1.32±1.01	0.26±1.19	0.25	-1.15
T-test	P 0.78 t 0.27	P 0.60 t -0.51	P 0.89 t -0.12		
Estradiol (pg/ml): Fasting	0.98±0.96	1.21±0.63	0.23±0.84	*0.008	-2.83
Non-fasting	1.05±1.20	1.32±1.01	0.26±1.19	*0.004	-3.20
T-test	P 0.78 t -0.27	P 0.60 t -0.51	P 0.89 t -0.12		

*FSH: Follicle-stimulating Hormone
*DHEA: Dehydroepiandrosterone
*LH: Luteinizing Hormone
*Significant P value

Furthermore, the changes in the serum levels of LH before and after Ramadan were not significantly different between the two study groups (P=0.50). After controlling the confounding effect of the Tanner stage, LH levels were not observed to be significantly different between the two groups (P=0.53, F=0.39), and no correlation was found between the Tanner stage and fasting (P=0.97, F=0.02).

According to the data presented in Table 3, progesterone levels had no significant increase during the time of study in the two groups (P=0.13, P=0.25). In addition, the changes in the serum levels of progesterone before and after Ramadan were not considered to be significantly different between the two study groups (P=0.89). After controlling the confounding effect of the Tanner stage, the levels of progesterone were not observed to be significantly different between the two groups (P=0.75, F=0.1); however, there was a correlation between the Tanner stage and fasting (P=0.04, F=3.25).

On the other hand, the serum levels of estradiol had a significant increase in both groups (P=0.008, P=0.004), and the difference between the groups was not considered to be statistically significant (P=0.73). After controlling the confounding effect of the Tanner stage, estradiol levels were not observed to be significantly different between the two groups (P=0.97, F=0.001), and no correlation was found between the Tanner stage and fasting (P=0.46, F=0.77).

Discussion

According to the commands of Islam and several clergies, women are expected to start fasting at the age of 9. During the month of Ramadan, changes in the lifestyle may have metabolic and hormonal effects on the body (3, 24-26).

Normal growth during childhood largely depends on adequate nutrition, desirable mental environment, absence of diseases and secretion of sufficient amounts of metabolic hormones (27-29). In this regard, sex hormones are known to play a pivotal role in normal puberty and sexual development of individuals (16, 17, 30).

In the current study, similar changes were observed in the serum levels of DHEA, progesterone and estradiol in both study groups during Ramadan; however, it should be noted that the changes in FSH and LH levels were statistically different between the fasting and non-fasting groups.

There are conflicting results regarding the effects of Ramadan fasting on the secretion of sex hormones. According to some studies, prolonged food deprivation may not affect the serum levels of FSH and testosterone (12). Moreover, serum concentrations of LH and the associated response to GnRH while fasting might not change significantly, while FSH levels are likely to decrease (31).

In one study, Bogden et al. reported no changes in the rhythms of LH and FSH in fasting individuals. Furthermore, they claimed that the mean concentration of LH had no significant changes during Ramadan, while FSH concentrations declined significantly (32).

In another study, Cameron et al. observed that after 48 hours of starvation, serum levels of LH and FSH decreased significantly (33), while Mebahzadeh et al. concluded that FSH levels significantly increased at the 20th day of Ramadan, and LH concentrations had no significant differences in fasting individuals.

In a study by Shahabi et al., it was declared that Islamic fasting caused no significant changes in the secretion of estrogen, progesterone, FSH and LH (34). The results of the present study indicated that the serum levels of progesterone after Ramadan had no significant difference compared to the beginning of the month in both study groups. However, estradiol levels significantly increased in both groups, which could be due to the differences in the age of the participants.

According to the results of the current study, serum levels of FSH and LH increased significantly in the non-fasting group, and it could be concluded that Ramadan fasting had no significant effects on the levels of these hormones.

Other studies in this regard have indicated that decreased levels of insulin could lead to increased concentrations of sex hormone binding globulin (SHBG) (35-37), which may enhance total testosterone levels and reduce the level of free testosterone. Furthermore, increased SHBG levels may result in the reduction of free testosterone, diminishing the prohibitory effect of this hormone on GnRH, which increases the levels of GnRH and LH (35, 37, 38).

In the current study, serum level of insulin decreased in both group after Ramadan. Although this change was not considered significant, it could be the main cause of the enhancement in the serum level of LH in the fasting group.

Inconsistencies in the findings of different studies regarding the effects of Ramadan fasting on sex hormones are due to a variety of factors such as unique food habits in different countries, differences in the duration of fasting and variations in the coinciding season with Ramadan, differences in study methods, race, gender, age and physiological status of subjects (e.g. puberty and Tanner stage), and time and date of sampling before and after Ramadan, which could affect the circadian rhythms of sex hormones (39, 40). Other influential factors include differences in sleep patterns, which is an essential component affecting the secretion of sex hormones (4, 41, 42), and psychological and social habits.

With respect to the aforementioned factors, a definite conclusion could not be drawn on the exact effects of Ramadan fasting on sex hormones secretion, especially in pre-menarche girls. Therefore, further comprehensive studies with controlled conditions are required as to provide more information on this subject.

Study Limitations

This study was conducted on a particular population (individuals supported by Imam Khomeini Relief Foundation), and it is suggested that the study be repeated on various groups of people.

Conclusion

According to the similar changes observed in the serum levels of DHEA, progesterone and estradiol in both study groups, it could be concluded that fasting may not be the major influential factor in these parameters, and other aspects of the different lifestyle during Ramadan might be responsible for the changes in the serum levels of LH and FSH.

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