LEPTIN LEVELS IN THE HYPERTENSIVE BLACK AFRICAN PARTURIENT

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September 2001

Submitted in partial fulfilment of the requirements for the Masters of Medicine degree in the Department of Obstetrics and Gynaecology.

DECLARATION

This is the candidate's own work, with assistance from the Department of Chemical Pathology, Nelson R Mandela Medical School, University of Natal. This work has not been previously submitted to the University of Natal or any other University.

Signed ..... Date 21/11/2001

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PUBLICATION

Kafulafula G, Moodley J. Leptin Levels in the obese African Parturient. Journal of Obstetrics and Gynaecology 2001; **21**:228-31.

ETHICS

The Faculty of Medicine Ethics Committee, University of Natal, and King Edward Hospital gave ethical approval for the study to be conducted.

ACKNOWLEDGEMENT

I am deeply grateful to the following:

- Professor J Moodley, Head, Department Of Obstetrics & Gynaecology, Nelson R Mandela School Of Medicine, University of Natal, Durban, for his motivation, continued guidance and support in this work and untiring editorial effort on the manuscript
- Professor P Ojwang, Head; and the team in the Department of Chemical Pathology, Nelson R Mandela Medical School, Durban, for the leptin assays
- Mr H Kagoro, Faculty Statistician, Department of Community Health, Nelson R Mandela School of Medicine for his help with statistical analysis of the data
- All the women who accepted to be part of this study
- The administration of King Edward VIII Hospital for granting permission to conduct this study at this hospital.
- Mrs Vanessa Tombe for her editorial efforts on the manuscript

DEDICATION

I dedicate this work to Ursula, and our two sons, Emmanuel and Mateo for the neglect they endured in my academic pursuance.

TABLE OF CONTENTS

Chapter II

Chapter III

Chapter IV

Index of Tables and Figures

ABSTRACT

Background: Leptin is a new adipose-derived hormone discovered in 1994. It is vital in energy balance and weight regulation in humans. During pregnancy the placenta is an extra source of leptin. The role of leptin in pregnancy is not established. This has generated a lot of interest in leptin research in pregnancy. Leptin is being examined in pathological states that may have origin in adipose tissue and the placenta such as pre-eclampsia, intrauterine growth restriction and obesity.

Aim and Method: This study measured concentrations of serum leptin in Black African women during late pregnancy in 68 women with pre-eclampsia, 92 healthy normotensive pregnant women and in 32 healthy non-pregnant women. In each group leptin levels were compared between obese (body mass index, BMI = or > than 30 kgm^{-2}) and lean women. Serum leptin concentrations were measured by radioimmunoassay (RIA) technique.

Results: Serum leptin levels were higher in pregnancy compared to non-pregnant women (26.66+/-16.13 ng/ml, 25.89+/-15.83 ng/ml vs 17.97+/-11.98 ng/ml, p=0.02). This is due to firstly, the extra fat accumulated as part of the maternal adaptation to pregnancy and secondly, to the placenta-derived leptin. Other pregnancy hormones such as insulin, hcG, prolactin and oestrogen may modulate the serum levels of leptin in pregnancy.

Simple anthropometric parameters (weight, BMI, circumferences of the mid upper arm (MAC), waist (WC), hip (HC), and thigh (TC) and waist-hip ratio (WHR)) were used to explore the relationship between leptin concentrations and obesity. All the parameters

showed a positive correlation with serum leptin concentration in all the groups with the exception of WHR. Weight and BMI showed the greatest correlation both in pregnant ($r=0.61$ and $r=0.58$, respectively, $p<0.001$) and non-pregnant ($r=0.74$ and 0.79, respectively, $p<0.001$) women.

However we did not find a significant difference in the concentrations of leptin between women with and those without pre-eclampsia (26.66 ng/ml vs 25.89 ng/ml, $p=0.95$). This probably means that adiposity is the predominant factor influencing levels of leptin in pregnancy. The other factors mentioned above play only a minor role. Indeed the mean serum leptin levels were higher in obese compared to lean women in both pregnant and non-pregnant women.

Conclusion: Pregnancy is a hyperleptinaemic state. There is no difference in serum leptin levels between women with pre-eclampsia and healthy normotensive pregnant women. Serum leptin concentration is largely determined by the degree of adiposity both in and outside pregnancy.

CHAPTER I

1.1 INTRODUCTION

Leptin is a novel peptide hormone, discovered in 1994[1] as the obese (*ob*) gene product. Its name is derived from the Greek word *leptos*, which means thin[2]. This name is appropriate as the gene that encodes the hormone was simplistically thought to be responsible for 'thinness' in mammalian species including humans. The hormone is produced primarily in adipocytes and it is involved in the maintenance and regulation of body weight in mice and in a more complex mechanism in humans[3]. Much of the research on leptin has been conducted in animals, in particular mice, and this knowledge has been extrapolated to gain insight into the role of leptin in human physiology and pathology and thus clinical research.

1.2 HISTORICAL BACKGROUND

1.2.1 Energy Balance and Body Weight Regulation: As far back as the 16th century energy balance and body weight maintenance in mammals was, thought to be, determined by some physiological factor(s)[4]. The hypothalamus, in particular the ventro-medial nucleus (VMN) component, was shown to be a crucial site in the central nervous system (CNS) for the regulation of appetite and energy expenditure[5]. One of the theories to link the body energy stores and the hypothalamus was the lipostatic theory[6]. This theory asserted that the CNS, via some fat metabolite (circulating in plasma) that interacts with the VMN in the

hypothalamus, determined the amount of fat reserves in the body. The nature of the postulated biochemical signal was not clear. In 1978 Coleman[7] suggested that the circulating signal could be a product of an *obese* (*ob*) gene.

1.2.2 The discovery of Leptin: It was not until 1994 when the elegant work by Zhang and his colleagues[1] in USA employing genetic engineering techniques (positional cloning) that the nature of the mouse *ob* gene and its human homologue were elucidated. Both in humans and mice, the mRNA expression of the gene was virtually confined to adipose tissue. They thus showed that the *ob* gene protein product is secreted exclusively by adipose tissue. They suggested that the *ob* gene encodes the circulating biochemical (hormonal) signal. A mutation of the *ob* gene has now been shown to be the cause of leptin deficiency and morbid obesity in these mice and the gene mutation is designated as Lep^{ob} [8].

The identification of the *ob* gene set the scene for further work to unravel the mechanism regulating adiposity and body weight in humans. This work has stimulated the explosion of publications on leptin research in the hope of finding a cure for human obesity. So far this has not been realised due not only to the complexity of the aetiology of human obesity but also to the incomplete understanding of the mechanism of action of leptin. Nonetheless much has been learnt regarding the physiology of leptin and its association with several human pathological conditions.

1.3 STRUCTURE AND BIOACTIVITY OF LEPTIN

Leptin is a 16-kDalton protein consisting of 167 aminoacids forming a helical structure[9]. This structure is similar to the structure of cytokines such as IL-1 and IL-6. Indeed it is now regarded as a cytokine and this may explain the potential role of leptin in some human diseases that have an inflammatory (both infective and non-infective) basis. These include pre-eclampsia[10] and Human Immunodeficiency Virus infection (HIV/AIDS)[11]. The bioactivity of leptin seems to 'reside' in an inter-cysteine disulphide bond at positions 96 and 146[1,11].

1.4 LEPTIN RECEPTOR, BINDING PROTEINS AND CLEARANCE

1.4.1 Receptors: It is now clear that the satiety centre in the hypothalamus is the target site for leptin. The strongest evidence is the localisation and abundance of leptin receptors in the hypothalamus[12]. At least 5 isoforms of the receptor (initially designated as OB-R_a, OB-R_b, OB-R_c, OB-R_d and OB-R_e) have been characterised. The OB-R_b is the functional receptor. Similar to the other isoforms the OB-R_b has three components - an extracellular, a transmembrane and an intracellular domain. The difference in the isoforms is due to the differences in the intracellular component. The signalling pathway of the receptor is complex involving Janus kinases (JAK) and signal transducers and activators of transcription (STAT)[11]. A *db* gene encodes the receptor. A mutation of *db* gene causes hypothalamic leptin receptor deficiency and this has also been shown to be the pathology underlying morbid obesity in mice. The gene mutation is now designated $Lepr^{db}$ [8].

1.4.2 Binding Proteins: Houseknecht *et al.*, (1996)[13] demonstrated the presence of 2 serum leptin binding proteins (macromolecules) in humans. Leptin in the circulation is either in the bound or the free state. Generally the majority of leptin is bound. There are differences in the proportion of free and bound leptin in the circulation between lean and obese individuals. Obese subjects have relatively elevated free leptin while in non-obese individuals leptin is predominantly in the bound state. Leptin binding does not only serve transport function but also influences leptin bioactivity because crucial conformational changes essential for ligand-receptor interaction takes place when leptin binds to the binding proteins. For example, the apparent leptin resistance in certain pathological conditions and the observed gender differences in the serum leptin levels and physiology may be due to this association between leptin and the binding proteins.

1.4.3 Clearance: The efficiency with which leptin is cleared from the circulation explains the very short half life of both endogenous and exogenous leptin[14,15]. Such a short half life would pose a problem to the leptin therapy dosing regime. Indeed poor compliance was noted in a study testing the efficacy of exogenous recombinant leptin in decreasing weight in obese individuals[16]. The poor compliance was attributed to the quantity and frequency of recombinant leptin that had to be given during the study.

1.5 MECHANISM OF ACTION

The interaction of leptin with the hypothalamus is complex. It has now been shown in animal studies that leptin acts on the feeding centres in the hypothalamus, via mediators, to inhibit food intake[17] and stimulate energy expenditure[18]. Neuropeptide Y (NPY) is a peptide neurotransmitter (for the neurones) in the arcuate and paraventricular nuclei of the hypothalamus. Overexpression of mRNA and, therefore, elevated levels of NPY causes overfeeding and lowering of energy expenditure[19,20]. In experiments on morbidly obese mice (with congenital leptin deficiency) administration of exogenous leptin has been shown to inhibit the arcuate-paraventricular nucleus NPY system. This was not the case in similarly obese mice with congenital leptin resistance at the hypothalamic NPY neurone level[17]. These authors propose a leptin negative feedback loop involving NPY as a mediator in the control of adiposity: increased hypothalamic NPY activity will increase adiposity, which will lead to increased leptin secretion. Leptin will in turn inhibit NPY activity in the hypothalamus. Weight loss will cause decreased leptin secretion thus removing the inhibition on the NPY activity and promoting weight gain, and fat deposition. Unfortunately the model is not comprehensive, as factors other than NPY have been implicated in leptin-hypothalamus interaction in the regulation of weight and adiposity. These include corticotrophin releasing hormone, melanocyte-stimulating hormone and its receptor (melanocortin-4)[11]. None the less this is a useful model for research in human obesity and obesity related pathologies.

1.6 HUMAN OBESITY AND LEPTIN

1.6.1 Leptin and Types of Obesity: In humans, failure of leptin action on the hypothalamus, due to target tissue resistance to the hormone is associated with the condition of obesity[21,22]. This has been the conventional rationale to explain the observation that increased adiposity in humans is associated with elevated serum levels of leptin[3,17,21,22]. However, the picture will certainly change with the recent finding of two children with early onset morbid obesity and a single nucleotide mutation in the gene that encodes leptin[23]. The hormone was absent in the circulation of the two children in the report. Both had the clinical problem of hyperphagia. It seems that eventually two types of obesity may emerge: 1) early onset obesity associated with congenital deficiency of leptin as a result of a leptin gene mutation and 2) obesity associated with leptin target tissue resistance. This is a situation similar to diabetes mellitus.

1.6.2 Complications of Human Obesity: Obesity is a global problem particularly affecting women. For example, 15-25% of adult women in Europe are obese compared to 10-20% of men[24]. Therefore, not surprisingly obesity is a relatively common problem in pregnancy. 10% of pregnant women have been reported to be obese[25]. The dangers and complications of obesity associated with pregnancy are well known[26,27]. Obese pregnant women are at increased risk of medical diseases such as diabetes mellitus and hypertension[28]. Further antenatal and intrapartum assessment of both the obese mother and her foetus is difficult[26,29]. Macrosomia and its sequelae are also commoner in obese

women[26,30,31], and morbidity and mortality associated with pregnancy may be two to three times that of non-obese women[32].

Genetic and racial factors are known to influence obesity. For example it is more prevalent among African-American women (37%) in USA compared to white women (22%)[33,34].

1.6.3 Human Pregnancy, Obesity and Leptin Research: The discovery of leptin has revived research interest in the various human conditions associated with obesity. These include pregnancy and its complications such as pre-eclampsia and intrauterine growth impairment. There has been a proliferation in the literature of studies on leptin and human pregnancy. Butte *et al.*, (1997) compared serum leptin levels at 36 weeks gestation to 3 and 6 months postpartum in 65 Caucasian women[35]. Serum leptin levels fell following delivery and correlated well with serum prolactin. A positive correlation of leptin with oestrogen and human chorionic gonadotrophic hormone (hcG) in pregnancy was found in a longitudinal study of 6 non-obese Caucasian women[36]. The findings in these two studies imply a possible role of prolactin, hcG and oestrogen in regulating serum leptin levels and therefore adiposity in pregnancy.

1.7 LEPTIN AND THE PLACENTA

Non-adipocyte sources of leptin in pregnancy were established when Masuzaki *et al.*, (1997)[37] demonstrated production of leptin by the trophoblast of term placentae in Japanese women. This was confirmed by others[38]. Montzoros *et al.*, (1997)[39] compared umbilical cord leptin levels of 50 term and 12 preterm

babies whose mothers were smokers. The differences were profound among the preterm babies. These findings emphasize the potential therapeutic role of leptin in neuroendocrine pathophysiology in babies of smoking mothers.

1.8 LEPTIN AND HYPERTENSION

The role of leptin in the development of hypertensive disease is suggested by several studies[12,40,41]. Narkiewicz *et al.*, (1999)[42] recently measured serum leptin levels in men with established essential hypertension. They failed to show a correlation between leptin and blood pressure. They however found a positive correlation between leptin and maternal pulse rate. Tartaglia *et al.*, (1995)[12] demonstrated in animal studies the presence of and the role of central nervous system leptin receptors in the control of circulatory function. Villarreal *et al.*, (1998)[40] in their study on the renal effects of leptin in rats were able to show that exogenous leptin increases sodium loss. Therefore leptin may be a potential factor in regulating salt excretion and play a role in the pathophysiology of hypertension. The relative risk of women with chronic hypertension developing pre-eclampsia is high. Ness and Roberts (1996)[43] in their critical review of the literature on the epidemiology and pathology of pre-eclampsia reported that among women with chronic hypertension the relative risk of developing pre-eclampsia was10. They proposed chronic hypertension as one of the independent causes of the pre-eclampsia syndrome. This was particularly so for the early onset pre-eclampsia. Therefore chronic hypertension is one of the

postulated theories for the development of pre-eclampsia. It is yet to be investigated whether leptin could be contributory to this pathological process.

1.9 LEPTIN AND PRE-ECLAMPSIA

This deranged physiological control may explain in part the development of pre-eclampsia. Shek *et al.*, (1998)[41] studied the influence of leptin in the development of essential hypertension in African-Americans. They concluded that leptin does not seem to play an important role in influencing the development of essential hypertension in this racial group.

Indeed the role of leptin in pre-eclampsia remains unresolved. Sattar *et al.*, (1998)[44] found that pre-eclampsia did not affect serum leptin levels. On the contrary Kokot *et al.*, (1999)[45] demonstrated significantly higher levels of leptin in women with pre-eclampsia. Both studies were conducted in Caucasian populations. These conflicting findings underlie the need for further research in the relationship between leptin and pre-eclampsia.

CHAPTER II

2.1 THE STUDY

Such research, as illustrated above, is beneficial towards the elucidation of the pathophysiology underlying the problem of human obesity. It should also help in the development of leptin based therapy for the obese and their offspring as demonstrated not only in animal studies[19] but also in humans[46]. Leptin may also play a significant role in the prediction and management of pre-eclampsia in the near future. Leptin research has mainly involved Caucasians and very little is known about leptin in pregnant Black African women in whom obesity and pre-eclampsia are common.

2.2 AIM

We therefore determined serum leptin levels in Black African pregnant women with the problems of pre-eclampsia and obesity and explored the correlation between simple clinical (anthropometric) measurements with serum leptin levels in pregnancy.

2.3 METHOD

2.3.1 Setting and design: Institutional ethical permission was obtained and informed written consent was obtained from all subjects. Pregnant African women receiving antenatal care at King Edward VIII Hospital, Durban, were enrolled. They were assigned to one of the two groups: Group I (pregnant

women with pre-eclampsia), Group II (pregnant normotensive healthy women, controls). Non-pregnant healthy women were similarly enrolled from the family planning and general gynaecology outpatient clinics. These were allocated to Group III (non-pregnant controls).

2.3.2 Definitions of Obesity and Pre-eclampsia: Obesity was defined as a body mass index of at least 30 kilograms per square metre (kgm^{-2})[31]. Pre-eclampsia was defined as a blood pressure of at least 140/90 mm Hg recorded at least six hours apart after 20 weeks of gestation with proteinuria of 300 mg or more in a 24 hour urine specimen (Davey and MacGillivray, 1988)[47].

2.3.3 Data collection: For all groups basic demographic data was obtained. This included hospital number, age, parity, gestational age, marital status, and level of education and occupation. Women with diabetes mellitus, multiple pregnancy, known polycystic ovary syndrome and those who smoke were excluded from the study.

2.3.4 Anthropometry: The following anthropometric measurements were taken: total body weight in kilograms (kg), height in metres (m) and circumferences of the mid-arm (MAC), waist (WC), hip (HC) and thigh (TC) in centimetres (cm). The MAC was taken with a plastic tape measure at a midpoint between the olecranon and the acromion process of the right arm. Similarly the hip circumference was measured at the level of the iliac crest. The waist circumference (WC) was taken at the level of the umbilicus and TC at the midpoint between the anterior superior iliac spine and the medial femoral

condyle. Venous blood (5ml) was obtained by venepuncture in heparinised containers from each woman, at 0800 hours following an overnight fast, for leptin measurement. The blood samples were transported on ice to the chemical pathology laboratory of the hospital. The blood was immediately centrifuged at 4 degrees Celsius (°C) for ten minutes. The plasma was stored at –20 °C pending batch analysis.

2.3.5 Radioimmunoassay of leptin in human plasma: Serum leptin levels were determined in the plasma samples by radioimmunoassay (RIA) using a commercial Human Leptin RIA kit (LINCO Research, Inc. Missouri, USA). This assay utilises radioactive ^{125}Iodine-labelled human leptin antiserum to determine the concentration of human leptin in the serum or plasma samples by a double antibody/PEG technique. The antibody was raised against highly purified human leptin and the standard and tracer used in the assay were both prepared with human leptin. This makes the assay completely homologous for human leptin.

2.3.5.1 Reagents: The kit was commercially acquired with the following reagents that were refrigerated upon arrival:

1. Assay buffer (0.05M Phosphosaline pH 7.4 containing 0.025M EDTA, 0.1% Sodium azide, 0.05% Triton X-100 and 1% RIA grade BSA). 40ml.
2. Human Leptin Antibody, 26ml.
3. ^{125}I-Human Leptin Label (<3micro CI). Lyophilized for stability. Hydrate using entire contents (27ml) of "label hydrating buffer."

4. Label Hydrating Buffer (containing normal rabbit IgG as a carrier). ^{125}I-Leptin must be hydrated with entire contents of label hydrating buffer.

5. Human Leptin Standards, 1 ml each (0.5,1,2,5,10,20,50,100 ng/ml)

6. Quality Controls I(low) and II(high). 2 ml each.

7. Precipitating Reagent, 260 ml.

2.3.5.2 Procedure: 12 x 75 mm polypropylene tubes were used for the assay set up, which was as follows:

1. 300 µl, 200 µl, and 100 µl of ***assay buffer*** were pipetted to non-specific binding (NSB) tubes(labelled 3-4), to reference (B_o) tubes (5-6) and to tubes 7 through the end of the assay respectively.

2. 100 µl of ***standards*** and ***quality controls*** were pipetted in duplicate.

3. 100 µl of ***samples*** was pipetted in duplicate.

4. 100 µl of ***^{125}I-Leptin*** was pipetted to all the above tubes.

5. 100 µl of ***leptin antibody*** was pipetted to all the above tubes except totals (1-2) and NSB (3-4).

6. The tubes were covered with vortex and ***incubated*** overnight at 4 °C

7. The next morning:

8. 1.0 ml of cold ***precipitating reagent*** was added to all tubes (except totals) and again incubated at 4 °C for 20 minutes.

9. The tubes were then centrifuged at 3000 xg at 4 °C

10. The supernatant was decanted immediately, the tubes were drained for 1 minute and the pellet was automatically counted using a gamma counter. The results were reported as ng/ml human leptin.

The assay has a sensitivity limit of 0.5ng/ml. This is the lowest concentration of leptin that can be detected by the assay. Its specificity is 100%. This is the ability of the kit to selectively measure leptin in the presence of other similar components in the serum.

2.3.6 Data Analysis: As a measure of obesity the body mass index (BMI) was calculated using the formula: $BMI = wt/h^2$ where *wt* is the pregnant body mass in kg and *h* is the height in metres. Waist to hip ratio (WHR) was computed. Statistical analysis was performed using the statistical programs Epi Info Version 6 & 2000 and Statistical Package for the Social Sciences (SPSS). Using the same program the appropriate sample sizes to detect a significant difference in leptin levels between the two-paired groups with a power of 90% are 70 (Group I), 70 (Group II), 40(Group III). One-way ANOVA was employed to test significance between the groups. Correlation between serum leptin and the anthropometric parameters were performed by the Pearson's test. All values are presented as means $\pm$ standard deviation (SD) unless otherwise stated. Statistical significance was set at $p < 0.05$.

CHAPTER III

3.1 Results

The results of the demographic parameters, anthropometric measurements and serum leptin measurements are summarised in Tables I – XI and Figs. 1-3 below.

3.2 Demographic factors

192 women were enrolled in the study. 68 had pre-eclampsia, 92 were pregnant controls and 32 were non-pregnant controls. The demographic parameters between the groups were similar (Table I). The mean age and parity for the

Table I. Demographic parameters for the women in the three groups.

	Pre-eclampsia	Pregnant control	Non-pregnant control	P-value
Age (years)	28.1(5.5)	30.5(5.6)	29.5(5.1)	0.56
Parity	1.8(1.5)	2.3(1.5)	2.2(1)	0.12
Gestation (weeks)	34.4(8.3)	35.5(4.6)	Not applicable	0.44

three groups were 28.1(range, 18-40) years and 2(range, 0-7), 30.5 (range, 19-43) years and 2(range, 0-7) and 29.5(range, 19-38) years and 2(range, 1-4) for the pre-eclampsia, pregnant control and the non-pregnant control group respectively. The means were not significantly different (p=0.56, p=0.12 respectively). The gestation age for the pre-eclampsia and the pregnant control groups were 34.4(8.3) weeks and 35.5(4.7) weeks respectively. There was no statistically significant difference between the two (p=0.44).

The three demographic parameters did not show a significant correlation with serum leptin levels (Table Ia).

Table Ia. The correlation values (r) for the relationship between the demographic parameters (age, parity, gestation) and serum leptin levels in the three groups of women

Parameter	**Pearsons Correlation coefficient (r)**		
	Pre-eclampsia	Pregnant control	Non-pregnant control
Age	0.02 (p=0.13)	0.10 (p=0.38)	0.15 (p=0.25)
Parity	0.23 (p=0.17)	0.13 (p=0.24)	0.20 (p=0.32)
Gestation	-0.09 (p=0.10)	-0.15 (p=0.06)	Not applicable

3.3 Anthropometry

Table II gives a summary of the anthropometric parameters and serum leptin levels for the three groups of women.

Table II. The anthropometric parameters and serum leptin levels for women in the three groups.

	Pre-eclampsia	Pregnant Control	Non-pregnant Control	P Value
Weight (kg)	88.7 (18.9)	91.1 (21.3)	85.1 (19.7)	0.16
Height (m)	1.62 (0.06)	1.57 (0.08)	1.61 (0.07)	0.26
BMI (kgm^{-2})	35.0 (7.5)	37.1 (8.5)	38.5 (9.7)	0.18
MAC (cm)	30.9 (4.3)	31.2 (4.8)	30.7 (5.8)	0.42
WC (cm)	104.8 (15.5)	106.9 (14.6)	83.6 (13.8)	<0.01*
HC (cm)	111.4 (12.9)	115.8 (14.5)	107.8 (11.6)	0.27
WHR	1.05 (0.09)	0.92 (0.05)	0.78 (0.08)	<0.01*
TC (cm)	58.2 (10.6)	60.4 (8.1)	56.5 (7.7)	0.34
Leptin (ng/ml)	26.66 (16.13)	25.89 (15.83)	17.97 (11.98)	0.02*

* Correlation statistically significant

3.3.1 Pre-eclampsia Group: The women with pre-eclampsia had a mean weight of 88.7 (18.9) kg, height of 1.62 (0.06) m and body mass index (BMI) of 35.0 (7.5) kgm^{-2}. The MAC, WC, HC and TC were 30.9 (4.3) cm, 104.8 (15.5) cm and 111.4 (12.9) cm and 58.2 (10.6) cm respectively. The mean WHR was 1.05. The mean serum leptin level was 26.66 (16.13) ng/ml.

There was strong positive correlation among the various anthropometric parameters, with the exception of WHR (Table III). The correlation between serum leptin and the anthropometric parameters are shown in Table IV. All the parameters showed a positive correlation with serum leptin level. This was greatest for BMI ($r=0.61$, $p< 0.001$) and TC the showed the least significant correlation ($r=0.39$, $p=0.001$). WHR had a non-significant correlation ($r=0.035$, $p=0.78$).

Table III. The Pearson Correlation, r, between the various anthropometric parameters for women with pre-eclampsia

	Weight	BMI	MAC	WC	HC	WHR	TC
Weight	--	0.95*	0.85*	0.91*	0.85*	0.004	0.71*
BMI		--	0.87*	0.90*	0.82*	-0.01	0.71*
MAC			--	0.83*	0.80*	-0.05	0.65*
WC				--	0.91*	-0.00	0.69*
HC					--	0.1	0.6*
WHR						--	-0.6*
TC							--

* Correlation is statistically significant (2-tailed test)

The correlation values for weight, MAC, WC and HC were 0.55 ($p<0.001$), 0.57 ($p<001$), 0.52($p<0.001$) and 0.52 ($p<0.001$) respectively.

Table IV. The correlation between leptin and the anthropometric parameters for women with pre-eclampsia

	Correlation (r)
Weight	0.55*
BMI	0.61*
MAC	0.57*
WC	0.52*
HC	0.52*
WHR	0.04
TC	0.34*

*Statistically significant correlation

The BMI-leptin relationship is depicted in Fig. I.

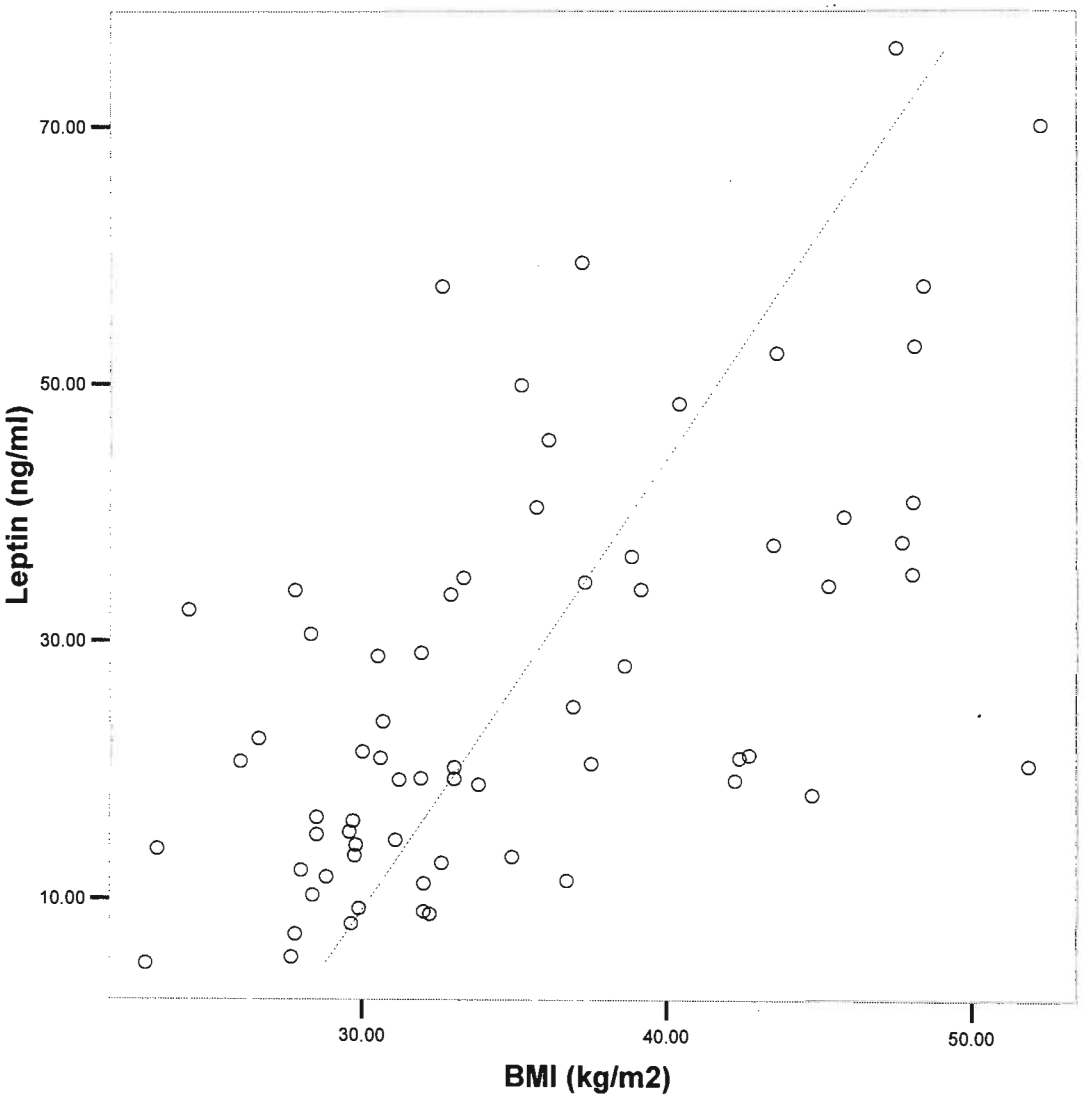

Fig 1. Scatter graph showing the correlation between BMI (kgm^{-2}) and leptin (ng/ml) in patients with pre-eclampsia.

3.3.2 Pregnant control Group: In this group the mean weight was 91.1 (21.3) kg and the mean height was 1.57 (0.08) m. The mean body mass index (BMI) was 37.1 (8.5) kgm^{-2}. The corresponding circumferences for the mid-arm (MAC), waist (WC), hip (HC) and thigh (TC) were 31.2 (4.8) cm, 106.9 (14.6) cm, 115.9 (14.5) cm and 60.4 (8.1) cm. The mean serum leptin level was 25.89 (15.8) ng/ml. Table II shows the summary of these results.

The correlations among the anthropometric parameters were strong except for WHR (Table V). The parameters also showed significant ($p< 0.001$) positive correlation with leptin, with the exception of WHR ($r=0.12$, $p=0.27$) (Table VI). The correlation was moderate for weight ($r=0.58$), BMI and WC ($r=0.56$) and mild for TC ($r= 0.50$) and MAC ($r=0.42$). The relationship between BMI and serum leptin is shown in Fig. 2.

Table V. The Pearson Correlation, r, between the various anthropometric parameters for the pregnant control group

	Weight	BMI	MAC	WC	HC	WHR	TC
Weight	--	0.88*	0.87*	0.91*	0.91*	0.16	0.89*
BMI		--	0.82*	0.87*	0.86*	0.20	0.88*
MAC			--	0.81*	0.82*	0.11	0.84*
WC				--	0.92*	0.39*	0.86*
HC					--	0.01	0.92*
WHR						--	0.04
TC							--

*Statistically significant correlation

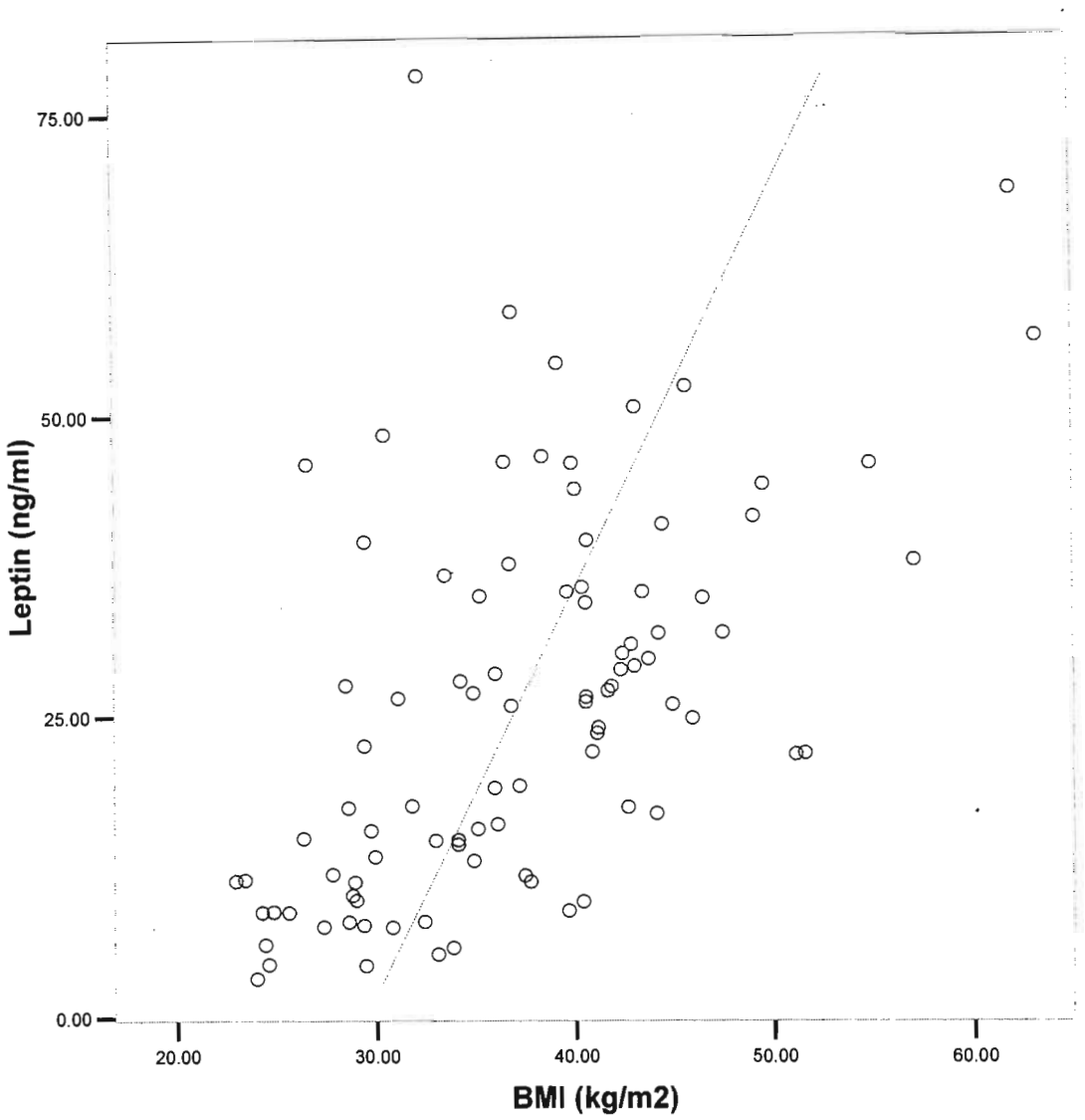

Fig 2. Scatter graph showing correlation between BMI (kgm^{-2}) and leptin (ng/ml) in patients without pre-eclampsia (pregnant controls).

Table VI. The correlation values (r) between the anthropometric parameters and leptin for the pregnant control group

Parameter	Correlation (r)	p- value
Weight	0.58*	<0.001
BMI	0.56*	<0.001
MAC	0.42*	<0.001
WC	0.56*	<0.001
HC	0.54*	<0.001
WHR	0.12	0.27
TC	0.50*	<0.001

*Statistically significant correlation

3.3.3 Non-pregnant controls: In this group 11 women were obese and 21 were non- obese. The mean weight, height, BMI, MAC, WC, HC, WHR and TC were 85.1 (19.7) kg, 1.61 (0.07) m, 38.5 (9.7) kgm^{-2}, 30.7 (5.7) cm, 83.6 (13.8) cm, 107.9 (11.6) cm, 0.78 (0.08) and 56.5 (7.7) cm respectively. The mean serum leptin level was 17.97 (11.98) ng/ml.

There was positive correlation among all the anthropometric parameters (Table VII) and the parameters showed positive correlation with leptin (Table VIII).

Similar to the pre-eclampsia group, the parameter correlating the strongest with leptin was BMI (r=0.79, p<0.001). This is graphically portrayed in Fig 3. The respective correlation coefficients (r) for the other parameters with leptin were 0.74 (p<0.001), 0.71(p<0.001), 0.77(p<0.001), 0.49(p=0.005), 0.71(p<0.001) and 0.63 (p<0.001) for weight, MAC, WC, HC, WHR and TC, respectively. The correlation was moderate for TC and HC but strong for the other 4 parameters.

Table VII The Pearsons Correlation (r) values among the various anthropometric parameters for the group of non-pregnant women

	Weight	BMI	MAC	WC	HC	WHR	TC
Weight	--	0.96*	0.95*	0.90*	0.68*	0.69*	0.84*
BMI		--	0.95*	0.95*	0.71*	0.73*	0.78*
MAC			--	0.89*	0.63*	0.72*	0.77*
WC				--	0.78*	0.74*	0.73*
HC					--	0.15	0.56*
WHR						--	0.54*
TC							--

* Statistically significant correlations

Table VIII *The correlation values (r) between the anthropometric parameters and leptin for non-pregnant women*

	Correlation (r)
Weight	0.74*
BMI	0.79*
MAC	0.71*
WC	0.77*
HC	0.49*
WHR	0.71*
TC	0.63*

* Statistically significant correlation

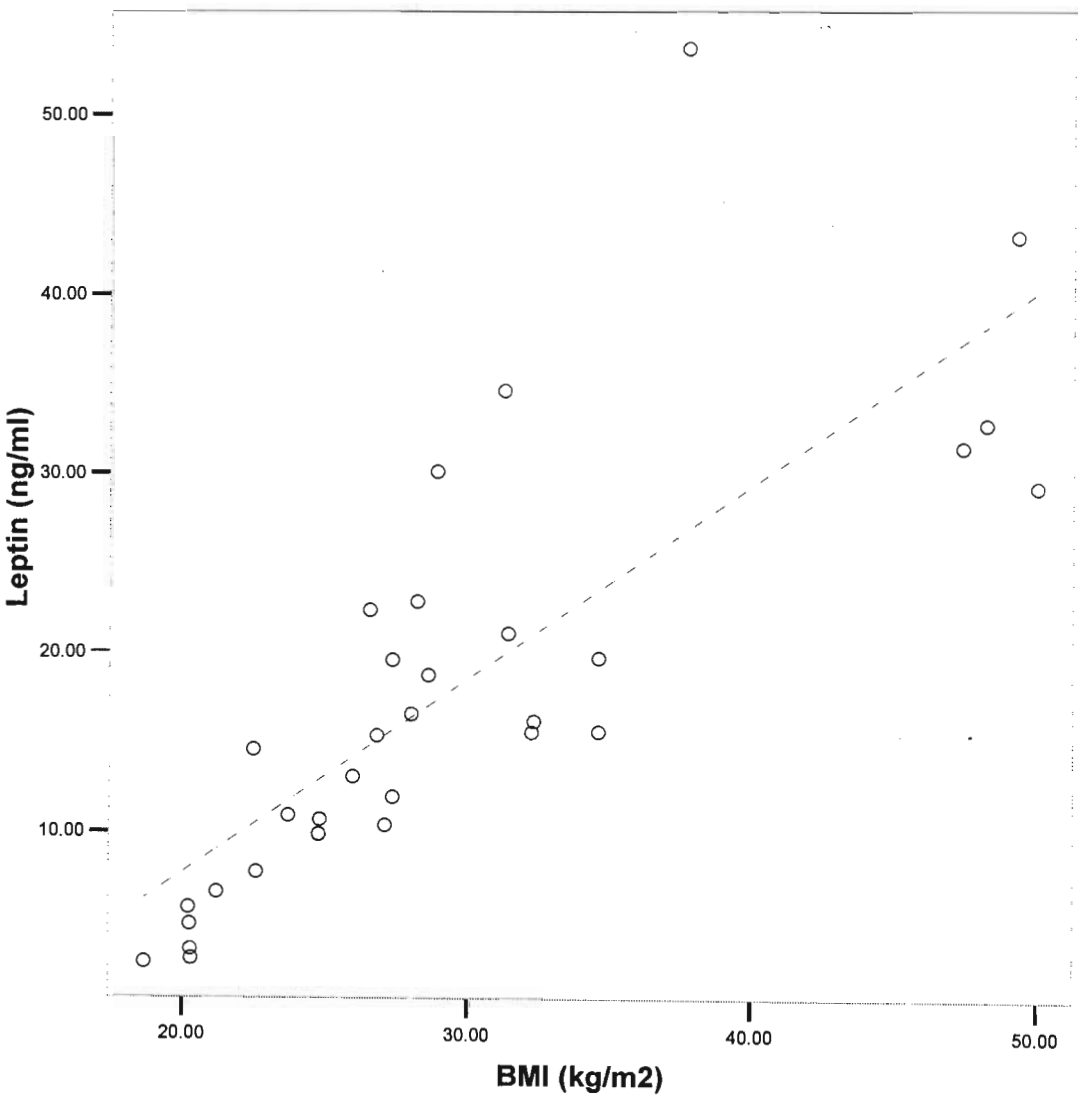

Fig.3. Scatter graph showing the correlation between BMI (kgm^{-2}) and serum leptin (ng/ml) in non-pregnant (control) women.

3.4 Leptin and Obesity in pregnancy

The mean age, parity and gestation age for the obese and lean women in the pregnant controls were similar; 31.3 (4.8) vs 29.8 (6.2) years, 2.6 (1.2) vs 2.3 (0.9) and 34.9 (5.1) vs 35.4 (4.6) weeks, respectively. There was also no statistically significant difference in the mean height; 1.61 (0.2) m for the non-obese group and 1.59 (0.3) for the obese women. The other anthropometric parameters were, as expected, higher for the obese compared to the lean group. These were a mean weight of 100.4 (16.5) vs 66.8 (8.1) kg ($p<0.001$), a BMI of 40.54 (7.1) vs 27.3 (2.4) kgm^{-2} ($p<0.001$), a MAC of 32.5 (4.7) vs 26.8 (2.0) cm ($p<0.001$), a WC of 113.0 (11.7) vs 90.7 (8.6) cm ($p<0.001$), an HC of 120.2 (10.4) vs 99.6 (7.7) cm ($p<0.001$) and a TC of 65.6 (6.0) vs 52.4 (3.8) cm ($p<0.001$). The WHR was similar for obese and lean women; 0.94 (0.6) vs 0.91 (0.2) ($p=0.72$). These are summarised in Table IX.

Serum leptin levels, in all the three groups, were significantly higher in obese compared to lean women (Table X). In the pre-eclampsia group the leptin level was 31.60(16.38) ng/ml in the obese compared to 15.60(8.27) ng/ml ($p <0.001$)

Table IX. The clinical characteristics and leptin levels of obese and lean women in the pregnant control group.

Clinical Characteristic	Obese	Lean	P-value
Age (years)	31.3 (4.8)	29.8 (6.2)	0.32
Parity	2.6 (1.2)	2.3 (0.9)	0.18
Gestation (weeks)	34.9 (5.1)	35.4 (4.6)	0.08
Weight (kg)	100.4 (16.5)	66.8 (8.1)	<0.001*
Height (m)	1.61 (0.2)	1.59 (0.3)	0.74
BMI (kgm^{-2})	40.54 (7.1)	27.3 (2.4)	<0.001*
MAC (cm)	32.5 (4.7)	26.8 (2.0)	<0.001*
WC (cm)	113.0 (11.7)	90.7 (8.6)	<0.001*
HC (cm)	120.2 (10.4)	99.6 (7.7)	0.001*
WHR	0.94 (0.6)	0.91 (0.2)	0.72
TC (cm)	65.6 (6.0)	52.4 (3.8)	<0.001*
Leptin (ng/ml)	30.15 (15.20)	13.79 (10.55)	<0.001*

*Statistically significant difference

in lean women. Obese and lean women in the pregnant control group had leptin levels of 30.15 (15.2) and 13.79 (10.55) ng/ml (p<0.001) respectively. The respective serum leptin levels were 28.46 (12.37) and 12.47 (7.33) ng/ml (p=0.002) for the obese and lean women respectively, in the non-pregnant group.

Table X. The mean (SD) serum leptin levels in obese and lean women in the three groups

	Pre-eclampsia		Pregnant Control		Non-preg control	
	Obese	**Lean**	**Obese**	**Lean**	**Obese**	**Lean**
Leptin (ng/ml)	31.60 (16.38)*	15.60 (8.27)*	30.15 (15.20)♣	13.79 (10.55)♣	28.46 (12.37)#	12.47 (7.33)#

*Statistically significant (p< 0.001) ♣Statistically significant (p<0.001)

#Statistically significant (p=0.002)

The correlations between the anthropometric parameters in the obese and lean pregnant control women are summarised in Table XI. For the obese women weight, BMI and WC show the strongest correlation with the corresponding r values of 0.39 (p<0.001), 0.37 (p=0.001) and 0.37 (p<0.001). HC (r = 0.30, p<0.01) and TC (r = 0.28, p<0.01) correlated weakly with serum leptin. Height, MAC, and WHR correlated the least with leptin.

Table XI. The correlation coefficients (r) for the anthropometric parameters of obesity with leptin in the pregnant control women.

Clinical Characteristic	Correlation coefficient (r)	
	Obese	Lean
Weight (kg)	0.39	0.31
Height (m)	0.11	0.17
BMI (kgm^{-2})	0.37	0.24
MAC (cm)	0.18	0.37
WC (cm)	0.37	0.37
HC (cm)	0.30	0.55
WHR	0.18	-0.08
TC (cm)	0.28	0.58

In the lean group the anthropometric parameters that showed the greatest correlation with serum leptin are the TC ($r = 0.58$, $p<0.001$) and the HC ($r = 0.55$, $p<0.001$), with MAC ($r = 0.37$, $p<0.001$), WC ($r = 0.37$, $p<0.001$) and weight ($r= 0.30$, $p<0.01$) correlating rather moderately with the hormonal levels. BMI in this group also correlated weakly ($r = 0.24$, $p<0.001$) with serum leptin level.

3.5 Leptin and Pre-eclampsia

The serum leptin level was higher in the group of pregnant women with pre-eclampsia, 26.66(16.13) ng/ml, than those without pre-eclampsia (pregnant controls), 25.89 (15.83) ng/ml. However this difference did not reach statistical significance (p=0.95).

CHAPTER IV

4.1 DISCUSSION

In this study we measured the serum levels of the novel peptide hormone leptin in both pregnant and non-pregnant Black African women. Among pregnant women the mean serum leptin level in late pregnancy was 26.66 (16.13) ng/ml in women with pre-eclampsia and 25.89 (15.83) ng/ml in those without pre-eclampsia. These serum leptin levels in pregnancy were much higher than in non-pregnant women. The mean serum leptin level was 17.97 (11.9) ng/ml in the non-pregnant control group.

The serum leptin level in pregnancy in this study is similar to our earlier study on serum leptin in obese Black African parturients[48]. We found a serum leptin level of 24.7 (15.9) ng/ml in late pregnancy using the same technique of leptin assay. The first report, in 1997, on the concentration of leptin in pregnancy found a maternal serum level of 13.4 (8.13) ng/ml[49]. This is much lower than our finding. The difference may be explained in two ways. Firstly, their sample size was small, consisting of only 20 women. Secondly, they measured serum leptin at 20 weeks gestation. It is interesting to note that this same group of researchers found similarly lower maternal leptin levels (11.8 ng/ml) at 10-20 weeks gestation in a subsequent report[50]. It is clear from longitudinal studies that leptin levels in pregnancy increase with advancing gestation[36,51].

Our result is however, in keeping with other numerous studies on leptin levels in late pregnancy. Butte *et al.* (1997)[35], found a serum leptin level of 29.8 (17.0) ng/ml in late pregnancy. Serum leptin levels in pregnancy may be as high as 4 times that in normal non-pregnant women[52]. Although there are variations in the reported levels of serum leptin in late pregnancy in other, mainly Caucasian, population groups most of the studies are consistent in finding mean serum levels between 25.2 and 38.4 ng/ml[35,36,53,54]. Our finding being consistent with these reports suggest that there are no racial differences in leptin levels in late pregnancy. However it should be noted that racial differences in leptin levels have been reported outside pregnancy in postmenopausal women[55].

Although their conclusion may be limited by a small sample size, Hardie *et al.*, (1997)[36] demonstrated the trend in maternal leptin during pregnancy. In the 5 women they followed up through out pregnancy serum leptin increased steadily with increasing gestation until the second trimester when the levels peaked. Subsequently the levels either declined or remained the same during the rest of the pregnancy. This pattern mirrors that of maternal adipose tissue accumulation during pregnancy as observed by Chesley (1944)[56]. This suggests a possible role of leptin in metabolic regulation. Alternatively the elevated leptin levels may just be a reflection of total accumulated fat mass in pregnancy.

Indeed the causal-effect relationship between leptin and adipose tissue in pregnancy is not resolved at the moment. Butte *et al.*, (1997)[35] studied the pattern of other 'pregnancy' hormones and found that oestrogen, progesterone and the placental hormones human chorionic gonadotrophin and human

placental lactogen may modulate maternal leptin levels during gestation. This was supported by other workers[36].

Normal pregnancy is a state of insulin resistance[57]. Hyperinsulinaemia has been shown in rodent studies to increase leptin mRNA expression in adipose tissue[58]. This observation was confirmed in human studies where a chronic hyperinsulinaemic state induces hyperleptinaemia[59]. Therefore the hyperleptinaemia of pregnancy may be secondary to insulin resistance of pregnancy and not simply a reflection of the increased adiposity. In a cross-sectional study such as ours it is not possible to explore these possible explanations regarding the observed elevated leptin levels associated with pregnancy. This is an area for further research as it may have some bearing on future leptin based therapies for obese gravidae and their progeny. During pregnancy the placenta is an additional source of leptin[37,38]; therefore the association between elevated leptin and adipose tissue in pregnancy may be casual rather than causal. However this is rather unlikely in view of the strong correlation between leptin and measures of obesity which holds true both in pregnancy and the non-pregnant state.

The role of leptin in human pregnancy has recently been reviewed[60,61]. The hyperleptinaemia of pregnancy has been clearly demonstrated as discussed earlier and later in this treatise. Leptin may either reflect the normal physiology of pregnancy or serve a pathophysiological role. The former indicates the association with fat mass accumulated in pregnancy while the latter is either a result or a cause of certain obstetric disorders such as obesity, pre-eclampsia,

insulin resistance syndrome and intrauterine growth restriction (IUGR). Kratzsch *et al.*, (2000)[61] propose that the hyperleptinaemia of late pregnancy may cause uncoupling of feeding behaviour and diminished responsiveness of the leptin receptors as fat reserves are amassed for foetal growth and lactation. The rapid decline in leptin levels postpartum will then stimulate eating behaviour[62] suggesting that leptin may play a role in the excessive weight gain experienced by some women following delivery. Of further interest is the observation that abnormally low levels of leptin in the first trimester have been associated with increased risk of spontaneous miscarriage[62]. Increased knowledge in the regulation of leptin and its function in pregnancy may ameliorate perinatal mortality and morbidity associated with conditions of pre-eclampsia and IUGR.

Obesity is clearly a significant health problem. However it is not easy to define particularly for research purposes. Various definitions have been used in the past based on absolute body weight, percentage increase over the ideal body weight or an absolute cut-off point of body mass[28].

The problems are even greater in defining obesity in pregnancy. A maternal body weight greater than 90 kg was the commonest definition in the past[31]. This did not take into account the woman's height. Currently the body mass index (BMI) rather than absolute weight is used. BMI is a better predictor of fat proportion in the body than weight alone. Even with BMI there is no consensus regarding the definite cut off point to define obesity. For this study a BMI = or greater than 30 kgm^{-2} was used to define obesity[31]. Obese women had much higher serum leptin levels compared to the lean women in all the three groups in

our study (Table X). This is in agreement with findings in previous studies conducted both in men and women and in pregnancy and outside pregnancy[3,22,63]. The association of leptin with adiposity explains the observed higher leptin levels in the obese compared to the lean women in all the groups. Most studies however measured leptin levels in non-obese women or women with a wide range of BMIs. Our study is unique in measuring leptin concentrations in predefined obese and lean women in pregnancy (pre-eclampsia and normotensive controls) and outside pregnancy. The anthropometric parameters, apart from height and WHR were greater in the obese than in the lean women. This is to be expected as the parameters are a reflection of the degree of adiposity. The parameter that showed the strongest correlation with leptin in obese women is weight and TC in lean women. WC, which has proved a reliable measure of intra-abdominal fat in men, also showed good correlation in pregnancy. This may be a result of the characteristic central, rather than peripheral, fat accumulation that occurs in pregnancy. WC may therefore be an appropriate index of adiposity in the pregnant state. If these findings were confirmed in further studies it may be prudent to use the appropriate parameters for particular ranges of BMIs when studying obesity and leptin in pregnancy. Height and WHR would seem to be poor parameters and thus not recommended.

Most studies on leptin in pregnancy have been conducted among Caucasian women[35,36,50,64], and most have demonstrated a positive correlation between serum leptin and the degree of adiposity both in non-pregnant and pregnant

populations[3,50,62,65]. Some of these studies employed complex body composition analytical techniques to estimate total fat mass or its proportion to the total body mass and relate these to leptin levels. These complex measures give a more accurate estimate of adiposity. They however are not only cumbersome to perform and inconvenient to the study subjects but expensive as well. In this study, the first to report serum leptin levels in Black African pregnant women with the problems of obesity and pre-eclampsia, the less sophisticated and cheaper methods of assessing body fat were used. The anthropometric parameters used in this study were body weight, height, BMI, MAC, WC, HC, WHR and TC. The serum leptin concentrations showed a positive correlation with all these parameters both in pregnancy and outside pregnancy except WHR in pregnancy. This is expected as there was strong correlation among the various anthropometric parameters apart from WHR in all the three groups. In pregnancy, weight and BMI showed the greatest correlation. This was true for both the pre-eclampsia and the pregnant control group. This is in keeping with previous studies[3,35]. In the non-pregnant controls WHR also correlated strongly with leptin levels. This is rather a surprising result. One would have expected WHR to be a more sensitive measure of adiposity in pregnancy than outside pregnancy[48].

The syndromes of pre-eclampsia and obesity share certain clinical and metabolic characteristics. These include hyperlipidaemia, insulin resistance and glucose intolerance[66]. Thus it may be expected that the hyperleptinaemia observed in obese women may be realised in women with pre-eclampsia. Logically the clue

to the prediction and/or management of pre-eclampsia may lie in leptin research. The women with pre-eclampsia in our study had similar serum leptin level as healthy pregnant women without pre-eclampsia. This is in contrast with a report by McCarthy *et al.*, (1999)[64] who found markedly elevated serum leptin level of 45.6 ng/ml in women with pre-eclampsia compared to 27.0 ng/ml in the control group of white women matched for body mass index, and gestational age. Several reasons may explain this difference. The criteria to define pre-eclampsia do vary. While proteinuria in our study was defined as total protein of 300mg or more in a 24-hour urine collection they used a cut off of 500 mg or more in a 24-hour urine collection. This might have contributed to the difference between the results in the 2 studies. Secondly the trend in serum leptin during the third trimester is not well established. While some authors report a continuing rise others found that the levels actually plateau and yet others found that leptin actually declined just before delivery[67]. There is ample evidence now that leptin levels decline abruptly following delivery[52]. The mean gestation age in our study was 34.5 weeks compared to 38 weeks in the study by McCarthy and colleagues. Furthermore they used pre-pregnant BMI (this may be liable to recall bias and hence inaccuracies) while we computed and utilised the pregnancy BMI. This may also have contributed to the lack of correlation between BMI and leptin levels in their study. Pre-pregnancy BMI has been found in some studies not to correlate with leptin as well as pregnancy BMI[67]. The very small sample size (n=24) in their study may also explain this lack of correlation.

Williams *et al.*, (1999)[67] in a nested case-control study measured second trimester leptin concentration in 38 women with pre-eclampsia and 192 normotensive controls. They found that pre-eclamptic women had either higher or lower leptin levels than the controls depending on whether the BMI was 25 kgm^{-2} and below or was above 25 kgm^{-2} respectively. This finding was interpreted to mean that the normal leptin-adiposity relationship during pregnancy is disrupted by pre-eclampsia and that other factors other than adiposity determine leptin levels in pregnancies complicated by pre-eclampsia. This possibility and the fact that they measured leptin in the second trimester (we studied leptin in the third trimester) may explain the disparity between these results and the results of our study.

Anim-Nyame *et al.*, (2000)[68] recently conducted a longitudinal study to determine the timing of the elevation in serum leptin during pregnancy. Eight women in the study went on to develop pre-eclampsia and it was noted that in this group the concentration of leptin was consistently higher compared to another group of 7 women who did not develop pre-eclampsia. They also noted that from 20 weeks gestation leptin concentration rose gradually in both groups up to 32 weeks. Subsequently there was a slight decline in the normal group as opposed to a clear increase in the pre-eclampsia destined group. The increase occurred before clinical manifestation of pre-eclampsia. This is an important and interesting finding. If these results were confirmed by other studies with greater numbers the clinical value of assaying serum leptin in the prediction of pre-

eclampsia would be established. Their study design and its findings are different from ours.

However our results are similar to the results of Sattar *et al.*, (1998)[44]. They found that, although the level of leptin in the third trimester was higher than in non-pregnant controls, the levels between pre-eclamptic and normotensive controls were similar.

The hyperleptinaemia of pregnancy may be due to either the increased adiposity in pregnancy, placental source of leptin, pregnancy hormone-induced modulation of leptin synthesis/secretion or a combination of these factors. It was not possible to explore these mechanisms in our study because of the cross-sectional design. This area requires further research.

We were unable to demonstrate elevated serum leptin concentrations in women with pre-eclampsia. This may be due to the fact that adiposity has the predominant influence on serum leptin levels overriding all other factors both in and outside pregnancy. The strong correlation, demonstrated in this study, between the various anthropometric parameters (especially BMI and weight) and leptin levels both in pregnancy and outside pregnancy is consistent with this explanation.

Indeed studies that have demonstrated hyperleptinaemia in pre-eclampsia[52,64] are limited firstly by a failure to demonstrate the well established correlation between BMI and leptin, secondly the small sample sizes. None the less possible explanations for this observation have been proposed. Pre-eclampsia

may be complicated by reduced renal clearance and reduced intravascular volume. Both of these can cause hyperleptinaemia. The other possibility of course is that leptin may be the cause (similar to other cytokines) rather than the result of pre-eclampsia[67,69]. There is need to explore the cause-effect relationship between leptin and pre-eclampsia.

4.2 CONCLUSION

Leptin is a novel, adipocyte-derived hormone discovered in 1994. There has been an explosion of publications on leptin research both in pregnancy and outside pregnancy since its discovery seven years ago. The placenta is an additional source of leptin in pregnancy. Pregnancy is a hyperleptinaemic state with the levels of serum leptin correlating with the degree of adiposity as measured by pregnancy weight, BMI, and circumferences of the mid upper arm, waist, hip and thigh.

Although some researchers have demonstrated that pre-eclampsia and other pathological conditions such as obesity and intrauterine growth restriction (IUGR) are associated with even more elevated concentrations of leptin in pregnancy, this study did not confirm the findings that pre-eclampsia is a pathological hyperleptinaemic condition.

4.3 RECOMMENDATIONS

Increased knowledge in the regulation of leptin and its function in pregnancy may decrease perinatal morbidity and mortality associated with conditions of pre-

eclampsia, obesity and IUGR. There is need to quantify the various sources of leptin in pregnancy. However there is also great need for further research with larger sample sizes to establish the clinical value of (serum) leptin (assays) in the prediction or treatment of pre-eclampsia and obesity.

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