Lipid and Hematological Parameters in Hyperleptinemic Healthy Arab Male Youth in Jordan

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Abstract: To analyze the influence of hyperleptinemia on fasting lipid and hematological parameters in healthy Arab male youth in Jordan, this cross-sectional study was carried out in April 2009 on a sample of 120 students aged 18-24 years. Subjects were stratified by fasting leptin into two groups (control, <12.7 ng mL⁻¹ vs. hyperleptinemic, e<sub>2</sub> < 12.7 ng mL⁻¹) and BMI (normal weight, <25 kg m⁻² vs. overweight/obese, BMI > 25 kg m⁻²). Fasting serum leptin, blood glucose, lipid profile and hematological parameters values were determined by standard kit methods. Mean serum leptin concentrations were more than five times as high in hyperleptinemic subjects than in control subjects (p < 0.001). Compared with control group, significant elevations (p < 0.01) were observed in the means total cholesterol, LDL cholesterol and triglyceride levels of hyperleptinemic group whereas no significant differences was detected in HDL-cholesterol. Except the changes of WBC count, MCH and slightly MCHC, there were no differences between both groups in any other term of hematological parameters. In conclusion, changes in lipid variables and some hematological parameters may increase plasma viscosity as a step during atherosclerosis pathogenesis in male youth at risk for dyslipidemia and cardiovascular diseases. Thus, hyperleptinemia could be a useful index in identifying healthy youth male subjects but this hypothesis needs further investigation.

Key words: Hyperleptinemia, lipid profile, hematological parameter, dyslipidemia

INTRODUCTION

Leptin, the peptide encoded by the obesity gene, is secreted by adipose cells and plays an important role in regulating food intake and energy expenditure (Ambhurit, 2008; Himuy et al., 2010). Plasma leptin concentration is proportional to body adiposity and is markedly increased in obese individuals (Beltowski, 2006). Obesity is a strong risk factor for the development of type 2 diabetes mellitus and cardiovascular disease CVD but the pathophysiological mechanisms that link obesity with CVD are poorly defined.

An enhanced lipid profiles rise occurs in some conditions that are associated with an increased risk of vascular disease such as hypertension, (Beltowski, 2006; Ren, 2004) diabetes mellitus DM (Boersma et al., 2011) and obesity (Himuy et al., 2010).

Although recent reviews have identified the importance of preventive medicine (McKee et al., 2011; Toledo-Corral et al., 2011; Hankinson et al., 2010), significant percentage of those studies have been conducted on a patient population and show an association between hyperleptinemia and increased TC, LDL and TG in CAD and diabetes patients (Packard, 2006; Obzanecka et al., 2010; Shankar and Xiao, 2010; West et al., 2009).

Such as those highlighted the same association among healthy and young population are less consistent and controversial. Wu et al. (2001) showed that children with higher plasma leptin levels have significantly higher TG, LDL and Apo B levels than those with relatively lower leptin levels. Furthermore, Okada et al. (2010) also reported that leptin/leptin receptor gene polymorphisms may partly contribute to serum lipid profile in Japanese
obese children. These results were not confirmed by Reinheimer et al. (2009) who found that baseline leptin concentrations were not associated with blood lipids in multiple regression analyses. Haluzik et al. (2000) also noted that a relationship between leptin concentrations and lipid or lipoprotein levels was not statistically significant.

On the other hand, almost no data are available assessing a possible link between lipid and hematological parameters with hyperleptinemia in youth. Most limited available data, however, from studies involving patients and healthy samples were less consistent and controversial. Wang et al. (2004) showed that increased white blood cells and red blood cells counts were associated with a variety of metabolic syndrome features in a Taiwan Chinese population. Similarly, in hemodialysis patients, Nasri (2007) found a positive association between serum leptin and lymphocytes. Recently but in children with steady-state sickle cell disease, Sexas et al. (2010) found a positive association of HDL cholesterol with hemoglobin and hematocrit with total cholesterol. In healthy subjects, positive correlations were observed between hyperleptinemia and Mean Corpuscular Hemoglobin Concentration (MCHC) and Mean Corpuscular Volume (MCV) in Thai overweight subjects. Tungtrongchit et al. (2000) also, with platelets count in obese adolescents according to Foschini et al. (2008) results and finally with WBC count in obese Jordanian male youth (Abu-Samuk et al., 2008). In contrast, no relationships were observed between serum leptin with leukocyte or platelets counts in healthy term infants (Koc et al., 2001). Also in a study by Tungtrongchit et al. (2000), there was a negative correlation between serum leptin with hemoglobin and hematocrit in overweight and obese subjects. That is, obesity gene product, leptin, is gaining more importance, since clarify the mechanisms leading to T2DM and CAD during early stages of youth life are more comprehensible as indicated in the previous studies. Thus, the aim of the study was to examine whether lipids and hematological parameters vary by serum leptin levels in Arab youth males in Jordan.

MATERIALS AND METHODS

This was a cross sectional study carried out in the Applied Science University, Amman, Jordan during the period from January to April 2009. This study was performed using a protocol for the protection of human subjects approved by the Applied Science University Ethical Committee, Amman, Jordan. Written informed consent and demographic characteristics and current medications were obtained from each subject. To avoid confounding factors known to affect leptin levels, subjects with chronic disease such as diagnosed cardiovascular diseases, cerebrovascular disease, dyslipidemia, stable hypertension treated by drugs, chronic hepatic, renal, or taking any kind of medications during the previous 2 months were excluded.

One hundred and twenty Jordanian male nursing students aged 18-24 years were stratified by fasting leptin into two groups. Control group, subjects, (n = 70) with normal serum leptin levels <9.4 ng mL⁻¹ and hyperleptinemic subjects (n = 50) considered to have hyperleptinemia if they had values of >9.4 ng mL⁻¹. BMI, used as an index of general obesity (nonobese, <25 kg m⁻² vs. overweight / obese, BMI.

Fasting venous blood samples were obtained, centrifuged and stored at -20°C until assayed. The following parameters were measured: blood glucose levels (using one touch test, Lifescan; Johnson and Johnson, Palmitas, CA, USA), serum leptin levels (by enzyme immunoassay; ELISA kit, DRG Diagnostics, Marburg, Germany), triglycerides, total cholesterol and high density lipoprotein cholesterol (HDL) by enzymatic colorimetric kits, Linear Chemicals, Barcelona, Spain). Low density lipoprotein cholesterol (LDL) was calculated from the (Friedewald et al., 1972) equation.

Clinical hematology parameters were measured for all volunteers, platelets count, total leukocyte count, differential leukocyte counts, hematocrit, hemoglobin and RBC indices (Mean Cell Hemoglobin [MCH], Mean Cell Volume [MCV], Mean Cell Hemoglobin Concentration [MCHC]), mean platelets count. Complete blood count was performed on the (COBAS MICROS OT 18, Roche, France).

Statistical analysis: Statistical analyses were performed using the STATISTICA 6.0 for Windows software (StatSoft, Tulsa, Oklahoma). Data were expressed as Means±SD. Differences were considered significant at p<0.05.

RESULTS

Table 1 summarizes the anthropometric measurements, fasting biochemical variables and clinical characteristics of the 120 healthy subjects in the present study. Mean age, height and fasting blood glucose were similar in both groups. The mean of ages for all subjects (n = 120) was 21.98±1.78 years and ranged from 18-24 years. Mean BMI and weight were significantly higher in subjects with hyperleptinemia compared with those without hyperleptinemia (p<0.0001).
Table 1: Subjects characteristics of the two study groups subdivided by the serum levels of leptin (Mean±SD)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normal leptin level</th>
<th>Hyperleptinemic</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight</td>
<td>71.4±9.2</td>
<td>89.8±13.4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Height</td>
<td>173.0±6.0</td>
<td>173.8±5.8</td>
<td>0.519</td>
</tr>
<tr>
<td>BMI</td>
<td>23.3±2.4</td>
<td>29.1±4.4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>TG</td>
<td>116.0±41.1</td>
<td>157.2±50.8</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Chol</td>
<td>169.6±29.3</td>
<td>186.7±30.3</td>
<td>0.002</td>
</tr>
<tr>
<td>HDL</td>
<td>51.7±8.2</td>
<td>48.5±7.1</td>
<td>0.032</td>
</tr>
<tr>
<td>LDL</td>
<td>97.0±28.6</td>
<td>117.2±24.7</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>WBC</td>
<td>5.4±1.4</td>
<td>6.7±1.7</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>RBC</td>
<td>3.4±0.7</td>
<td>5.2±0.6</td>
<td>0.131</td>
</tr>
<tr>
<td>Hg</td>
<td>13.8±3.1</td>
<td>16.1±1.3</td>
<td>0.153</td>
</tr>
<tr>
<td>PCV</td>
<td>46.7±4.5</td>
<td>46.2±4.7</td>
<td>0.631</td>
</tr>
<tr>
<td>MCV</td>
<td>86.7±4.7</td>
<td>88.0±3.7</td>
<td>0.094</td>
</tr>
<tr>
<td>MCH</td>
<td>29.4±3.0</td>
<td>31.1±2.1</td>
<td>0.001</td>
</tr>
<tr>
<td>MCHC</td>
<td>34.1±2.4</td>
<td>35.0±1.7</td>
<td>0.020</td>
</tr>
<tr>
<td>FBC</td>
<td>87.2±8.1</td>
<td>86.2±8.7</td>
<td>0.540</td>
</tr>
<tr>
<td>Lymph</td>
<td>53.9±8.2</td>
<td>36.2±6.4</td>
<td>0.817</td>
</tr>
<tr>
<td>Mono</td>
<td>6.3±1.7</td>
<td>6.1±1.8</td>
<td>0.651</td>
</tr>
<tr>
<td>Granuloc</td>
<td>58.3±5.8.7126</td>
<td>57.9±7.4</td>
<td>0.753</td>
</tr>
<tr>
<td>Platelet</td>
<td>255.32±68.548</td>
<td>260.1±43.1</td>
<td>0.674</td>
</tr>
<tr>
<td>Age</td>
<td>21.94±1.665</td>
<td>22.2±1.6</td>
<td>0.430</td>
</tr>
</tbody>
</table>

The values of clinical and laboratory features of the leptin and blood lipids for hyperleptinemic subjects (n = 50) as well as for controls (n = 70) are given in Table 2.

Mean serum leptin concentrations were more than seven times as high in hyperleptinemic subjects than in control subjects (28 vs 4) (p<0.0001). Significant differences were noted in serum levels of LDL-C (p<0.0001), TG (p<0.0001) and TC (p = 0.0002) between the two study groups, whereas, the difference in the serum high HDL-C was less significant (p = 0.032). Of all hematological parameters, only significant differences were observed between means of WBC (p<0.0001), MCH (p<0.0001) and MCHC (p = 0.0002) in study groups.

Correlation of hyperleptinemia with anthropometric, lipid and hematological parameters: The data indicates that irrespective of the levels of leptin in both groups are completely mediated by subject’s body weight and the relationship seems to be more stronger in subjects with hyperleptinemia (pearson correlation coefficient: 0.546 vs. 0.360) (Table 2). The data also reveals that many other subjects’ variable in addition to body weight may have an effect on the serum level of leptin. To establish a relationship between leptin serum levels and subjects’ characteristics, linear regression analysis was carried out considering the following independent variables: body weight, MCHC, TG, cholesterol, HDL, FBC, RBC-count and WBC-count (Table 3). Five different models had significance value of the F statistic (ANOVA) less than 0.0001 which means that the variation explained by any of the model is not due to chance. The only variables that remained in the models were body weight, MCHC, TG, RBC-count and WBC-count. The models were: 1. (body weight), 2. (body weight, MCHC), 3. (body weight, MCHC, TG), 4. (body weight, MCHC, RBC), 5. (body weight, MCHC, RBC, WBC). The multiple correlation coefficients of the five models had large values (0.816, 0.876, 0.884, 0.888 and 0.849) which indicated strong relationships (Fig.1). The squared value of the multiple correlation coefficients showed that about two thirds of the variation in leptin serum levels time is explained by the models (0.67, 0.76, 0.78, 0.78 and 0.79). As a further measure of the strength of the model fit, the standard errors of the estimate in the models were compared to the standard deviation of leptin serum level (9.3). All the linear regression models, the errors of the estimates were lower (8.0, 6.7, 6.6, 6.5 and 6.3).

One of the major flaws in the models was that dependent predictors were inter-related as can be seen in Table 2. Running the collinearity diagnostics confirmed that there were serious problems with multi-co-linearity. A Variance Inflation Factor (VIF) greater than 2 is usually
considered problematic and the smallest VIF in the regression analysis for the included parameters was 12. Several eigenvalues were close to 0, indicating that the predictors are highly inter-correlated and that small changes in the data values may lead to large changes in the estimates of the coefficients. The square roots of the ratios of the largest eigenvalue to each successive eigenvalue were calculated (condition indices). Values greater than 30, indicates serious co-linearity problem.

Seven of these indices were larger than 30, suggesting a very serious problem with co-linearity. The co-linearity problem was fixed by re-running the regression using z scores of the variables (body weight, TG, MCHC, WBC and RBC) and the stepwise method of model selection. This is in order to include only the most useful variables in the model. The process ended with a single model with a single variable; the body weight.

**DISCUSSION**

In this study, we report here that LDL cholesterol, triglycerides and total cholesterol are strongly associated with fasting hyperleptinemia in healthy Arab male youth in Jordan. While the same association did not hold true for HDL-Cholesterol.

Present results confirm the results a number of previous studies among different ethnicities. Thai children and adolescents (Poprak et al., 2008), Colombian male school children (Poveda et al., 2007), Canadian youth aged from 12-15 years (Mahmud et al., 2009) and finally in Japanese male high schools aged 16 to 17 years (Nakatani et al., 2008). The strong association which we found is that hyperleptinemia is not mediated only by body weight but also by lipid parameters except HDL-cholesterol.

This observation may explain the fact that leptin is an independent risk factor for coronary heart disease as was shown in the WOSCOPS study (Wallace et al., 2001) and conversely to Baratta et al. (2004) who noted that the same association was entirely mediated by BMI.

It is difficult to answer, whether leptin is a major mediator or a reflection of other more critical endocrine and growth-related processes (Alexe et al., 2006), therefore, we studied leptin influence at an earlier ages of youth life which may clarify a part of leptin gene activity during this period.

The age of an individual, when the disease is diagnosed, is an important factor in determining the influence of particular risk factor of the disease (Yoon et al., 2003) which reflects and emphasizes the importance of early life environment in programming the susceptibility to chronic diseases in later life (Hales and Barker, 2001) but inability to undertake longitudinal studies from early to late life makes it difficult to directly evaluate the existence of such associations (Alexe et al., 2006). The strength of this study its homogenous sample in terms of age, gender and ethnicity which may explain why the results of some previous studies for hematological (Wang et al., 2004; Seixas et al., 2010) and lipid (Baratta et al., 2004; Gannage-Yared et al., 2006) parameters differ from our results. Notably, recent studies have demonstrated that leptin modulates lipid transport in rodents and human. Some reports have been conducted that leptin amplifies the pro-atherogenic properties of human monocytes (Ngai et al., 2010; Konstantinidis et al., 2009). Similarly, Hongo et al. (2009) suggested that leptin accelerates cholesteryl ester accumulation in human monocyte-derived macrophages by increasing ACAT-1 expression, thereby suppressing cholesterol efflux. Subsequently, Kosztaczky et al. (2007) have noted that leptin enhances cholesterol synthesis through a statin-sensitive pathway in circulating monocytes. In contrast to previous studies, Gannage-Yared et al. (2006) found, in healthy elderly male population, a weak relationship of leptin to lipid but strongly with insulin resistance, this is not confirmed by Galili et al. (2007) who found that early obesity is characterized by endothelial dysfunction in association with increased levels of leptin occurs before the development of insulin resistance.
In recent molecular studies, it has been found leptin/leptin receptor gene polymorphisms may partly contribute to serum lipid profile in Japanese obese children (Okada et al., 2010) and partly to testosterone effect on leptin gene expression in adipocytes according to Horenburg et al. (2008). Therefore, the major limitations of current study were: First, the study was cross-sectional and thus it is not possible to discern cause and effect using this design. Future studies of the impact of lipid and hematological parameters on longitudinal changes in leptin levels will be of value. Second, we did not have access to direct, detailed measures of some outcome variables such as waist circumference, physical inactivity, testosterone levels and blood viscosity. Third, subjects were volunteers and hence, this might lead to biased associations compared with a random sample of the population.

Finally, almost no data are available assessing a possible link between lipid and hematological parameters with leptin levels in healthy Arab. However, our findings were in consistent with the limited available previous studies that have shown a positive associations between leptin levels with selected hematological parameters, particularly MCHC (Tungtrongchitr et al., 2000) platelet count (Foschini et al., 2008) and WBC count (Abu-Samak et al., 2008). Interestingly, our data for hematological parameters, partly, are in agreement with previous by Moriarity and Gibson (2005) who reported that the ability of HDL - cholesterol to reverse atherosclerosis and reduce cardiovascular disease via improving all of the rheological mediators.

CONCLUSION

Present data support the hypothesis that leptin may affect serum lipids independently. These results suggest that change in lipid variables and some hematological parameters may increase plasma viscosity as a step of atherosclerosis pathogenesis mechanism in healthy male youth but this hypothesis needs further investigation.

REFERENCES


