

# Subclinical Eating Disorder, Polycystic Ovary Syndrome- Is There Any Connection Between These Two Conditions Through Leptin- A Twin Study

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## Summary

The genetic property of subclinical eating behaviour (SEB) and the link between SEB and polycystic ovary syndrome (PCOS) has been studied before but the role of leptin within this connection has never been investigated. The objective of this study was 1) to study the genetic property of SEB. 2) To find a link between leptin, SEB and PCOS. One hundred and fifty four (77 pairs) female-female Iranian twins including 96 MZ individuals (48 pairs) and 58 DZ individuals (29 pairs) participated in the study. Clinical, biochemical and ultrasound tools were used to diagnose polycystic ovary syndrome. BITE questionnaire was filled out for subjects. Eight percent of subjects were diagnosed for subclinical eating disorder. No significant difference was found between intraclass correlation of MZ and DZ ( $z = 0.57$ ,  $P = 0.569$ ). Serum leptin level correlated significantly with bulimia score ( $P < 0.007$ ). The mean ( $\pm$ SD) value for bulimia score was found to be higher among PCOS<sup>positive</sup> subjects ( $3.27 \pm 5.51$ ) in comparison with PCOS<sup>negative</sup> subjects ( $2.06 \pm 4.48$ ) ( $P < 0.001$ ). The genetic property of subclinical eating disorder was not confirmed as shared environment might have played a major role in likeliness of DZ twins as well as MZ. Leptin is linked with both subclinical eating disorder and PCOS.

**Key Words:** Subclinical eating behaviour, Polycystic ovary syndrome, Leptin

## Introduction

Both polycystic ovary syndrome (PCOS) and subclinical eating behaviour (SEB) are commonly found among female population<sup>1, 2</sup>. Symptoms such as acne, unovulation, and amenorrhea are found in both endocrinopathies<sup>3, 4</sup>. A genetic link for both conditions has been defined extensively through family pedigrees and twin studies<sup>5, 6</sup>. Twin studies have confirmed that both PCOS and SEB are familial<sup>7, 8, 9, 10</sup>. In a study performed in 34 female-female Australian twin pairs, a significant contribution of additive genetic effects and unique environmental factors were found in these conditions<sup>11</sup>. The magnitude of the contribution of

shared environment was less clear in the previous twin study. Thus, the first objective of this study was to use greater statistical power in a much larger population of twins, within the younger population so that the possibility of shared environment would be greater.

SEB has been associated with impaired satiety, decreased resting metabolic rate, and abnormal neuroendocrine regulation<sup>12</sup>. Preclinical studies suggest that such alterations could be associated with impaired leptin function<sup>13</sup>. These results are consistent with the hypothesis that decreased leptin function may be associated with alterations in eating patterns, metabolic rate, and neuroendocrine regulation in SEB<sup>14</sup>.

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Alteration in leptin level has been also reported in PCOS<sup>15, 16</sup>. The second objective of our study was to find a link between these two conditions and leptin level.

### Materials and Methods

Since Iran has no twin registry, subjects were recruited through mass media, posters and advertisements in magazines and newspapers. A poster was designed including a short introduction of PCOS, inviting female-female twins, aged between 15-45 to have a free ultrasound, blood test and an examination. An offer was also made to pay their traveling costs. Five thousand posters were distributed to public and private hospitals, major universities in Tehran and also girls' high-schools. Private medical officers and midwives were also notified through the Medical Council by leaflets and advertisements in monthly journals distributed to the private practices throughout the capital city, Tehran.

The study was done in AVECINA Research Center, situated in a former National University (Shahid-Beheshti), a well-known university in Tehran. Subjects could contact the center during working hours. Full-time research assistance was available to attend to the phone calls. A preliminary questionnaire including contact number, addresses and descriptive data was filled out by telephone. Appointments were made for twin sisters during their early follicular phase if they had normal regular menstrual cycles. If they were amenorrhoeic, the appointment was made at the first convenient time. Subjects were instructed to fast overnight and start drinking 5 glasses of water an hour prior to their appointment for ultrasound examination.

Those who were on contraceptive pills, any other hormonal medications, pregnant or were breastfeeding their babies were excluded from the study. High level of 17-hydroxy progesterone was also considered an exclusion criteria. Because study analysis was not possible if the data from one of them was missing, both twins were asked to participate in the study. In some cases several phone calls were made to ensure that subjects kept their appointments, and were familiar with the preparatory instructions.

Subjects were briefed upon their arrival. A consent form was signed. A self-rating scale for bulimia known as bulimia investigation test (Edinburgh) (BITE) was filled out for each subject. The questionnaire consisted

30 questions to investigate habits of dieting and symptoms and behavior associated with binge eating. Subjects were scored and classified according to their score. Those with score above 20 were considered at high risk of having bulimia (severe), subjects with a score of 10-19 were considered to have a subclinical eating disorder (moderate) and lastly those with score less than 10 were classified as subjects with normal eating patterns. This questionnaire has 100% sensitivity and 95% specificity with a positive predictive value of 89% and a false positive rate of 5%<sup>17</sup>.

A standard questionnaire was filled out containing the result of their examination. Fasting blood was drawn using vacutainer syringes. Blood was then centrifuged at the speed of 2000 RPM for 10-20 minutes, immediately after withdrawal and serum was placed in 2cc plastic containers before they were marked and stored in -80C freezer. Subjects were then asked to drink a syrup containing 75mg of glucose. Two hours later a blood test was performed. Pelvic ultrasound was performed to visualize the ovaries using Prie-Medial machine and 3.5 MHz trasabdominal and 5 MHz transvaginal transducer when appropriate. Adam's criteria were adopted to diagnose polycystic ovaries<sup>18</sup> namely the existence of more than 10 peripheral follicles (2-8 mil in diameter) associated with an increase in ovarian stroma. The ovaries were measured in three planes and the volume was calculated using the formula: length × width × thickness × 0.5. Vaginal ultrasound was performed in case of obesity when necessary. The sonographer was blind to the clinical and biochemical findings.

The subjects' height and weight were measured and BMI was calculated. Hirsutism was evaluated using the Ferriman and Gallway Scoring System<sup>19</sup>. The Marynick score system was adopted to examine acne severity<sup>20</sup>. Oligomenorrhea was defined as less than 8 cycles per year and amenorrhea as 0-2 cycles per year. Regular menstrual cycles were defined as having regular periods every 21 to 35 days.

Clinical symptoms were considered positive if subjects were suffering from hyperandrogenemia (hirsutism, acne) and chronic anovulation (amenorrhea, oligomenorrhea or irregular menstruation).

Biochemical measurements included the following: Testosterone (T), Luteinizing hormone (LH), Follicle stimulating hormone (FSH), Dehydroepiandrosterone Sulfate (DHEAS), Sex hormone Binding Globulin

(SHBG), 17-hydroxy progesterone (17-OHP), Insulin, Fasting blood sugar, 2 hours blood sugar, and leptin.

Serum hormone levels were quantified by well-established RIA methods using BIO Source Europe S.A. Leptin was measured by a direct RIA kit (DRG Instruments GmbH, Germany). In all assays intra-assay and inter-assay coefficients of variation did not exceed 7% and 15%, respectively. Biochemical findings were considered positive if T was more than 1.1 ng/ml and/or the ratio of LH/FSH was more than 2.

The zygosity of twin pairs was determined by asking about their physical similarity, frequency of confusion as children by parents, teachers, and strangers. In a population-based sample of female-female adult twin pairs, self-reported zygosity in both members of the twin pair agreed with assigned zygosity in about 85% of pairs<sup>21</sup>.

One hundred and fifty four (77 pairs) female-female twins aged 15-45 participated in the study. They were 96 MZ individuals (48 pairs) and 58 DZ individuals (29 pairs) who all lived in Tehran, the capital city of Iran.

Student's t-test and Chi-square test were used to compare quantitative and qualitative variables, respectively. Fisher test was also used when appropriate.  $P < 0.05$  was considered statistically significant. Results are expressed as means  $\pm$  SD. Details of twin statistical analysis has been explained elsewhere<sup>11</sup>.

## Results

The mean  $\pm$  SD value for age in the whole group was  $21.77 \pm 6.71$ . Overall, 31.8% (49/154) of subjects had hirsutism (4.3% severe, 7.1% moderate and 7.1% mild), 48.7% had acne (38.3% mild, 10.4% moderate and none severe), 2.6% were amenorrhic, 7.7% had oligomenorrhea and 29.9% had irregular menstruation.

No subject had severe bulimia (score more than 20). Only 8% of subjects were diagnosed for subclinical eating disorder and 92% had normal eating behaviour. As it has been shown in Table I, BMI was higher in subjects with subclinical eating disorder. Moreover, amenorrhea (16.7% vs 1.4%,  $P < 0.001$ ) and anovulation (33.3% vs. 5.6%,  $P < 0.001$ ) were found to be significantly higher in the group with eating disorder as

compared with subjects with normal eating habit. Although irregular menstruation was higher among subjects with eating disorder it was not significantly different from the normal group (50% vs 28.2%,  $p = 0.113$ ).

The mean value of bulimia score was found to be higher among MZ ( $4.00 \pm 1.85$ ) twins as compared with DZ ( $5.4 \pm 3.07$ ) ( $P < 0.002$ ). Correlation between the bulimia score for MZ twins (Twin1-Twin2) resulted in  $r_{MZ} = 0.527$  ( $P = 0.001$ ). This means that there is a similarity between the bulimia score of MZ twin sets (Figure 1). The corresponding value for DZ twins was  $r_{DZ} = 0.418$  ( $P = 0.024$ ) (Figure 2), suggesting that ( $r_{MZ} > r_{DZ}$ ). A comparison between these two correlations suggests no significant difference ( $z = 0.57$ ,  $P = 0.569$ ).

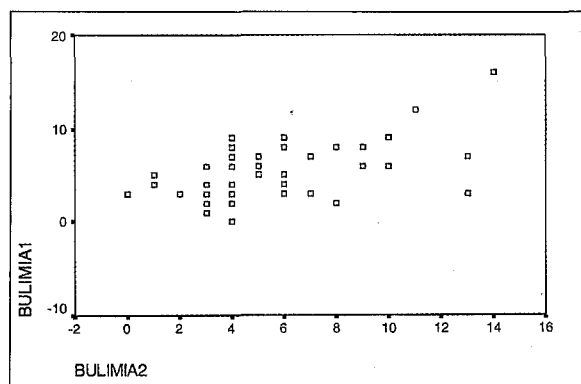
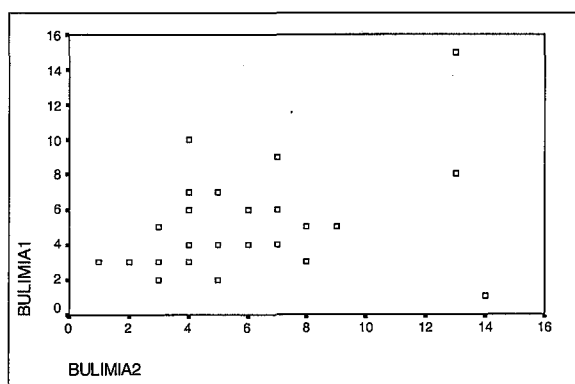
Using clinical and biochemical measures to diagnose PCOS, 16.2% of the subjects were diagnosed as PCOS positive (25/154). When clinical symptoms were used to diagnose PCOS, the mean ( $\pm$ SD) value for bulimia score was found to be higher among PCOS<sup>positive</sup> subjects ( $3.27 \pm 5.51$ ) in comparison with PCOS<sup>negative</sup> subjects ( $2.06 \pm 4.48$ ) ( $P < 0.001$ ). However, when using other measures (clinical, biochemical and ultrasound), classification of subjects with or without PCOS was done no significant difference was found between the two groups of PCOS<sup>positive</sup> and PCOS<sup>negative</sup> for bulimia score.

Bulimia score was found to be positively correlated with BMI ( $P < 0.001$ ), acne ( $P < 0.0001$ ), amenorrhea ( $P < 0.001$ ), number of periods per year ( $P < 0.02$ ). No significant correlation was found between bulimia score and age ( $P < 0.592$ ), age of menarche ( $P < 0.290$ ), hirsutism ( $P < 0.627$ ). From biochemical measurements, serum leptin level was the only parameter which was correlated significantly with bulimia score ( $P < 0.007$ ). Leptin itself, however was negatively correlated with SHBG ( $P < 0.001$ ), and positively correlated with fasting blood sugar ( $P < 0.002$ ), fasting insulin level ( $P < 0.001$ ) and the level of sugar 2 hours after drinking 70gr of syrup ( $P < 0.0001$ ).

When the serum level of leptin was compared between the two groups of PCOS<sup>positive</sup> and PCOS<sup>negative</sup> irrespective of their zygosity, the mean $\pm$ SD values were  $11.62 \pm 7.67$  and  $13.84 \pm 9.92$  respectively. Comparing the mean values, no significant difference was found ( $P = 0.073$ ).

**Table 1: A comparison between mean  $\pm$ SD values of demographic parameters for subjects with (n= 12) and without (n= 142) subclinical eating disorder.**

Variable	Normal eating habits (n=142)	Subclinical eating disorder(n=12)	P value
Age (year)	21.7 $\pm$ 6.86	22.58 $\pm$ 4.58	-
Menarche (year)	12.62 $\pm$ 1.23	12.5 $\pm$ 1.62	-
BMI (kg/m <sup>2</sup> )	22.79 $\pm$ 4.65	26.37 $\pm$ 5.14	0.012
Hirsutism score	4.11 $\pm$ 5.66	4.83 $\pm$ 3.97	-
Acne score	0.54 $\pm$ 0.67	1.25 $\pm$ 0.62	0.001

**Fig. 1: Correlation between the bulimia score Monozygotic Twins****Fig. 2: Correlation between the bulimia score of the Dizygotic Twins**

## Discussion

This twin study was performed in female-female twin pairs living in Tehran in order to find the genetic or environmental property of bulimia. Moreover, a link between PCOS and SEB through leptin levels was also sought.

Because the subjects were younger, mostly studying in high school or university, only 6.5% of them were married. The rest were living with their parents in a shared environment. Twin analysis on bulimia score suggests that although the similarity between MZ twins was more than that of the DZ, this difference was not significant indicating the importance of environmental factors. The value for correlation of coefficient was high for DZ twins. It is interesting to note that only one pair of DZ twins in this study were married and living in different environment. Living in a similar environment can have a psychological effect on eating patterns leading to the more similarity between DZ and obscuring the genetic factor.

Explanation on the hormonal imbalance in PCOS and SEB lie in the relationship between metabolism and reproduction. Such substances as insulin, amino acids and IGFBP-I have been proposed as signals of body mass fat on the female endocrine axis<sup>22, 23</sup>. Today this role is claimed by leptin, a protein hormone decoded from the obesity gene and secreted exclusively from adipose tissue<sup>24, 25</sup>. This hormone acts on the central nervous system to result in the suppression of food intake and increase in energy consumption<sup>26</sup>. Leptin, also interacts with the reproductive axis at multiple sites, with stimulatory effects at the hypothalamus and pituitary and inhibitory actions at the gonads<sup>27</sup>.

Studies show plasma leptin concentration decreases in individuals subjected to binge eating patterns<sup>28, 29</sup>. This may suggest that decreased leptin function may be associated with alterations in eating patterns. Thus, with decreased leptin function, feeding may be stimulated more readily, resulting in increased food intake which can lead to constant weight problems.

Weight gain may lead to various clinical and biochemical alterations consistent with PCOS condition.

The second objective of this study was to investigate the relation between leptin, SED and PCOS.

There was a significant correlation between bulimia score and the leptin level ( $P < 0.007$ ). However, leptin was not found to be significantly different in subjects with or without PCOS. This result is in the light of the fact that BMI has been adjusted for comparative traits.

Correlation between bulimia and BMI has been shown in many studies<sup>30, 31</sup>. Our study also shows the same results. The correlation between clinical symptoms such as acne and amenorrhea (which are also common symptoms of PCOS) and SEB suggests the relation between these two conditions. It also explains the higher bulimia score for PCOS<sup>positive</sup> subjects when their diagnosis is based on the clinical symptoms. In the Australian twin study a significant correlation was found between the severity of bulimia score and the hirsutism<sup>32</sup>. In the current study, no subject had severe bulimia and no significant correlation was found between hirsutism and bulimia score. It may suggest that hirsutism occurs in a chronic status of eating disorder. Acne score however was higher in subjects with subclinical eating disorder when compared with the normals.

The prevalence of sub clinical eating disorder in our study was found to be less than other similar studies<sup>11</sup>. Thus, younger age of the subjects may be the main reason. SEB is known as a chronic disorder. As we age, the rate of metabolism slows down. Weight gain worsens the disorder. It can be postulated that with time the severity of eating disorder develops leading to clinical symptoms and biochemical alterations common to both SEB and PCOS. This spectrum can be set to reverse itself by losing weight. A recent study has shown when patients with bulimia nervosa were treated, and when their weight gaining process has been reversed, the PCO morphology of their ovaries returned to normal<sup>33</sup>. This can be due to regaining control over leptin production. As it has been shown, weight losing exercises can normalises the level of leptin<sup>34</sup> which in time can lead to biochemical changes such as alteration in the level of SHBG, insulin and sugar content of the blood.

## Conclusion

The genetic property of bulimia nervosa was not found in this twin study. The reason can be the younger age of our study group. Leptin was linked with both SEB and PCOS conditions.

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