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ABSTRACT

Objective: **To examine the determinants for elevated plasma leptin concentration in normal weight (NW), obese (OB), and morbidly obese (MO) individuals in Tanzania.**
Design: **Cross-sectional epidemiological study, the CARDIAC study.**
Setting: **Three areas in Tanzania; Dar es Salaam, urban(U), Handeni, rural(R) and Monduli, pastoralists(P), in August 1998.**
Subjects: **Five hundred and forty five participants from a random sample of 600 people aged 46-58 years.**
Main outcome measures: **Plasma leptin concentrations, height, weight, body mass index (BMI), lipid profiles, haemoglobin Alc (HBA1c), and blood pressure (BP).**
Results: **Plasma leptin concentrations were higher in women than in men (women; 16.0 ng/mL, men; 3.1 ng/mL; $p<0.0001$). Women showed a higher mean body mass index (BMI), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) than men. In both genders, plasma leptin concentration, total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), triglycerides (TG), systolic BP (SBP) and diastolic BP (DBP) were significantly higher in OB than in NW participants. MO women had significantly higher leptin concentration, SBP and DBP compared with the other two groups. In NW men, log leptin concentrations showed a direct correlation with weight, BMI, HBAlc, TC, LDL-C, TG, SBP and DBP (all $p<0.0001$ except TG; $p<0.001$), while among NW women and OB men, weight and BMI correlated positively with log leptin (all $p<0.05$). OB women observed a positive correlation between log leptin and weight, BMI and LDL-C. Regression analysis indicated that among NW subjects, gender, BMI and TC explained 53.9% of the variation in log leptin. In OB subjects, gender, BMI and LDL-C explained 51.7% of the variability in leptin levels. No relationship was found between log leptin and CVD risk factors among MO subjects.**
Conclusion: **The most important determinants for hyperleptinaemia in NW participants were gender, BMI, TC, while in addition to these LDL-C, was an important determinant of leptin concentration in OB individuals. In MO women, the high leptin concentrations did not reflect the amount of adipose stores.**

INTRODUCTION

Leptin, a 16-kilodalton protein encoded by the ob gene is produced mainly by white adipose tissue(1). In humans, serum leptin levels are strongly associated with parameters of body composition like body weight, body mass index (BMI) and total body fat(2). Despite this strong association, large variations in leptin levels are observed for a certain BMI or fat mass, which cannot be explained by weight or body composition(3). Also there is a striking sexual dimorphism in serum leptin levels, with women having 2 to 3-fold higher levels compared to men. Although this was initially attributed to a higher relative fat mass in women(4), more recent studies have found that serum leptin levels remain higher in women even after adjustment for BMI or absolute fat mass(5). This could be related, in part, to differences in fat distribution between men and women, with visceral fat producing less leptin than subcutaneous fat(6). Attempts have been made to find other factors, which may be associated with leptin concentrations in various populations. Studies conducted to identify physical and biochemical determinants of leptin in African populations are few and inconclusive(7).

Therefore, this study examines anthropometric and

metabolic factors, which may influence elevated plasma leptin concentrations in an African population and further evaluate the relationship between leptin levels and cardiovascular (CVD) risk factors in morbidly obese subjects.

MATERIALS AND METHODS

The WHO-CARDIAC study: World Health Organization Cardiovascular Disease and Alimentary Comparison(WHO CARDIAC) study was conducted in Tanzania in 1998, according to the CARDIAC study protocol. The details of the study design have been described in our previous publication(8). In summary, the study was a cross sectional epidemiological survey to investigate the relationship between dietary factors and blood pressure. The survey was conducted in three centres of Dar es Salaam (U, urban), Handeni (R, rural) and Monduli (P, pastoralists). Participants were selected randomly from an administrative list of all men and women aged 30 years and above. Invitation letters were then sent to 100 men and 100 women aged 46-58 years. Five hundred and forty five subjects are included in this analysis. The Medical Ethical Committee of the Muhimbili University College of Health Sciences approved the study protocol.

Anthropometric measurements included weight and height, which were measured with participants wearing light clothing and without shoes. After a 5-10 minutes rest, blood pressure (BP) was measured three times for each subject and the mean value was used in this analysis. In order to remove observer bias, we used an automated BP measurement system (KW machine) (8).

Participants reported in the morning after an overnight fast. After informed consent was obtained, fasting blood samples were drawn for measurement of plasma leptin, lipid profile and haemoglobin Alc percent (HBA1c). Blood samples were flown on dry ice (-20°C) to Japan. The blood measurements were done at the WHO Collaborating Center for Research on Primary Prevention of Cardiovascular Diseases, Graduate School of Human and Environmental Studies, Kyoto University. Frozen samples were stored at -80°C until they were analysed in a batch. Plasma leptin was measured by a radioimmunoassay technique (RIA) (Linco, St Charles, MO). The intra-assay and inter-assay coefficients of variation were less than 6% and a lower detection limit of 0.5ng/ml. Standardised biological methods were used to analyse total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and triglycerides (TG) (enzymatic methods, Kit determiner TC 555, Kyowa Medics, Tokyo, Japan). HBAlc was determined by high performance liquid chromatography (HPLC) (HLC-723Ghb, Toyosoda, Tokyo). Low density lipoprotein cholesterol (LDL-C) was calculated according to Friedewald's Formula:

LDL-C = [TC- HDL-C-(TG/5)] (9).

The participants were stratified into three groups depending on their BMI levels(10). Normal weight (NW) group included subjects with BMI < 25 kg/m^2. Subjects with BMI of 25 kg/m^2 to 39.9 kg/m^2 were considered as obese (OB) and morbidly obese (MO) subjects had BMI of >40 kg/m^2.

Statistical analysis: All analyses were done using Stat view statistical software(11). To approximate normal distribution, plasma leptin was logarithm (log) transformed and used in correlation and regression analyses. Descriptive statistics of demographic characteristics are presented as mean ± standard error of the mean (S.E). ANOVA with Scheffe's test were used to compare the results between NW, OB and MO groups. Simple correlation analysis using the Pearson method allowed the assessment of univariate relationships. Stepwise multiple regression analysis was used to calculate the different contribution of each of the co-variates to the variability in plasma leptin levels among the three groups.

RESULTS

A total of 545 subjects were included in this analysis (259 men and 286 women). The mean age was 51.6±0.2 years for men and 51.7±0.2y for women. Subjects with higher HBAlc percent had significantly higher plasma leptin levels than those with HBAlc levels lower than 7%(13.9±3.1 ng/mL versus 9.3±0.7 ng/mL; p< 0.05).

The characteristics of the population on the basis of gender are shown in Table 1. Plasma leptin concentration was higher among women than men (women 16.0±1.2 ng/mL; men 3.1±0.3 ng/mL,

Table 1

Mean plasma leptin concentration, anthropometric measurements and metabolic parameters of the population stratified by gender

Parameter	Men (n=259)	Women (n=286)	P-value
Weight Adjusted Leptin (ng/mL)	3.1±0.3	16.0±1.2	<0.0001
Age (years)	51.6±0.2	51.7±0.2	0.8697
Height (cm)	165.5±0.6	155.9±0.4	<0.0001
Weight (kg)	62.1±0.8	60.0±1.0	0.1089
Body Mass Index (kg/m^2)	22.5±0.3	24.7±0.4	<0.0001
Glycocylated Haemoglobin(%)	5.1±0.1	5.2±0.1	0.6391
Total Cholesterol (mmol/L)	4.2±0.1	4.9±0.1	<0.0001
Low Density Lipoprotein C (mmol/L)	2.7± 0.1	3.3±0.1	<0.0001
Triglycerides (mmol/L)	2.5±0.1	2.5±0.1	0.8749
High Density Lipoprotein C (mmol/L)	1.1±0.02	1.2±0.03	0.0057
Systolic Blood Pressure (mmHg)	127.4±1.5	127.7±1.5	0.8829
Diastolic Blood Pressure (mmHg)	72.5±0.9	74.7±1.0	0.1092

Values are presented as Mean±S.E (Standard Error)
C=Cholesterol

p<0.0001). Significant gender differences were also observed for height, BMI, TC, LDL-C and HDL-C. In all these parameters, women observed significantly higher levels than men did.

Table 2 shows the characteristics of subjects on the basis of BMI. Only women participants (n=9) had BMI of≥40 kg/m^2 (MO) with an average BMI of 44.7±1.1 kg/m^2. Mean plasma leptin concentration increased from 5.6±0.5 ng/ml. among NW women (BMI < 25 kg/m^2) to 57.3±10.9 ng/mL in the MO women (p<0.0001). Mean serum TC, LDL-C and TG were significantly higher in OB (BMI of 25 to 39.9 kg/m^2) among men and women.

Table 2

Mean differences in plasma leptin concentration, anthropometric measurements, blood parameters and blood pressure levels among normal weight, obese and morbidly obese men and women

	Men		Women		
Parameter	Normal Weight (NW) (n=195)	Obese (OB) (n=62)	Normal Weight (NW) (n=170)	Obese (OB) (n=101)	Morbidly Obese(MO) (n=9)
Age (years)	51.4±0.3	52.3±0.5	51.7±0.3	51.5±0.4	52.7±1.3
Adj. Leptin (ng/mL)	1.7±0.2	8.0±0.7***	5.6±0.5	30.0±2.1***	57.3±10.9***/###
Height (cm)	165.2±0.7	166.8±0.8	155.9±0.6	155.7±0.6	157.4±1.8
Weight (kg)	56.7±0.6	79.6±1.1***	48.8±0.5	74.2±1.0***	110.8±3.5***/###
BMI (kg/m^2)	20.6±0.2	28.6±0.03***	20.1±0.2	30.6±0.4***	44.7±1.1***/###
HBAlc (%)	5.0±0. 1	5.4±0.2*	5.1±0.1	5.3±0.1	5.2±0. 2
TC (mmol/L)	4.0±0.1	5.0±0.2***	4.6±0.1	5.5±0.1***	5.2±0.5
LDL-C (mmol/L)	2.5±0.1	3.1±0.2*	3.1±0.9	3.6±0.1*	3.5±0.5
TG (mmol/L)	2.1±0.1	3.9±0.4***	2.0±0.1	3.3±1.8*	2.8±0.5
HDL-C (mmol/L)	1.1±0.03	1.2±0.04	1.2±0.04	1.2±0.04	1.1±0.11
SBP (mmHg)	123.6±1.6	140.3±2.8***	120.1±1.8	138.7±2.4***	149.9±10.0**
DBP (mmHg)	70.2±1.0	80.5±1.7***	70.8±1.3	79.9±1.4***	96.5±5.4***/#

Values are expressed as Mean±S.E= BMI: Body Mass Index; TC=Total Serum Cholesterol; HDL-C=Low Density Lipoprotein Cholesterol; HDL-C=High density Lipoprotein Cholesterol; SBP=Systolic Blood Pressure; DBP=Diastolic Blood Pressure; Normal weight (NW): BMI<25kg/m^2; Obese (OB): BMI 25-39.9kg/m^2; Morbidly obese (MO): BMI > 40kg/m^2; ***Versus Normal Weight; ###Versus Obese

*/#P<0.05; **/## P<0.001; ***"'P<0.0001

Table 3

Coefficients of correlation (r) between logarithm leptin concentrations and anthropometric and metabolic parameters between normal weight, obese and morbidly obese subjects

	Men		Women			All Subjects
Parameter	Normal Weight (n=195)	Obese (n=62)	Normal Weight (n=170)	Obese (n=101)	Morbidly Obese (n=9)	(n=545)
Gender						0.571
Age (years)	-0.047	-0.196	0.049	-0.012	-0.261	0.01
Weight (kg)	0.354***	0.279*	0.420***	0.250**	0.119	0.551***
BMI(kg/m^2)	0.419***	0.309*	0.395***	0.367***	0.155	0.709***
HBA lc (%)	0.150	-0.208	0.008	0.048	0.675	0.095
TC (mmol/L)	0.368***	0.206	0.152	0.320*	0.276	0.458***
LDL (mmol/L)	0.330***	0.252*	0.158	0.322*	0.124	0.394***
TG (mmol/L)	0.228**	-0.086	-0.030	0.035	0.047	0.242***
HDL (mmol/L)	0.127	-0.035	0.054	0.055	0.616	0.174**
SBP (mmHg)	0.311	0.185	-0.040	-0.118	0.127	0.259***
DBP (mmHg)	0.380	-0.002	-0.018	0.121	0.407	0.307

* p< 0.05
** p< 0.001
*** P< 0. 0001

Only OB men showed a higher mean HBAlc% ($p<0.05$), no difference was found among women. The mean systolic blood pressure (SBP) and diastolic blood pressure (DBP) were higher in OB men and women (all $p<0.0001$ vs. NW) and reached the hypertensive level in MO women (SBP; 149.9±10.0mmHg; DBP; 96.5±5.4mmHg).

Correlation coefficients between log leptin and anthropometric and metabolic parameters are shown in Table 3. NW men showed a strong positive correlation between log leptin and body weight, BMI, TC, LDL-C, TG, SBP and DBP. NW women and OB men showed a direct correlation between log leptin and weight and BMI. Obese women showed a positive correlation with weight, BMI, TC and LDL-C. MO women did not observe any significant correlation between log leptin and anthropometric and metabolic parameters. When the correlation was done to all participants combined (results not shown), it was found that, gender, weight, BMI, HBAlc, TC, LDL-C, HDL-C, TG, SBP and DBP both correlated strongly with log leptin concentration (all $p<0.0001$, except HBAlc $p=0.0412$ and HDL-C $p=0.0001$ respectively).

The results of a stepwise multiple regression analysis is shown in Table 4. The model comprised of gender, weight, BMI, HBAlc, TC, LDL-C, HDL-C, TG, SBP and DBP. Among the NW participants, gender, BMI and TC explained 53.8 % of the variability in plasma leptin concentration independent of other factors ($p<0.0001$). While among OB subjects, 51.7% of the difference in leptin concentration was explained by the differences in gender, BMI and LDL-C concentration ($p<0.0001$). MO subjects did not show any significant relationship with either of the variables in the regression model, indicating the possibility of leptin resistance in excessive obesity. When all subjects were combined together, it was found that gender, BMI and TC were important contributors to elevated leptin concentrations in this population ($p<0.0001$, $R^2 = 0.727$).

Table 4

Multiple regression analysis with logarithm plasma leptin concentration as a dependent variable for normal weight and obese subjects

Parameter	All Subjects (All) (n=420) β	Normal Weight (NW) (n=286) β	Obese Subjects (OB) (n=126) β
Age (years)	0.067	0.090	-0.004
Gender	0.497***	0.530***	0.460***
Weight (kg)	0.029	0.036	-0.064
BMI (kg/m^2)	0.057***	0.057***	0.036***
HBA1C (%)	0.031	0.083	-0.125
TC (mmol/L)	0.080***	0.067***	-0.033
LDL-C (mmol/L)	-0.049	-0.078	0.067***
TC (mmol/L)	0.050	0.080	-0.054
SBP (mmHg)	0.048	0.075	-0.088
DBP (mmHg)	0.068	0.099	-0.035

Values are regression coefficients (β) and significant levels. For all subjects $R^2=0.727$; Normal weight individuals $R^2=0.538$; and for obese participants $R^2=0.517$; *** $p<0.0001$

DISCUSSION

The findings of this study indicate that gender; body mass index, total cholesterol and low density lipoprotein cholesterol were important determinants for elevated plasma leptin concentration among all, NW and OB subjects. In contrast, for MO subjects, no relationship was observed between leptin concentration and body composition, or any of the biochemical variables. These findings add valuable information to the determinants of elevated plasma leptin concentration in Africans and confirm that leptin resistance exists in obese individuals, such that, persistently higher than normal leptin levels in obese individuals, may contribute to its adverse effects in human obesity.

The gender dimorphism in plasma leptin concentration found in our study was previously reported by several other studies (5,6). It is not clear whether this gender difference is oestrogen-dependent. Studies, which evaluated the influence of oestrogens or progesterone on serum leptin levels, have shown conflicting results (12,13). Van Harmelen *et al*,(14), provided evidence on the differences in leptin synthesis in the two fat compartments. Their study showed that subcutaneous tissue is two to three times more effective in synthesizing leptin than omental tissue. Therefore, the gender-related difference in plasma leptin, which cannot be fully accounted for by differences in the hormonal milieu, could partly be explained by the presence of more abundant subcutaneous fat in women and of visceral fat in men.

The strong correlation between leptin and BMI, observed in our study is supported by several other studies, (2,5,6). Gender, BMI and TC contributed to 53.8% of the variations in plasma leptin concentration among NW subjects. This indicates that, factors other than the degree of adiposity may be involved in the variation in plasma leptin levels. In MO women, we did not observe any significant correlation between log leptin and BMI or biochemical parameters. This indicates that, in excessive obesity the higher than normal plasma leptin concentrations do not reflect the amount of adipose stores(15). Hence, in our subjects, at levels of obesity ≥40kg/m^2 sensitivity to leptin was greatly impaired. We observed a strong positive correlation between log leptin and TC and TG in NW men and with TC and LDL-C in OB women. Matsubara *et al*(16) found that leptin levels were elevated in subjects with hypercholesterolaemia and hypertriglyceridaemia compared with normolipidaemic individuals. One other study reported no correlation between leptin and lipid parameters, (17). However, recent evidence suggested that leptin plays a role in lipid metabolism. Because of deficiency and unresponsiveness to leptin, results in the ectopic over accumulation of lipids secondary to under-expression of PPARα (Peroxisome-Proliferator Activated Alpha Receptors) and enzymes of fatty acids oxidation, it is possible that loss of sensitivity to leptin in obese individuals causes ectopic accumulation of un-oxidized lipids(18).

Since leptin binding sites have been found in regions of the brain that are important in cardiovascular control, it has been suggested that leptin plays a role in blood pressure regulation through its central nervous system effects(19).

Only in all subjects and NW men did we observe a direct correlation between log leptin and both SBP and DBP. The fact that leptin may explain obesity-related hypertension has been discussed previously(19). Several murine models of obesity, including agouti obese mice, exhibit resistance to the anorexic and weight-reducing effects of leptin. Hypertension in agouti mice has been attributed to hyperleptinaemia(20). These observations pose a seemingly paradox. We had showed that in MO women the mean blood pressure levels were actually hypertensive levels. Thus, in regard to leptin resistance in obese individuals, it may be that it operates in a selective manner, with resistance to the metabolic actions but with preservation of the sympatho-excitatory actions. This concept of "selective leptin resistance" may have potential implications for human obesity, which as we have shown in MO women, was associated with excessively higher leptin levels. Therefore, if leptin resistance is selective in obese humans, then leptin could contribute to sympathetic over-activity and its adverse consequences in human obesity.

Therefore, we conclude that important determinants for hyperleptinaemia in NW African subjects were gender, BMI and TC while in addition to these parameters, in OB subjects, LDL-C was associated with elevated plasma leptin levels. In MO women, increased plasma leptin concentrations did not reflect the amount of adipose stores and those MO subjects exhibited the novel concept of selective leptin resistance.

Furthermore, we suggest the use of leptin levels as a marker of good health, as well as a useful motivational aid towards achieving and maintaining a health lifestyle among Africans.

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