Serum leptin concentration, obesity, and insulin resistance in Western Samoans: cross sectional study

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See editorial
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Abstract

Objective—To measure serum leptin concentrations in the Polynesian population of Western Samoa and to examine epidemiological associations of leptin with anthropometric, demographic, behavioural, and metabolic factors in this population with a high prevalence of obesity and non-insulin dependent diabetes mellitus.

Design—Cross sectional study, leptin concentration being measured in a subgroup of a population based sample.

Subjects—240 Polynesian men and women aged 28–74 years were selected to cover the full range of age, body mass index, and glucose tolerance.

Main outcome measurements—Serum leptin, insulin, and glucose concentrations; anthropometric measures; physical activity; and area of residence.

Results—Leptin concentrations were correlated with body mass index ($r = 0.80$ in men, 0.79 in women) and waist circumference ($r = 0.82$ in men, 0.78 in women) but less so with waist to hip ratio. At any body mass index, leptin concentration was higher in women than men (geometric mean adjusted for body mass index 15.3 ± 3.6 pg/l, $P < 0.001$). Leptin concentration also correlated with fasting insulin concentration ($r = 0.63$ in men, 0.64 in women) and insulin concentration 2 hours after a glucose load ($r = 0.58$ in men, 0.52 in women). These associations remained significant after controlling for body mass index; effects of physical activity and of rural or urban living on leptin concentration were eliminated after adjusting for obesity, except values remained high in urban men. 78% of variance in leptin was explained by a model including fasting insulin concentration, sex, body mass index, and a body mass index by sex interaction term. Similar results were obtained if waist circumference replaced body mass index.

Conclusions—The strong relation of leptin with obesity is consistent with leptin production being proportional to mass of adipose tissue. The relation with insulin independent of body mass index suggests a possible role for leptin in insulin resistance or hyperinsulinaemia.

Introduction

The positional cloning of the obese (ob) gene¹ and the subsequent preparation of its encoded product, leptin,^{2,3} have provided renewed stimulus for research into obesity. Halaas *et al* have suggested that leptin, a 16 kilodalton protein, may act as a sensing hormone, or "lipostat," responding to the mass of adipose tissue in a feedback loop between adipose tissue and leptin receptors in the hypothalamus.² Recently, leptin receptors have been located in the hypothalamus of the diabetic (db) mouse,⁴ providing further support for the feedback

hypothesis that was generated over 20 years ago by Coleman from the results of parabiosis experiments in obese (ob/ob) and diabetic (db/db) mice.⁵

In obese people, unlike obese mice,¹ leptin deficiency and mutations in the ob gene do not seem to have a major role.^{6,7} Hyperleptinaemia has been shown in animals such as *Psammomys obesus*⁸ and normal mice made obese by a high fat diet,⁹ as well as in obese people.^{10,11} These results suggest that leptin receptors in the central nervous system are either downregulated or defective if the brain is assumed to be the location of the apparent resistance to leptin. A defect in feedback occurs in diabetic mice,¹² and this could be the mechanism of human obesity, or of some forms of it.

Animal studies suggest that leptin may have a role in regulating appetite and energy expenditure and possibly in modulating insulin sensitivity.^{2,3} A major question is whether these observations apply to humans or whether blood leptin concentration is solely a reflection of the amount of adipose tissue.

Population based studies are useful in examining the importance of leptin in human obesity and non-insulin dependent diabetes mellitus. In the South Pacific island of Western Samoa the prevalence of obesity and non-insulin dependent diabetes mellitus have escalated because of changes in lifestyle during the second half of the 20th century.^{13–15} We report the epidemiological associations between leptin concentration and anthropometric, demographic, behavioural, and metabolic risk factors in this Polynesian population.^{14,15}

Subjects and methods

Detailed information on Western Samoan geography, history, and socioeconomic conditions have been published.^{13,14} Lifestyles range from semi-subsistence agriculture and fishing in rural areas to urban lifestyles with low amounts of physical activity and dependence on imported food.

SUBJECTS

Subjects were drawn from a large, population based study performed in 1991.¹⁴ For leptin analysis, a subgroup of subjects with diabetes was selected at random from each category of body mass index (≤ 25 , 25–29.9, 30–34.9, 35–39.9, and ≥ 40 , measured in kg/m^2). Subjects with normal or impaired glucose tolerance were then chosen to match as closely as possible for age and body mass index. The 240 subjects in the leptin part of the study came from Apia, the capital (104 subjects); Poutasi, a rural village with road access to the capital (68); and Tuasivi, a more isolated rural village (68). These areas were chosen to represent differing degrees of modernisation. The subjects studied were representative of the total 1991 study population in sex distribution, area of residence, and level of physical activity. Diabetic men selected for leptin assay were younger and had a higher fasting glucose concentration than men with diabetes in the original study ($P < 0.005$).

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Table 1—Characteristics of 240 Western Samoan men and women with normal, or impaired glucose tolerance and diabetes. Values are means (ranges) unless stated otherwise

	Men		Women	
	Normal or impaired glucose intolerance	Diabetes	Normal or impaired glucose tolerance	Diabetes
No of subjects	86	28	95	31
Age (years)	42.6 (28-74)	51.5 (35-66)	44.0 (28-73)	53.3 (35-72)
Body mass index (kg/m ²)	30.5 (21.2-47.8)	32.0 (22.5-44.2)	32.1 (19.1-54.9)	35.9 (18.4-52.9)
Waist to hip ratio	0.902 (0.799-1.056)	0.960 (0.863-1.083)	0.838 (0.732-1.106)	0.891 (0.795-1.031)
Waist circumference (cm)	97.7 (73.2-145.2)	106.1 (78.5-137.0)	94.9 (65.7-137.2)	104.2 (80.0-138.5)
Insulin (μU/ml)*:				
Fasting	8.1 (0.9-69.8)	10.8 (0.9-66.5)	8.8 (0.9-67.0)	14.8 (1.8-94.1)
2 Hours after glucose load	19.2 (0.9-253.5)	17.7 (3.3-80.0)	33.5 (3.8-239.5)	43.8 (3.4-192.3)
Glucose (mmol/l)*:				
Fasting	5.7 (4.6-7.8)	13.0 (6.3-20.5)	5.4 (3.3-8.5)	11.3 (5.9-20.0)
2 Hours after glucose load	5.4 (3.5-10.1)	18.4 (12.6-29.2)	5.8 (3.4-10.5)	16.6 (11.4-32.0)

*Geometric mean (range).

Non-diabetic men with leptin measurements were also younger than their counterparts ($P < 0.01$), whereas in all other studied variables and for all women there were no differences between the subsample and the full study population (data not shown).

The study protocol was approved by the Alfred Healthcare Group Ethics Committee.

SURVEY PROCEDURE AND ANALYSES

Subjects presented to a survey site between 0730 and 1000 after an overnight fast. Oral glucose tolerance testing (75 g glucose as dextrose monohydrate) was used to determine glucose tolerance status according to the criteria of the World Health Organisation^{14,16} in those who were not using hypoglycaemic drugs. Fasting blood glucose concentrations and those 2 hours after the glucose load were measured on site using a glucose analyser (YSI, Yellow Springs, Ohio, USA). Serum samples were aliquoted, frozen at -20°C , and transported in dry ice to Melbourne, Australia, for long term storage.

After four years of storage leptin concentration was measured in fasting serum samples with a solid phase double antibody enzyme immunoassay¹⁷ with affinity purified polyvalent antibodies. Concentrations were calculated from standard curves generated with recombinant human leptin. The limits of detection for the leptin assay were 20 pg/ml in serum or plasma. The interassay coefficient of variation was 8.45% and the intra-assay coefficient of variation 7.7% for the high standard and 10.5% for the low standard.

Insulin was measured using a modification of the method of Soeldner and Slone.¹⁸

Height, weight, and waist and hip girths were measured as described^{14,15} and used to calculate body mass index (kg/m^2) and the waist to hip ratio. Data on leisure and occupational physical activity were collected by trained interviewers using separate four point scales.¹⁴

STATISTICAL ANALYSIS

All analyses were performed using the statistical package for the social sciences.¹⁹ The distributions of leptin, glucose, and insulin concentrations were normalised by log transformation, and geometric means are presented. Differences in mean values of continuous variables between non-diabetic (normal or impaired glucose tolerance) and diabetic groups were assessed by an unpaired t test. Covariance analysis was used to adjust means for body mass index, and the significance of the differences between groups was assessed by the F statistic. Men and women were divided into low and high physical activity groups on the basis of the summed activity score, which ranged from 2 to 8. Low activity

was defined as a score of 4 or less and high activity as a score greater than 4. Multiple linear regression was used to identify the independent effects of variables associated with variations in leptin concentrations.

Results

Table 1 shows the characteristics of the 240 subjects. Similar proportions of men and women fell into each category of glucose tolerance, with 70% of men (80/114) and 69% of women (87/126) having normal glucose tolerance, 5% (6/114) and 6% (8/126) having impaired glucose tolerance, 18% each having newly diagnosed diabetes (6/114 and 8/126 respectively), and 7% each having known diabetes (8/114 and 9/126 respectively). Although leptin concentration seemed higher in men and women with diabetes than in those without, there was considerable overlap (fig 1). Differences in leptin concentration between the groups were not significant ($P > 0.05$) and were attenuated after adjusting for body mass index in both sexes. Small differences in fasting and 2 hour insulin concentrations between diabetic and non-diabetic men and women were also reduced and were not significant after adjusting for body mass index (data not shown).

Body mass index, waist circumference, and both fasting and 2 hour insulin concentrations were strongly positively correlated ($r > 0.52$ in all cases) with leptin concentration in men and women (table 2). Waist to hip ratio was less strongly correlated with leptin concentration. Fasting glucose correlated with leptin concentra-

Fig 1—Distribution of leptin concentrations in all subjects according to glucose tolerance. Box plots represent 10th, 25th, 50th, 75th, and 90th centiles

Table 2—Crude correlations (two tailed test) of leptin concentration with anthropometric and metabolic variables in men and women

	Men (n = 114)		Women (n = 126)	
	r	P value	r	P value
Age	-0.09	0.318	-0.014	0.106
Body mass index	0.80	<0.001	0.79	<0.001
Waist to hip ratio	0.53	<0.001	0.28	<0.001
Waist circumference	0.82	<0.001	0.78	<0.001
Insulin (μ U/ml):				
Fasting	0.63	<0.001	0.64	<0.001
2 Hours after glucose load	0.58*	<0.001	0.52 †	<0.001
Glucose (mmol/l):				
Fasting	0.19	0.044	0.03	0.761
2 Hours after glucose load	0.16*	0.100	0.03 †	0.714

*n = 104.

†n = 117.

tion only weakly in men but not in women, whereas 2 hour glucose concentration and age did not show significant correlations in either men or women. The correlation of leptin concentration with fasting insulin concentration was similar in subjects with diabetes ($r = 0.54$ in men, $P = 0.003$; $r = 0.58$, $P = 0.001$ in women) and subjects without diabetes ($r = 0.64$, $P < 0.001$ in men; $r = 0.67$, $P < 0.001$ in women). Also, when subjects with normal glucose tolerance were compared with subjects with impaired glucose tolerance or diabetes, similar correlations between leptin and fasting insulin concentrations were found. Owing to the lack of association between leptin and glucose concentration, grouping of the subjects according to glucose tolerance was omitted in further analyses.

Geometric mean leptin concentrations in subjects from rural areas with lower degrees of modernisation were slightly lower than those in subjects from the urban area. These differences were reduced after adjusting for body mass index (data not shown), but rural men still had lower leptin concentrations than urban men (2.7 v 4.2 pg/l, $P = 0.006$). Physically active men and women tended to have lower serum leptin concentrations than those with a low activity score, but these differences were not significant (data not shown).

Waist circumference and waist to hip ratio were used as markers of body fat distribution, and their associations with leptin concentration independent of body mass index were examined by linear regression. In women waist circumference ($P = 0.006$) and body mass index ($P = 0.001$) contributed independently to variations in leptin values (R^2 for model 0.64). In models including waist to hip ratio and body mass index, only body mass index was significant and explained variations in leptin concentration in both sexes (data not shown).

Figure 2 shows the relation of both body mass index and waist circumference to the log of leptin concentration in men and women. Leptin concentrations in women were higher than in men for the same body mass index or waist circumference. However, at higher body mass indices the leptin concentrations in men and women converged. In an overall model assessing variables that contribute independently to the variation

Table 3—Linear regression model ($R^2 = 0.78$) for variables associated with leptin concentration in men and women combined

Variable	Beta	SE	P value
Body mass index	1.108	0.113	<0.001
Fasting insulin	0.193	0.039	<0.001
Sex	1.244	0.135	<0.001
Body mass index $\times$ sex	-1.037	0.182	<0.001

Fig 2—Association of serum leptin concentration with body mass index and waist circumference in non-diabetic and diabetic men and women

in leptin concentrations a large proportion (78%) of the variation was explained by fasting insulin concentration, sex, body mass index, and the interaction term body mass index $\times$ sex (table 3). A similar result was obtained when waist circumference was used instead of body mass index as a measure of obesity. Substituting fasting insulin concentration with the 2 hour measurement did not change the results. Non-linear models for the relation between body mass index and leptin concentration were also tested but not found to be better.

Discussion

LEPTIN AND OBESITY

This is one of the first studies to examine leptin concentrations in humans from a population based sample. Among these obese people leptin concentration strongly correlated with measures of obesity in both men and women. These data agree with the results in other recent reports^{10 11} and are consistent with leptin concentration being directly related to mass of adipose tissue. However, leptin concentration seems to vary considerably between people with similar degrees of obesity in this and other studies.^{10 11} This suggests the potential importance of other variables that may regulate blood leptin concentration, including physical activity, nutritional factors, genotype, fat distribution, and insulin or other hormones. The independent effects of body mass index and waist circumference on leptin values suggest that the distribution of body fat may also be an important determinant of leptin concentration. However, limitations in assessing absolute mass of body fat with measures used in epidemiological studies, as well as variations in leptin concentrations due to methodological factors, have to be considered as contributing to the observed variability as well.

Animal studies suggest that ob RNA concentrations are higher in adipose tissue from central fat deposits than from other sites.²⁰ Our finding that waist circumference was, independently of body mass index,

Key messages

- Serum leptin concentrations in men and women were strongly positively correlated with body mass index and waist circumference
- They were also strongly correlated with serum insulin concentrations even after adjusting for obesity in both sexes
- Concentrations were higher in women than men, even at the same body mass index or waist circumference
- Resistance to the effects of leptin may be important in human obesity
- Leptin may simply reflect the size of adipose tissue stores, but the independent association with insulin concentration suggests a possible role in insulin resistance or hyperinsulinaemia

associated with leptin concentration is consistent with this observation. The weaker association of waist to hip ratio with leptin value, and its lack of association independent of body mass index, may reflect the fact that waist to hip ratio does not measure the absolute amount of intra-abdominal fat.

LEPTIN AND SEX, PLACE OF RESIDENCE, AND PHYSICAL ACTIVITY

Leptin concentrations were significantly higher in women than in men at all body mass indices. However, these differences disappeared when leptin value was compared across similar body fat percentages.^{10,11} This is consistent with a higher body fat content of women at any body mass index. Women also had higher concentrations of leptin than men for the same waist circumference, which may reflect the overall different patterns of fat distribution between the sexes.

We have already reported on the rural-urban differences in obesity in Western Samoa with lower body mass index in rural Polynesians.¹⁵ This factor explains, to a large extent, the trend for leptin concentration to be lower in rural subjects. Increased energy or fat intake and reduced physical activity in urban compared with rural subjects may contribute to increased obesity and hence indirectly to higher leptin values. Certainly there was no effect of physical activity independent of obesity on leptin concentration.

Physical activity could have a more direct effect on leptin values that may have been missed in this study because the degree of activity was measured inaccurately. Exercise could reduce leptin resistance as is the case with insulin resistance, or it could act to improve insulin sensitivity.²¹

LEPTIN AND INSULIN

Leptin and fasting insulin concentrations were strongly correlated in Western Samoans, and results in animal studies suggest that insulin may directly affect leptin concentration²²⁻²⁴ or that leptin reduces insulin values.²⁵

Although glucose tolerance does not seem to be associated with serum leptin concentration, insulin concentration is clearly associated with leptin concentration, even after correction for obesity. In animals the expression of the *ob* gene is increased by insulin²⁴ and reduced when insulin deficiency is induced by streptozotocin.²² Other studies have also highlighted the association between insulin and leptin.^{2,24,25,26} This relation held even in subjects with diabetes, supporting the decision to combine data from diabetic and non-diabetic subjects.

CONCLUSION

In conclusion, our data show large differences between leptin concentrations in normal and obese subjects; a progressive increase in leptin concentration with increasing body mass index; and significant independent correlations of leptin concentration with body mass index, waist circumference, and fasting insulin concentration. These results strongly support an important role for leptin in human metabolism and obesity. Since the publication of evidence against a mutation in leptin, or a leptin deficiency in obese people,⁶ subsequent groups have reported consistently raised concentrations of leptin^{10,11} or mRNA from the *ob* gene^{27,28} in obese subjects. However, apart from one study comparing Pima Indians with other Americans,¹⁰ ethnic group has not been specified. By confirming previous results in a different population, specifically one with a high prevalence of obesity and non-insulin dependent diabetes mellitus, our study provides important evidence against the major form of human obesity being analogous to that in obese mice—that is, due to leptin deficiency.

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Conflict of interest: MN, MS, JM, AM, JL are full time employees of Amgen, which conducts research on and manufactures leptin.

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Cell mediated immunity after measles in Guinea-Bissau: historical cohort study

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Abstract

Objective—To investigate whether children who have had measles have reduced general cell mediated immunity three years later compared with vaccinated children who have not had measles.

Design—Historical cohort study.

Setting—Bissau, Guinea-Bissau.

Subjects—391 children aged 3-13 years who were living in Bissau during a measles epidemic in 1991 and still lived there. These included 131 primary cases and 139 secondary cases from the epidemic and 121 vaccinated controls with no history of measles.

Main outcome measures—General cell mediated immunity assessed by measurement of delayed type hypersensitivity skin responses to seven recall antigens. Anergy was defined as a lack of response to all antigens.

Results—82 out of 268 cases of measles (31%) were anergic compared with 20 of the 121 vaccinated controls (17%) (odds ratio adjusted for potential confounding variables 2.2 (95% confidence interval 1.2 to 4.0); $P = 0.009$). The prevalence of anergy was higher in secondary cases (33% (46/138)) than in primary cases (28% (36/130)), although this difference was not significant. Anergy was more common in the rainy season (unadjusted prevalence 31% (91/291)) than in the dry season (11% (11/98)) (adjusted odds ratio 4.8 (2.2 to 10.3)). This seasonal increase occurred predominantly in the cases of measles.

Conclusions—Reduced general cell mediated immunity may contribute to the higher long term mortality in children who have had measles compared with recipients of standard measles vaccine and to the higher child mortality in the rainy season in west Africa.

Introduction

Measles kills more than one million children in developing countries each year.¹ This estimate is based on the numbers of deaths in the acute phase of the disease and does not take account of the longer term effects of measles. In west Africa, where acute death rates for measles are particularly high, a phenomenon of "delayed mortality" has been described. Children who survive acute measles (the first month) are more likely to die during the subsequent months than are vaccinated children of a similar age.^{2,3} Studies of the pattern of exposure to measles virus have shown that acute and delayed death rates are higher in secondary cases (those infected by someone living in the same house) than in primary cases (those infected by someone outside the house).⁴⁻⁶ Many communities in west Africa are polygamous and have large extended

families, which leads to extreme overcrowding in houses. Children are therefore intensively exposed to measles at home, and this results in a high proportion of secondary cases.

The mechanism underlying delayed death is not known. One possibility is that general cell mediated immunity may be depressed for many months after measles, which increases the risk of other infections. Anergy, or loss of delayed type hypersensitivity on skin testing with antigens such as tuberculin, is well recognised during acute measles.⁷ Although most studies have found that this does not persist for longer than a few months,^{8,9} one study from South Africa suggested that it might last as long as one year.¹⁰ An alternative hypothesis to explain the difference in long term mortality between children with measles and vaccinated children is that measles vaccination stimulates general immunity, thus protecting against other infections.^{6,11,12}

We investigated the relation between measles and subsequent cell mediated immunity in children in Guinea-Bissau, where measles is particularly severe. The aims were, firstly, to see whether children who had measles had lower cell mediated immunity three years later compared with children who had been vaccinated and not had measles, and, secondly, to see whether cell mediated immunity was more impaired in secondary than primary cases of measles.

Subjects and methods

The study area was on the outskirts of Bissau, the capital of Guinea-Bissau, and comprised two semirural districts, Bandim 1 and Bandim 2, and an urban district, Belem. The houses are multifamily dwellings of mud brick, and polygamy is common. The Papel is the largest of the ethnic groups living in the study area.

Measles has been studied in Bissau since 1979. Epidemics have occurred every few years, and cases of measles have been ascertained by clinical examination or interviews with mothers during the epidemics. In Guinea-Bissau mothers can recognise measles infection reliably.^{13,14} Measles vaccination has also been documented. Most vaccinated children have received the standard Schwarz vaccine, but a few have received medium or high doses of Edmonston-Zagreb vaccine.

A major measles epidemic occurred between October 1990 and June 1991. Measles cases were ascertained by daily contact with the health centre and paediatric department of the hospital, by contact tracing whenever a new house with measles cases was detected, by ongoing weekly morbidity surveys in some houses in the study area, by demographic survey of all houses every three months, and by four specific measles surveys during and after the peak of the epidemic. The timing of the rash of multiple cases in each house was documented to determine whether children were primary or secondary

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