Interplay of leptin with obesity and polycystic ovarian syndrome

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Abstract: Objectives. To determine whether polycystic ovarian syndrome (PCOS) is a causative factor in dysregulation of leptin levels in obese subjects and to identify effect of PCOS on the relationship of serum leptin with hormonal, metabolic and anthropometric parameters in obese PCOS women. **Methods**: A cross sectional study in Jinnah Postgraduate Medical Center Karachi, Pakistan recruited twenty nine obese (BMI >30 kg/m^2) and 20 normal-weight (BMI <25 kg/m^2) PCOS women together with twenty seven normal cycling (NC) obese (BMI >30 kg/m^2) and 25 normal-weight females (BMI <25 kg/m^2). Serum leptin, prolactin, follicle stimulatimg hormone (FSH), lutenizing hormone (LH), free testosterone, estradiol (E2) and fasting blood glucose (FBG) levels were measured by Radioimmunoassay. **Results**: Mean serum leptin levels were higher in obese compared to normal-weight PCOS (54.00 ± 9.33 vs. 26.08±2.44 ng/ml; p <0.01) and in obese compared to normal-weight NC (51.54 ±9.19 vs. 25.39 ± 2.37 ng/ml; p <0.01). Significantly lower level of FSH, LH, E2 (p <0.01), was observed in both groups of PCOS as compared to non-PCOS while higher levels of testosterone, progesterone and FBG were found in obese PCOS in comparison to obese NC (p <0.01). Leptin levels correlated with BMI in all four groups. There was a significant correlation of FBG with obese NC (r=0.42, p<0.05) and normal-weight and obese groups of PCOS (r=0.45, p<0.05; r=0.58, p<0.05 respectively). The leptin level correlated with E2 and testosterone in obese PCOS subjects (p<0.05; p<0.05 respectively). **Conclusion**: It seems that leptin has a role in pathogenesis of PCOS in obese patients.

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Key words: Leptin, obesity, PCOS

Introduction:

Leptin classified under "tumor necrosis factor" family of cytokines, is an amino acid peptide consisting of 167 amino acids. (Ramanand et al., 2014) It is secreted from fat cells in adipose tissue and moves in the circulation as free or bound to the soluble isoform and its levels are directly proportional to the amount of adipose cells. (Buettner et al., 2008) The main role in the human body appears to control body weight through the regulation of appetite and thermogenesis. It has been established that hyperleptinemia is responsible for stimulating the appetite center and may also result in reduction of thermogenesis. (Enriori et al.,2011).

Obesity has emerged as an epidemic with increased prevalence in developing countries of the world. (Rehman et al.,2013) It is documented that 1% of the Pakistani population in the reproductive age of 15-24 years are overweight to obese. Obesity (OB) gene is mainly expressed in adipocytes and works by playing a central role through signaling the amount of energy stores. Genetic correlation of OB gene expression to leptin levels has been studied. (Lui et al., 2012). The conventional gauge for measurement of obesity is body mass index (BMI). It measures body weight against standard height and gives an index of health risks for metabolic disorders and reproductive functions.

The association of leptin and obesity have been proved by reduction of body fat tissue and hence, body weight by injection of leptin in animals. Increased leptin in circulation, acts on its hypothalamic receptors resulting in reduction of appetite and food intake. Lack of existence of physiological feedback mechanism is supposed to be involved in development of obesity. (Ramanand et al., 2012, Buettner et al., 2008) Additionally, several observations in animals and human studies suggest that leptin plays a key role in signaling for adequate energy stores, which are available for reproduction. Circulating leptin concentrations can be used as predictors of menstrual function in addition to BMI, fat mass or percentage body fat. (Baranova et al., 2013, El-Gharib et al., 2014). This phenomenon indicates the relationship between leptin, adipose cells and reproductive capability.

Congenital deficiency of leptin in ob/ob mice results in obesity as well as infertility. (Wang et al., 2011) The leptin treatment of these mice increases

luteinizing hormone (LH) concentrations and weight of the reproductive organs. (Wang et al., 2012) In pituitary gland, the growth hormone, LH, follicle stimulating hormone (FSH), and thyroid stimulating hormone (TSH) producing cells have receptors for adiponectin, and leptin. (Lui et al., 2012, Psilopanagiotio et al., 2009) androgens and estrogens have effect on some adipokines secretion. (Mammi et al., 2012, Merki-Feld et al., 2011). Literature suggests that there is mutual relationship between endocrine function of adipose tissue and hypothalamic-pituitary-gonadal axis.

Polycystic ovarian syndrome (PCOS) is a common endocrine disorder in women with 6-7% prevalence in the world. PCOS produces symptoms in approximately 5% to 10% of women of reproductive age (12–45 years old). (Yildiz et al., 2013) The heterogeneous disorder is associated with menstrual dysregularities, hyperandrogenemia, obesity, increased insulin levels and insulin resistance features that are linked to leptin and its receptors. (Rojas et al., 2014) This syndrome affects the reproductive capability of women due to chronic anovulatory cycles and is also associated with increase in BMI. It is also reported that women with PCOS had higher leptin concentrations as compared to the non-PCOS group stating that this is due to higher BMI. (Olszanecka-Glinianowicz et al., 2013) Thus, leptin, body weight, PCOS, androgens and insulin level show some variant of inter linkage and may be dependent on one another. However, the relationship between leptin, obesity and pathogenesis of PCOS is still controversial. Several studies have been reported in favor and against their interaction. The high leptin concentration in PCOS with obesity and abnormal ovarian functions pursued us to find whether PCOS is a causative factor in dysregulation of leptin levels in obese subjects and what's the effect of PCOS on the relationship of serum leptin with hormonal, metabolic and anthropometric parameters in obese PCOS women.

Methods

Patient Selection

This study was approved by the Board of Advanced Studies and Research (BASR), Karachi University, Karachi, to conduct according to Helsinki declaration of human rights. We used the CONSORT checklist (http:// www.consort-statement.org/#12a) for designing and conduct of this study. For the present cross sectional study, from total 49 females with PCOS (mean age 29.13 ±2.18 years) were selected from Gynecology and Obstetrics Department and Infertility Clinic, Jinnah Postgraduate Medical Center (JPMC), Karachi, Pakistan after written informed consent.They were divided into two groups according to their BMI (29 obese PCOS females with BMI >30 and 20 normal-weight PCOS females with BMI <25). Fifty two normally menstruating (NC), fertile healthy females (mean age 28.52 ±3.14 years) were selected from the general population and divided into two groups, according to their BMI (27 obese females with BMI >30 and 25 normal-weight females with BMI <25).

Inclusion criteria for NC subjects

The subjects in NC groups had normal menstrual cycle and there was no sign of hirsuitism and no finding of polycystic ovaries on ultrasonographic examination. Female NC subjects had normal thyroid profile and they had not been using oral contraceptive pills or any hormonal medication within the six month preceding the study.

Exclusion criteria for NC subjects

Females with diabetes mellitus or Cushing syndrome or taking any other hormonal medication were excluded from the study.

Inclusion criteria for PCOS patients

The diagnostic criteria of Rotterdam consensus group was used to select PCOS subjects (Rotterdam ESHRE, 2004). Females with lacking continuity or regularity in menses or oligomenorrhea (menses failure for 35-182 days) or amenorrhea (menses failure for >182 days), hirsutism and laboratory confirmation of excess of androgen (i.e., high serum free testosterone), ultrasonographic records confirming not less than 12 follicles (two to nine mm in diameter) placed peripherally around a dense core of ovarian stroma or spread throughout and increased quantity of stroma were registered for the study.

Exclusion criteria for PCOS patients

PCOS subjects with systemic illness including diabetes mellitus, thyroid disorder, acromegaly, Cushing syndrome, hyperprolactinaemia, congenital adrenal hyperplasia, systemic inflammatory diseases, functional hypothalamic amenorrhea, or using oral contraceptive pills and any other hormonal medication, were excluded from the study.

A questionnaire regarding their demographic data and sign and symptoms were also filled from by all study subjects. Height and weight of all participants was measured and BMI was computed [BMI = body weight (kg)/height (m^2)].

Laboratory Measurements

The blood samples were collected in NC group on 12th day of menstrual cycle, while samples from PCOS patients were collected irrespective of the day of menstrual cycle (because of irregularity in menstrual cycle) in the morning after 12-14 hours fasting. Blood was centrifuged and serum was separated and stored at -20°C for further analyses. Prolactin, T3, T4, TSH, FSH, LH, free testosterone, and estradiol (E2) levels were measured with standard Radioimmunoassay (RIA) on Gamma counter (Capintec, USA). Immunoradiometric (IRMA) kit (Diagnostic Systems

Laboratories, USA) was used for assaying serum leptin levels. The sensitivity of leptin IRMA kit (lowest detectable limit) was 0.1 ng per milliliter and intra and inter assay coefficients of variations were less than 8% and it was highly specific because there was no any detectable cross-reactivity with peptide, protein or other related agent. Glucose oxidase method was used to determine blood glucose levels.

Statistical analysis

Statistical analysis was executed by SPSS 16 (SPSS inc, Chicago, IL) program. Analysis of variance (ANOVA) was used for calculating comparison between groups. Pearson correlation analysis was done to determine linear correlations between the variables. Analysis of covariance (ANCOVA) was used to find out the correlation of serum leptin concentration with other variables (fasting glucose, TSH, E2) and testosterone after adjusting BMI (because leptin levels and BMI are positively related with each other) and $p<0.05$ was taken as significant.

Results:

The mean values of BMI, serum leptin and FSH, LH, FSH/LH ratio, testosterone, E2, progesterone, prolactin, TSH, and FBG are shown in table 1. Mean serum leptin levels were significantly higher in the obese PCOS women compared to normal-weight PCOS (54.0 ± 9.3 ng/ml vs 26.08 ±2.44 ng/ml; p=<0.01) and in obese NC women compared to normal-weight (51.5 ±9.1 ng/ml vs 25.3 ± 2.3 ng/ml; p=<0.01). But there was no significant difference found when obese PCOS compared with obese NC women and normal-weight PCOS with normal-weight NC women (table 1).

When the hormone profile parameters of normal-weight and obese PCOS compared with normal-weight and obese NC women, there was significantly lower level of FSH, LH, E2 ($p<0.01$) in both groups of PCOS as compared to non-PCOS while considerably higher levels of testosterone, progesterone and FBG were found in obese PCOS in comparison to obese NC ($p<0.01$).Leptin levels correlated strongly with BMI in obese and normal-weight PCOS ($r = 0.48$, $p<0.008$ and $r = 0.5$, $p<0.02$, respectively) and in obese and normal-weight NC women ($r = 0.48$, $p<0.01$ and $r = 0.54$, $p<0.006$, respectively) (figure 1& 2).

There was a significant correlation of FBG with obese NC ($r=0.4$, $p<0.05$) and in normal-weight and obese groups of PCOS ($r= 0.4$, $p<0.05$; $r=0.5$, $p<0.05$). The leptin level correlated with E2 and testosterone levels in obese PCOS subjects ($r=0.3$, $p<0.05$, $r=0.3$; $p<0.05$ respectively) (table 2). There was no significant correlation found between leptin levels and other hormonal parameter in normal-weight and obese PCOS and NC women (table 2, figure 3).

Discussion:

The combination of obesity, insulin resistance, hyperandrogenemia, and infertility in PCOS has been observed by number of researchers.(Baranova et al.,2013, El-Gharib et al.,2014) The phenotypic presenation of PCOSvaries with respect to selection from a referral population or through unselected screening. (Rotterdam ESHRE, 2004) The cause effect relationship of obesity, disorders in PCOS and hyperleptinemia can be solved only if we correlate metabolic, anthropometric and hormonal parameters in sub groups of obese NC and PCOS women.

Present study found no significant difference in serum leptin levels in obese and normal-weight groups of PCOS as compared to obese and normal-weight groups of NC women. These results are inconsistent with Olszanecka-Glinianowicz et al (2013) who reported significantly higher serum leptin level in obese PCOS compare to obese non- PCOS. We found considerably higher levels of leptin in obese females with and without PCOS than in all normal-weight females in both groups. This finding is compareable with several studies.(Lecke et al., 2013, Svendsen et al., 2012) So, these results demonstrate that increased BMI is associated with increased leptin levels independently of the presence of PCOS.

In our study, NC group showed a positive correlation of leptin levels with increased BMI which is comparable to few other studies.(Rojas et al., 2014, Tariq et al., 2014) The main action of leptin on nutrition could be seen through the hypothalamic-pituitary gonadal axis that moderates secretion of gonadal steroids by signals sent to neural networks with respect to volume of energy reserves required to activate reproduction.(Christian et al.,2008)

Leptin has important effects to stimulate luteinizing hormone release hormone (LHRH) from hypothalamus and also stimulates FSH and LH release from the pituitary glands in experimental animals by a direct action. The administration of recombinant leptin to these animals resulted in resumption of fertility, which might indicate that low leptin levels are associated with reduction in reproductive hormones. (Kalra et al., 2008) Our study has revealed low concentration of FSH and LH in normal-weight to obese PCOS and obese NC women with lack of significant correlation in both groups. This is in contradiction to high LH levels in PCOS observed by Telli et al and similar to observation reported by Pehlivanov et al. (Baig et al., 2014, Pehlivanov et al. 2009)

Leptin receptors on granulosa cells interact with glucocorticoids to encourage steroidogenesis as well as ovarian folliculogenesis. (Chakrabarti et al., 2013) Reduction in E2 level was observed in PCOS irrespective of BMI and in obese females of NC group.

The presumption that greater the body weight, less leptin would stimulate E2 production as detected in our study. Low levels of serum E2 correlated with leptin in PCOS patients which is comparable to other researchers and correlation persisted after adjusting BMI. (Buettne et al., 2008)

The androgenic activity at the levels of the pituitary, ovaries and adrenals may contribute to the etiology of PCOS with increase in rate of testosterone production. (Hendrix et al., 2014) The extent of androgen concentration imitates the degree of sympatho-excitation, acne formation and hirsuitism which is associated with the degree of PCOS severity, phenotypic abnormalities and defective OB production. Present study found higher levels of testosterone in both groups of normal-weight and obese PCOS and obese control. But, when serum testosterone was examined for correlation with serum leptin, no significant association was observed in either of the groups which is similar to other study. (Chakrabarti et al., 2013)

Table 1: Comparison of variables in PCOS and normal cycling (NC) subjects

Parameters	**PCOS subjects BMI<25**n=20	**NC subjects BMI<25**n=25	**PCOS subjects BMI>30**n=29	**NC subjects BMI>30n=27**
BMI(kg/m^2)	23.32± 1.36	22.51± 1.39	32.91 ± 2.72$^{\alpha}$	33.16± 2.84 §
Leptin (ng/ml)	26.08± 2.44	25.39± 2.37	54.00 ± 9.33 $^{\alpha}$	51.54± 9.19 §
Follicle stimulating hormone (IU/L)	1.86± 0.51	10.01 ± 1.57*	1.75 ± 0.49	10.20 ± 1.89 †
Luteinizing (IU/L)	6.22 ± 1.05	22.53± 4.38*	6.12± 1.15	25.53± 4.43†§
LH/ FSH ratio	3.59± 1.13	2.56±2.74	3.75± 1.20	2.80±2.37
Testosterone(ng/ml)	1.07 ± 0.26	0.24 ±0.15	1.13± 0.35	0.64 ± 0.39†§
Estradiol (pg/ml)	166.00± 7.41	216.82±5.73*	170.80±5.21	285.12±6.81†§
Progesterone (ng/ml)	2.04± 0.16	0.33 ± 0.04	1.98±0.09	0.35±0.03†
Prolactin (ng/ml)	9.64 ± 1.97	10.42± 1.89	10.15± 2.60	9.98 ± 2.59
TSH (mIU/L)	1.59 ± 0.07	1.63 ± 0.08	2.28±0.07	2.65±0.08§
Fasting blood sugar (mg/dl)	91.81±1.92	79.13±1.27*	94.77±1.65 $^{\alpha}$	84.33±1.31 †§

Results are shown as mean ± SD (standard deviation), n=number of subjects
*p<0.01 when lean PCOS compared with lean control
† p<0.01 when obese PCOS compared with obese control
$^{\alpha}$ p<0.01 when lean PCOS compared with obese PCOS
§ p<0.01 when obese NC compared with lean control

Table 2: Correlation coefficient of serum leptin with different variables in PCOS and normal cycling subjects in all groups

Parameters	PCOS subjects BMI<25n=20	NCsubjects BMI<25n=25	PCOS subjects BMI>30 n=29	NCsubjects BMI>30n=27
FSH (IU/L)	-0.089	-0.292	0.227	-0.066
Estradiol (pg/ml)	-0.050	0.090	0.363*	0.270
Progesterone (ng/ml)	-0.02	0.078	0.063	0.091
Testosterone (ng/ml)	0.246	0.102	0.317*	0.065
Prolactin (ng/ml)	-0.082	-0.008	-0.144	-0.170
TSH (mIU/L)	0.340	0.357	0.213	0.306
Fasting blood glucose (mg/dl)	0.45*	0.21	0.58*	0.42*

Values are given as correlation coefficient (R), *P<0.05,

PCOS females present with various metabolic abnormalities of which insulin resistance and hyperinsulinemia are noteworthy. Leptin contributes to the maintenance of glucose homeostasis in addition to body weight. (Koch et al., 2010) It has also been reported that obesity is a major risk factor for the development of diabetes Mellitus and insulin resistance. Insulin resistance can occur due to hereditary aberrations of insulin or insulin receptor genes (Enriori et al., 2011) in the absence of which, leptin offers a rational description for obesity related insulin resistance. It is documented that obesity, hyperleptinemia, insulin resistance, and hyperinsulinemia further increases leptin production by a self-perpetuating vicious circle of events.(Enriori et al., 2011) Strong correlation of leptin with FBG level was observed in PCOS groups irrespective of obesity which is similar to others. (Baranova et al., 2013) The strong correlation of FBG with leptin in PCOS abolished after adjustment of BMI which supports relation of obesity and insulin resistance in normal as well in PCOS.

Serum leptin levels correlated with BMI in females with and without PCOS emphasizing its role in communicating information on the body's fat stores/energy reserves to the brain which is comparable to other studies. (Baig et al., 2014) The correlation of serum leptin with E2 and testosterone in obese PCOS indicates its involvement in the PCOS. It may be claimed that leptin has a role in pathogenesis of PCOS in only the obese patients but further studies are needed to verify the interplay of serum leptin with obesity and PCOS.

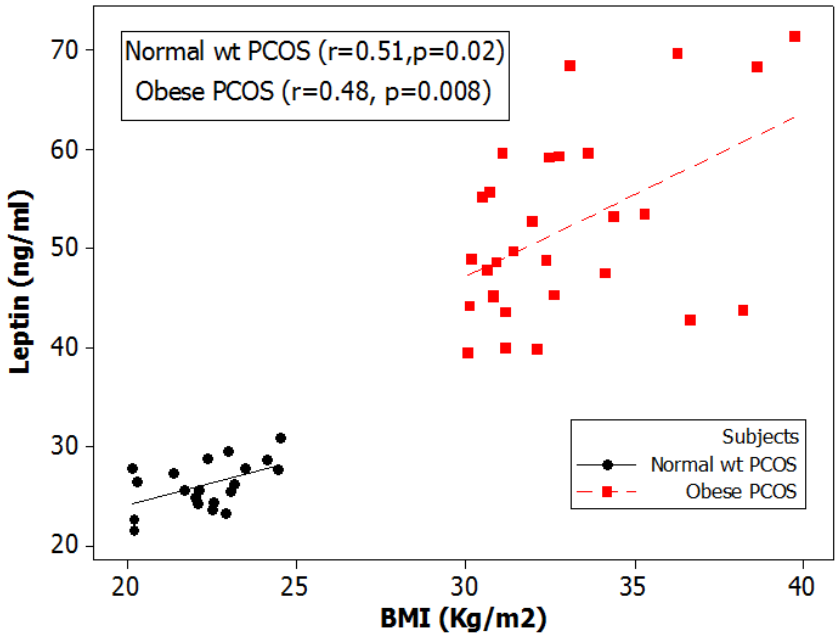

Figure 1: Correlation between serum leptin concentration and BMI in normal-weight and obese PCOS subjects.

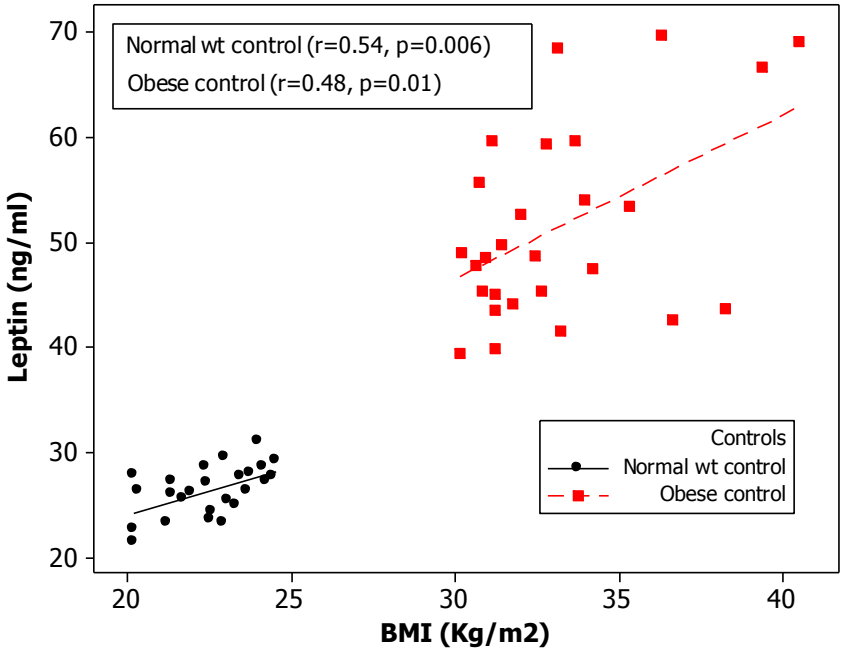

Figure 2: Correlation between serum leptin concentration and BMI in normal-weight and obese normal cycling groups.

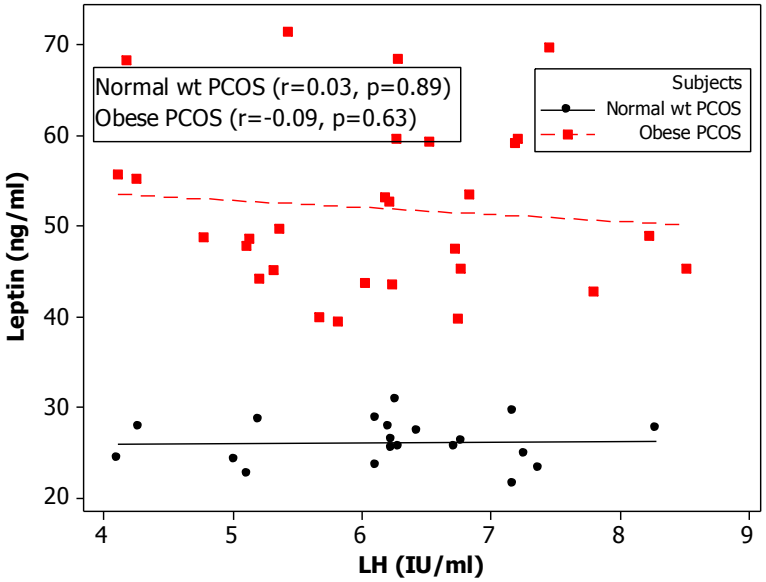

Figure 3: Correlation between serum leptin concentration and LH in normal-weight and obese PCOS subjects.

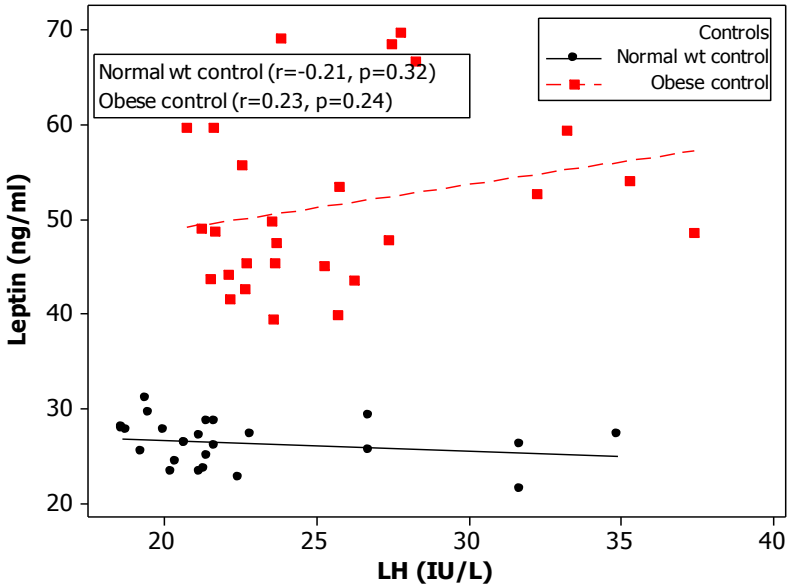

Figure 4: Correlation between serum leptin concentration and LH in normal-weight and obese normal cycling subjects.

Limitations Of Study :

The study is limited in terms of sample size, the sense that insulin resistance was determined by fasting glucose only and adrenal androgen could not be measured. To see the association between leptin and PCOS, authors also did not adjust BMI in the regression model

Conflict Of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Running title: leptin, obesity & PCO

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