Leptin in the General Population, Differences in Sex Hormones, Blood Lipids, Gender and Life Style Characteristics

Reidun Olstad[1,5], Jon Florholmen[2,5], Johan Svartberg[3,5], Jan H. Rosenvinge[4] and Grethe Støa Birketvedt[*,6]

[1]*Psychiatric Research Centre of North Norway,* [2]*Department of Gastroenterology, Department of Internal Medicine,* [3]*Section of Endocrinology, University Hospital of North Norway, 9291 Tromsø, Norway,* [4]*Institute of Psychology, Faculty of Social Science,* [5]*Institute of Clinical Medicine, Faculty of Medicine, University of Tromsø, 9037 Tromsø, Norway,* [6]*Center of Morbid Obesity, Oslo University Hospital, Aker Hospital, Oslo, Norway*

Abstract: *Introduction*: Fat mass is the main predictor of the serum concentration of leptin, a 167-amino acid peptide hormone encoded by the obesity gene. There is no general agreement about other determinant factors of serum leptin, but attention has been focused on gender and sex hormones. The objective of this study was to describe gender differences in a population study and evaluate the individual contribution of life style characteristics, blood lipid pattern and sex hormones as determinants of serum leptin. *Materials and Methodology*: In a multipurpose, cross-sectional general health survey, ("Tromsø IV"- N = 27.159), conducted during the years 1994/95, measurement of leptin was performed in a sub sample of 1812 subjects. Multiple regression analysis was performed in order to establish the importance of each predictor. *Results*: Gender differences in leptin increased with increasing BMI, and the gender predicted 33 % of the variation of leptin. Differences in life style factors between men and women could not explain gender differences. Smoking, physical activity, testosterone and SHBG were independent negative predictive factors. Estradiol was positive predictive factor of serum leptin. However, all contributed to a small variation in serum leptin. A gender dimorphism was observed between serum leptin and serum testosterone and between serum testosterone and BMI. *Conclusions*: BMI and gender were strong predictors in variations in serum leptin. Life style factors and sex hormones were less predictive. Gender dimorphisms between leptin, testosterone and BMI indicate a complex relationship between body fat distribution and the two hormones.

Keywords: Body fat, physical activity, smoking, testosterone.

INTRODUCTION

Leptin is secreted mainly by adipocytes and plays an important role in regulating food intake, energy expenditure and adiposity [1]. It has evolved from being a satiety signal to a regulatory and integrated hormone that communicates with both central and peripheral endocrine pathways resulting in metabolic effects on peripheral tissue (for review, see [2, 3]). However, no human disease except for some casual reports on severe familiar adiposity has so far been directly linked to leptin [4, 5].

Despite intensive general population research on serum leptin, only a few determining factors are well documented. It is generally accepted that serum leptin reflects the total adipocyte mass, and that serum leptin increases with increasing body fat mass [6, 7]. Moreover, the gender differences in serum leptin concentrations are 3-4 times higher in females than in males [8]. The mechanism behind this gender differences is not fully understood, but sex hormones have been claimed to be candidates [9, 10]. Serum leptin level may be influenced by factors such as physical activity [11], smoking [12], alcohol [13], body fat distribution [14], age [9] pre- and postmenopausal changes [9] and cholesterol/fat components [15], though conflicting results have been reported. Small sample size, variation in adjustments for covariates, selected populations according to gender [7, 16] and age [16], are all factors that may contribute to conflicting results.

Therefore, using a large general population sample is a major step to gain further knowledge about determinants of serum leptin. The aims of the present study are,

- To describe gender differences in serum leptin level in the general population.

- To explore whether gender differences in serum leptin values can be explained by life style characteristics or blood lipid pattern when controlled for Body Mass Index (BMI), age and body shape.

- To explore whether sex hormones act as determinants of serum leptin values when life style characteristics, BMI, body shape, blood lipid pattern and age are controlled for.

MATERIALS AND METHODOLOGY

Subjects

A large multipurpose cross-sectional general health survey, ("Tromsø IV"), was conducted during the year 1994/95. The study is described in detail elsewhere [17].

*Address correspondence to this author at the Center of Morbid Obesity, Oslo University Hospital, Aker Hospital, Oslo, Norway; Tel: 47 90224754; E-mail: gsb42nor@aol.com

1874-2300/11

Subjects aged 25 or more in the municipality of Tromsø (N=35 443), were invited to participate in the first phase of the survey and 27 159 (77 %) attended. The first phase of the study included simple clinical measurements as well as completion of two questionnaires (Phase I). A subset of individuals, all men and women aged 55 – 74 years and 5 – 10% of the remaining age groups were invited to a second phase of the study (Phase II). In phase II 7965 individuals (78% of eligible) attended.

A strategic sub-sample from the Phase II population was selected for leptin analysis (N= 1812). The selection of individuals covered both gender, including both premenopausal and postmenopausal women. Blood samples taken throughout the year were further analysed. The characteristics of the Tromsø IV – Phase II/ TROST study and the population selected for leptin analyses are described in Table **1**.

Table 1. **Characteristics of the Population Selected for Leptin Analysis Compared with the Tromsø IV Study**

Characteristics	Tromsø IV respondents phase II N= 7965	Population selected for leptin analyses N=1812
Males Females Male- female ratio	3397 4568 0.74	746 1066 0.69
Age (Mean±SD) Range	 58.9 ± 10.3 25 - 84	 57.5 ± 11.9 25 – 80
BMI (kg/m^2) (Mean ± SD) Range	 26.0 ± 4.0 11.9 - 58.9	 25.8 ± 4.0 15.4 – 52.1

Questionnaires

Besides gender and age the questionnaire included number of cigarettes smoked, hours of easy and vigorous physical activity per week and alcohol consumption. A physical activity score was outlined by adding together the hours of easy and vigorous physical activity, giving the number of hours of vigorous activity double weight. [18]. The score had a range from 3 – 12 and was normally distributed. Female respondents were also asked to record whether they still menstruated and if not, the age at menopause was recorded.

Blood Samples

Between 0800 h and 1600 h, blood was drawn by venipuncture on a non-fasting basis. Sex hormones were analysed on Immulite 2000 (Diagnostic Products Corp.Los

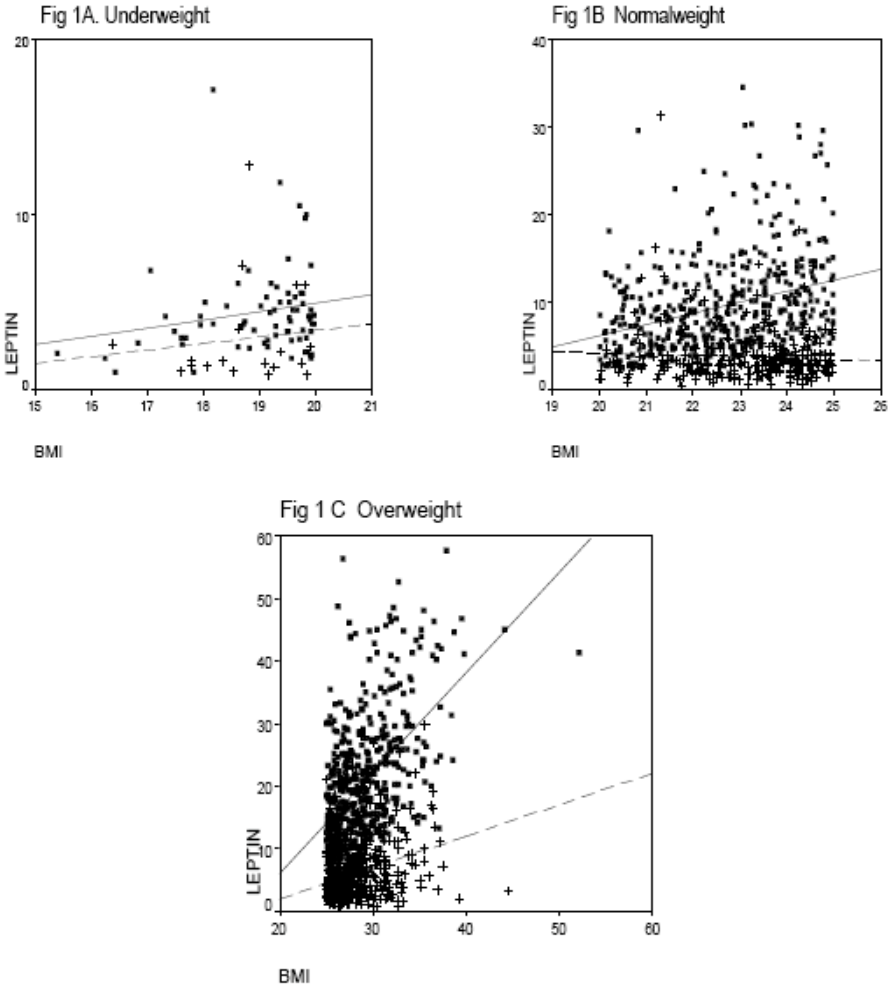

Fig. **(1)**. Scatterplot of leptin *versus* BMI for underweight, normal weight and overweight women (□) and men (+). Separate regression lines for prediction of Leptin by BMI for women (—) and men (----) are added.

Angeles, CA, USA). Intra and inter assay variation coefficients based on assays of pooled human sera run in parallel each day, were in general low [18]. Leptin was analysed by a commercially available radio- immunoassay kit as described in a previous report [19]. Serum lipids were analysed as described previously [20].

Physical Examination

Height and weight were measured in light clothing without shoes. BMI was calculated as weight in kilograms divided by the square of the height in meter (kg/m^2). Waist circumference was measured at the umbilical line, hip – girth at the widest circumference, and waist/hip – ratio was calculated.

Statistical Analyses

All data were analysed in SPSS statistical package for Windows, version 11.0 (SPSS Inc. Chicago, IL, USA). Descriptive analysis of all the key variables in men and women are shown in Table **2**. For regression analysis, missing values were substituted by means of EM algorithm in SPSS. In general, number of missing variables was about 5%. 20% of the answers were missing in the questions about alcohol consumption. All continuous variables were checked for distribution, and logarithmically transformed if skewness was detected. Using logleptin as the dependent variable, all variables previously shown to influence leptin levels, were added as explanatory variables in a multiple linear regression analysis. In some cases, gender specific analyses were made, in other cases, separate analyses according to subgroups of the sample were used.

The study was approved by the Regional Committee of Research Ethics and the Norwegian Data Inspectorate and performed according to the Declaration of Helsinki.

RESULTS

Gender Differences in Serum Leptin Level

In Table **2**, the gender difference in serum leptin level is shown. As expected, the leptin levels were significantly higher in women than in men. Moreover, gender differences in underweight, normal weight and overweight individuals were examined (Fig. **1 A, B** and **C**). Fig. (**1**) shows that gender differences in serum leptin level were small in underweight (BMI<20) and normal weight individuals (BMI 20 – 25), but increasing with increasing overweight (BMI >25). For all groups, the difference in mean value of leptin between men and women was statistically significant in all weight groups (data not shown). It should be noted that serum leptin increased with increasing BMI in all groups except for normal weight men.

As men and women differ in several life style characteristics and blood lipid pattern (Table **2**), these characteristics may explain some of the gender differences in leptin values. All these variables were entered simultaneously in the multiple regression analysis. Table **3** shows that gender differences persist when lifestyle, BMI, body shape and blood lipid pattern were controlled for. However, BMI, physical activity level and smoking also entered the equation, but age, alcohol drinking and serum lipid pattern did not. The model explained 57.9 % of the total variance in leptin. The results were identical when the variables were entered in a forward stepwise regression procedure. Gender

Table 2. Gender Differences in Key Variables in the Sample Selected for Leptin Analyses. Except for Smoking, Mean Values and SD are given

	Men N= 746	All women N= 1066	Premenopausal women N= 253	Postmenopausal women N=772	P- value* (F- value)
Leptin (nmol/l) (N = 1812)	5.2 ± 4.2	14.6 ± 9.9	11.3 ± 7.5	15.8 ± 10.4	< 0.000 (F = 600.0)
BMI (kg/m^2) (N =1811)	26.2 ± 3.5	25.5 ± 4.3	24.1 ± 3.9	26.0 ± 4.3	0.001 (F = 11.5)
Waist/hip ratio (N = 1671)	0.92 ± 0.06	0.82 ± 0.07	0.78 ± 0.05	0.83 ± 0.07	< 0.000 (F = 1118.7)
Percent smoking on a daily basis (N = 1803)	33.9%	33.1%	40.3%	30.6%	NS
Physic activity score (N = 1789)	6.75 ± 2.48	6.19 ± 2.36	7.31± 2.57	5.80 ± 2.15	< 0.000 (F = 22.9)
Number of times drinking alcohol per month (N = 1471)	3.7± 5.0	2.3 ± 3.4	2.6 ± 2.7	2.1 ± 3.7	< 0.000 (F = 44.6)
Serum cholesterol (mmol/l) (N = 1810)	6.49 ± 1.16	6.67 ± 1.43	5.50 ± 1.04	7.10 ± 1.31	0.007 (F = 7.21)
Serum triglycerides (mmol/l) (N = 1810)	1.78 ± 1.07	1.51 ± 0.93	1.11 ± 0.63	1.66 ± 0.97	< 0.000 (F = 32.1)
Serum HDL – cholesterol (nmol/l) (N = 1810)	1.39 ± 0.36	1.67 ± 0.42	1.67 ± 0.37	1.67 ± 0.44	<0.000 (F = 216.5)
SHBG (nmol/l) (N = 1729)	49.8 ± 24.0	76.6 ± 35.0	78.7 ± 39.5	75.8 ± 33.0	<0.000(F = 332.1)
FSH (IU/liter) (N = 1728)	9.7 ± 10.2	51.2 ± 35.2	11.5 ± 16.1	66.2 ± 28.0	< 0.000 (F = 971.2)
LH (IU/liter) (N =1729)	5.4 ± 3.9	21.6 ± 13.9	10.8 ± 12.9	25.7 ± 12.1	<0.000 (F = 953.8)
Estrogen (pmol/l) (N = 1728)	0.05 ± 0.03	0.15 ± 0.52	0.41 ± 1.00	0.05 ± 0.08	<0.000 (F = 24.3)
DHEA (µmol/l) (N = 1729)	3.5± 2.1	2.3 ± 1.6	3.7 ± 1.8	1.8 ± 1.1	< 0.000 (F = 195.6)
Testosterone (nmol/l) (N = 1705)	14.1 ± 6.1	0.63 ± 0.89	0.89 ± 0.85	0.53 ± 0.89	< 0.000 (F = 5177.5)

*Men compared with all women. ANOVA. NS = non significant.

alone predicted 33 % of the variance. Adding BMI to the model increased the explained variation to be 55.8 %. The rest of the predicting variables contributed to 2 % of the variation in serum leptin level.

Table 3. **Determinants of Serum Leptin Level in a General Population Sample by Multiple Linear Regression and Logleptin as the Dependent Variable (N= 1812)**

Independent variables	B	T-value	P-value
Gender (men =1, women =2)	0.49	28.8	< 0.000
Age	0.00	-0.37	NS
BMI	0.042	22.95	< 0.000
Waist/hip ratio	0.15	1.25	NS
Physical activity	- 0.11	- 4.17	< 0.000
Smoking on a daily basis (no=1, yes = 2)	- 0.05	- 3.40	= 0.001
Number of times drinking alcohol per month	0.02	1.88	NS
Cholesterol (mmol/l)	0.006	1.11	NS
Triglycerides (mmol/l)	0.02	1.24	NS
HDL – cholesterol (mmol/l)	0.02	0.83	NS

For analysis of sex hormone contribution, separate linear multiple regression analysis for each gender was performed, due to the large differences in gender hormones in the two sexes. Due to the large number of respondents, subgroups could be analysed according to BMI.

As for analysis in men, all lifestyle variables, BMI, WHR, age, blood lipids and sex hormones were entered in the regression. Serum estrogen was at an undetectable level for a large percent of the men, and could not be entered into the analyses. Separate analyses were conducted for normal weight and overweight men (Table **4**). In addition to BMI and testosterone, SHBG predicted leptin for overweight men, but the effect of SHBG was only found for those over 60 years (data not shown). None of the variables entered in the analyses predicted serum leptin level in normal weight men older than 60 years. BMI started to predict leptin in men over 65 years with BMI values > 27 kg/m^2 (Data not shown). In general, the model could predict only a small percent of the variance in serum leptin level. Using a stepwise regression procedure including all men, the contribution of testosterone in predicting leptin was only about 1 %.

Using total level of sex hormones as predictor variable, it is possible that biologically active free hormone fraction could produce a different result. To check this possibility, free testosterone level was calculated [18] and entered as predictor variable. The results were comparable with the results using total testosterone. However, DHEA predicted leptin level in addition to BMI and free testosterone, but the contribution of DHEA to explain variance was marginal (0.8 %).

Significant predictors for women are shown in Table **5**. In addition to subgroups by weight, subgroups according to menstrual status were created. Due to low number of individuals in the premenopausal overweight group (N=90), only variables which predicted leptin with a p value < 0.1 in all women, were included in this analysis. The percentages of variance in leptin level explained by the models were substantially higher compared with that in men. Different subgroups according to body weight and menstrual status, showed a different pattern with respect to which factors that

Table 4. **Prediction of Serum Leptin Level in Men from Tromsø IV (N= 746)**

	All men N= 746			Normal weight N= 262			Overweight N=463		
Adjusted R square	**16.8 %**			**4.8 %**			**11.1 %**		
Variables	**B**	**T**	**P**	**B**	**T**	**P**	**B**	**T**	**P**
Age	0.0002	0.18	NS	0.002	0.864	NS	-0.003	-1.5	NS
BMI	0.03	6.5	<0.000	0.0001	0.009	NS	0.02	3.5	<0.0000
Waisthip - ratio	0.33	1.36	NS	0.249	0.6	NS	0.5	1.60	NS
Physical activity	- 0.008	-1.8	0.08	-0.007	-0.95	NS	-0.008	-1.4	NS
Smoking on a daily basis	-0.002	-0.07	NS	-0.02	-0.5	NS	-0.02	-0.64	NS
Alcohol* (times drinking /month)	-0.000006	0.000	NS	-0.06	-2.7	0.009	0.02	1.2	NS
Cholesterol (mmol/l)	0.007	0.72	NS	0.03	1.5	NS	-0.006	-0.52	NS
HDL – cholesterol (nmol/l)	0.06	1.77	0.07	0.09	1.7	0.08	0.05	1.1	NS
Triglyceride* (mmol/l)	0.05	1.87	0.06	0.06	1.5	NS	0.04	1.4	NS
SHBG*(nmol/l)	0.03	0.784	NS	-0.08	-1.4	NS	0.09	2.2	0.025
LH*(IU/l)	- 0.001	-0.05	NS	-0.05	-1.1	NS	0.04	0.915	NS
FSH*(IU/l)	-0.04	-1.52	NS	-0.03	-0.88	NS	-0.05	-1.6	NS
DHEA (µmol/l)	-0.02	-0.73	NS	-0.02	-0.65	NS	-0.03	-0.79	NS
Testosterone* (nmol/l)	-0.09	-3.2	0.001	0.06	1.2	NS	-0.15	-4.5	< 0.000

*ln transformed due to scewness

Table 5. Prediction of Serum Leptin Level in Women from Tromsø IV (N= 1066)

	All women N = 1066			Premenopausal Normalweight N = 176			Premenopausal Overweight** N = 92			Postmenopausal Normalweight N = 282			Postmenopausal Overweight N = 441		
Adjusted R square	**53.2 %**			**12.3 %**			**21.9 %**			**21.7 %**			**32.6 %**		
Variables	**B**	**T**	**P**	**B**	**T**	**P**	**B**	**T**	**P**	**B**	**T**	**P**	**B**	**T**	**P**
Age	0.000	- 0.38	NS	-0.007	-2.4	0.02	0.001	0.24	NS	-0.007	-2.8	0.005	0.005	2.7	0.007
BMI	0.05	23.4	<0.000	0.03	2.8	0.005	0.03	4.22	<0.000	0.05	4.85	<0.000	0.03	10.4	<0.000
Waist-hip- ratio*	0.338	1.4	NS	1.1	1.4	NS				0.54	1.2	NS	-0.24	-0.73	NS
Physical activity	- 0.01	- 4.43	<0.000	-0.02	-2.4	0.02	-0.001	-0.09	NS	-0.026	-4.02	<0.000	-0.01	-2.88	0.004
Alcohol* (times drinking pr. month)	0.03	2.81	0.005	0.01	0.5	NS	0.006	0.21	NS	0.02	0.72	NS	0.03	2.18	0.03
Smoking	- 0.06	- 4.18	<0.000	-0.04	-1.1	NS	- 0.07	-1.43	NS	-0.088	-2.96	0.003	-0.02	0.74	NS
Cholesterol (mmol/l)	0.01	1.9	0.06	0.04	2.1	0.04	0.01	0.47	NS	-0.006	-0.54	NS	0.007	0.84	NS
HDL – cholesterol (nmol/l)	- 0.002	- 0.11	NS	-0.01	-0.3	NS				- 0.02	-0.77	NS	0.06	2.11	0.04
Triglycerides* (mmol/l)	0.000	- 0.01	NS	0.009	0.22	NS				0.04	1.1	NS	-0.04	-1.83	0.07
SHBG (nmol/l)	- 0.001	- 5.63	<0.000	-0.001	-1.5	NS	<0.000	-0.822	NS	-0.001	-1.73	0.08	-0.02	-6.51	<0.000
Testosterone* (nmol/l)	-0.05	- 2.41	0.016	-0.03	-0.6	NS	- 0.02	-0.42	NS	- 0.05	- 1.19	NS	-0.04	-1.29	NS
DHEA* (µmol/l)	-0.006	- 0.26	NS	0.03	0.48	NS				0.04	0.869	NS	-0.02	-0.69	NS
Estrogen* (pmol/l)	0.06	3.5	<0.000	0.04	1.0	NS	0.05	1.48	NS	0.06	1.55	NS	-0.02	-2.20	0.03
LH (IU/l)	<0.0001	-0.005	0.996	0.003	0.1	0.92				- 0.03	- 0.81	NS	-0.001	-0.05	NS
FSH (IU/l)	0.000	0.89	0.372	<0.000	0.21	0.835				0.001	1.5	NS	-0.001	-1.1	NS

* Ln transformed due to scewness. ** a limited number of independent variables was tested due to low number of individuals.

predicted leptin level. While BMI was a strong predictor in all groups, the life style variables, sex hormones and blood lipid pattern predicted leptin in different subgroups of the women.

Some of the women currently used HRT. Therefore, the analyses were repeated, resulting in slightly different results (data not shown). For all premenopausal women, estrogen predicted leptin (B = 0.07, t = 2.0, p=0.03), and the variance in leptin level explained by the model increased to 46.6%.

As testosterone predicted leptin in a different pattern in overweight men and women, the relationship between testosterone and BMI was explored by plotting testosterone *versus* BMI for both genders (Fig. **2**). Increasing BMI was associated with decreased testosterone in men, but not in women. In the subgroups of normal and overweight men, there were still an inverse relation between testosterone and BMI. In women, no relationship between testosterone and BMI in normal weight subjects was found. In overweight subjects the relationship turned to be positive (data not shown).

DISCUSSION

This study has shown that in the whole general population sample, BMI and gender were strong independent predictors of serum leptin. Serum leptin gender differences increased with increasing BMI. On the other hand, the effect was modest with respect to physical activity and smoking, whereas alcohol and age did not at all predict the variance in serum leptin. Neither life style factors nor serum lipid pattern

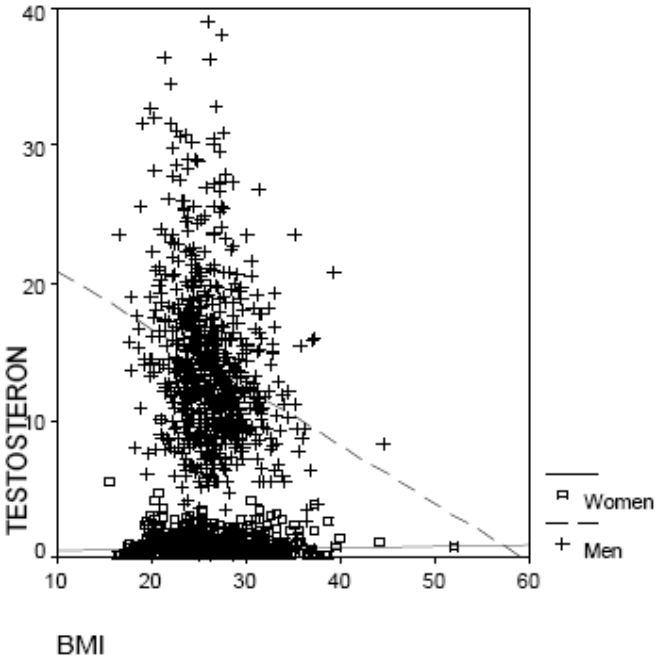

Fig. (2). Scatterplot of serum testosterone level *versus* BMI in women (□) and men (+). Separate regression lines for prediction of leptin by BMI for women (—) and men (----) are added.

could explain the gender difference. In general, the contributions of sex hormones were only marginal when corrected for life-style variables and serum lipid pattern. A much larger percent of the variance in serum leptin level could be explained in women compared to men, and in overweight individuals compared to normal weight individuals.

An important limitation of this study is that the cross-sectional design will only provide associations between variables, not casual relationships. The sample size to some extent represents a limitation, as it was impossible to detail specific areas. The blood samples were non-fasting, but it is generally accepted that the effect of individual meals on serum leptin level is small [19]. More time consuming measures of body fat distribution would have been useful. On the other hand, the fact that the study was a multipurpose study represents a strength, as a large amount of variables are collected simultaneously at different levels, such as life-style variables, blood samples and physical examination. Moreover, the sample size allows for simultaneous study of many variables, both in the total sample as well as in subgroups.

It is generally accepted that BMI as a global indicator of body fat, is a predictor of serum leptin [6, 21]. This is also confirmed by our study. In our model, BMI alone explained 49.2 % of the variances of serum leptin in all women, but as low as 14.7 % in all men. In other population studies performing more exact measures of body fat, the models may explain the variance in serum leptin up to 70 % [22].

When controlled for all other variables, smoking was an independent negative prediction factor of serum leptin in the total population sample, also among postmenopausal women. There are no general agreement on the relationship between serum leptin and smoking [12, 13].

Moreover, physical activity was an independent negative predictive factor of serum leptin in the whole population sample. When adjusted for BMI, other studies have also shown a negative relationship between serum leptin and physical activity, as well as moderate intensity exercise training in women/girls, but not in men/boys [23, 24]. The explanation of this gender difference is unknown, but if testosterone is included in the total model for both genders in our material, the effect of activity is unchanged. This indicates that variation in testosterone level can not explain the gender differences.

The serum concentrations of cholesterol, HDL-cholesterol and triglyceride, were not predictive factors of serum leptin neither in the whole group nor in the subgroups. An exception was for postmenopausal women where HDL-cholesterol at statistically significance level. This finding is in accordance with a previous report [25]. In some studies, [15] and especially in children [26], independent predictive factor of the serum lipids on the serum leptin have been reported. The findings in our study are to some extent unexpected. Leptin has been proposed to have an important role in multiple metabolic pathways (for review, see [2]). Moreover, in experimental models leptin administration induces changes in blood lipids [27].

Differences in lifestyle could not explain the gender difference in serum leptin level reported by us. This finding is in agreement with other reports [8, 28]. In our study the global fat measure BMI was associated with serum leptin in all weight groups of women and overweight men, but not in normal weight men. The expression of mRNA leptin is higher in subcutaneous fat than in visceral adipose fat [29], and females have a significant greater proportion of their fat mass in the subcutaneous fat depots [30]. The highly reduced contribution of BMI to predict serum leptin in men compared to female observed in our study, can be attributed to lower amount of subcutaneous fat in men. When using more specific measures of subcutaneous fat, such as sum of 4 skin fold test, these measures can explain around 70 % of the variations of serum leptin both in women and men [22]. However, there are still controversial whether gender dimorphism in serum leptin is accounted for by differences in adipose tissue distribution [8, 14].

In agreement with the majority of previous reports (for review see [16, 31, 32]), we found that testosterone levels were inversely associated with leptin levels in men. In addition a negative association between testosterone and leptin was also observed in women. In one report [33] this inverse association was observed in normal-weight women, but changed to a positive association in obese women. The discrepancy is hard to explain.

It is well established that testosterone levels are reduced in obese men and especially in men with abdominal obesity [34]. This was also confirmed in our data, as increased BMI was associated with a substantial reduction in testosterone in men. In women, however, an association between testosterone and BMI was only found in overweight women. Previous studies in women have indicated that abdominal obesity was positively associated with testosterone [35], whereas in a cross sectional study [36], the association was only found in postmenopausal women. In our study, the larger number of participants from a general population might explain these diverging results. Androgens are known to play an important role in normal fat distribution [37]. Lower testosterone levels have been reported to predict visceral obesity [38], and replacement doses of testosterone decreases abdominal fat mass in men with low testosterone levels [39]. It is likely that testosterone plays a causal role in visceral fat accumulation. Therefore, it is interesting to note that subcutaneous fat, but not visceral fat, predicts serum leptin [40, 41], and that there is a greater secretion of leptin in the former fat depots [42]. Thus, it is likely that the gender dimorphism in testosterone in subjects with increasing BMI, may reflect a greater deviation of fat accumulation towards abdominal fat rather than subcutaneous fat in men, compared with women. The same differentiation in fat depots between (obese) men and women could also explain the gender dimorphism of serum leptin with low and high leptin secreting adipocytes. Unfortunately our study was not designed to differentiate between fat deposits. Whether the gender dimorphisms in both leptin and testosteron secretion reflect negative opposing effects between these to hormones, is an interesting hypothesis that needs [43, 44] further investigation.

In women, estrogen was positively associated with leptin in agreement with previous reports [45, 46], while no association was found between leptin and DHEAS in either gender. The relative contributions of sex hormones were only marginal, and may explain only 1% of the variance in serum leptin level for both genders.

It is well established that high BMI and obesity are associated with metabolic disturbances such as Type 2 diabetes, lipid disturbances and cardiovascular disease. Our and previous reports indicating BMI as the main predictor of leptin, may indicate that leptin is a potential mediator of these metabolic disturbances. So far there are no evidence of this causal relationship. Our study was a cross-sectional study without possibility to study potential causal link of leptin, but in the Tromsø 6 study just finished we are able to compare leptin levels in a prospective design and the correlations to the development of metabolic disturbances.

CONCLUSIONS

The present results confirm previous results, that BMI as a global measure of fat explains only some of the variations in serum leptin. The prediction is especially low in men. Gender differences increase with increasing BMI, and differences in life style parameters are independent, but weak predictors of serum leptin. The gender dimorphism is observed between serum leptin and serum testosterone, and between testosterone and BMI, suggesting a complex relationships between body fat distribution and the two hormones.

ACKNOWLEDGEMENTS

We are grateful to Ingrid Christiansen and Odd Sverre Moen at the Department of Gastroenterology, University of Tromsø, Tromsø, Norway for technical assistance.

SOURCES OF SUPPORT

The project was supported by grant from the non-profit organization - Norwegian Foundation for Health and Rehabilitation. Grant number 2000/2/0009.

ABBREVIATIONS

BMI = Body mass index

DHEA = Dehydroepiandrosterone

FSH = Follicle stimulating hormone

HDL = High density lipoprotein

IU = international units

LH = Luteinizing hormone

SD = Standard deviation

SHBG = Sex hormone-binding globulin

REFERENCES

[1] Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. Nature 1994; 372: 425-32.

[2] Auwerx J, Staels B. Leptin. Lancet 1998; 351: 737-42.

[3] Sandoval DA, Davis SN. Leptin: metabolic control and regulation. J Diabetes Complications 2003; 17: 108-13.

[4] Sahu A. Minireview: A hypothalamic role in energy balance with special emphasis on leptin. Endocrinology 2004; 145: 2613-20.

[5] Bates SH, Myers MG. The role of leptin receptor signaling in feeding and neuroendocrine function. Trends Endocrinol Metab 2003; 14: 447-52.

[6] Considine RV, Sinha MK, Heiman ML, *et al.* Serum immunoreactive-leptin concentrations in normal-weight and obese humans. N Engl J Med 1996; 334: 292-5.

[7] Lissner L, Karlsson C, Lindroos AK, *et al.* Birth weight, adulthood BMI, and subsequent weight gain in relation to leptin levels in Swedish women. Obes Res 1999; 7: 150-4.

[8] Rosenbaum M, Pietrobelli A, Vasselli JR, Heymsfield SB, Leibel R. Sexual dimorphism in circulating leptin concentrations is not accounted for by differences in adipose tissue distribution. Int J Obes Relat Metab Disord 2001; 25: 1365-71.

[9] Baumgartner RN, Waters DL, Morley JE, Patrick P, Montoya GD, Garry PJ. Age-related changes in sex hormones affect the sex difference in serum leptin independently of changes in body fat. Metabolism 1999; 48: 378-84.

[10] Roemmich JN, Clark PA, Berr SS, *et al.* Gender differences in leptin levels during puberty are related to the subcutaneous fat depot and sex steroids. Am J Physiol 1998; 275: E543-551.

[11] Kraemer RR, Chu H, Castracane VD. Leptin and exercise. Exp Biol Med 2002; 227: 701-8.

[12] Li MD, Parker SL, Kane JK. Regulation of feeding-associated peptides and receptors by nicotine. Mol Neurobiol 2000; 22: 143-65.

[13] Perkins KA, Fonte C. Effects of smoking status and smoking cessation on leptin levels. Effects of smoking status and smoking cessation on leptin levels. Nicotine Tob Res 2002; 4: 459-66.

[14] Ruhl CE, Everhart JE, Ding J, *et al.* Aging, and Body Composition Study. Am J Clin Nutr 2004; 80: 576-83.

[15] Njelekela M, Kuga S, Hiraoka J, *et al.* Determinants of hyperleptinaemia in an African population. East Afr Med J 2003; 80: 195-9.

[16] Luukkaa V, Pesonen U, Huhtaniemi I, *et al.* Inverse correlation between serum testosterone and leptin in men. J Clin Endocrinol Metab 1998; 83: 3243-6.

[17] Rosvold Berntsen GK, Fonnebo V, Tollan A, Sogaard AJ, Joakimsen RM, Magnus JH. The Tromsø study: determinants of precision in bone densiometry. J Clin Epidemiol 1999; 53(11): 1104-12.

[18] Svartberg J, Midtby M, Bønaa KH, Sundsfjord J, Joakimsen RM, Jorde R. The associations of age, lifestyle factors and chronic disease with testosterone in men. The Tromsø Study. Eur J Endocrinol 2003; 149: 145-52.

[19] Birketvedt GS, Florholmen J, Sundsfjord J, Osterud B, Dinges D, Bilker W, Stunkard A. Behavioral and neuroendocrine characteristics of the night-eating syndrome. JAMA 1999; 282: 657-63.

[20] Wilsgaard T, Jacobsen BK, Schirmer H, *et al.* Tracking of Cardiovascular Risk Factors. The Tromsø Study, 1979 – 1995. Am J Epidemiol 2001; 154 (5): 418-26.

[21] Sumner AE, Falkner B, Kushner H, Considine RV. Relationship of leptin concentration to gender, menopause, age, diabetes, and fat mass in African Americans. Obes Res 1998; 6: 128-33.

[22] Ruhl CE, Everhart JE. Leptin concentrations in the United States: relations with demographic and anthropometric measures. Am J Clin Nutr 2001; 74: 295-301.

[23] Romon M, Lafay L, Bresson JL, *et al.* Relationships between physical activity and plasma leptin levels in healthy children: the Fleurbaix-Laventie Ville Sante II Study. Int J Obes Relat Metab Disord 2004; 28(10): 1227-32.

[24] Perusse L, Collier G, Gagnon J, *et al.* Acute and chronic effects of exercise on leptin levels in humans. J Appl Physiol 1997; 83: 5-10.

[25] Ostlund RE Jr, Yang JW, Klein S, Gingerich R. Relation between plasma leptin concentration and body fat, gender, diet, age, and metabolic covariates. J Clin Endocrinol Metab 1996; 81: 3909-13.

[26] Kavazarakis E, Moustaki M, Gourgiotis D, *et al.* Relation of serum leptin levels to lipid profile in healthy children. Metabolism 2001; 50(9): 1091-4.

[27] Shimabukuro M, Koyama K, Chen G, *et al.* Direct antidiabetic effect of leptin through triglyceride depletion of tissues. Proc Natl Acad Sci USA 1997; 94: 4637-41.

[28] Havel PJ, Kasim-Karakas S, Dubuc GR, Mueller W, Phinney SD. Gender differences in plasma leptin concentrations. Nat Med 1996; 2: 949-50.

[29] Montague CT, Prins JB, Sanders L, Digby JE, O'Rahilly S. Depot- and sex-specific differences in human leptin mRNA expression:

implications for the control of regional fat distribution. Diabetes 1997; 46: 342-7.

[30] Leenen R, Kooy Kvd, Deurenberg P, *et al.* Visceral fat accumulation in obese subjects: relation to energy expenditure and response to weight loss. Am J Physiol 1992; 263: E913-919.

[31] Mann DR, Johnson AO, Gimpel T, Castracane VD. Changes in circulating leptin, leptin receptor, and gonadal hormones from infancy until advanced age in humans. J Clin Endocrinol Metab 2003; 88: 3339-45.

[32] Mayes JS, Watson GH. Direct effects of sex steroid hormones on adipose tissues and obesity. Obes Rev 2004; 5: 197-216.

[33] Soderberg S, Olsson T, Eliasson M, *et al.* A strong association between biologically active testosterone and leptin in non-obese men and women is lost with increasing (central) adiposity. Int J Obes Relat Metab Disord 2001; 25: 98-105.

[34] Svartberg J, von Mühlen D, Sundsfjord J, Jorde R. Waist circumference and testosterone levels in community dwelling men. The Tromsø Study. Eur J Epidemiol 2004; 19: 657-63.

[35] Garaulet M, Perex-Llamas F, Fuente T, Zamora S, Tebar FJ. Anthropometric, computed tomography and fat cell data in an obese population: relationship with insulin, leptin, tumor necrosis factor-alpha, sex hormone-binding globulin and sex hormones. Eur J Endocrinol 2000; 43: 657-66.

[36] Lukanova A, Lundin E, Zeleniuch-Jacquotte A, *et al.* Body mass index, circulating levels of sex-steroid hormones, IGF-I and IGF-binding protein-3: a cross-sectional study in healthy women. Eur J Endocrinol 2004; 150: 161-71.

[37] Björntorp P. Male fat distribution and cardiovascular risk. Blood Press Suppl 1992; 4: 17-9.

[38] Khaw KT, Barrett-Connor E. Lower endogenous androgens predict central adiposity in men. Ann Epidemiol 1992; 2: 675-82.

[39] Rebuffe-Scrive M, Mårin P, Björntorp P. Effect of testosterone on abdominal adipose tissue in men. Int J Obes 1991; 15: 791-5.

[40] Dua A, Hennes MI, Hoffmann RG, *et al.* Leptin: a significant indicator of total body fat but not of visceral fat and insulin insensitivity in African-American women. Diabetes 1996; 45: 1635-7.

[41] Langendonk JG, Pijl H, Toornvliet AC, *et al.* Circadian rhythm of plasma leptin levels in upper and lower body obese women: influence of body fat distribution and weight loss. J Clin Endocrinol Metab 1998; 83: 1706-12.

[42] Montague CT, Prins JB, Sanders L, *et al.* Depot-related gene expression in human subcutaneous and omental adipocytes. Diabetes 1998; 47: 1384-91.

[43] Castrogiovanni D, Perello M, Gaillard RC, Spinedi E. Modulatory role of testosterone in plasma leptin turnover in rats. Endocrine 2003; 22: 203-10.

[44] Kaukua J, Pekkarinen T, Sane T, Mustajoki P. Sex hormones and sexual function in obese men losing weight. Obes Res 2003; 11: 689-94.

[45] Isidori AM, Strollo F, More M, *et al.* Leptin and aging: correlation with endocrine changes in male and female healthy adult populations of different body weights. J Clin Endocrinol Metab 2000; 85: 1954-62.

[46] Shimizu H, Shimomura Y, Nakanishi Y, *et al.* Estrogen increases *in vivo* leptin production in rats and human subjects. J Endocrinol 1997; 154: 285-92.

Received: December 7, 2010 Revised: February 15, 2011 Accepted: February 28, 2011