METABOLISM CLINICAL AND EXPERIMENTAL 60 (2011) 987–993

available at www.sciencedirect.com

ScienceDirect

www.metabolismjournal.com

Soluble leptin receptor and leptin are associated with baseline adiposity and metabolic risk factors, and predict adiposity, metabolic syndrome, and glucose levels at 2-year follow-up: the Cyprus Metabolism Prospective Cohort Study

Ole-Petter R. Hamnvik[a,*], *Xiaowen Liu*[b], *Michael Petrou*[c], *Huizhi Gong*[b], *John P. Chamberland*[b], *Esther H. Kim*[b], *Costas A. Christophi*[c,d], *Stefanos N. Kales*[d], *David C. Christiani*[d], *Christos S. Mantzoros*[d,e]

[a] *Division of Endocrinology, Diabetes, and Hypertension, Department of Medicine, Brigham and Women's Hospital, and Harvard Medical School, Boston, MA, USA*

[b] *Division of Endocrinology, Diabetes, and Metabolism, Department of Medicine, Beth Israel Deaconess Medical Center, and Harvard Medical School, Boston, MA, USA*

[c] *Cyprus International Institute for Environmental and Public Health in association with Harvard School of Public Health, Cyprus University of Technology, Limassol, Cyprus*

[d] *Department of Environmental Health, Harvard School of Public Health, Boston, MA, USA*

[e] *Section of Endocrinology, Boston VA Healthcare System, Boston, MA 02130, USA*

ARTICLE INFO

Article history:
Received 20 August 2010
Accepted 20 September 2010

ABSTRACT

We examined the relationship between serum levels of leptin-binding protein (soluble leptin receptor [sOB-R]) and leptin with metabolic parameters at baseline and prospectively at 2-year follow-up in young healthy men. A total of 916 eighteen-year-old men were examined at baseline, with a subgroup of 91 participants examined again 2 years later. Anthropometric and metabolic measurements were performed at baseline and at follow-up. In the cross-sectional study, levels of sOB-R were significantly inversely correlated with all baseline measures of obesity and metabolic risk factors (blood pressure, total and low-density lipoprotein cholesterol, and fasting glucose), and significantly positively correlated with high-density lipoprotein cholesterol. After correcting for age, smoking status, and waist-to-hip ratio, the inverse correlation remained statistically significant for all measures of adiposity, fasting glucose, and the metabolic syndrome score. Correlations for leptin were similar in magnitude but opposite in direction to correlations for sOB-R. In prospective analyses, baseline levels of sOB-R were predictive at 2-year follow-up of fasting glucose, the metabolic syndrome score, and measures of adiposity in both unadjusted and adjusted models. Similarly, leptin was predictive of fasting glucose, the metabolic syndrome score, adiposity, and systolic blood pressure. We confirm correlations of leptin and sOB-R levels

Disclosure statement: The authors have no relevant conflict of interests.

CSM derived the hypothesis and conceived the study design; MP, CAC, SNK, and DCC planned and organized the collection of the data; XL, HG, and LPC performed the laboratory analyses; OPRH, XL, EHK, and CSM collated the data and planned and did the statistical analyses, OPRH, XL, MP, EHK, CAC, SNK, DCC, and CSM contributed to the interpretation and discussion of results; OPRH wrote the manuscript. This report was critically reviewed and subsequently approved by all authors.

* *Corresponding author*. Division of Endocrinology, Diabetes, and Hypertension, Brigham and Women's Hospital, Boston, MA 02215, USA. Tel.: +1 617 732 5666; fax: +1 617 732 5469.

E-mail address: ohamnvik@partners.org (O.-P.R. Hamnvik).

0026-0495/$ – see front matter
doi:10.1016/j.metabol.2010.09.009

with measures of adiposity and metabolic risk factors at baseline, and demonstrate for the first time prospectively the role of sOB-R as an independent, although weak, predictor of metabolic syndrome and fasting glucose in young men.

1. Introduction

Leptin is the prototypical adipose tissue–secreted hormone [1], and its levels are directly associated with the percentage of body fat [2]. Leptin exerts its effects by binding to several isoforms of the leptin receptor, a class I cytokine receptor widely distributed in peripheral organs as well as the central nervous system [3]. Activation of the leptin receptor stimulates multiple signaling pathways that modulate neuroendocrine functions, body weight, and metabolic processes [3,4].

Circulating leptin binds to a leptin-carrying protein in plasma, which circulates in relatively higher levels (and thus results in a higher proportion of bound leptin) in lean subjects than in obese subjects [5,6]. The major leptin-binding protein in plasma has been identified as the extracellular cleaved part of the leptin receptor, that is, the soluble leptin receptor (sOB-R) [7]. This binding protein may act as regulator of leptin's physiologic effects by influencing leptin's interactions at its cell-bound receptor or by modulating leptin's half-life [8]. A range of prior cross-sectional studies suggest that levels of sOB-R also appear to be an indicator of leptin activity as well as a predictor of diabetes among older women [9].

These studies have found that sOB-R levels are higher in healthy young men than in women, and are inversely related to body mass index (BMI), adiposity, and waist circumference [8]. Levels of sOB-R have also been found to be inversely correlated with other components of the metabolic syndrome, such as systolic/diastolic blood pressures, fasting insulin/glucose, and triglycerides, while it is positively associated with age, total cholesterol, and high-density lipoprotein (HDL) cholesterol [10,11]. Weight loss, low-energy diets, and regular exercise, which decrease circulating leptin levels, are also associated with higher sOB-R levels [12,13]. However, to date, most studies examining the associations between sOB-R and anthropometric and metabolic measurements have been relatively small.

Only one published study has evaluated sOB-R prospectively as a predictor of the development of type 2 diabetes mellitus. This study, a case-control study nested in the prospective Nurses' Health Study, found that higher levels of sOB-R, but not leptin, were associated with a lower risk of developing type 2 diabetes mellitus in women, even after correcting for BMI [9]. So far, no prospective data are available in men and no study has yet assessed the role of sOB-R as a predictor of developing other metabolic risk factors in the future. To assess whether sOB-R and leptin are useful markers of risk for developing metabolic abnormalities, we studied a cohort of 18-year-old men in Cyprus.

2. Methods

The study has received approval from both the Harvard School of Public Health Institutional Review Board and the Cyprus National Bioethics Committee.

2.1. Cross-sectional cohort

The study population consists of 18-year-old men who were screened and enrolled in the Cyprus Metabolism Study at the start of their 2-year mandatory service in the Cypriot Army.

All recruits who were inducted into the army the previous day and who were 18 years or older were eligible for enrollment. Exclusion criteria included health reasons prohibiting military service, such as most chronic diseases. The conscripts were briefed on the study and provided informed consents in a group setting. During the first recruitment phase of the study (July 2006), 417 participants were enrolled in the study. An additional 639 participants were enrolled in the second phase of the study (July 2007), yielding a total of 1056 participants.

Of these 1056 participants, a total of 140 were excluded from this report, mostly because of lack of sample for sOB-R assay or incomplete anthropometric measurements. Thus, a total of 916 participants were included in this cross-sectional analysis. Because military service at 18 years of age is obligatory in Cyprus, the study provides a representative sample of the 18-year-old healthy male population of Cyprus, with participants coming from all regions of Cyprus and all socioeconomic levels. They were all at the same educational level, having completed high school.

2.2. Prospective cohort

All of the 417 participants recruited in the first phase of the study in July 2006 were invited for the follow-up study 2 years later. Of these, 115 agreed to participate. The follow-up of participants took place in the Nicosia General Hospital in July 2008. Twenty-three participants were excluded because of lack of a baseline sample for sOB-R assay. One other participant was excluded because no follow-up weight was available. This resulted in 91 participants eligible for inclusion in the prospective analyses.

2.3. Anthropometric, cardiovascular, and metabolic measures

After an overnight fast, several measurements were done to set up a comprehensive database of baseline health information; these included height, weight, body fat composition, resting heart rate, and systolic and diastolic blood pressure. For all participants, measurements were performed using the same equipment and by the same group of technicians trained to perform standardized measurements. Body composition was measured using the Tanita Bioelectrical Impedance Analysis. To ensure reliability, all measurements were done twice, and if the measurements differed by more than a prespecified value, a third measurement was performed and the average was used. Questionnaires were also administered to collect information including smoking status. Blood samples were collected and analyzed at Nicosia General Hospital using routine automated laboratory methods (Olympus

AU2700 Chemistry-Immuno Analyzer, Olympus, Center Valley, PA) for glucose, total cholesterol, HDL cholesterol, low-density lipoprotein (LDL) cholesterol, and triglycerides.

2.4. *Measurement of soluble leptin receptor and leptin*

Samples were frozen and stored as plasma at –80°C until assayed in duplicate. Levels of the soluble leptin receptor were measured using a commercially available enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN), with a sensitivity of 0.057 ng/mL, an intra-assay coefficient of variation of 2.2% to 6.1%, and an inter-assay coefficient of variation of 5.3% to 8.6%. Leptin concentrations were measured by commercially available radioimmunoassay kits (Millipore, Billerica, MA) as previously described [9], with a sensitivity of 0.5 ng/mL, an intra-assay coefficient of variation of 3.4% to 8.3%, and an inter-assay coefficient of 3.0% to 6.2%.

2.5. *Metabolic risk factor score*

A metabolic risk factor score based on the International Diabetes Federation (IDF) definition of metabolic syndrome

Table 1 – Baseline characteristics of participants in the cross-sectional study and the prospective study

Continuous variables	All participants (N = 916)		Follow-up group (n = 91)		*P* for difference (entire cohort vs follow-up group)	Follow-up group (n = 91)		*P* for difference (baseline vs follow-up)
	At baseline		At baseline			At 2-y follow-up		
	Mean	SEM	Mean	SEM		Mean	SEM	
Age at enrollment visit	18.41	0.02	18.22	0.01	<.001			
Height (m)	1.753	0.002	1.746	0.006	.29	1.751	0.007	<.001
Weight (kg)	72.4	0.5	68.8	1.4	<.001	72.9	1.4	<.001
BMI (kg/m^2)	23.5	0.1	22.5	0.4	<.001	23.7	0.4	<.001
Waist circumference (cm)	81.8	0.4	79.1	1	.01	83.8	1.0	<.001
Hip circumference (cm)	96.9	0.3	95.7	.8	0.12	97	0.8	.001
WHR	0.842	0.002	0.826	0.005	<.001	0.862	0.004	<.001
Percentage body fat	14	0.2	12.8	0.6	<.001	15.6	0.6	<.001
Heart rate (beats/min)	70.3	0.3	66.1	0.9	<.001	71.4	1.0	<.001
Systolic blood pressure (mm Hg)	108.7	0.3	106.3	1.2	<.001	111.6	1.0	<.001
Diastolic blood pressure (mm Hg)	65.1	0.3	61.7	0.8	<.001	71.4	0.7	<.001
Mean arterial pressure (mm Hg)	79.7	0.3	76.6	0.8	<.001	84.8	0.8	<.001
Total cholesterol (mg/dL)	156.7	1.1	143.9	2.7	<.001	159.5	2.9	<.001
HDL (mg/dL)	47.6	0.3	47.1	0.9	.53	46	0.9	.09
LDL (mg/dL)	102.9	0.9	101.6	2.4	.57	98.4	2.5	.07
Triglycerides (mg/dL)	61.9	0.9	58.7	2.3	.29	75.8	4.3	<.001
Fasting glucose (mg/dL)	82.9	0.3	78.2	0.7	<.001	86.9	1.0	<.001
Leptin (ng/mL)	2.5	0.1	1.8	0.2	<.001			
Leptin-binding protein (ng/mL)	23.2	0.2	25	0.5	<.001			
Categorical variables	n	Percent	n	Percent		n	Percent	*P*
Smoking status					.27			
Current	546	59.6	29	31.9				
Former	46	5.0	2	2.2				
Never	323	35.3	60	65.9				
Waist circumference >94 cm	129	14.1	7	7.7	.06	12	13.2	.06
WHR >1	2	0.2	0	0	.64	0	0	NA
BMI >25 kg/m^2	270	29.5	17	18.7	.02	22	24.2	.10
Systolic hypertension (>140 mm Hg)	5	0.5	2	2.2	.02	1	1.1	.32
Diastolic hypertension (>90 mm Hg)	1	0.1	0	0	.74	1	1.1	.32
Hypertension (>140/90 mm Hg)	6	0.7	2	2.2	.06	1	1.1	.32
Impaired fasting glucose (>100 mg/dL)	16	1.7	0	0	.18	2	2.2	.16
Total cholesterol >200 mg/dL	80	8.7	2	2.2	.02	7	7.7	.06
HDL <40 mg/dL	164	17.9	14	15.4	.48	22	24.2	.045
LDL >130 mg/dL	135	14.7	12	13.2	.64	9	9.9	.32
Triglycerides >150 mg/dL	11	1.2	1	1.1	.92	6	6.6	.03
Dyslipidemia (any of the above)	279	30.5	26	28.6	.64	34	37.4	.07
Metabolic syndrome (IDF criteria)	11	1.2	0	0	.27	3	3.3	.08
Family history of hypertension	99	10.8	10	11.0	.95			
Family history of myocardial infarction	17	1.9	2	2.2	.80			
Family history of hyperlipidemia	166	18.1	21	23.1	.20			
Family history of stroke	9	1.0	21	23.1	.91			
Family history of diabetes mellitus	42	4.6	5	5.5	.66			

NA indicates not applicable.

was created, where one point was added for the presence of, and one point subtracted for the absence of, each of the following: waist circumference >94 cm (37 in), triglycerides >150 mg/dL, HDL cholesterol <40 mg/dL, blood pressure >130/85 mm Hg, and fasting glucose >100 mg/dL [14].

2.6. Statistical analysis

All statistical analyses were calculated using SPSS version 17.0 (SPSS, Chicago, IL).

Baseline characteristics of the follow-up group were compared to those of the entire cohort using *t* test for continuous variables and Pearson χ^2 test for categorical variables. Characteristics of participants for the prospective follow-up study at baseline and at follow-up were compared using paired-sample *t* test for continuous variables and χ^2 test for qualitative characteristics.

Pearson coefficients were used to evaluate the univariate associations between leptin or sOB-R levels and the anthropometric and metabolic measurements of interest (as continuous variables). In addition, partial correlation analysis was performed for both leptin and sOB-R, controlling for age, smoking status (never, previous, current), and waist-to-hip ratio (WHR). For sOB-R, a final partial correlation analysis, controlling for these parameters as well as baseline leptin, was also performed. The data were also analyzed by quartiles of sOB-R using 1-way analysis of variance.

A multivariate linear regression model was used to estimate associations between baseline leptin or sOB-R and follow-up values of continuous parameters. In addition to the unadjusted model, we also examined models adjusting for age and smoking, as well as adjusting for age, smoking, and baseline WHR. As we wanted to compare the predictive power of sOB-R with that of leptin, the standardized coefficient β was calculated, as well as R^2 for the unadjusted model.

For highly skewed variables, a logarithmic transformation was performed for correlation and regression analyses, although the nontransformed data were used for description of baseline characteristics. All quantitative data are presented as means ± SE, or percentages for qualitative data. All *P* values for the cross-sectional study are 2-sided. We report 1-sided *P* values for the prospective study only because our a priori hypothesis was that in this population of young men, similar to the prior study of women, sOB-R would be negatively associated with future fasting glucose as well as with other adverse metabolic risk factors. *P* values less than .05 were used to infer statistical significance.

3. Results

3.1. Cohort description

Descriptive characteristics of the study population are presented in Table 1. As expected from a young population, all mean values were within the normal range. However, 29.5% of the participants had a BMI greater than 25 kg/m^2. Dyslipidemia was seen in 30.5% of the participants, mainly elevated LDL or low HDL cholesterol. Eleven subjects had metabolic syndrome by the IDF criteria, defined as either waist circumference ≥94 cm (37 in) or BMI ≥30 kg/m^2, and, in addition, 2 or more of the following: blood pressure ≥130/85 mm Hg, triglyceride level ≥150 mg/dL, HDL cholesterol level <40 mg/dL, and fasting glucose ≥100 mg/dL [14]. Three participants had developed

Table 2 – Correlation between leptin-binding protein and $\log_{10}$ leptin with baseline values of variables

Variable	Unadjusted model				Adjusted model 1				Adjusted model 2	
	sOB-R (ng/mL)		$\log_{10}$ leptin		sOB-R (ng/mL)		$\log_{10}$ leptin		sOB-R (ng/mL)	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*
Age at enrollment visit	−0.04	.19	0.02	.55						
Weight (kg)	−0.47	<.001	0.74	<.001	−0.38	<.001	0.63	<.001	−0.24	<.001
BMI (kg/m^2)	−0.46	<.001	0.81	<.001	−0.35	<.001	0.71	<.001	−0.19	<.001
Waist circumference (cm)	−0.47	<.001	0.81	<.001	−0.38	<.001	0.73	<.001	−0.23	<.001
Hip circumference (cm)	−0.47	<.001	0.80	<.001	−0.39	<.001	0.73	<.001	−0.24	<.001
WHR	−0.31	<.001	0.56	<.001						
Percentage body fat	−0.44	<.001	0.84	<.001	−0.33	<.001	0.76	<.001	−0.15	<.001
Heart rate (beats/min)	−0.20	<.001	0.24	<.001	−0.15	<.001	0.17	<.001	−0.10	.002
Systolic blood pressure (mm Hg)	−0.23	<.001	0.44	<.001	−0.15	<.001	0.33	<.001	−0.05	.11
Diastolic blood pressure (mm Hg)	−0.18	<.001	0.29	<.001	−0.12	<.001	0.20	<.001	−0.05	.11
Mean arterial pressure (mm Hg)	−0.22	<.001	0.39	<.001	−0.14	<.001	0.28	<.001	−0.06	.07
Total cholesterol (mg/dL)	−0.09	.01	0.35	<.001	−0.01	.86	0.24	<.001	0.08	.02
HDL (mg/dL)	0.24	<.001	−0.10	.004	0.19	<.001	0.001	.99	0.20	<.001
LDL (mg/dL)	−0.10	.003	0.35	<.001	−0.01	.82	0.23	<.001	0.07	.03
Triglycerides (mg/dL)	−0.20	<.001	0.35	<.001	−0.11	.001	0.25	<.001	−0.04	.27
Fasting glucose (mg/dL)	−0.18	<.001	0.32	<.001	−0.15	<.001	0.29	<.001	−0.06	.07
$\log_{10}$ (leptin)	−0.42	<.001			−0.32	<.001				
Composite metabolic syndrome score	−0.31	<.001	0.44	<.001	−0.21	<.001	0.28	<.001	−0.13	<.001

Adjusted model 1: adjusted for age, smoking, and WHR. Adjusted model 2: adjusted for age, smoking, WHR, and $\log_{10}$ leptin. The Pearson correlation coefficient is denoted by *r*.

metabolic syndrome on follow-up; none of the 3 had metabolic syndrome at baseline.

Table 1 also shows the baseline and follow-up characteristics of the 91 participants who took part in the prospective study. The follow-up group differed significantly from the baseline group in several of the parameters at baseline. However, when compared to the group of participants who were invited to follow-up but declined, only waist circumference, WHR, LDL cholesterol, and leptin were significantly different (data not shown); our adjusted models correct for WHR and leptin. In addition, there were statistically significant adverse changes over the 2-year period between visits in most of the variables examined, including BMI.

3.2. *Cross-sectional study*

Findings from the cross-sectional portion of the study are summarized in Table 2. Results when examining quartiles of sOB-R were largely similar to those found when using sOB-R as a continuous variable, so only the continuous data are reported here. We found an inverse relationship between levels of sOB-R and measures of adiposity (weight, BMI, waist circumference, hip circumference, WHR, percent body fat), as well as protective associations in relation to other metabolic measurements (systolic and diastolic blood pressure, mean arterial pressure, HDL cholesterol, LDL cholesterol, triglycerides, fasting glucose, and the metabolic syndrome score).

For our adjusted models, we adjusted for WHR, rather than for BMI or percentage body fat, as a marker of body composition. Leptin is much more closely associated with measures of fat mass than with measures of body fat distribution. By including 2 variables that are as closely related as leptin and BMI or percentage body fat in the models, colinearity would be introduced. In addition, WHR has been found to be more predictive of the components that constitute the metabolic syndrome than BMI [15].

After correction for age, smoking, and WHR, sOB-R remained significantly inversely correlated with weight (r = –0.38), BMI (r = –0.35), waist circumference (r = –0.38), hip circumference (r = –0.39), heart rate (r = –0.15), systolic blood pressure (r = –0.15), diastolic blood pressure (r = –0.12), mean arterial pressure (r = –0.14), triglycerides (r = –0.11), fasting glucose (r = –0.15), and the composite metabolic syndrome score (r = –0.21). A statistically significant direct correlation was observed with HDL cholesterol (r = 0.19). After also correcting for leptin, the correlation coefficients changed little and most remained significant.

When compared with leptin, the association of sOB-R to the above measures is less strong except for HDL, and opposite in direction.

3.3. *Prospective cohort study*

The prospective analysis is summarized in Table 3. Logistic regression studies showed results similar to the linear regression studies, and the data are therefore not presented here. In the age, smoking, and WHR adjusted model, sOB-R is significantly predictive of fasting glucose and the composite metabolic syndrome score (β = –.18 for both). It is also predictive of most measures of adiposity, such as weight (β = –.38), BMI (β = –.31), waist and hip circumferences (β = –.35

Table 3 – Multivariable linear regression models of leptin-binding protein or leptin levels (per one unit increase) predicting metabolic risk factors at follow-up

Dependent variable	Unadjusted model						Adjusted model 3				Adjusted model 1			
	sOB-R (ng/mL)			Log_{10} leptin			sOB-R (ng/mL)		Log_{10} leptin		sOB-R (ng/mL)		Log_{10} leptin	
	β	P	R^2	β	P	R^2	β	P	β	P	β	P	β	P
Weight (kg)	–.44	<.001	0.20	.67	<.001	0.44	–.47	<.001	.66	<.001	–.38	<.001	.54	<.001
BMI (kg/m^2)	–.41	<.001	0.17	.69	<.001	0.48	–.41	<.001	.68	<.001	–.31	<.001	.54	<.001
Waist circumference (cm)	–.45	<.001	0.20	.75	<.001	0.57	–.46	<.001	.75	<.001	–.35	<.001	.59	<.001
Hip circumference (cm)	–.48	<.001	0.23	.68	<.001	0.46	–.51	<.001	.68	<.001	–.44	<.001	.63	<.001
WHR	–.26	.01	0.07	.64	<.001	0.41	–.23	.02	.64	<.001	–.09	.11	.35	<.001
Percentage body fat	–.40	<.001	0.16	.70	<.001	0.49	–.40	<.001	.69	<.001	–.30	<.001	.57	<.001
Heart rate (beats/min)	–.29	.003	0.08	.20	.03	0.04	–.27	.01	.19	.04	–.28	.01	.24	.03
Systolic blood pressure (mm Hg)	–.09	.19	0.01	.28	.004	0.08	–.13	.12	.29	.003	–.10	.17	.29	.01
Diastolic blood pressure (mm Hg)	–.05	.32	0.003	.23	.02	0.05	–.12	.14	.25	.01	–.07	.26	.17	.09
Mean arterial pressure	–.08	.24	0.01	.27	.004	0.08	–.14	.10	.30	.002	–.09	.20	.24	.02
Total cholesterol (mg/dL)	–.12	.13	0.02	.19	.04	0.04	–.14	.10	.19	.04	–.11	.17	.13	.15
HDL (mg/dL)	–.09	.20	0.01	.002	.49	<0.01	–.10	.18	.004	.49	–.09	.28	–.06	.31
LDL (mg/dL)	–.09	.19	0.01	.16	.06	0.03	–.12	.15	.17	.05	–.08	.23	.10	.22
Triglycerides (mg/dL)	–.02	.41	0.001	.13	.11	0.01	–.02	.45	.13	.12	–.03	.40	.23	.04
Fasting glucose (mg/dL)	–.15	.08	0.02	.20	.03	0.04	–.17	.05	.20	.03	–.18	.049	.29	.01
Composite metabolic syndrome score	–.24	.01	0.06	.42	<.001	0.17	–.23	.02	.31	.002	–.18	.04	.32	.01

Adjusted model 3: adjusted for age and smoking. Adjusted model 1: adjusted for age, smoking, and WHR. β denotes the adjusted regression coefficient.

and −.44, respectively), and percentage body fat (β = −.30), as well as heart rate (β = −.28). No significant predictive value was noted for blood pressure or cholesterol.

Leptin exhibited a similar pattern to sOB-R with opposite direction, although its predictive power was stronger as demonstrated by a higher absolute β value for weight (0.54), BMI (0.54), waist and hip circumference (0.59 and 0.63, respectively), percentage body fat (0.57), fasting glucose (0.29), and metabolic score (0.32). In addition, leptin was significantly predictive of WHR, systolic blood pressure, mean arterial pressure, and triglyceride levels, for which sOB-R had no significant predictive value.

Interaction models revealed significant interactions between sOB-R or leptin and BMI in predicting metabolic factors in this study, but the number of subjects with BMI higher than 25 kg/m^2 was relatively small preventing any further evaluation of these. For example, we confirm prior findings of a positive association with leptin for participants with a BMI less than 25 kg/m^2 [9], but we found null association in those with a BMI higher than 25 kg/m^2. However, only 17 subjects had a BMI higher than 25, and only 3 had a BMI greater than 30 kg/m^2. When correcting for BMI instead of WHR, the strength of the associations were weaker. Consequently, in the prospective study, sOB-R remained significantly associated only with weight, waist circumference, hip circumference, and heart rate at follow-up; leptin remained significantly associated only with waist circumference, WHR, total cholesterol, and LDL cholesterol.

4. Discussion

We first confirmed, using data from the cross-sectional arm of this study of young Cypriot men, the previously described inverse correlation between levels of sOB-R and leptin, anthropometric measurements of obesity, fasting glucose, and a composite score reflecting the components of the metabolic syndrome. These correlations were consistently weaker for sOB-R than for leptin, but persisted even after adjusting for WHR.

In the cohort study, we found for the first time that sOB-R is predictive of fasting glucose in men, and it is the first prospective study in either men or women looking at leptin and sOB-R as predictors of anthropometric variables, cholesterol profile, and blood pressure. We found sOB-R to be predictive of most measurements of obesity, but no significant association was found between sOB-R and blood pressure or cholesterol.

Our data are consistent with previously published findings of an association between sOB-R and diabetes mellitus [9]. In addition, our study shows that leptin also is predictive of fasting glucose at the 2-year follow-up, a finding that was not seen in the prior study in women [9]. Prior studies have showed a sex difference in the predictive power of leptin for type 2 diabetes mellitus, with one study showing a significant prediction for men but not for women [16]. There may also be differences between the American and Cypriot populations: genetic, environmental, or dietary. Finally, the young age of our study subjects might have allowed us to detect associations with serum glucose, whereas in older people higher blood glucoses might have been translated into diabetes.

The importance of sOB-R predicting the development of elevated fasting glucose lies in the underlying relationship of fasting glucose with diabetes and metabolic syndrome. As recently described [17], elevated fasting glucose is predictive of diabetes mellitus, in addition to being a diagnostic criterion for metabolic syndrome. Development of the metabolic syndrome is in turn associated with increased risk of cardiovascular disease morbidity and mortality [17].

There are several plausible reasons for why sOB-R might be predictive of fasting glucose and other metabolic markers. In slimmer people, the higher level of sOB-R may prolong the half-life of leptin [18] and may also potentiate leptin action at the leptin receptor [19]. In addition, the higher serum level of sOB-R likely reflects higher levels of available cell-bound receptors [20]. All of these would potentially lead to increased physiological effect of leptin, which through its central and peripheral effects could reduce further weight gain and prevent development of an adverse metabolic profile. It is also possible that sOB-R exerts direct effects on the mechanisms underlying diabetes mellitus and the metabolic syndrome, but no such effect has yet been described.

Comparing sOB-R with similar hormone-binding proteins can also help elucidate its importance. Most hormonal systems are characterized by the presence of binding proteins; these act as a hormone reservoir, delay clearance of the hormone from the circulation, and may also have other effects. In some cases, the main binding protein consists of the cleaved extracellular portion of the membrane receptor of the hormone or cytokine. This is the case, for example, for growth hormone and tumor necrosis factor (TNF) α [21,22]. In the case of growth hormone, there is indirect evidence that serum levels of the high-affinity growth hormone binding protein roughly mirror the levels of the membrane bound growth hormone receptor [23]. Hence, levels are low in states of growth hormone resistance and elevated in states of growth hormone sensitivity. In the case of TNF-α, multiple studies have shown that levels of its soluble receptors (sTNFR-I and II) correlate with the activation status of the TNF-α system [24,25].

It is our current understanding that similarly, levels of sOB-R correlate with the activation status of the leptin system. In states of leptin sensitivity (states of lower fat mass), leptin binds to its cell-bound receptor leading to upregulation of the receptor and increased cleavage of the receptor into the extracellular space. This in turn leads to increased serum levels of the receptor, which is what we are measuring as sOB-R.

However, whereas measuring the receptor levels for TNF-α and growth hormone are useful because of the lability of TNF-α and the pulsatile secretion of growth hormone, leptin is a stable molecule and can be measured in human serum relatively easily. Moreover, our study finds that leptin has a stronger association with fasting glucose and metabolic syndrome at the 2-year follow-up than its circulating receptor levels in young men. Both make routine measurements of sOB-R less useful.

Strengths of this study include the number of participants in the cross-sectional analysis, which is larger than any prior study investigating the associations between sOB-R and metabolic risk factors. In addition, this is the first prospective study examining the association between serum sOB-R levels and future metabolic characteristics in men. We adjusted for

other potential confounders in our analysis, and the study results were in keeping with prior results in women. Although exercise and diet were not analyzed, the participants were in a setting where their diet and exercise was nearly identical. Finally, the prospective nature of the study incorporates the time-sequence criterion for causality, but cannot fully prove causality; this would require a prospective interventional trial.

The limitations of our study include a young and healthy population and a relatively short follow-up time of 2 years. Only 28% of those invited participated in the 2-year follow-up, and they had significantly different LDL cholesterol, waist circumference, WHR, and leptin from those who did not follow up. Although attrition bias could affect the generalizability of the study, systemic attrition from death or development of disease is unlikely to be present and introduce bias, as the population was healthy and young. The follow-up group was relatively small at 91 subjects, but statistical significance was still maintained for several measures, indicating sufficient power. Errors in laboratory measurements are possible, but laboratory variables were measured blindly and without knowledge of the underlying hypotheses. In addition, random misclassification would have suppressed effect estimates and could not have been responsible for significant results shown herein.

The lack of a significant predictive effect on other metabolic measures such as blood pressure and cholesterol may be related to insufficient variation in these parameters between participants and between the 2 time points. In contrast, sOB-R remained predictive of measures of adiposity, which had changed significantly for the worse at the 2-year follow-up visit. Because findings in women and in older adults may differ from the results from this population of all young males, similar studies in an older cohort with a longer follow-up time would be helpful to confirm or refute our findings and expand our observations to other parameters of the metabolic syndrome, diabetes, and cardiovascular disease.

Acknowledgment

The authors would like to thank Mary Yannakoulia, Harokopio University, Athens, Greece, with assistance with translating documents from Greek.

REFERENCES

[1] Masuzaki H, Ogawa Y, Isse N, et al. Human obese gene expression. Adipocyte-specific expression and regional differences in the adipose tissue. Diabetes 1995;44:855-8.
[2] 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.
[3] Kelesidis T, Kelesidis I, Chou S, et al. Narrative review: the role of leptin in human physiology: emerging clinical applications. Ann Intern Med 2010;152:93-100.
[4] Ahima RS, Flier JS. Leptin. Annu Rev Physiol 2000;62:413-37.
[5] Hill RA, Margetic S, Pegg GG, et al. Leptin: its pharmacokinetics and tissue distribution. Int J Obes Relat Metab Disord 1998;22: 765-70.
[6] Sinha MK, Opentanova I, Ohannesian JP, et al. Evidence of free and bound leptin in human circulation. Studies in lean and obese subjects and during short-term fasting. J Clin Invest 1996;98:1277-82.
[7] Lammert A, Kiess W, Bottner A, et al. Soluble leptin receptor represents the main leptin binding activity in human blood. Biochem Biophys Res Commun 2001;283:982-8.
[8] Chan JL, Bluher S, Yiannakouris N, et al. Regulation of circulating soluble leptin receptor levels by gender, adiposity, sex steroids, and leptin: observational and interventional studies in humans. Diabetes 2002;51:2105-12.
[9] Sun Q, van Dam RM, Meigs JB, et al. Leptin and soluble leptin receptor levels in plasma and risk of Type 2 diabetes in US women: a prospective study. Diabetes 2010;59:611-8.
[10] Ingelsson E, Larson MG, Yin X, et al. Circulating ghrelin, leptin, and soluble leptin receptor concentrations and cardiometabolic risk factors in a community-based sample. J Clin Endocrinol Metab 2008;93:3149-57.
[11] Ogawa T, Hirose H, Yamamoto Y, et al. Relationships between serum soluble leptin receptor level and serum leptin and adiponectin levels, insulin resistance index, lipid profile, and leptin receptor gene polymorphisms in the Japanese population. Metabolism 2004;53:879-85.
[12] Laimer M, Ebenbichler CF, Kaser S, et al. Weight loss increases soluble leptin receptor levels and the soluble receptor bound fraction of leptin. Obes Res 2002;10:597-601.
[13] Tsao TH, Yang JB, Yang CB, et al. The soluble leptin receptor of regular exercisers. Int J Sports Med 2007;28:732-5.
[14] International Diabetes Federation. The IDF consensus worldwide definition of the metabolic syndrome. Accessed at http://www.idf.org/webdata/docs/MetSyndrome_FINAL.pdf on 21 August 2010.
[15] Lee CMY, Huxley RR, Wildman RP, et al. Indices of abdominal obesity are better discriminators of cardiovascular risk factors than BMI: a meta-analysis. J Clin Epidemiol 2008;61: 646-53.
[16] Soderberg S, Zimmet P, Tuomilehto J, et al. Leptin predicts the development of diabetes in Mauritian men, but not women: a population-based study. Int J Obes (Lond) 2007;31:1126-33.
[17] Simmons RK, Alberti KG, Gale EA, et al. The metabolic syndrome: useful concept or clinical tool? Report of a WHO Expert Consultation. Diabetologia 2010;53:600-5.
[18] Huang L, Wang Z, Li C. Modulation of circulating leptin levels by its soluble receptor. J Biol Chem 2001;276:6343-9.
[19] Cohen SE, Kokkotou E, Biddinger SB, et al. High circulating leptin receptors with normal leptin sensitivity in liver-specific insulin receptor knock-out (LIRKO) mice. J Biol Chem 2007;282:23672-8.
[20] Maamra M, Bidlingmaier M, Postel-Vinay MC, et al. Generation of human soluble leptin receptor by proteolytic cleavage of membrane-anchored receptors. Endocrinology 2001;142: 4389-93.
[21] Leung DW, Spencer SA, Cachianes G, et al. Growth hormone receptor and serum binding protein: purification, cloning and expression. Nature 1987;330:537-43.
[22] Diez-Ruiz A, Tilz GP, Zangerle R, et al. Soluble receptors for tumour necrosis factor in clinical laboratory diagnosis. Eur J Haematol 1995;54:1-8.
[23] Amit T, Youdim MB, Hochberg Z. Clinical review 112: does serum growth hormone (GH) binding protein reflect human GH receptor function? J Clin Endocrinol Metab 2000;85:927-32.
[24] Rogy MA, Coyle SM, Oldenburg HS, et al. Persistently elevated soluble tumor necrosis factor receptor and interleukin-1 receptor antagonist levels in critically ill patients. J Am Coll Surg 1994;178:132-8.
[25] Van Zee KJ, Kohno T, Fischer E, et al. Tumor necrosis factor soluble receptors circulate during experimental and clinical inflammation and can protect against excessive tumor necrosis factor alpha in vitro and in vivo. Proc Natl Acad Sci U S A 1992;89:4845-9.