ORIGINAL ARTICLE

Free leptin index as a marker for leptin action in diabetes and obesity: leptin and soluble leptin receptors relationship with HbA1c

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Abstract

OBJECTIVES: Glycosylated haemoglobin (HbA1c), leptin, leptin soluble receptors (sOB-R) and free leptin index (FLI) may all be influenced by diabetes, but their associations remain unclear. Therefore, we put forward a hypothesis that serum leptin, sOBR and FLI might be parallel to Hb1c, as they all reflect the metabolic status.

DESIGN AND SETTING: We measured leptin and sOB-R concentrations in 97 obese non-diabetic (47 women and 50 men), and 65 obese diabetic (32 women and 33 men) humans, and examined whether they were related to HbA1c. Under the condition, the presence of diabetes was the only differentiating factor between two groups of frankly obese humans.

RESULTS: Non-diabetic vs. diabetic, median and interquartile range, respectively: Leptin (ng/ml), 30.83, 37.27vs.28.24, 23.34; $p>0.05$; sOB-R (ng/ml), 17.62, 17.05vs.21.81, 16.61, $p<0.05$; FLI, 231.23, 310.00vs.131.76, 157.68, $p<0.05$. To investigate the influence of HbA1c on leptin and sOB-R, both groups were divided into tertiles based on HbA1c. In diabetics, leptin did not differ between the high, intermediate, and low HbA1c levels subgroups, $p>0.05$, and leptin was not influenced by HbA1c levels: r=0.086; $p>0.05$. For sOB-R, respectively: $p>0.05$; r=0.080; $p>0.05$. In non-diabetics, respectively: $p<0.05$; r=0.2923; $p<0.05$ for leptin; and $p<0.0001$, r=0.5103; $p<0.0001$, for s-OB-R.

CONCLUSIONS: Not leptin alone but serum sOB-R and FLI are the markers of leptin action impairment in type 2 diabetes. Further, HbA1c is not associated with metabolic status of leptin in obese diabetic patients, whereas this association is found in obese non-diabetic humans.

Abbreviations:

BMI	- the body mass index	HOMA	- the homeostasis model assessment of insulin resistance
FLI	- the free leptin index		
HbA1c	- glycosylated haemoglobin A1c	sOB-R	- soluble leptin receptors

INTRODUCTION

Facing an epidemic of type 2 diabetes, clinicians seek valuable markers that could serve as a tool in the management of this disorder. Glycosylated haemoglobin (HbA1c), leptin and leptin soluble receptors (sOB-R) may all be influenced by diabetes and insulin treatment (Aas *et al.* 2009), and thus their measurements may help manage diabetic patients. Nonetheless, whereas HbA1c is the cornerstone of a modern approach to diabetes, the role of leptin and sOB-R in diabetes arouses controversy (Al-Shoumer KA *et al.* 2008; Kratzsch *et al.* 2004).

Leptin, encoded by the *ob* gene, is a protein that regulates body weight by suppressing food intake and increasing energy expenditure (Ahima & Flier 2000; Campfield *et al.* 1995; Considine *et al.* 1995; Halaas *et al.* 1995; Myer *et al.* 2008; Sinha *et al.* 1996a; Zhang *et al.* 1994). Serum leptin levels show a positive correlation with body fat, being increased in obesity (Considine *et al.* 1995; Hamilton *et al.* 1995; Lonnqvist *et al.* 1995) and decreased in states of severe malnutrition such as anorexia nervosa (Ferron *et al.* 1997); furthermore, serum leptin decreases postprandially (Stirban *et al.* 2008) but is not influenced by exercise (Malkova *et al.* 2008). Therefore, leptin is an adipocyte-synthesized hormone, the role of which is to inform the brain of the amount of the adipose tissue present in the body (Jeanrenaud & Jeanrenaud 1996). In addition, leptin has also a range of other physiological functions including regulation of puberty, placental function, peripheral insulin sensitivity, and interaction with other hormonal mediators and regulators (Meier 1996).

Leptin binds to its receptor, the leptin receptor that exists as membrane-bound isoforms and a soluble form (sOB-R). The latter, of the reported molecular mass of approximately 140 kDa (Liu *et al.* 1997), has been proposed to be a binding protein, and an important regulator of leptin activity (Zastrow *et al.* 2003). The soluble form of the leptin receptor is generated by cleavage of the membrane-bound forms (Maamra *et al.* 2001). It has been proposed that the balance between leptin and sOB-R, or the free leptin index (FLI), might be a useful tool to assess leptin activity (Kratzsch *et al.* 2002).

Elevated concentrations of circulating leptin have been associated with type 2 diabetes, obesity and insulin resistance (Ruhl *et al.* 2001; Zimmet el al. 1996; Koebnick *et al.* 2008; Widjaja *et al.* 1997), whereas data regarding the correlations of sOB-R and FLI with obesity and diabetes are more limited, and conflicting particularly with regard to the markers of glycaemic control in diabetes (Kiess *et al.* 1998; Kratzsch *et al.* 2004). Therefore, we have put forward a hypothesis that serum leptin, sOBR and FLI might be parallel to Hb1c, as they all reflect the metabolic status. To prove this hypothesis, we aimed to measure leptin and sOB-R concentrations in non-diabetic and diabetic humans with a similar range of trunkal obesity, and to examine whether they were related to the levels of HbA1c. Under the condition, the presence of diabetes was the only differentiating factor between two groups of frankly obese humans

MATERIALS AND METHODS

The study group consisted of 97 obese non-diabetic subjects (47 women and 50 men), and 65 obese and diabetic subjects (32 women and 33 men), recruited by local advertising. Both groups were sex- and age-matched. Their age was 51±6.9, and 52±6.7 years, respectively. Obesity was defined according to the WHO criterion (BMI, >30 kg/m^2). In all non-diabetics, diabetes was excluded by means of the standard oral glucose tolerance test: 75 grams of glucose was administered orally and the plasma glucose concentration was measured in 2 hours. In the diabetes group, all subjects presented with type 2 diabetes mellitus. Diabetes was defined according to the WHO criterion (fasting glucose of 126 mg/dL, or 6.99 mmol/L, on two various days). All subjects were treated with oral sulfonylureas. All subjects were otherwise healthy according to history, clinical examination, and routine laboratory findings. In particular, none of the studied subjects had evidence of acute or chronic inflammatory disease, had a history of alcohol overconsumption, was completely sedentary, or involved in athletics. All subjects were examined in the morning (at 08:00) after an overnight fast. For women, the examination was made in the middle of the menstruation cycle according to self report. All women had no history of menstrual disturbances or infertility and showed no clinical features of hyperandrogenism or PCOS. Height and weight were measured, and the percentage of body fat was determined with a bioimpedance technique (Bodystat 1500, by BODYSTAT Isle of Man Ltd., U.K.). Repeated bioimpedance tests were performed at the same time after an overnight fast (08:00 A.M.). Exercise, and alcohol or caffeine consumption was banned 24 hrs prior to the bioimpedance test. Premenstrual women and women during menstruation were excluded, as well as patients using diuretics. A venous blood sample was obtained for the measurement of plasma levels of glucose (expressed as mmol/litre, mmol/l) at the hospital's routine chemistry laboratory. Serum levels of leptin, sOB-R and insulin were determined at the Department of Clinical Biochemistry of the Poznań University of Medical Sciences using commercial ELISA kit (R&D Systems Inc., MN, USA); microplater reader SunriseTM (Tecan Group Ltd., Männedorf, Switzerland), and were expressed as nanograms per millilitre (ng/ml), and as milliunits per litre (mU/l), for leptin and sOB-R, and for insulin, respectively. Leptin and sOB-R were measured according to the manufacturer's recommended protocol – in duplicate, with a sensitivity of 0.0078 ng/ml and 0.057 ng/ml, respectively. Intra-assay coefficients of variation (CV) were respectively for leptin and sOB-R: 4.87% and 5.20%, whereas inter-assay CVs were 5.97% for leptin

and 6.13% for sOB-R. The homeostasis model assessment (HOMA) approach was used to calculate the *in vivo* insulin sensitivity with the formula: fasting plasma glucose (mmol/l) × fasting plasma insulin (mU/l) × 22.5^{-1} (Matthews *et al.* 1985).

The values are given as the median and interquartile range. Mann-Whitney test, Kruskal-Wallis test, Dunn test (for post hoc analysis), and Spearman correlation analysis were used for statistical examinations. Standard computer software (STATISTICA 6.0 by Statsoft, Inc.) was applied to the statistical analyses. *p*-values of less than 0.05 were considered statistically significant.

The study was approved by the ethics committee of the Poznań University of Medical Sciences. All subjects gave informed consent to participate.

RESULTS

The clinical data of the diabetic and non-diabetic subjects are shown in Table 1. Both study groups were of similar age, and their body mass indices and percent body fat did not differ. However, as expected, diabetic subjects showed higher levels of HbA1c and plasma glucose. FLI and sOB-R concentrations differed between diabetic and non-diabetic subjects, $p<0.05$, whereas leptin did not, $p>0.05$.

To investigate the influence of HbA1c on serum concentrations of leptin and sOB-R, both study groups were divided into tertiles according to their HbA1c levels. In diabetics, as seen in Table 2, except HbA1c, all parameters did not differ between the tertile subgroups. The plasma concentrations of leptin did not differ significantly between the high (H), intermediate (I), and low (L) HbA1c levels subgroups (in Kruskal-Wallis test: $p=0.3613$; Dunn test: H vs. I, $p=0.4615$; H vs. L, $p=1.000$; I vs. L, $p=1.000$, respectively). Moreover, the serum concentration of leptin was not influenced by HbA1c levels: in Spearman's correlation coefficient analysis, the serum leptin levels were not correlated with HbA1c levels (r=0.086; $p=0.5179$). Moreover, the plasma concentrations of sOB-R in diabetic patients did not differ significantly between the high (H), intermediate (I), and low (L) HbA1c levels subgroups (in Kruskal-Wallis test: $p=0.9171$; Dunn test: H vs. I, $p=1.000$; H vs. L, $p=1.000$; I vs. L, $p=1.000$, respectively). In addition,

Tab. 1. Clinical characteristics of obese diabetic and non diabetic subjects examined. Data are given as median and interquartile range. The abbreviations used are as follows: n, number of subjects; BMI, the body mass index; P, plasma; HbA1c, glycosylated haemoglobin; sOB-R, soluble leptin receptors; FLI, free leptin index.

	Non-diabetics	Diabetics	*p*-value
n	**97**	**65**	
BMI (kg/m^2)	35.35, 10.30	36.20, 8.50	0.996
Body fat (%)	44.8, 13.50	43.85, 12.80	0.663
P glucose (mmol/liter)	5.5, 0.90	7.30, 2.63	**<0.001**
HbA1c	5.90, 0.90	7.40, 1.30	**<0.0001**
P insulin (mU/liter)	25.72, 26.05	20.25, 22.61	0.163
Leptin (ng/ml)	30.83, 37.27	28.24, 23.34	0.574
sOB-R (ng/ml)	17.62, 17.05	21.81, 16.61	**<0.05**
FLI	231.23, 310.00	131.76, 157.68	**<0.05**

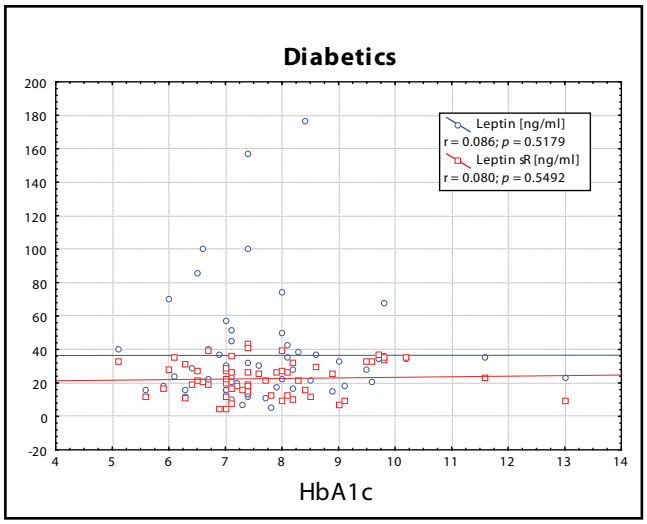

Fig. 1. Correlations between leptin and soluble leptin receptors (Leptin sR), and HbA1c in diabetic subjects. Spearman correlation analysis was used, and *p*-values of less than 0.05 were considered statistically significant.

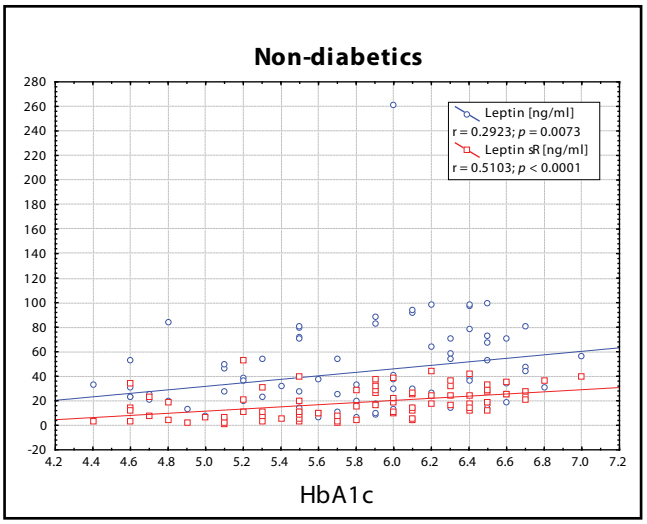

Fig. 2. Correlations between leptin and soluble leptin receptors (Leptin sR), and HbA1c in non diabetic subjects. Spearman correlation analysis was used, and *p*-values of less than 0.05 were considered statistically significant.

Tab. 2. Diabetic subjects divided into tertiles based on their HbA1c levels. Data are given as median and interquartile range. The abbreviations used are as follows: n, number of subjects; BMI, the body mass index; P, plasma; HbA1c, glycosylated haemoglobin; sOB-R, soluble leptin receptors; FLI, free leptin index.

	high	intermediate	low	*p*-value
n	**21**	**22**	**22**	
BMI (kg/m^2)	36.80, 7.60	35.10, 9.00	35.35, 10.60	0.854
Body fat (%)	48.80, 7.50	41.90, 1 4.70	42.45, 9.80	0.135
P glucose (mmol/liter)	9.27, 0.01	7.51, 2.62	6.70, 1.20	0.301
HbA1c	9.00, 1.40	7.40, 0.50	6.50, 0.90	**<0.0001**
P insulin (mU/liter)	22.86, 42.24	21.78, 20.10	18.31, 13.74	0.451
Leptin (ng/ml)	34.63, 15.96	21.50, 35.28	27.79, 24.18	0.361
sOB-R (ng/ml)	25.82, 21.47	20.33, 10.78	21.04, 14.44	0.917
FLI	159.95, 98.63	113.67, 150.45	131.76, 145.39	0.376

Tab. 3. Non-diabetic subjects divided into tertiles based on their HbA1c levels. Data are given as median and interquartile range. The abbreviations used are as follows: n, number of subjects; BMI, the body mass index; P, plasma; HbA1c, glycosylated haemoglobin; sOB-R, soluble leptin receptors; FLI, free leptin index.

	high	intermediate	low	*p*-value
n	**28**	**36**	**33**	
BMI (kg/m^2)	39.75, 7.30	33.50, 10.20	36.60, 12.40	**<0.05**
Body fat (%)	50.0, 7.55	38.25, 17.85	43.30, 12.00	**<0.01**
P glucose (mmol/liter)	6.0, 1.0	5.55, 1.10	5.56, 1.10	**<0.05**
HbA1c	6.45, 0.30	5.90, 0,25	5.20, 0.60	**<0.0001**
P insulin (mU/liter)	27.03, 21.37	28.54, 31.32	25.49, 24.65	0.692
Leptin (ng/ml)	55.66, 38.73	25.11, 31.47	27.74, 30.36	**<0.01**
sOB-R (ng/ml)	24.83, 14.78	15.22, 18.96	7.96, 14.89	**<0.0001**
FLI	250.9, 225.8	182.5, 295.4	328.5, 508.0	**<0.05**

the serum concentration of sOB-R was not influenced by HbA1c levels: in Spearman's correlation coefficient analysis, the serum sOB-R levels did not correlate with HbA1c levels (r=0.080; p=0.5492). FLI did not differ significantly between the high (H), intermediate (I), and low (L) HbA1c levels subgroups (in Kruskal-Wallis test: p=0.3761; Dunn test: H vs. I, p=0.6238; H vs. L, p=1.0000; I vs. L, p=0.7495, respectively), and it did not correlate with HbA1c levels (r=0.008; p=0.9521).

The results in non-diabetic subjects are shown in Table 3. Unlike in diabetics, leptin was influenced by HbA1c levels because it differed between the high (H), intermediate (I), and low (L) HbA1c levels subgroups (in Kruskal-Wallis test: P =0.0047; Dunn test: H vs. I, p=0.0073; H vs. L, p=0.0256; I vs. L, p=1.000, respectively), and it correlated with HbA1c levels (r=0.2923; p=0.0073). Further, sOB-R was influenced by HbA1c levels, as it differed between the high (H), intermediate (I), and low (L) HbA1c levels subgroups (in Kruskal-Wallis test: p=0.0000; Dunn test: H vs. I, p=0.0242; H vs. L, p=0.00002; I vs. L, p=0.2408, respectively), and it correlated with HbA1c levels (r=0.5103; p<0.0001). FLI did not differ between the high (H), intermediate (I), and low (L) HbA1c levels subgroups (in Kruskal-Wallis test: P =.0431; Dunn test: H vs. I, p=1.0000; H vs. L, p=0.1422; I vs. L, p=0.0667, respectively). However, FLI it correlated with HbA1c levels (r=–0.239; p=0.0294).

In order to evaluate the influence of sex on leptin and sOB-R levels, and gender relation to HbA1c, we estimated all correlations for diabetic and no diabetic females and males separately, in an identical manner. Results were similar to those in the whole cohort. However, due to relatively low numbers of participants we decided not to present the data as reliable conclusions could not have been drawn on their basis.

DISCUSSION

In this study in a homogenous group of obese human subjects with and without type 2 diabetes mellitus, we demonstrate that serum concentrations of leptin are similar despite the presence of type 2 diabetes, whereas the levels of soluble receptors for leptin are significantly higher in diabetic humans as compared with non diabetic individuals. Consequently, the ratio of leptin to its soluble receptors, or the free leptin index (FLI), is significantly decreased in diabetic individuals. That sOB-R levels are higher in diabetic patients is discordant with previous reports (Sun *et al.* 2010). Furthermore, it is also contrasting with the fact that sOB-R has been reported lower in obese individuals as compared with lean subjects (Considine *et al.* 1996; Maffei *et al.* 1995; Sinha *et al.* 1996b), because obesity is a well established risk factor for the development of type 2 diabetes mellitus.

As a consequence of the above findings, FLI levels were lower in diabetic patients as compared with non-diabetic subjects. As FLI reflects leptin activity, one may conclude that leptin activity was lower in diabetic patients, in spite of similar leptin concentrations. This finding may be explained not only by pure mathematical division. Rather, we postulate that some regulatory

mechanisms are impaired in obesity with concomitant diabetes: with similar levels of leptin, higher sOB-R levels are needed to guarantee sufficient leptin action in diabetic subjects. Leptin action would be impaired in those patients not only by the presence of obesity, but also by the presence of diabetes.

What we add to the current knowledge, is that we advocate that it is not leptin alone, and not s-OBR alone that should be measured in diabetes. As sOB-R has been reported to function as inhibitor or stabilizer of leptin (Lammert *et al.* 2001; Liu *et al.* 1997; Sinha *et al.* 1996b), its changes might reflect the proposed leptin resistance in obesity and diabetes (Arch *et al.* 1998; Martin *et al.* 2000; Zimmet *et al.* 1999). In this setting, FLI seems to be the best indicator of metabolic abnormalities that develop in diabetes. The relative imbalance between leptin and its receptors may be one of the foundations of type 2 diabetes pathogenesis.

Further, we analyzed whether leptin, sOB-R and FLI were associated with HbA1c, which is a well established marker of diabetes control. Interestingly, we found no correlations between leptin, sOB-R, FLI and HbA1c in the diabetic group but we observed those associations in non diabetics. This was surprising as one would rather expect parallel increases of both leptin resistance and HbA1c in diabetes. This finding seems to be odd at first sight, however it may be explained with the following theory: in type 2 diabetes, many regulatory mechanisms fail to function correctly. Under the circumstance, the precise interactions between adipocytokines and various serum proteins are disturbed, and presumably so is the interaction between sOB-R and leptin, and HbA1c. The latter, with physiological concentrations in non diabetics, seems to be parallel to leptin and sOB-R, whereas non-physiologically secreted in diabetic humans may not be parallel to changes of adipocytokines concentrations. In addition, there also may be some statistical reasons for this finding in our study: in the non-diabetic group, but not in the diabetic group, BMI and the amount of fat tissue differed between various HbA1c subgroups. As a result, BMI differences might influence leptin, sOB-R and FLI in non obese patients, which in turn influenced the overall statistical results in this group. HbA1c might be associated with BMI, and along with this association, with leptin, sOB-R and FLI.

In conclusion, we demonstrate that serum sOB-R levels are higher, FLI lower, whereas leptin concentrations are similar in diabetic patients as compared with non diabetic subjects with the same degree of obesity. Under the circumstance, FLI seems to be a better marker of leptin action impairment in type 2 diabetes mellitus that leptin alone. Moreover, leptin, sOB-R and FLI did not correlate with HbA1c in diabetics, but they all did in non diabetics. Our findings suggest that HbA1c levels are not associated with the metabolic status of leptin in diabetic patients. Instead, they are correlated with Hb1c in obese non diabetic patients. The loss of this association that occurs in obesity after the development of diabetes remains unclear, but presumably it may be related to the presence of a strong relationship between HbA1c and BMI in non diabetic obese humans and the absence of this associations in diabetes that we also show in this study.

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