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ABSTRACT

Objective: **To determine the serum leptin concentration in a cohort of healthy rural Africans, it's relation to the commonly used anthropometric measures of obesity and its relation to the patterns of distribution of fat in the body.**
Design: **A cross-sectional population survey.**
Setting: **Baziya area, Transkei region, South Africa.**
Subjects: **One hundred and thirty five(79 females and 56 males) healthy adults from the Baziya location, Transkei aged 17-70 years were selected by stratified random sampling.**
Measurements: **Anthropometric measurements of height, weight, and skinfold thickness at the biceps, triceps, subscapular and suprailiac sites and derived total body fat and ratios of fat distribution. Fasting serum leptin using the sandwich ELISA method.**
Results: **Skinfold measurement was significantly higher in the females than the males throughout the age range. Centralisation of body fat to the trunk was significantly greater in the males than in the females. Serum leptin concentration was higher in the females (mean = 13.5 ng/ml; 95% confidence interval = 10.0 - 16.8) than in the males (mean = 5.2 ng/ml; 95% confidence interval = 2.8 - 7.6) ($p<0.001$). The gender difference in leptin concentration persists when expressed as serum leptin per kilogram of fat mass (serum leptin (ng/ml)/FM). The mean value for the males was 5.1 ng/ml/kg (95% confidence interval = 2.9 - 7.3) compared to the mean value for females of 6.9 ng/ml/kg (95% confidence interval = 5.4 - 8.3)($p<0.05$). In the females BMI and body fat were significant contributors to the variance in serum leptin. In the males the upper-to-lower trunk skinfold thickness ratio and BMI were the significant contributors to the variance in serum leptin concentration. Deposition of fat in the abdomen did not have a significant contribution to the variance in circulating leptin in both sexes.**
Conclusion: **Serum leptin concentration in rural Africans is similar to that observed in other communities with the exception that regional fat distribution has a significant influence on the leptin levels in the males.**

INTRODUCTION

Leptin is a peptide hormone, which is predominantly produced by the adipocytes(1). The placenta and the gastrointestinal tract have also been positively identified as sites of leptin synthesis(2,3). The physiological role of leptin in the human body is currently an area of major research interest. The major role of leptin is in the regulation of body weight. Leptin regulates body weight predominantly through the negative effect it has on food intake, and possibly through increased thermogenesis as well(4). Evidence is accumulating of a physiological role for leptin in reproduction and in immunological response(5,6).

Serum leptin concentration is primarily a function of the fat content of the human body. Serum concentration of leptin is positively correlated with the two commonly used anthropometric indicators of obesity; namely the body mass index (BMI) and the total body fat content. Obesity is paradoxically associated with high concentrations of serum immunereactive leptin. It has been suggested that obesity might be a result of tissue insensitivity to leptin - a case of failure in leptin signalling pathway(7). There are other factors besides obesity that influence the concentration of leptin in serum. These factors include gender, race, and the distribution of fat in the body. For any given measure of obesity, leptin levels are higher in women than in men(8). Leptin levels have been shown to be lower in African-American women than in Caucasian women with a similar degree of adiposity(9). Furthermore, leptin has been found to increase the resting energy expenditure (REE) in African-American women but not in the Caucasian women(9).

There is a scarcity of information on serum leptin and its determinants in rural African communities. Apart from its role in obesity, leptin has also been implicated in the pathophysiology of a diverse array of diseases. Elevated levels of leptin have been demonstrated in essential hypertension, depression and pre-eclampsia independent of the fat mass(10-11). These are some of the diseases that are becoming common in the rural African communities(12). The purpose of this study was to

determine the serum leptin concentration in a cohort of healthy rural Africans, its relation to the commonly used anthropometric measures of obesity, and its relation to the patterns of distribution of fat in the body. It is essential that the normal parameters be established before the role of leptin in the pathophysiology of these metabolic diseases in developing communities is investigated.

MATERIALS AND METHODS

Study population: The study was carried out in the Baziya area of the Xhosa speaking people of the Transkei region of South Africa. This is a defined rural community that forms part of the teaching complex of the University of Transkei. Homesteads within a radius of one kilometre from the Baziya health centre were included in the study. Altogether there were 137 homesteads. Fifty homesteads were randomly selected for the study. Adults (ages 17 and above) within the homesteads were invited to participate in the study. Informed consent was obtained from each participant. A research assistant interviewed each participant to obtain the medical history, and information on the personal habits including smoking and alcohol consumption. Initially 150 subjects out of the 162 approached consented to participate in the study. Fifteen of the volunteers were eliminated from the study because of the presence of chronic and debilitating illnesses like tuberculosis, pulmonary fibrosis, diabetes, and hypertension. The subjects were asked to fast overnight and report to the health centre where the anthropometric measurements were done, and blood samples withdrawn. The ethics committee of the University of Transkei Medical Faculty approved the study.

Anthropometric measurements: Single measurements of height were done, to the nearest centimetre (e.g. 0.4 would be rounded off to 0, whereas 0.5 would be rounded off to 1), with the subjects wearing underclothes only, barefooted, feet together, back and heels firmly against the upright of the height scale. Weight was measured on a balance scale to the nearest kilogram (e.g. 0.4 would be rounded to 0, whereas 0.5 would be rounded to 1). The subjects were weighed with their underclothes and with no shoes on. A single measurement of abdominal girth was taken at the level of the umbilicus at the end of expiration with the subject supine and breathing quietly. The hip girth was measured at the level of maximal protrusion of the gluteal muscles while the participant stood erect and the weight distributed equally over both feet.

One observer measured the skinfold thickness throughout the study. A Lange Calliper (Cambridge Scientific Industries) calibrated to give a constant pressure of 10 g/mm^2 throughout the full range was used throughout the study. The skinfold thickness was read to the nearest 0.5mm. The skinfold thickness was measured over the biceps (BCP), triceps (TRCP), subscapular (SSCP), and the suprailiac (SPIL) areas of the non-dominant side of the body. The sum of the skinfold thickness was used to derive percent body fat using the tables of Durnin and Womersley(13). Fat mass (FM) was calculated as body weight x percent body fat. The body mass index (BMI) was derived using the Quetlet index (mass (kg)/height (m)^2.

The following parameters were used to determine the regional distribution of body fat.

Measures of abdominal adiposity: Dividing the hip girth with the abdominal girth derived the waist-to-hip ratio (WHR), a measure of abdominal adiposity. This measure does not have values that would allow for inter-individual comparisons. Another measure of abdominal adiposity, which allows for inter-individual comparisons, is the conicity index (CI) calculated according to the formula of Valdez(14):

$$CI = \frac{\text{abdominal girth}}{0.109 \times \sqrt{W/H}}$$

Where the abdominal girth is in metres, weight (W) in kilograms, and height (H) in metres. The greater the value of the index, the more double coned (two cones with a common base) the individuals shape will be as a result of fat deposition in the abdomen. The range of the conicity index is between 1.00 (a perfect cylinder) to 1.73 (a perfect double cone).

Limb-to-trunk skinfold thickness ratio (TLR): The sum of the subscapular and suprailiac skinfold thickness divided by the sum of triceps and biceps skinfold thickness (SSCP+SPIL)/(TRCP+BCP) gives the trunk to limb ratio (TLR). It is indicative of the relative distribution of fat between the trunk and the limbs.

Upper-to-lower trunk skinfold thickness ratio (ULTR): Subscapular skin fold thickness divided by the suprailiac skinfold thickness (SSCP/SPIL) gives the upper-to-lower trunk skinfold thickness ratio (ULTR). It is indicative of the relative fat distribution between the upper and the lower trunk.

Leptin assay: Fasting venous samples were collected in plain vaccutainer tubes and centrifuged at 3000 rpm for 30 minutes. The serum was transported to the laboratory on dry ice and stored at 40°C until the time of analysis. The serum leptin was measured, in duplicate samples, by solid phase ELISA technique using the R&D System Quantikine human leptin assay kits. The assay kit contained a low and a high leptin concentration control sera for quality control. The coefficient of variation in leptin values between the duplicate samples was 3.7%.

Statistical analysis: All the anthropometric variables did not have normal distribution. The distribution of the body weight, BMI, skinfold thickness, and percent body fat was relatively flat. The abdominal girth, WHR, trunk to limb ratio, upper to lower trunk skinfold thickness ratio, and conicity index showed a high degree of kurtosis with a right - tail skew. Log transformed serum leptin distribution was normal. Mann-Whitney U test was used to test the mean differences of the anthropometric variables between males and females. Interrelationships among the anthropometric variables were examined using Spearman rank correlation coefficients.

Log serum leptin was analysed with one-way analysis of variance (anthropometric variable as a factor) for each gender. Each of the anthropometric variables was divided into tertiles and each tertile was treated as a group. The R^2 obtained from general linear models were compared among anthropometric measurements to identify the major anthropometric determinants of circulating leptin. Mean levels (and the tertiles) of the anthropometric variables were contrasted between males and females. Student-Neuman-Kuel test was conducted to determine if the mean levels of serum leptin were different among tertiles of each anthropometric variable within the gender. Statistical analysis was carried out using the Statistical Analysis System (SAS)(15).

RESULTS

Anthropometric measurements: There were 79 females and 56 males recruited into the study. Table 1 shows the characteristics of the female and male study groups. The female subjects were older (mean age 34.9; 95% confidence interval = 29.9 - 38.8) than the male

subjects (mean age 30.1; 95% confidence interval = 26.9 - 34.8;)($p<0.05$). The females had a higher BMI (mean = 26.08, 95% confidence interval 24.3 - 28.8) than the male subjects (mean = 22.4; 95 % confidence interval = 20.6 - 24.2)($p<0.05$). Percent body fat was higher in the females (mean = 26.3; 95% confidence interval = 23.7 - 28.8) than in the males (mean = 14. 4; 95% confidence interval = 12.0 - 16.8)($p<0.001$). Females had a higher fat mass (mean = 16.9 kg; 95% confidence interval = 14.6 - 19.3) than males (mean = 9.4 kg; 95% confidence interval = 7.2 - 11.7)($p<0.01$). The trunk-to-limb subcutaneous fat thickness ratio was significantly higher in males (mean = 2.37; 95% confidence interval = 1.90 - 2.84) compared to females (mean = 1.83; 95% confidence interval 1.49 - 2.17)($p<0.05$). Mean values of weight, conicity index, waist circumference, waist-to-hip ratio, and upper-to - lower trunk subcutaneous fat thickness ratio were not significantly different between the sexes.

Table 1

Anthropometric profile of the study group: values (means ± S.D.)

Parameter	Female			Male		
	N	mean	s.d	N	mean	s.d.
Age (years)*	79	30.9	14.7	56	26.8	8.2
Weight (kg)	79	62.7	13.5	56	66.2	14.5
Height (m)**	79	1.57	0.09	56	1.68	0.07
BMI (kg/m^2)+*	79	26.1	6.3	56	22.4	3.9
Lean	65.4%			86%		
Overweight	21.2%			9%		
Obese	13.4%			5%		
%Body fat**	79	26.3	9.1	56	13.9	5.0
1st tertile	27	15.0	3.8	17	8.4	2.6
2nd tertile	25	27.2	2.7	24	14.6	2.0
3rd tertile	27	35.5	3.2	15	19.5	3.2
Waist circumference (cm)	79	81.7	17.4	56	76.1	12.7
1st tertile	26	66.3	3.4	19	65.5	3.7
2nd tertile	27	78.6	3.9	19	73.5	1.0
3rd tertile	26	100.6	17.4	18	89.5	13.2
WHR	79	0.79	0.12	56	0.77	0.10
1st tertile	24	0.68	0.05	18	0.69	0.02
2nd tertile	28	0.77	0.01	19	0.75	0.12
3rd tertile	27	0.91	0.12	19	0.88	0.13
Conicity index	79	1.18	0.17	56	1.13	0.1
1st tertile	27	1.03	0.07	15	1.01	0.05
2nd tertile	21	1.13	0.02	21	1.12	0.03
3rd tertile	31	1.32	0.17	20	1.24	0.10
TLR*	79	1.83	0.78	56	2.37	0.55
1st tertile	26	0.89	0.19	20	1.55	0.32
2nd tertile	27	1.41	0.15	18	2.16	0.20
3rd tertile	26	3.24	1.35	18	3.39	0.80
ULTR	79	1.12	0.36	56	1.08	0.47
1st tertile	26	0.55	0.18	20	0.60	0.13
2nd tertile	29	0.94	0.13	18	0.92	0.08
3rd tertile	22	2.01	0.64	18	1.71	0.47

*$p<0.05$
**$p< 0.005$
+Note: For Females: Lean, <27.3 kg/m^2; overweight, 27.3 - 32.29 kg/m^2; obese, ≥32.3 kg/m^2
For Males: Lean, <27.8 kg m^2; overweight 27.8 - 31.1 kg m^2; obese, ≥31.1 kg m^2.

Table 2

Anthropometric variables and serum leptin by age group and gender (values unadjusted means ± standard deviation)

Age group	Female								Male							
	No.	BMI*	%BF*	WHR	C-index	TLR*	ULTR	Leptin (ng/ml)	No.	BMI	%BF	WHR	C-index	TLR*	ULTR	Leptin (ng/ml)
<20	20	23.0	20.7	0.75	1.16	1.91	1.46	12.1	16	22.8	14.0	0.76	1.15	1.70	0.95	6.6
		(4.5)	(8.1)	(0.08)	(0.10)	(1.2)	(0.6)	(1.1)		(5.7)	(5.8)	(0.07)	(0.09)	(0.5)	(0.6)	(7.2)
20-29	30	26.1	25.4	0.79	1.14	2.20	1.02	14.1	28	21.2	12.6	0.77	1.11	2.52	1.09	5.1
		(6.7)	(9.0)	(0.10)	(0.09)	(1.6)	(0.5)	(1.5)		(1.9)	(3.9)	(0.13)	(0.13)	(0.7)	(0.3)	(4.9)
30-39	10	29.8	32.1	0.88	1.29	1.65	0.94	14.0	12	26.2	18.7	0.79	1.14	2.97	1.14	5.2
		(5.1)	(6.6)	(0.19)	(0.32)	(0.4)	(0.7)	(1.2)		(4.9)	(6.2)	(0.08)	(0.12)	(1.5)	(0.8)	(1.4)
40+	19	28.6	30.7	0.86	1.21	1.38	1.03	13.9								
		(7.0)	(7.6)	(0.26)	(0.23)	(0.5)	(0.8)	(1.4)								

*$p<0.05$

Table 3

Serum leptin levels by tertiles of anthropometric variables and by gender (values unadjusted means± standard deviation).

Variable	Female 1st tertile	2nd tertile	3rd tertile	p-value	Male 1st tertile	2nd tertile	3rd tertile	p-value
BMI	4.9±4.4	11.4±10.8	22.0±14.2	0.0001	3.4±0.7	4.8±4.0	8.6±5.8	0.02
%body fat	5.5±7.7	10.6±9.1	24.4±13.9	0.0000	2.7±1.2	3.0±1.0	5.1±3.5	0.18
Waist circumference	6.9±11.7	11.6±10.9	21.8±13.1	0.003	5.3±3.4	3.2±8.7	7.4±8.4	0.52
WHR	13.6±13.8	11.5±14.2	15.8±12.1	0.62	3.7±1.1	5.6±6.0	6.5±7.3	0.16
Conicity index	13.2±14.9	11.8±13.0	13.8±10.7	0.89	5.2±3.4	2.7±1.0	6.1±7.5	0.4
TLR	9.2±10.0	17.8±14.3	13.6±12.8	0.12	4.0±1.1	2.7±1.4	4.5±3.9	0.44
ULTR	16.6±14.3	14.3±11.0	9.5±14.2	0.28	2.6±1.3	3.2—0.7	5.4±1.7	0.05

Table 2 shows anthropometric measurements and the serum leptin concentrations by age group. In the females, BMI, body fat, waist-to-hip ratio rose with age and peaked in the 30-39 years age group. In the males, body fat and waist-to-hip ratio rose with age. But there was no clear-cut age pattern for BMI and conicity index in the males. Age had a contrasting effect on the trunk-to-limb skinfold thickness ratio between the sexes. In females, the ratio decreased with age, whilst in males the ratio rose with age. Similarly, the upper-to-lower trunk skinfold thickness ratio decreased with age in females, and rose with age in males.

Serum leptin: Serum leptin concentration was higher in the females (mean = 13.5 ng/ml; 95% confidence interval = 10.0 - 16.8) than in the males (mean = 5.2 ng/ml; 95% confidence interval = 2.8 - 7.6)($p<0.001$). The gender difference in leptin concentration persists if when expressed as serum leptin per kilogram of fat mass (serum leptin (ng/ml/FM). The mean value for the males was 0.51 ng/ml/kg (95% confidence interval = 0.29 - 0.73) compared to the mean value for females of 0.69 ng/ml/kg (95% confidence interval = 0.54 -0. 83)($p<0.05$).

Depicted in Table 3 are the serum leptin concentrations by tertiles of anthropometric variables and by gender. The mean value of serum leptin in the first tertile of BMI was significantly lower than that of the third tertile in both sexes. The mean value of leptin in the first tertile of percent body fat was significantly lower than the mean values in the other tertiles in the females but not in the males. Similarly, the mean value of leptin in the third tertile of the waist circumference was significantly higher than the mean values in the other tertiles in the females, but not in the males. In the males, the mean leptin concentration increased significantly with upper-to-lower skinfold thickness ratio increases ($p<0.05$). This relationship was not evident in the females.

There were no significant differences in the mean values of serum leptin between the tertiles of waist-to-hip ratio, conicity index, trunk-to-limb skinfold thickness ratio in both sexes. As shown in Table 2, serum leptin concentrations showed no significant variation with age in both sexes

Table 4

Percent variance explained by one factor analysis[1] of variance on log leptin by gender

Variable	Females	Males
BMI	0.286**	0.093
Body fat	0.339**	0.079
Waist circumference	0.165*	0.008
WHR	0.001	0.001
Conicity index	0.002	0.020
TLR	0.023	0.018
ULTR	0.018	0.186*

[1]Factor is tertiles of anthropometric measurements
*$p<0.05$
**$p<0.01$

Table 4 shows the results of a general linear regression model that was used to determine the effect of the anthropometric variables on the variance in the log-transformed concentration of serum leptin. In the females BMI and body fat were significant contributors to the variance in serum leptin. In the males the upper-to-lower trunk skinfold thickness ratio and BMI were the significant contributors to the variance in serum leptin concentration. Thirty six percent of the variance in leptin levels was explained by tertiles of percent body fat in the females ($p<0.01$). In the males, twenty two percent of the variance in leptin levels was explained by the tertiles of the upper-to-lower trunk skinfold thickness ratio. In both sexes, central deposition of fat as evidenced by the waist circumference, waist-to-hip ratio, and the conicity index did not have a significant influence on the variance in leptin levels.

DISCUSSION

The data from this study have once again confirmed the results of earlier studies of an emerging problem of obesity in women in rural African communities(16-17). Up to one third of the women studied could be classified as overweight, or obese on the basis of BMI. Females were close to three times more likely to be obese than males. In contrast to women, obesity was not shown to be of major

significance in the male study group. The BMI in females rose with age and peaked in the 30-39 years age group and declined thereafter. This relationship was not evident in the male study group. This could be due to the smaller sample size and younger age distribution in this group.

Anatomical location of fat has been shown to be an important risk factor in obesity-related illnesses. Central obesity (abdominal body fat), for example, has been shown to influence both mortality and morbidity in obesity, diabetes, and hypertension(18). In the present study, there was little abdominal fat deposition in both males and females as indicated by the low conicity index scores and the waist-to-hip ratios. This study, however, showed that proportionately more fat was deposited in the limbs than in the trunk in females. This was in contrast to males where fat deposits were centralised on the trunk. The truncal fat was concentrated more in the upper torso in the males as opposed to the lower torso in the females. This gender dichotomy in fat distribution was accentuated by age.

To the best of my knowledge, this study provides the first data on serum leptin concentration in rural African communities. The serum concentrations are similar to those obtained in other communities(8,19-21). The data from this study show that in females circulating leptin is primarily a function of adiposity. This relationship was not evident in the male population probably because of the narrow and generally low levels of adiposity in this rural sample. This would then suggest that the relationship between circulating leptin and adiposity is not a continuum, but that the relationship exists above a certain level of body adiposity. The data also confirms the presence of gender dichotomy in the relationship between leptin and body fat mass in the humans. For an equivalent fat mass, the females had more circulating leptin than the males. Regional distribution of fat influenced circulating leptin in the males but not in females. Deposition of fat in the upper trunk regions raised the levels of circulating leptin in the males. The significance of this observation is not very clear and should be subjected to further study. The absence of a strong relationship between leptin levels and regional fat distribution in women has been documented in several studies(8,19-22).

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