

## PAPER

# Relationship between high plasma leptin concentrations and metabolic syndrome in obese pre-pubertal children

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**OBJECTIVE:** To evaluate the relationship between serum leptin levels and metabolic syndrome, fasting insulin level and anthropometric index in obese pre-pubertal children.

**DESIGN:** A cross-sectional study was carried out on obese children.

**SUBJECTS:** A study was made of 41 obese children (aged 6–9 y) and the same number of non-obese children (control group), matched by age and sex.

**METHODS:** Body mass index (BMI), waist/hip ratio (WHR) and blood pressure were determined in each child. Serum leptin, glucose, insulin, lipid profile, sex hormone binding globulin (SHBG), plasminogen activator inhibitor-1 (PAI-1), tissue-plasminogen activator (t-PA) and fibrinogen were all measured.

**RESULTS:** The serum leptin level was significantly higher in obese children (15.47 vs 4.73 ng/ml). In the obese group, leptin showed a positive correlation with BMI ( $P < 0.001$ ), insulin ( $P < 0.001$ ), triglycerides ( $P < 0.05$ ), PAI-1 ( $P < 0.05$ ) and t-PA ( $P < 0.05$ ), and correlated negatively with SHBG ( $P < 0.01$ ), apolipoprotein A-I ( $P < 0.05$ ) and high-density lipoproteins cholesterol (HDL-C) ( $P < 0.05$ ). Corrected for BMI and WHR, leptin ( $P$  partial = 0.002) is only an independent predictive factor for basal insulin. Using multivariate regression analysis, only insulin ( $P$  partial = 0.003) and BMI ( $P$  partial = 0.018) were independent predictive factors for leptin.

**CONCLUSION:** For this age group, high leptin resistance may be another component of metabolic syndrome, and may be involved in its etiopathogenesis. The involvement of leptin in this syndrome may be indirect, modulating the insulin's action. *International Journal of Obesity* (2003) 27, 13–18. doi:10.1038/sj.ijo.0802154

**Keywords:** leptin; obesity; children; metabolic syndrome; cardiovascular risk

### Introduction

Obesity is a health risk frequently associated with complications such as type 2 diabetes, dyslipidemia, high blood pressure, abnormal fibrinolysis and cardiovascular disease.<sup>1–4</sup> The syndrome known as metabolic syndrome or insulin resistance is associated with high plasma leptin concentrations<sup>5</sup> and/or leptin resistance.

Insulin resistance is one of the main disorders present in obese individuals.<sup>6</sup> The presence of hyperinsulinism and

insulin resistance has been described in obese children of both sexes (mean age 10 y), using a hyperinsulinemic euglycemic clamp.<sup>7</sup> Some of the disorders related to this syndrome have been described in children, suggesting that it may have a pre-pubertal origin.<sup>8–14</sup>

A positive correlation has been described in children between insulin and plasma leptin concentrations.<sup>15</sup> Independent of obesity, insulin resistance is associated with a high concentration of leptin,<sup>16</sup> which suggests that persistently high plasma levels of this hormone stimulate leptin production.

*In vitro* leptin, meanwhile, lessens the action of insulin on isolated liver cells,<sup>17</sup> increases the oxidation of fatty acids and the depletion of triglycerides in adipocytes,<sup>18</sup> reduces the union of insulin and its receptors in adipocytes,<sup>19</sup> and possibly inhibits the secretion of insulin from the pancreas.<sup>20</sup>

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Leptin may therefore play a role in modulating the action of insulin. The independent correlation between leptin and insulin sensitivity has been described. Leptin has been related to different variables of metabolic syndrome, however the results concerning the possible relationship between these variables and leptin have not been sufficiently addressed,<sup>21–24</sup> particularly in pre-pubertal obese children.

A recent study shows that leptin is an independent risk factor for coronary heart disease<sup>25</sup> and may participate in metabolic syndrome.

Hyperleptinemia and/or leptin resistance may be an important component in the etiopathogenesis of metabolic syndrome by participating in its origin either directly or by regulating insulin sensitivity.

The present study aims to analyze the variables involved in the increase of plasma leptin concentration in child obesity, and to assess the influence of leptin on the various components of metabolic syndrome.

## SUBJECTS AND METHODS

### Subjects

A case-control study was carried out in obese children of both sexes. One group comprised 41 obese children (body mass index (BMI) over 90th percentile in growth curves for the study population,<sup>26</sup> and the control group comprised the same number of non-obese children paired by age and sex (aged six–nine y). All subjects were at Tanner stage 1.

Several schools in the area were informed of the study. All parents submitted written consent and the study was authorized by the ethical investigation committee of our hospital.

Children with primary hyperlipidemia, hypertension, diabetes or glucose intolerance, and secondary obesity were excluded from the study. Any child receiving pharmacological treatment was also excluded. All children had similar lifestyles, with no significant physical training program.

### Blood sampling and analysis

Blood samples were collected after 12 h of fasting from a vein in the antecubital fossa, without venous occlusion. All collections were made between 8.00 and 9.00 am. Whole blood specimens were collected in different tubes to obtain serum and plasma. The samples were separated into aliquots and frozen immediately at  $-45^{\circ}\text{C}$  until analyses could be performed.

The basal leptin concentration was determined for all the children, as well as different variables related to metabolic syndrome (insulin, lipids, blood pressure, hydrocarbonate metabolism, hemostasia, sex hormone binding globulin (SHBG), uric acid and biochemical markers of the intra-abdominal fat deposit).

Glucose, uric acid, alanine aminotransferase (ALT), cholinesterase (ChE), cholesterol and triglycerides (TG) were determined in a random access analyser (Axon, Bayer

Diagnostics, Tarrytown, NY, USA) with Bayer Diagnostics reagents.

High-density lipoprotein cholesterol (HDL-C) was determined after precipitation of chylomicrons, very low-density lipoproteins and low-density lipoproteins, with phosphotungstic acid and magnesium ions. The concentration of low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald formula.<sup>27</sup> The non-esterified fatty acids (NEFA) were quantified by a colorimetric enzyme assay (NEFA C ACS-ACOD Method, Wako Chemicals GmbH, Nissanstr, Germany).

Insulin was quantified using a microparticle immunoassay (IMx system Insulin, Abbott Laboratories, Chicago, IL, USA) in an IMx automatic analyser (Abbott Laboratories, Chicago, IL, USA). Apolipoprotein A-I (Apo A-I) and apolipoprotein B (Apo B) were measured by nephelometry (N Antisera to Human Apolipoprotein A-I and Apolipoprotein B reagent, Behringwerke AG, Marburg, Germany) in a Dade Behring Analyzer II Nephelometer.

The sex hormone binding globulin was quantified using an enzyme immunoassay (Radim S.A., Liège, Belgium) in a microtiter plate analyser (Labotech, Chemila, Rome, Italy).

Antigenic immunoassay methods were used for the quantification of tissue-plasminogen activator (t-PA) (Coliza t-Pa, Chromogenix AB, Mölndal, Sweden), plasminogen activator inhibitor-1 (PAI-1) (Asserachrom PAI-1, Stago Diagnoses, Asnieres-south-Seine, France) and leptin (Quantikine human leptin, RD systems, Wiesbaden-Nordenstadt, Germany), carried out in a microtiter plate analyser (Labotech, Cormédica, Barcelona, Spain). Fibrinogen was measured by quantitative assay using thrombin in an automatic analyser (Electra 1600, Ortho Clinical Diagnostics, Madrid, Spain).

### Anthropometric measurements

Weight was measured to the nearest 0.1 kg and height to the nearest 0.1 cm. The BMI was calculated as weight (kg)/height (m)<sup>2</sup>. Waist circumferences were measured at the level of the umbilicus and hip circumferences at the level of the greater trochanters and pubic symphysis to the nearest 0.1 cm.

### Blood pressure

The blood pressure of all the children was measured with a mercury sphygmomanometer (Pymah Corporation, Somerville, NJ, USA) after 20 min of rest and in a supine position. One measurement was taken on each of three days, and the mean calculated.

### Statistical analysis

Statistical assessment was conducted using Microstat (Ecosoft, Indianapolis, Inc.) or GraphPAD InStat (GraphPAD Software, San Diego, CA). Abnormal values (outliers) were excluded. Results were expressed as mean  $\pm$  s.e.m. with a 95% confidence interval (95% CI). The distribution of each

variable was tested for departure from Gaussian distribution and variance equality was controlled by Snedecor's *F*-test. The mean values of the groups were compared using Student's unpaired *t*-test. Statistical significance was set at  $P < 0.05$ .

Correlation between variables was evaluated using Pearson's correlation coefficient and regression analysis. Multivariate regression analysis was performed using the Stepwise method. For each variable, potential confounding factors ( $0.05 < P < 0.2$ ) were evaluated by an analysis of raw and adjusted regression coefficients.

## Results

Table 1 shows the anthropometric data of both groups. The median age was 7.89 y (obese) and 7.85 y (control), with a range of 6–9 y.

Mean plasma leptin concentration was significantly higher in the obese children, at 15.47 ng/ml (95% CI 12.9–18.05), in comparison to the control group, at 4.73 ng/ml (95% CI 3.39–6.08; Table 1). In the obese group, sex-related differences in plasma leptin concentrations were evaluated (boys 12.93 ng/ml  $\pm$  2.08 vs 17.10 ng/ml  $\pm$  1.64 girls;  $P = 0.122$ ). The comparisons between the biochemical parameters related to metabolic syndrome are summarized in Table 2.

### Leptin vs insulin and BMI

In the single linear correlation, for the obese group, plasma leptin concentration was positively correlated with insulin ( $r = 0.5779$ ;  $P < 0.001$ ) and BMI ( $r = 0.5382$ ;  $P < 0.001$ ), but not with WHR. Using multivariate regression analysis, insulin ( $P$  partial = 0.003) and BMI ( $P$  partial = 0.018) were independent predictive factors for plasma leptin concentration ( $R = 0.449$ ;  $P < 0.001$ ). Leptin ( $P$  partial = 0.002), corrected for BMI and WHR, is also an independent predictive factor for basal insulin concentration ( $R = 0.3452$ ;  $P < 0.001$ ).

In the control group, insulin ( $r = 0.5838$ ;  $P < 0.001$ ) and BMI ( $r = 0.5193$ ;  $P < 0.001$ ) also correlated with leptin, and

**Table 1** Descriptive statistic and selected biochemical parameters of the study groups

<i>n</i>	Control 41	Obese 41	<i>P</i>
Male/Female	16/25	16/25	
Age (y)	7.84 $\pm$ 0.12	7.79 $\pm$ 0.14	0.787
Weight (kg)	28.08 $\pm$ 0.72	38.30 $\pm$ 0.95	< 0.001
Height (cm)	128.86 $\pm$ 1.06	130.19 $\pm$ 0.95	0.353
BMI (kg/m <sup>2</sup> )	16.80 $\pm$ 0.23	22.46 $\pm$ 0.33	< 0.001
WHR	0.842 $\pm$ 0.006	0.856 $\pm$ 0.007	0.133
Glucose (mg/dl)	87.17 $\pm$ 0.88	86.66 $\pm$ 0.91	0.688
Insulin ( $\mu$ U/ml)	5.68 $\pm$ 0.40	8.67 $\pm$ 0.65	< 0.001
Insulin/Glucose ratio $\times$ 100	7.91 $\pm$ 1.02	9.92 $\pm$ 0.71	0.109
Leptin (ng/ml)	4.73 $\pm$ 0.69	15.47 $\pm$ 1.31	< 0.001

Values are mean  $\pm$  s.e.m.; BMI = body mass index; WHR = waist/hip ratio.

**Table 2** Selected biochemical parameters related to metabolic cardiovascular syndrome

<i>n</i>	Control 41	Obese 41	<i>P</i>
Triglycerides (mg/dl)	50.86 $\pm$ 2.07	65.58 $\pm$ 3.83	0.001
Apolipoprotein A-I (mg/dl)	159.80 $\pm$ 2.82	158.75 $\pm$ 3.61	0.819
HDL cholesterol (mg/dl)	55.98 $\pm$ 1.82	49.33 $\pm$ 1.58	0.007
SBP (mmHg)	89.58 $\pm$ 1.29	94.66 $\pm$ 1.15	0.004
DBP (mmHg)	52.61 $\pm$ 1.29	56.29 $\pm$ 1.26	0.045
ALT (U/l)	18.20 $\pm$ 0.91	20.82 $\pm$ 0.92	0.046
Cholinesterase (U/l)	10619.11 $\pm$ 267.32	11770 $\pm$ 295.6	0.005
SHBG (nmol/l)	58.68 $\pm$ 2.65	48.09 $\pm$ 2.14	0.002
PAI-1 (ng/ml)	21.17 $\pm$ 2.65	38.98 $\pm$ 3.92	< 0.001
t-PA (ng/ml)	2.79 $\pm$ 0.19	3.47 $\pm$ 0.27	0.043
Fibrinogen (g/l)	2.25 $\pm$ 0.06	2.78 $\pm$ 0.09	< 0.001

Values are mean  $\pm$  s.e.m.; SBP = systolic blood pressure; DBP = diastolic blood pressure; ALT = alanine aminotransferase; SHBG = sex hormone binding globulin; PAI-1 = plasminogen activator inhibitor-1; t-PA = tissue plasminogen activator.

both are independent predictive factors. As occurs in the obese group, leptin ( $P$  partial < 0.001) corrected for BMI and WHR is an independent predictive factor for basal insulin ( $R = 0.4335$ ;  $P < 0.001$ ).

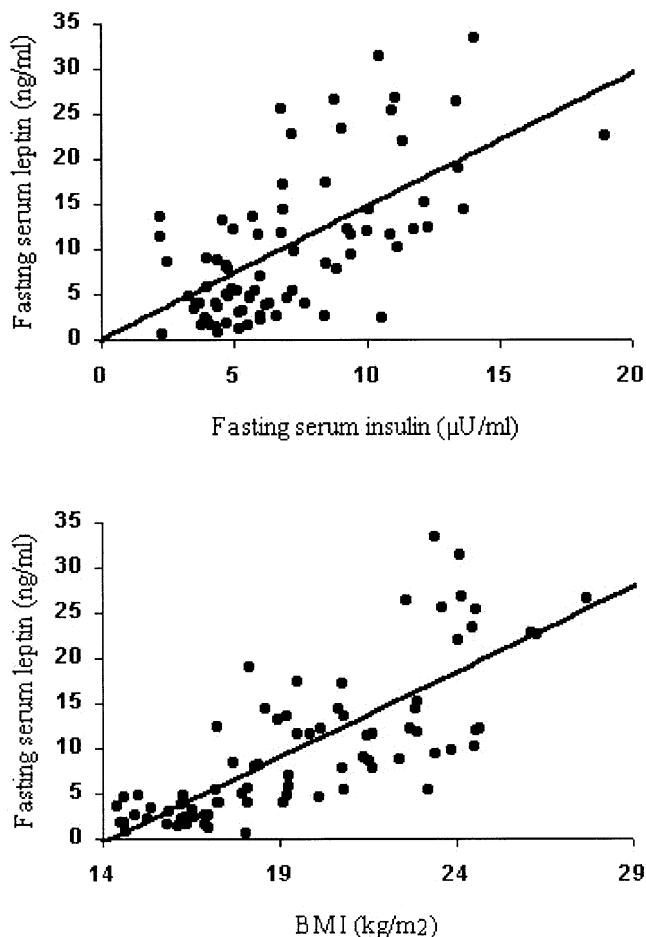
Figure 1 shows the single linear correlation of leptin with BMI and insulin for all of the children (obese and non-obese). In the combined group, corrected for BMI and WHR, leptin ( $P$  partial < 0.001) is an independent predictive factor for basal insulin ( $R = 0.4485$ ;  $P < 0.001$ ).

### Leptin and metabolic syndrome

With regard to the metabolic syndrome (MS) components, the univariate correlation analysis is summarized in Table 3 (obese) and Table 4 (obese and non-obese together). In the obese group, plasma leptin concentrations correlate positively with triglycerides, PAI-1 and t-PA, and negatively with Apo A-I, HDL-C and SHBG. Blood pressure, cholesterol, LDL-C, Apo B and NEFA levels did not correlate with leptin. The study with multivariate regression analysis showed that leptin corrected for BMI and insulin is not an independent predictive factor for the MS variables analysed.

In the obese group, using stepwise multivariate regression analysis, insulin was selected as a predictive variable for triglycerides, Apo A-I, ALT and SHBG, just as BMI was for HDL-C, ChE, PAI-1 and t-PA. Plasma leptin concentration was not selected as a predictive variable for the analysed metabolic syndrome parameters (with the exception of insulin).

In the control group plasma leptin concentration correlated positively with triglycerides ( $r = 0.4244$ ;  $P < 0.01$ ), ALT ( $r = 0.4227$ ;  $P < 0.01$ ), ChE ( $r = 0.3272$ ;  $P < 0.05$ ), t-PA ( $r = 0.3121$ ;  $P < 0.05$ ) and fibrinogen ( $r = 0.4981$ ;  $P < 0.01$ ), and negatively with SHBG ( $r = -0.4306$ ;  $P < 0.01$ ). As occurs with the obese children, in the control group plasma leptin concentration was not correlated with blood pressure, cholesterol, LDL-C or Apo B levels. The study with multivariate



**Figure 1** Serum leptin concentrations as a function of insulin ( $r = 0.6636$ ;  $P < 0.001$ ) and BMI ( $r = 0.7525$ ;  $P < 0.001$ ) in obese and non-obese (together) children.

**Table 3** Coefficients of simple correlation ( $r$ ) between different variables of the obese group

	Leptin	Insulin	Ins/Glc ratio	BMI	WHR
TG	0.3141 <sup>c</sup>	0.4084 <sup>b</sup>	0.4248 <sup>b</sup>	0.3318 <sup>c</sup>	NS
Apo A-I	-0.3239 <sup>c</sup>	-0.4940 <sup>a</sup>	-0.4899 <sup>a</sup>	NS	NS
HDL-C	-0.3168 <sup>c</sup>	NS	NS	-0.4162 <sup>b</sup>	-0.3517 <sup>c</sup>
SBP	NS	NS	NS	NS	NS
DBP	NS	NS	NS	NS	0.3223 <sup>c</sup>
ALT	0.2499	0.3771 <sup>c</sup>	0.4133 <sup>b</sup>	NS	NS
ChE	NS	0.3433 <sup>c</sup>	0.3507 <sup>c</sup>	0.4930 <sup>a</sup>	NS
SHBG	-0.4153 <sup>b</sup>	-0.4499 <sup>b</sup>	-0.4659 <sup>b</sup>	-0.3320 <sup>c</sup>	NS
PAI-1	0.3960 <sup>c</sup>	NS	NS	0.4709 <sup>b</sup>	NS
t-PA	0.3536 <sup>c</sup>	NS	NS	0.4654 <sup>b</sup>	NS
Fibrinogen	NS	NS	NS	NS	NS

<sup>a</sup>  $P < 0.001$ ; <sup>b</sup>  $P < 0.01$ ; <sup>c</sup>  $P < 0.05$ ; NS = not significant; Ins/Glc = Insulin/Glucose; BMI = body mass index; WHR = waist/hip ratio; TG = triglycerides; Apo A-I = apolipoprotein A-I; HDL-C = high-density lipoproteins cholesterol; SBP = systolic blood pressure; DBP = diastolic blood pressure; ALT = alanine aminotransferase; ChE = cholinesterase; SHBG = sex hormone binding globulin; PAI-1 = plasminogen activator inhibitor-1; t-PA = tissue-plasminogen activator.

**Table 4** Coefficients of simple correlation ( $r$ ) between different variables of the total group (obese and non-obese together)

	Leptin	Insulin	Ins/Glc ratio	BMI	WHR
TG	0.42636 <sup>a</sup>	0.4996 <sup>a</sup>	0.2756 <sup>c</sup>	0.4251 <sup>a</sup>	0.2639 <sup>c</sup>
Apo A-I	NS	NS	NS	NS	NS
HDL-C	-0.3546 <sup>b</sup>	-0.2026	-0.1955	-0.4306 <sup>a</sup>	-0.2506 <sup>c</sup>
SBP	0.2715 <sup>c</sup>	0.2975 <sup>b</sup>	0.2197	0.3535 <sup>b</sup>	NS
DBP	NS	NS	NS	0.2205	NS
ALT	0.3514 <sup>b</sup>	0.4049 <sup>a</sup>	0.2597 <sup>c</sup>	0.2838 <sup>c</sup>	NS
ChE	0.4042 <sup>a</sup>	0.4018 <sup>a</sup>	NS	0.5048 <sup>a</sup>	0.3213 <sup>b</sup>
SHBG	-0.4975 <sup>a</sup>	-0.4676 <sup>a</sup>	NS	-0.4663 <sup>a</sup>	NS
PAI-1	0.4900 <sup>a</sup>	0.3202 <sup>b</sup>	NS	0.4893 <sup>a</sup>	0.2380 <sup>c</sup>
t-PA	0.3875 <sup>a</sup>	0.3423 <sup>b</sup>	NS	0.3503 <sup>b</sup>	0.2966 <sup>b</sup>
Fibrinogen	0.4560 <sup>a</sup>	0.2908 <sup>c</sup>	NS	0.4983 <sup>a</sup>	0.2530 <sup>c</sup>

<sup>a</sup>  $P < 0.001$ ; <sup>b</sup>  $P < 0.01$ ; <sup>c</sup>  $P < 0.05$ ; NS = not significant; Ins/Glc = Insulin/Glucose; BMI = body mass index. WHR = waist/hip ratio; TG = triglycerides; Apo A-I = apolipoprotein A-I; HDL-C = high-density lipoproteins cholesterol; SBP = systolic blood pressure; DBP = diastolic blood pressure; ALT = alanine aminotransferase; ChE = cholinesterase; SHBG = sex hormone binding globulin; PAI-1 = plasminogen activator inhibitor-1; t-PA = tissue-plasminogen activator.

regression analysis showed that plasma leptin is not an independent predictive factor for the variables studied. In the combined group (obese and non-obese together), plasma leptin (Table 4) correlates positively with triglycerides, SBP, ALT, ChE, PAI-1, t-PA and fibrinogen, and negatively with HDL-C and SHBG. The study with multivariate regression analysis showed that plasma leptin corrected for BMI and insulin is not an independent predictive factor for the MS variables analysed.

## Discussion

Obesity is a chronic pathology with a high morbidity–mortality rate which, due to eating habits and the lifestyle of western civilization, may become a veritable epidemic in coming years. It is associated with hypertension and various metabolic disorders such as type 2 diabetes mellitus, dyslipidemia, hyperuricemia, inappropriate fibrinolysis and hyperinsulinemia.<sup>4,13,14,28–30</sup>

Hyperinsulinemia and/or insulin resistance have been described as the key factors underlying the association of this group of metabolic disorders, known as metabolic syndrome or insulin resistance syndrome (IRS).<sup>4,30</sup>

Peripheral insulin resistance is one of the main disorders present in obese subjects.<sup>6,7</sup> In obese children, high insulin levels and insulin resistance were described when compared with non-obese children.<sup>7,31</sup> Some metabolic alterations described in IRS are present in most obese children,<sup>8,14,32</sup> and begin at an early age.

As well as the disorders traditionally related to IRS we have described, in obese children in the same age group as those studied here, and in comparison with non-obese children, significantly high levels of PAI-1, t-PA, fibrinogen

and insulin.<sup>13</sup> We have also observed a significant decrease of SHBG.<sup>12</sup> All these parameters are related to IRS.

Obesity is frequently associated with high plasma leptin concentrations and leptin resistance. A positive correlation has been described in children between levels of insulin and leptin.<sup>15</sup> The concentration of leptin in serum is independently correlated with insulin sensitivity.<sup>21</sup> Elevated plasma leptin levels strongly predict first-ever acute myocardial infarction in men<sup>33</sup> and recently it has been shown as an independent risk factor for coronary heart disease.<sup>25</sup>

In our study we describe, in pre-pubertal obese children of both sexes, high concentrations of plasma leptin, which are dependent both on insulin concentration and on BMI. For the group of non-obese children, leptin is also correlated with insulin and BMI, suggesting that both parameters are determinants of leptin concentration in both physiological and pathological situations. In the combined group (obese and non-obese together), leptin is also correlated with insulin and BMI. For this age group, fat distribution (WHR) appears to have less influence on this syndrome. The leptin–insulin association therefore begins before puberty and may be connected to the onset of metabolic syndrome. In our results, leptin corrected for BMI and WHR is an independent predictive factor for the basal concentration of insulin.

Although PAI-1 has been correlated with leptin, indicating that leptin *per se* may increase PAI-1 concentration in adults aged 18–45 y,<sup>34</sup> other authors have not found it to be independently associated with the parameters of fibrinolysis.<sup>35</sup> Our results coincide with these findings, although our age group is lower than that studied by these other authors. In the single linear correlation analysis, for the obese group, leptin correlated positively with PAI-1, although in a multivariate regression analysis leptin is not an independent predictive factor for PAI-1.

Leptin has also been related to levels of glucose, uric acid and lipids.<sup>21–24</sup> For some authors leptin would intervene in the development of metabolic syndrome, even more than insulin.<sup>21–24</sup> Hypertension has also been related to high levels of leptin.<sup>36–38</sup>

With the group of obese children that we studied, in a single linear correlation analysis, leptin correlated with different metabolic syndrome variables (insulin, triglycerides, HDLc, Apo-AI, SHBG and PAI-1); however leptin was not an independent predictive factor for any of them (except insulin).

Chronic insulin increase, described in obesity, favours an increase in leptin. Due to the action of leptin on insulin sensitivity (leptin favours the development of resistance to insulin and hyperinsulinism), it would perpetuate the increase of insulin and favour the onset of insulin resistance.

The risk of developing obesity-related diseases may be in part a function of resistance to both insulin and leptin among susceptible persons.

To summarize, for this age group high plasma leptin concentrations depend on both insulin and BMI levels.

Hyperleptinemia and/or leptin resistance may be one more component of metabolic syndrome, and may be involved in its etiopathogenesis. The involvement of leptin in this syndrome may be indirect, modulating the insulin's action.

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### References

- 1 Turner RC. The role of obesity in diabetes. *Int J Obes Relat Metab Disord* 1992; **16**(Suppl 2): S43–S46.
- 2 Pi-Sunyer FX. Medical hazards of obesity. *Ann Intern Med* 1993; **119**: 655–660.
- 3 Kissebah AH, Freedman DS, Peiris AN. Health risks of obesity. *Med Clin North Am* 1989; **73**: 111–138.
- 4 Reaven GM. Role of insulin resistance in human disease. *Diabetes* 1988; **37**: 1595–1607.
- 5 Considine RV, Sinha MK, Heiman ML, Kriauciunas A, Stephens TW, Nyce MR, Ohannesian JP, Marco CC, McKee LJ, Bauer TL *et al*. Serum immunoreactive-leptin concentrations in normal weight and obese humans. *New Engl J Med* 1996; **334**: 292–295.
- 6 Monti LD, Brambilla P, Stefani I, Caumo A, Magni F, Poma R, Tomasini L, Agostini G, Galli-Kienle M, Cobelli C *et al*. Insulin regulation of glucose turnover and lipid levels in obese children with fasting normoinsulinaemia. *Diabetologia* 1995; **38**: 739–747.
- 7 Caprio S, Bronson M, Sherwin RS, Rife F, Tamborlane WV. Coexistence of severe insulin resistance and hyperinsulinaemia in pre-adolescent obese children. *Diabetologia* 1996; **39**: 1489–1497.
- 8 Csabi G, Torok K, Jeges S, Molnar D. Presence of metabolic cardiovascular syndrome in obese children. *Eur J Pediatr* 2000; **159**: 91–94.
- 9 Asayama K, Hayashibe H, Dobashi K, Uchida N, Kawada Y, Nakazawa S. Relationships between biochemical abnormalities and anthropometric indices of overweight, adiposity and body fat distribution in Japanese elementary school children. *Int J Obes Relat Metab Disord* 1995; **19**: 253–259.
- 10 Wattigney WA, Harsha DW, Srinivasan SR, Webber LS, Berenson GS. Increasing impact of obesity on serum lipids and lipoproteins in young adults. The Bogalusa Heart Study. *Arch Intern Med* 1991; **151**: 2017–2022.
- 11 Tokunaga K, Ishikawa K, Sudo H, Matsuzawa Y, Yamamoto A, Tarui S. Serum lipoprotein profile in Japanese obese children. *Int J Obes* 1982; **6**: 399–404.
- 12 Gascón F, Valle M, Martos R, Ruz FJ, Ríos R, Montilla P, Cañete R. Sex hormone-binding globulin as a marker for hyperinsulinemia and/or insulin resistance in obese children. *Eur J Endocrinol* 2000; **143**: 85–89.
- 13 Valle M, Gascón F, Martos R, Ruz FJ, Bermudo F, Ríos R, Cañete R. Infantile obesity: a situation of atherothrombotic risk? *Metabolism* 2000; **49**: 672–675.
- 14 Valle M, Gascón F, Martos R, Ruz FJ, Bermudo F, Morales R, Cañete R. Metabolic cardiovascular syndrome in obese pre-pubertal children: the role of high fasting insulin levels. *Metabolism* 2002; **51**: 423–428.
- 15 Kamoda T, Saitoh H, Nakahara S, Izumi I, Hirano T, Matsui A. Serum leptin and insulin concentrations in prepubertal lean, obese and insulin-dependent diabetes mellitus children. *Clin Endocrinol* 1998; **49**: 385–389.

- 16 Zimomet P, Hodge A, Nicolson M, Staten M, de Courten M, Moore J, Morawiecki A, Lubina J, Collier G, Alberti G, Dowse G. Serum leptin concentration, obesity, and insulin resistance in Western Samoans: cross sectional study. *Br Med J* 1996; **313**: 965–969.
- 17 Cohen B, Novick D, Rubinstein M. Modulation of insulin activities by leptin. *Science* 1996; **274**: 1185–1188.
- 18 Shimabukuro M, Koyama K, Chen G, Wang MY, Trieu F, Lee Y, Newgard CB, Unger RH. Direct antidiabetic effects of leptin through triglyceride depletion of tissues. *Proc Natl Acad Sci USA* 1997; **94**: 4637–4641.
- 19 Walder K, Filippis A, Clark S, Zimmet P, Collier GR. Leptin inhibits insulin binding in isolated rat adipocytes. *J Endocrinol* 1997; **155**: R5–7.
- 20 Emilsson V, Liu YL, Cawthorne MA, Morton NM, Davenport M. Expression of the functional leptin receptor mRNA in pancreatic islets and direct inhibitory action of leptin on insulin secretion. *Diabetes* 1997; **46**: 313–316.
- 21 de Courten M, Zimmet P, Hodge A, Collins V, Nicolson M, Staten M, Dowse G, Alberti KG. Hyperleptinaemia: the missing link in the metabolic syndrome? *Diabetic Med* 1997; **14**: 200–208.
- 22 Leyva F, Godsland IF, Ghatei M, Proudler AJ, Aldis S, Walton C, Bloom S, Stevenson JC. Hyperleptinemia as a component of a metabolic syndrome of cardiovascular risk. *Arterioscler Thromb Vasc Biol* 1998; **18**: 928–933.
- 23 Zimmet P, Boyko EJ, Collier GR, de Courten M. Etiology of the metabolic syndrome: potential role of insulin resistance, leptin resistance, and other players. *Ann NY Acad Sci* 1999; **892**: 25–44.
- 24 Fruehwald-Schultes B, Peters A, Kern W, Beyer J, Pfutzner A. Serum leptin is associated with serum uric acid concentrations in humans. *Metabolism* 1999; **48**: 677–680.
- 25 Wallace AM, McMahon AD, Packard CJ, Kelly A, Shepherd J, Gaw A, Sattar N. Plasma leptin and the risk of cardiovascular disease in the West of Scotland Coronary Prevention Study (WOSCOPS). *Circulation* 2001; **104**: 3052–3056.
- 26 Hernández M, Castell J, Narvaiza JL, Rincón JM, Ruiz E, Sánchez E. Curvas y tablas de crecimiento. Instituto de Investigación sobre Crecimiento y Desarrollo. Fundación Faustino Orbegozo. Madrid, Ed. Garsi 1988.
- 27 Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 1972; **18**: 499–502.
- 28 Krotkiewski M, Björntorp P, Sjöström L, Smith U. Impact of obesity on metabolism in men and women. Importance of regional adipose tissue distribution. *J Clin Invest* 1983; **72**: 1150–1162.
- 29 Bonora E, Targher G, Zenere MB, Saggiani F, Cacciatori V, Tosi F, Travia D, Zenti MG, Branzi P, Santi L, Muggeo M. Relationship of uric acid concentration to cardiovascular risk factors in young men. Role of obesity and central fat distribution. The Verona Young Men Atherosclerosis Risk Factors Study. *Int J Obes Relat Metab Disord* 1996; **20**: 975–989.
- 30 Ferrannini E, Haffner SM, Mitchell BD, Stern MP. Hyperinsulinemia: the key feature of a cardiovascular and metabolic syndrome. *Diabetologia* 1991; **34**: 416–422.
- 31 Moussa MA, Shaltout AA, Nkansa-Dwamena D, Mourad M, Al-Sheikh N, Agha N, Galal DO. Association of fasting insulin with serum lipids and blood pressure in Kuwaiti children. *Metabolism* 1998; **47**: 420–424.
- 32 Steinberger J. Insulin resistance and cardiovascular risk in the pediatric patient. *Prog Pediatr Cardiol* 2001; **12**: 169–175.
- 33 Soderberg S, Ahren B, Jansson JH, Johnson O, Hallmans G, Asplund K, Olsson T. Leptin is associated with increased risk of myocardial infarction. *J Intern Med* 1999; **246**: 409–418.
- 34 De Mitrio V, De Pergola G, Vettor R, Marino R, Sciaraffia M, Pagano C, Scaraggi FA, Di Lorenzo L, Giorgino R. Plasma plasminogen activator inhibitor-I is associated with plasma leptin irrespective of body mass index, body fat mass, and plasma insulin and metabolic parameters in premenopausal women. *Metabolism* 1999; **48**: 960–964.
- 35 Sudi KM, Gallistl S, Weinhandl G, Muntean W, Borkenstein MH. Relationship between plasminogen activator inhibitor-1 antigen, leptin, and fat mass in obese children and adolescents. *Metabolism* 2000; **49**: 890–895.
- 36 Aizawa-Abe M, Ogawa Y, Masuzaki H, Ebihara K, Satoh N, Iwai H, Matsuoka N, Hayashi T, Hosoda K, Inoue G, Yoshimasa Y, Nakao K. Pathophysiological role of leptin in obesity-related hypertension. *J Clin Invest* 2000; **105**: 1243–1252.
- 37 Sheu WH, Lee WJ, Chen YT. High plasma leptin concentrations in hypertensive men but not in hypertensive women. *J Hypertens* 1999; **17**: 1289–1295.
- 38 Uckaya G, Ozata M, Sommez A, Kinalp C, Eyiletlen T, Bingol N, Koc B, Kocabalkan F, Ozdemir IC. Plasma leptin levels strongly correlate with plasma renin activity in patients with essential hypertension. *Horm Metab Res* 1999; **31**: 435–438.