The impact of body mass index on serum androgen and leptin association in reproductive age women

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Abstract

Background and objective: Obesity has been associated with increased androgenicity in women. There are, however, major inconsistencies in available data concerning the possible association between androgenicity and leptin in humans.The objectives of this study were to evaluate the impact of body mass index on androgens and the potential contribution of leptin in determination of androgen levels in women.

Methods: The study included 80 healthy females with an established Body Mass Index (BMI). They were divided into 4 groups. First group, 30 normal weight subjects (BMI<25 Kg/m^2), second group, 25 overweight subjects (BMI=25–29.9 Kg/m^2) , third group,15 obese grade-I subjects (BMI=30–34.9 Kg/m^2) and forth group, 10 obese grade-II subjects (BMI>35 Kg/m^2). Serum, leptin, free testosterone, androstenedione, glucose, lipid profile and body mass index were measured.

Results: There was no statistically significant difference in median serum androstenedione, while free testosterone was significantly lower (14.5 pg/ml) in obese grade II and higher in normal weight (60 pg/ml) and statistically significant increase in serum leptin with increase in body mass index. Serum free testosterone was lower (23.5 pg/ml) in subjects with highest quartile serum leptin and higher (43pg/ml), in subjects with lowest quartile serum leptin, the difference was not significant statistically.

Conclusion: high body mass index negatively influence the circulating free testosterone in reproductive age women**.** An inverse association between circulating leptin and free testosterone was observed

Keywords: androstenedione, leptin, free testosterone.

Introduction

Interestingly, serum androgens are positively associated with body mass index (BMI) not only in polycystic ovary syndrome (PCOS) ,but also in women with simple obesity[1]. This, together with a fall in luteinizing hormone levels with increasing BMI[2,3], suggests that androgen synthesis may take place not only in adrenals and ovaries but also in adipose tissue. Some research focused on sex steroid conversion in human adipose tissue, specifically investigating the expression and activity of 17-hydroxysteroid dehydrogenases (17-HSDs), which represent a major switch regulating sex steroid activation and inactivation at the pre-receptor level [4-6]. The major androgens are testosterone, androstenedione, and dehydroepiandrosterone (DHEA). Half of the circulating testosterone is produced peripherally from androstenedione and other androgen precursors. In premenopausal women, testosterone is produced in the ovaries and adrenals, while in postmenopausal women, testosterone is produced by the adrenals and the peripheral conversion of androstenedione in adipose tissue [7]. Leptin, the hormone encoded by the obesity gene, is secreted mainly from adipocytes .[8] It regulates energy balance by reducing food intake and increasing energy expenditure[9]. A main function may be to initiate protective neuroendocrine responses during

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starvation [10]. As leptin levels rise due to obesity and other factors [11], the physiological regulation including androgens [12, 13] present in non-obese subjects may be altered. Some studies suggest that both hyperleptinemia[14,15] and relative alterations in androgen levels, ie hypoandrogenicity in men and hyperandrogenicity in women, may be associated with increased risk for cardiovascular diseases[16]. Since testosterone is involved in the regulation of fat mass, it may be speculated that the relation between testosterone and leptin is indirect. However, experimental data support the idea that testosterone acts directly on the adipocyte as expression and secretion of leptin is reduced in cultured adipocytes after coincubation with testosterone or dihydrotestosterone, suggesting a direct effect as well[17]. Our hypothesis was to examine the effect of BMI on circulating androgens (free testosterone, androstenedione) and influence of leptin on androgens in women reproductive age.

Methods

The study was based on the analysis of 80 healthy reproductive age females with an established BMI. They were divided into 4 groups. A group of 30 normal weight subjects (BMI<25Kg/m^2), second group 25 overweight subjects (BMI=25-29.9Kg/m^2), third group15 obese grade-I subjects (BMI=30-34.9Kg/m^2) and the fourth 10 obese grade-II subjects (BMI>35Kg/m^2). The exclusion criteria were: women on treatment affecting the body weight, gonadal, adrenal function, carbohydrate, lipid metabolism, dysfunction of, renal, liver, thyroid, polycystic ovary syndrome, hypertension, diabetes, and smoking. All subjects were seen in Razgary teaching hospital from January to May /2012 in laboratory department/blood sample collection unit.After overnight fasting blood sample were obtained for determination of serum, leptin (measured by a commercial ELISA Kit Diagnostics Biochem Canada Inc.), free testosterone , androstenedione (measured by commercial ELISA Kits from IBL. Immuno Biological Laboratories. IBL-America) ,glucose and lipid profile (measured through enzymetic colorimetric assays all these tests were estimated in Lab of Biochemistry / College of Pharmacy /Hawler Medical University. Body weight, body height were measured , body mass index was calculated as the ratio of body weight to body height in meter sequer expressed as (K/ m^2).

Statistical Analysis:

Data were translated into a computerized database structure. An expert statistical advice was sought for. Statistical analyses were done using SPSS version 20 computer software (Statistical Package for Social Sciences).Compliance of continuous random variables with Gaussian curve (normal distribution) was analyzed using the Kolmogorov-Smirnov test. Statistical significance of differences between averages for normally distributed variables between more than 2 groups was assessed using ANOVA. For parameters deviating from normal distribution (Serum leptin, andrstenedione and free testosterone) the nonparametric Kruskal-Wallis test was used. The non-normally distributed variables(study hormones) were described by median instead of mean and standard deviation which are reserved for normally distributed variables. P value of <0.05 was considered statistical significant. All analyzed tests were bilateral. The statistical significance, direction and strength of linear correlation between 2 quantitative variables, one of which being non-normally distributed was measured by Spearman's rank linear correlation coefficient[18]. The multiple linear regression model provides the following parameters:

1. P (model): In order to generalize the results obtained, the model should be statistically significant.
2. Unstandardized partial regression coefficient: Measures the amount of change expected in the dependent variable for each unit increase in the independent variable after adjusting for other

explanatory variables included in the model.

3. P for regression coefficient: reflects the statistical significance of the calculated partial regression coefficient of each explanatory variable included in the model.
4. R^2 (Determination coefficient): measures the overall performance of the model since it reflects the amount of variation in the dependent variable explained by the model. The closer its value to 100% the better the model fit.

Results

A total of 80 reproductive age women were enrolled in the study with an established BMI. They were divided into 4 groups. As shown in Table 1, there was no statistical significant difference in mean age between the study groups, their ages ranged from 18 to 38 years with mean ±SD (28.8±6.1) years. Descriptive statistics for serum study hormones concentrations are presented in Table 2 which shows there that was no statistically significant difference in median serum androstenedione, between the study subjects. The median of serum free testosterone was significantly lower (14.5 pg/ml) in morbid obese and significantly higher in normal weight (60 pg/ml) with p=0.001, with presence of statistically significant difference between study groups. While a statistically significant elevation was found in a median of serum leptin with increase in body mass index between the study groups with p<0.001.

Table 1: The difference in mean age between study groups categorized by body mass index .

	BMI (Kg/m2)-categories				
	Normal weight (<25) (N=30)	Overweight (25-29.9) (N=25)	Obese grade-I (30-34.9) (N=15)	Obese grade-II (>35) (N=10)	P
Age in years					0.58 [NS]
Range	(18 - 39)	(21 - 39)	(20 - 37)	(20 - 38)	
Mean ± SD	28.8±6.1	27.9±4.5	27±4.9	28±4.9	

Table 2: The difference in median of selected serum hormones between study groups categorized by body mass index .

	BMI (Kg/m2)-categories				
	Normal weight (<25) (N=30)	Overweight (25-29.9) (N=25)	Obese grade-I (30-34.9) (N=15)	Obese grade-II (>35) (N=10)	P
Serum Androstenedione (ng/ml)					0.22 [NS]
Range	(1.6 - 6.1)	(0.8 - 9.1)	(2.4 - 4.8)	(2.6 - 7.2)	
Median	2.95	2.8	3.3	2.85	
Inter-quartile range	(2.5 - 3.4)	(2.2 - 3.4)	(3.1 - 3.8)	(2.7 - 4.3)	
Serum free testosterone(pg/ml)					0.001
Range	(0.1 - 98)	(0.1 - 100)	(0.1 - 90)	(0.1 - 75)	
Median	60	35	49	14.5	
Inter-quartile range	(38 - 75)	(17 - 45)	(21 - 80)	(7 - 17)	
Serum leptin (ng/ml)					<0.001
Range	(3.3 - 74.8)	(4.9 - 65.1)	(20.2 - 95.5)	(3.5 - 86.8)	
Median	9.7	31	31.9	42.45	
Inter-quartile range	(4.6 - 21)	(23.3 - 37.8)	(25.3 - 34.5)	(30.8 - 52.5)	

A medium positive correlation was found between BMI and serum leptin (r=0.584 P<0.001) as shown in figure 1. While a weak correlation between BMI and androstenedione was found (r=0.146 P=0.19) as shown in figure 2.

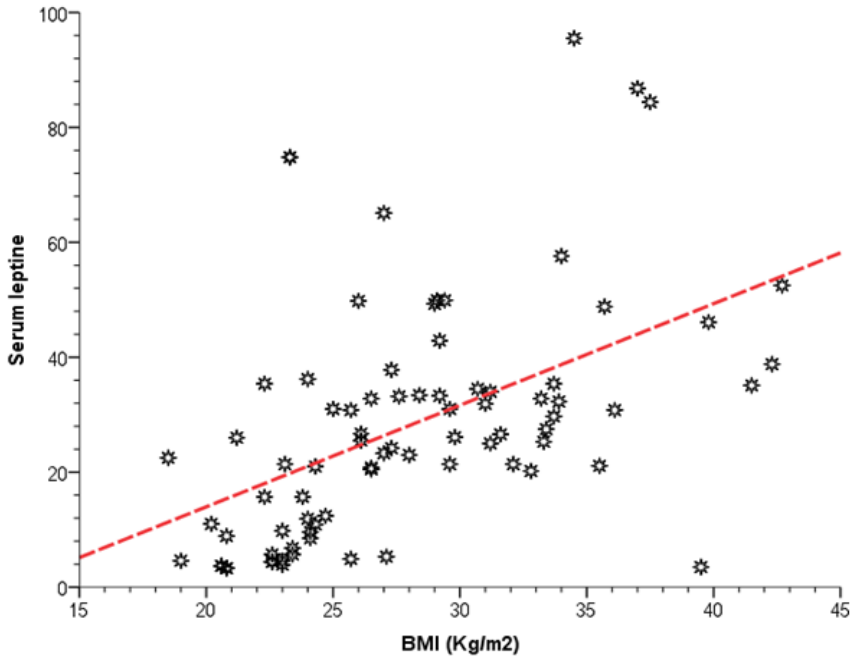

Figure 1: Scatter diagram showing the correlation (with fitted regression line) between BMI and serum leptin (r=0.584 P<0.001).

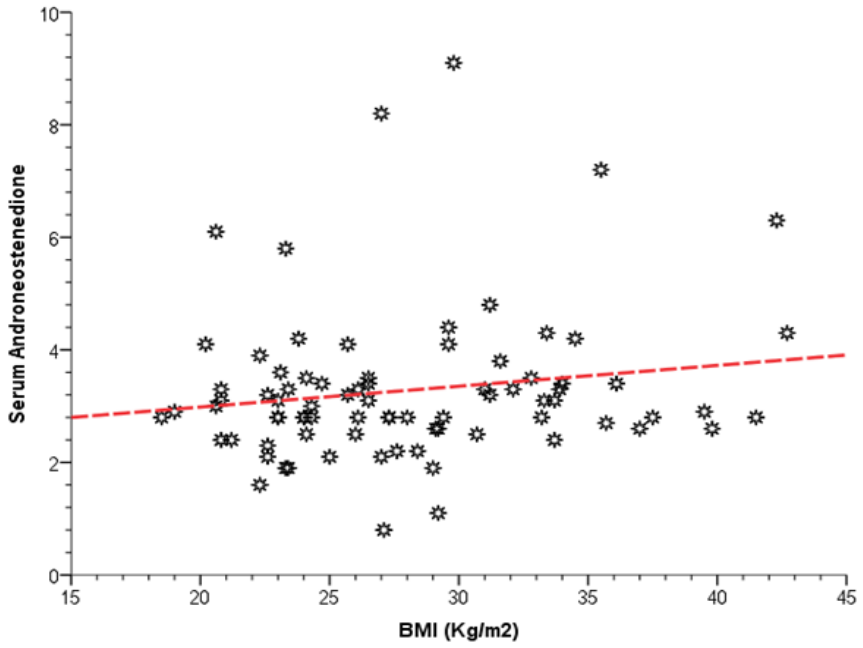

Figure 2: Scatter diagram showing the correlation (with fitted regression line) between BMI and serum androstenedione (r=0.146 P=0.19[NS]).

A hightly significant negative weak correlation was found between BMI and serum free testosterone in present study (r= -0.374 p=0.001) as shown in figure 3.

As shown in Table 3, no important or statistically significant differences between study groups for serum glucose and lipid profile.

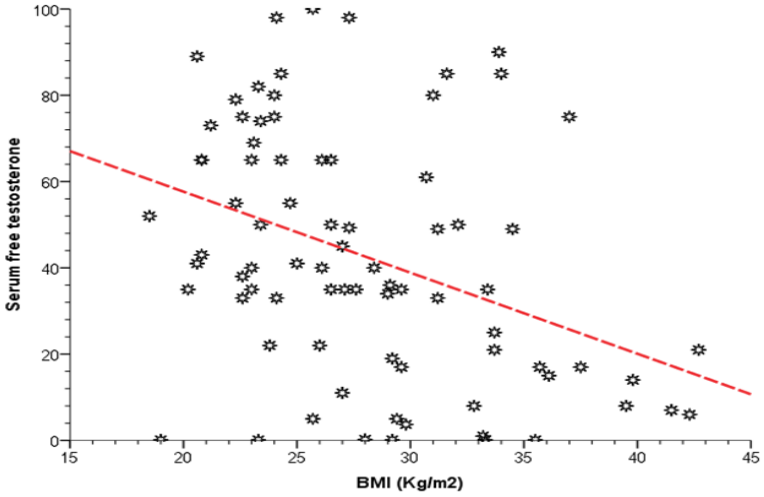

Figure 3: Scatter diagram showing the correlation (with fitted regression line) between BMI and serum free testosterone (r= -0.374 P =0.001).

Table 3: The difference in mean of selected serum parameters between study groups categorized by body mass index.

	BMI (Kg/m2)-categories				
	Normal weight (<25) (N=30)	Overweight (25-29.9) (N=25)	Obese grade-I (30 -34.9) (N=15)	Obese grade-II (>35) (N=10)	P
Serum fasting glucose (mg/dl)					0.12[NS]
Range	(75 - 127)	(72 - 180)	(81 - 140)	(81 - 136)	
Mean ± SD	93.7±12.4	104.6±23.9	104.7±16.6	104.5±17.2	
Serum total cholesterol (mg/dl)					0.34[NS]
Range	(80 - 220)	(105 - 245)	(105 - 210)	(95 - 202)	
Mean ± SD	158±36.9	156.7±43.1	141.5±28.8	149.2±36.3	
Serum TG (mg/dl)					0.07[NS]
Range	(40 - 230)	(26 - 290)	(62 - 220)	(35 - 237)	
Mean ± SD	85.4 ±44.3	120.5±70.8	110.1±42.3	127.2±56.1	
Serum HDL-Ch (mg/dl)					0.57[NS]
Range	(25 - 51)	(25 - 55)	(22 - 43)	(27 - 51)	
Mean ± SD	38.3 ±9.6	38.5 ±7.7	32.3 ±6.2	38.6 ±7.6	
Serum LDL-Ch (mg/dl)					0.23[NS]
Range	(54 - 206)	(75 - 183)	(82 - 137)	(86 - 156)	
Mean ± SD	105.6 ±37.4	120.2 ±24.7	109 ±14	122.6 ±23.8	
Serum VLDL-Ch (mg/dl)					0.21[NS]
Range	(9 - 64)	(13 - 59)	(14 - 44)	(15 - 48)	
Mean ± SD	19.9 ±11.8	25.4 ±13.2	22.5 ±8.7	26.4 ±10.1	

To study the net and independent effect of BMI on serum free testosterone after controlling for the possible confounding effect of age, serum total cholesterol, serum TG and fasting glucose, a multiple linear regression model was used. The model was statistically significant (P=0.002) and able to explain 22% (R^2=0.22) of observed variation in the dependent variable. Only BMI and serum fasting glucose had a statistically significant association with serum free testosterone. For each unit increase (Kg/m^2) in BMI the serum free testosterone is expected to decrease by an average of 2.2 *pg/ml* after controlling for the remaining independent variables included in the model. For each 1 unit (mg/dl) increase in serum fasting glucose the serum free testosterone is expected to increase by an average of 0.43 *pg/ml* after controlling for the remaining independent variables included in the model, Table 4. To study the net and independent effect of BMI serum Androstenedione after controlling for the possible confounding effect of age, serum total cholesterol, serum TG and fasting glucose a multiple linear regression model was used. The model was not significant statistically and none of the included explanatory variables showed an important or statistically significant association with the outcome variable, Table 5.

Table 4: Multiple linear regression model with serum free testosterone as the dependent (response) variable and selected explanatory variables.

	Partial regression coefficient	**P**
BMI (Kg/m2)	-2.2	<0.001
Serum fasting glucose (mg/dl)	0.43	0.013
Serum total cholesterol (mg/dl)	-0.08	0.34[NS]
Serum TG (mg/dl)	0.034	0.55[NS]
Age in years	0.50	0.39[NS]

P (Model) = 0.002
R^2= 0.22

Table 5: Multiple linear regression model with serum Androstenedione as the dependent (response) variable and selected explanatory variables.

Serum	**Partial regression coefficient**	**P**
BMI (Kg/m2)	0.033	0.25[NS]
Serum fasting glucose (mg/dl)	-0.004	0.65[NS]
Serum total cholesterol (mg/dl)	-0.008	0.08[NS]
Serum TG (mg/dl)	0.0001	0.95[NS]
Age in years	0.007	0.82[NS]

Table 6: Association between serum leptin and each of androstenedione and free testosterone.

	Serum leptin-categories			
	Lowest (first) quartile (<10.9) (N=19)	**Average (inter-quartile range (10.9-35.2) (N=41)**	**Highest (fourth) quartile (>35.2) (N=20)**	**P**
Serum Androstenedione(ng/ml)				0.039
Range	(0.8 - 6.1)	(2.1 - 9.1)	(1.1 - 6.3)	
Median	2.9	3.3	2.75	
Inter-quartile range	(2.4 - 3.3)	(2.8 - 3.8)	(2.3 - 3.25)	
Serum free testosterone(pg/ml)				0.22[NS]
Range	(0.1 - 100)	(0.1 - 90)	(0.1 - 98)	
Median	43	40	23.5	
Inter-quartile range	(35 - 74)	(19 - 65)	(12.5 - 65)	

Table 6 explores possible association between serum leptin and each of androstenedione and free testosterone. Serum leptin was transformed into an ordered categorical variable based on quartiles (dividing the sample into 4 quarters based on serum leptin after ordering it from minimum to maximum value). The resulting categorical variable will have 3 categories: The first one is the lowest quartile and the last one is the highest quartile. The remaining 2 central quartiles are merged into the "inter-quartile range) or what we call the central 50% of data. Serum free testosterone was lowest in subjects with highest quartile serum leptin (23.5 pg/ml) and higher in subjects with lowest quartile serum leptin (43 pg/ml). The difference observed however not statistically significant.

Discussion

The objective of the study was to identify the relationship between well-defined measures of body adiposity via body mass index and circulating androgens, leptin concentrations in healthy reproductive age women. Reproductive age women with higher levels of adiposity have higher concentrations of serum leptin (Table 2, Figure 1) this finding agreed with several previous reports which showed positive correlations between adiposity and leptin concentrations[19-22.] Higher body mass index was associated with higher serum leptin concentrations. Current study findings support the view that increased adiposity measured as BMI, percent body fat(central and peripheral fat) are all associated with increased leptin exposure in non-smoking women. Since increased BMI [23] and central fat [24,25] are associated with increased risk for breast cancer in prospective studies, increased leptin exposure associated with obesity and central adiposity could explain the greater incidence of breast cancer in overweight or obese postmenopausal women. This idea is also supported by the findings from several experimental studies in which leptin stimulated breast carcinogenesis [26,27]. However, very few epidemiologic studies have assessed leptin concentrations in relation to breast cancer risk. Two small case-control studies, one in postmenopausal [28] and the other in premenopausal [29] women, and a single prospective study [30] found no association between leptin and breast cancer. Thus, we recommend that more epidemiological studies are clearly needed to confirm the leptin-breast cancer association in reproductive age women. Many previous studies did not find an association of obesity with

levels of androstenedione [18,31-33] in either pre- or post-menopausal women. Our data are consistent with these observations. Most of studies in pre-menopausal women have shown an increase in free testosterone with increasing body Weight [31,34,35]. our results are inconsistent to these observations, but in agreement with other studies which confirm an inverse correlations in women between androgen and BMI, wist to hip ratio, although their statistic significance is less evident [16,36,37], however there are conflicting data on this subject. The matter of female obesity and androgens may still need in-depth research. It has been proposed that the relationship between BMI and androgens is mediated by obesity-related changes in insulin and bio available of insulin like growth factor I (IGF-I). In vitro studies have shown that both insulin and insulin like growth factor I (IGF-I) can stimulate ovarian androgen synthesis [38,39]. However, this gonadotropic effect of insulin and IGF-I may be of less significance before menopause mainly in reproductive age when circulating sex-steroid hormones are under the tight control of luteinizing hormone (LH) and follicle stimulating hormone (FSH) and regulated by powerful feed-back mechanisms this hypothesis is more pronounced to explain present result inconsistency with some others [31,34,35] and the choice of our subjects was women in reproductive age while the other studies focused primarily on postmenopausal women or a combination of pre and postmenopausal participants. Moreover, the effect of BMI may be less evident against the background of high testosterone hormone concentration after the cessation of ovulatory activity. In subjects investigated in the present study, the relationship between leptin and BMI was positive linear, as can be seen in Figure1. The findings showed the same results as previously reported studies in obese women [21,40]. There is a growing bulk of evidence suggesting that testosterone may infuence leptin levels. Testosterone administration reduces leptin levels in Hypogonadal [41,42] and eugonadal men [43] in adolescents with delayed puberty [44], and in female to male transsexuals [45]. Since testosterone is involved in the regulation of fat mass, it may be speculated that the relation between testosterone and leptin is indirect. However, experimental data support the idea that testosterone acts directly on the adipocyte as expression and secretion of leptin is reduced in cultured adipocytes after coincubation with testosterone or dihydrotestosterone [12,17], suggesting a direct effect as well, these observations may give an explanation for current finding which shows an inverse association between testosterone and leptin. We observed a significant association between serum androstenedione and serum leptin, androstenedione found to be higher in inter-quartile range of serum leptin this from one hand while from another hand serum free testosterone was lowest in subjects with highest quartile serum leptin, Table 6 this different behavioral of both androgens towered leptin hormone suggesting a complex relationships between these hormones.

Conclusion

The results of present study provide evidence that an increase in BMI influences the circulating levels of free testosterone hormone negatively, in the presence of the powerful feed-back mechanisms that control the synthesis of androgens and estrogens in reproductive age. A clear inverse association between circulating leptin and free testosterone was observed. Finally, recommend that the identification of potentially modifiable life-style or investigate study hormonal determinants is important because of the increasing epidemiological evidence linking these hormones to cancer risk.

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