Correlates of serum apelin-12 and adiponectin in obese non-diabetic youth females

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Abstract

The relationship between serum concentration of apelin-12 and adiponectin could be an important determinant in the pathophysiology of obesity-related diseases. The aim of this project is to evaluate serum apelin-12 concentration in non-diabetic obese youth Saudi females and display the relationship with serum adiponectin level. In this study, 163 students (19.3±2.5 years) of King Abdul Aziz University, KSA were stratified into four groups based on their BMI (Underweight <20 kg/m², normal weight between 20-25 kg/m², overweight >25 kg/m² and obese >30 kg/m²). The enzyme linked immunosorbent assay system (ELISA) was used to determine the serum apelin and adiponectin levels. In comparing serum apelin-12 level and adiponectin level in the four groups, the result showed that apelin-12 level did not differ significantly (P=1.00), whereas adiponectin level decreased with increased BMI (P=0.24). Pearson correlation analysis showed a high significant correlation between serum apelin-12 level and serum adiponectin level (P=0.008) in the obese group. Serum apelin-12 level did not correlate with anthropometric parameters, lipid profile, and glucose level in the four groups. The finding suggests that serum apelin-12 level does not reflect visceral or subcutaneous fat accumulation in youth Saudi females. It also proposes that regulation of circulating apelin-12 and adiponectin seems to be alike in obese non-diabetic young women.

Key words: Apelin, adiponectin, obese, non-diabetic, Saudi youth females.

INTRODUCTION

Apelin is an endogenous ligand of the previously discovered “orphan” receptor named APJ, isolated by Tatemoto and his team from bovine stomach extracts (Tatemoto et al., 1998). Apelin is a product of APLN gene that is located on chromosome Xq25–26.1 and translated as a 77 amino acid prepropeptide (Lee et al., 2000). It is processed into several active molecular forms with different biological activities (Kawamata et al., 2001; De Falco et al., 2002). Apelin belongs to the adipokines group because its mRNA expression has been demonstrated in mature adipocytes in rodents and humans (Boucher et al., 2005). APE peptide expression has been also demonstrated in several tissues (Habata et al., 1999; O’Carroll et al., 2000; Kawamata et al., 2001; De Falco et al., 2002; Wang et al., 2004; Boucher et al., 2005; Xie et al., 2006; Principe et al., 2008; Shimizu et al., 2009; Najafipour et al., 2012) and seems to have different regulatory functions, depending on the expressing tissue (Carpene et al., 2007). The apelinergic system distribution over such a variety of tissues has suggested that it might play relevant roles in human physiology. Apelin has been reported to have an effect on appetite, drinking behavior (Sunter et al., 2003), angiogenesis (Cox et al., 2006), and the cardiovascular system (Dai et al., 2006; Farkasfalvi et al., 2007). Apelin is regulated by insulin (Boucher et al., 2005). Apelin might be a novel target for preventing obesity and obesity-related diseases via enhancement of vascular integrity (Sawane et al., 2013). Several active apelin forms exist such as apelin-36, apelin-17, apelin-13, and apelin-12 (Mesmin et al., 2011). Apelin-12 is one of the most potent C-terminal fragments of the polypeptide that possesses a high affinity to APJ receptor and bioactivity in vivo (Langelaan and Rainey, 2009; Pisarenko et al., 2011; Ohno et al., 2012).

Adiponectin (AdipoQ) is another unique adipokine, which in human is encoded by the ADIPOQ gene.
Apelin was determined by Arita at the association between apelin, adiposity (Waki et al., 2003; Waki et al., 2003). The antibody of this concentration was determined by Arita at the association between apelin, adiposity (Waki et al., 2003; Waki et al., 2003). 

**MATERIALS AND METHODS**

**Subjects**

In this study, 163 healthy Saudi youth female students were enrolled from King Abdul Aziz University (KAU), Jeddah, KSA. There was no obligation or any direct/indirect forces for the students to enroll in the study. All subjects were recruited according to the criteria of fasting blood glucose. The volunteers were stratified into four groups based on their BMI (Underweight <20 kg/m², normal weight between 20-25 kg/m², overweight >25 kg/m² and obese >30 kg/m²). The age range was 14-28 years and the mean was 19.3±2.5 years. None of the volunteers had been on any medication treatments. Subjects, who were pregnant, had a history of irregular menstruation, high blood pressure, or high glucose fasting levels were excluded. The local ethical committee at KAU approved this study, and written informed consent was obtained from all participants.

**Samples collecting and preservation**

Blood samples were collected in the morning after an overnight fast and after centrifugation (3500g for 15 min) serum samples were stored at -80°C until assayed. Biochemical parameters were determined in fasting status.

**Anthropometric and biochemical measurements**

Standard methods were used to measure height, weight, waist circumferences (WC), and hip circumferences (HC). Body weight was measured with light clothing on, with up to 0.1kg precision. Height was measured up to 0.1cm precision. Body mass index (BMI) was calculated as weight (kg) divided by height in meters squared (m²). Waist-to-hip ratio (WHR) was also calculated as WC divided by HC. BMI is used to reflect the total body fat, while WC and WHR are indirect measurements of body fat centralization.

Serum apelin concentration was determined by using nonselective apelin-12 enzyme immunoassay kit (Phoenix Pharmaceuticals, Inc., USA). This test kit is effective in the range of 0.0 to 100 ng/mL. The antibody used in this apelin assay cross-reacts 100% with Apelin-12, -13, and -36. Serum adiponectin concentration was determined by using adiponectin enzyme immunoassay kit (ALPCO diagnostics, Salem, New Hampshire, USA). This test kit is effective in the range of 0.075 to 4.8 ng/mL with observed value of 80-120%. Duplicate measurements were performed in a single experiment.

In order to measure serum glucose, after collecting the samples in the morning after and overnight fast, the serum was separated and immediately analyzed. Serum glucose was determined by the glucose oxidase method. Serum concentrations of total cholesterol (TC), triglycerides (TG) and high-density lipoprotein-cholesterol (HDL-C) were measured using routine enzymatic methods with commercial kits purchased from Human Diagnostics (Wiesbaden, Germany). Low-density
lipoprotein cholesterol (LDL-C) levels were calculated using the Friedewald formula: (LDL-C= TC-HDL-C-TG/5 mg/dl)

Statistical analysis

Data are presented as means ± S.D. comparisons among the four groups were made by the Kruskal-Wallis test (nonparametric ANOVA). The correlation of serum apelin-12 level with adiponectin level, anthropometric parameters and metabolic parameters was analyzed by Pearson's linear correlation test. Differences with a P-value <0.05 were considered significant. All statistical analyses were carried out using the SPSS for Windows V16.0 (SPSS Inc., Chicago, IL, USA)

RESULTS

The physical and biochemical characteristics for the four groups, including the serum concentration of apelin-12 and adiponectin, are summarized in Table 1 and 2. The results showed a significant difference between the four groups in weight (P=0.0001), waist circumference (P=0.0001), hip circumference (P=0.0001), WHR (P=0.0001), and BMI (P=0.0001). There was no significant difference in age (P=0.45), height (P=0.40), cholesterol (P=0.56), triglycerides (P=0.54), HDL (P=0.31), LDL (P=0.16), and glucose (P=0.72). In regard to apelin serum level, there was no significant difference (P=1.00) between the underweight, normal weight, overweight and obese group and the level ranged between 0.11 and 2.83 ng/ml. On the other hand, adiponectin serum level decreased with increased BMI and showed no significant difference (P=0.24) between the four groups and the level ranged between 0.07 and 38.67 ng/ml.

In the correlation analysis, the results showed that serum apelin-12 level did not correlate with anthropometric parameters, lipid profile, and glucose level in the underweight, normal weight, overweight and obese group. In regards to the relationship between serum concentration of apelin-12 and adiponectin, the result showed a high significant correlation between serum apelin-12 level and serum adiponectin level (P=0.008) in the obese group (Figure 1).

DISCUSSION

The relationship between the serum apelin-12 and serum adiponectin could be an important determinant of the pathophysiological mechanism involved in the metabolic syndrome. The present study demonstrated that apelin levels were correlated significantly with adiponectin levels in obese non-diabetic youth females. The present study also showed that the level of apelin-12 did not change with obesity. Although the involvement of apelin in human obesity remains unresolved, the positive correlation between apelin and adiponectin suggest that the regulation of circulating apelin and adiponectin seems to be in the same manner in the young obese non-diabetic females. Therefore, we suggest that the increase in adiposity influenced the relationship between serum leptin and serum adiponectin in young obese non-diabetic females. This suggestion indicates clinical significance although the mechanism remains to be explained. It is important to note that future treatments of metabolic system associated disorders should focus on the regulation of adipocytokines, their relationships, and their modes of action. To further characterize obesity and metabolic disturbance aetiology, it is critical to extend adipokine analyses to earlier ages. To our knowledge, this is the first published report on serum apelin concentrations in youth Saudi females and the first to determined a relationship between apelin and adiponectin in obese non-diabetic young females.

Data about the behavior of apelin in obese youth females are limited. Only studies evaluating apelin in obese adult or children with obesity related diseases were conducted. Heinonen and his group observed significantly increased serum apelin-12 concentrations compared with normal-weight subjects and showed a positive correlation between apelin and BMI (Heinonen et al., 2005). Boucher and his team compared apelin expression in fat cells, evaluated its plasma levels in four different models of obesity in mice, and demonstrated a large increase in both apelin expression in fat cells and apelin plasma levels in all hyperinsulinemia-associated obesities. They also confirmed their results in obese men, showing elevated plasma apelin concentrations compared with lean controls (Boucher et al., 2005). Li and his group investigated whether plasma apelin levels were altered in normal, impaired glucose tolerance, and type 2 diabetic subjects and indicated the potential link of apelin with the pathogenesis of insulin resistance and type II diabetes. They also showed a positive correlation between apelin and BMI (Li et al., 2006). Other researchers assessed serum apelin-36 and apelin-12 concentrations in girls with anorexia nervosa (AN). They concluded that obese adolescents had elevated apelin-36 and apelin-12 due to excessive fat mass as well as increased apelin production in adipose tissue. Their research also showed that in participants with normal BMI, serum apelin-36 and apelin-12 concentrations correlated positively with BMI (Ziora et al., 2010). In contrast, a recent study investigated serum apelin levels in a large cohort of Italian subjects with T2D compared to non-diabetic controls and to patients with T1D. They demonstrated that T2D is a determinant of increased circulating apelin levels independently from the concomitant presence of obesity and other metabolic alterations (Cavallo et al., 2012). Our data is in an agreement with Cavallo et al., in regard to the fact that
Table 1. The physical parameters of the underweight, normal weight, overweight and obese non-diabetic youth females

<table>
<thead>
<tr>
<th>Variables</th>
<th>Underweight Mean±SD</th>
<th>Normal weight Mean±SD</th>
<th>Overweight Mean±SD</th>
<th>Obese Mean±SD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N=24</td>
<td>N=94</td>
<td>N=34</td>
<td>N=11</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>19.03±2.0</td>
<td>19.27±2.6</td>
<td>20.17±2.9</td>
<td>18.73±1.7</td>
<td>0.45</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>159.60±7.7</td>
<td>157.13±6.6</td>
<td>152.25±21.3</td>
<td>158.68±6.9</td>
<td>0.40</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>44.28±4.7</td>
<td>52.44±5.9</td>
<td>69.82±17.9</td>
<td>85.50±7.9</td>
<td>0.0001**</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>17.35±1.1</td>
<td>21.21±1.7</td>
<td>27.20±1.4</td>
<td>34.26±4.0</td>
<td>0.0001**</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>64.27±6.7</td>
<td>69.89±5.7</td>
<td>80.83±5.7</td>
<td>95.45±9.5</td>
<td>0.0001**</td>
</tr>
<tr>
<td>Hip (cm)</td>
<td>86.57±7.1</td>
<td>94.53±5.6</td>
<td>105.75±5.2</td>
<td>118.31±5.9</td>
<td>0.0001**</td>
</tr>
<tr>
<td>WHR</td>
<td>0.75±0.10</td>
<td>0.74±0.05</td>
<td>0.77±0.05</td>
<td>0.81±0.05</td>
<td>0.0001**</td>
</tr>
</tbody>
</table>

Kruskal-Wallis test was used for the analysis.
Values were represented as the mean ± standard deviation.
** Highly significant.
WHR: waist-to-hip ratio; BMI: body mass index.

Table 2. The biochemical parameters of the underweight, normal weight, overweight and obese non-diabetic youth females

<table>
<thead>
<tr>
<th>Variables</th>
<th>Underweight Mean±SD</th>
<th>Normal weight Mean±SD</th>
<th>Overweight Mean±SD</th>
<th>Obese Mean±SD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N=24</td>
<td>N=94</td>
<td>N=34</td>
<td>N=11</td>
<td></td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>147.14±22.9</td>
<td>151.58±23.1</td>
<td>150.43±23.7</td>
<td>134.60±23.9</td>
<td>0.56</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>59.00±21.5</td>
<td>58.93±23.8</td>
<td>75.71±31.9</td>
<td>56.00±14.5</td>
<td>0.54</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>67.07±10.8</td>
<td>64.45±11.7</td>
<td>62.43±13.5</td>
<td>65.40±12.1</td>
<td>0.31</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>68.27±21.7</td>
<td>75.34±18.8</td>
<td>72.86±23.1</td>
<td>58.00±15.8</td>
<td>0.16</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>78.79±12.1</td>
<td>79.30±10.0</td>
<td>75.43±7.4</td>
<td>75.80±13.5</td>
<td>0.72</td>
</tr>
<tr>
<td>Apelin-12 (ng/ml)</td>
<td>0.82±0.5</td>
<td>0.85±0.5</td>
<td>0.78±0.4</td>
<td>0.81±0.5</td>
<td>1.00</td>
</tr>
<tr>
<td>Adiponectin (ng/ml)</td>
<td>8.45±8.6</td>
<td>7.11±5.6</td>
<td>6.54±7.3</td>
<td>4.46±5.3</td>
<td>0.24</td>
</tr>
</tbody>
</table>

Kruskal-Wallis test was used for the analysis.
Values were represented as the mean ± standard deviation.
HDL: High-density lipoprotein; LDL: Low-density lipoprotein.

Figure 1. Correlation between serum apelin-12 level and serum adiponectin level in obese non-diabetic youth females.
the increase in apelin was not related to the obesity states (Cavollo et al., 2012).

In the current study, the finding revealed that the level of apelin-12 did not appear to correlate significantly with anthropometric parameters and lipid profiles in the non-obese, overweight and obese non-diabetic youth females. These findings suggest that the serum apelin-12 level does not reflect visceral or subcutaneous fat accumulation in healthy youth women. It is also possible that the obese non-diabetic females may have not yet developed the metabolic consequences of obesity. More research and long-term outcome studies are warranted to elucidate the active role of apelin-12 in the physiopathology of metabolic system associated disorders and its relationship with other adipokines to identify its clinical implications.

CONCLUSION

Our study observed no change of apelin level, a decrease in adiponectin level, and a significant correlation between serum apelin level and serum adiponectin level in the obese non-diabetic youth females. Whether the relationship between the serum apelin level and serum adiponectin level could be an important determinant of the pathophysiologival mechanism involved in the metabolic syndrome in obese individuals merits further investigation.

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REFERENCES


