Serum apelin levels and metabolic risk markers in obese women

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Abstract Background: Adipose tissue hormones, Adipokines, play an important role in obesity-associated complications. Apelin has recently been added to the family of adipokines. The aim of this study was to evaluate the relationship between serum apelin levels and metabolic abnormal parameters in Egyptian obese women.

Materials and methods: The study included 400 unrelated women; they were 200 obese women and 200 non-obese matched healthy women. All participants underwent clinical, anthropometric and biochemical examinations. Insulin resistance (IR) was determined by the homeostasis model assessment of insulin resistance (HOMA-IR). Serum apelin levels and obesity biomarkers were measured using enzyme-linked immunoassay (ELISA) kits. Fat mass was measured by Tanita Body Composition Analyzer.

Results: Obese women showed significant higher levels of serum apelin, leptin, triglycerides, LDL-C, total cholesterol, fasting insulin HOMA-IR and blood pressure levels than controls. Significant positive correlations between apelin and leptin levels with abnormal metabolic markers were noted in obese women.

Conclusion: The present study suggests the significant role that might be mediated by apelin for developing abnormal metabolic parameters among Egyptian obese women.

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metabolism are controlled by apelin [5]. In obesity, excess apelin might be one of the last guards before the appearance of obesity complications such as type 2 diabetes or cardiovascular dysfunctions or insulin resistance (IR) [6]. Leptin is a peptide that is strongly correlated with obesity and its complications [7]. Conflicting results have been obtained from the clinical studies regarding the role of fat distribution and concentrations of serum leptin [8]. In humans, measures of obesity and percentage of body fat are powerfully related with serum leptin levels. It is well known that leptin disturbs insulin action and causes insulin resistance [9]. Therefore, the aim of the present study was to evaluate relation between levels of serum apelin levels with metabolic markers in a sample of Egyptian obese women and evaluate biochemical features of the obese women comparing with healthy normal controls.

2. Subjects and methods

All the procedures used in this study were in accordance with the guidelines of the Helsinki Declaration on Human Experimentations. The study was approved by local ethics committee of the National Research Centre (No.: 13176); the purpose of the protocol was explained to the women, and written informed consent was obtained from them before beginning the study. The study included 400 unrelated women; they were 200 obese women and 200 age-matched healthy women. Their age was between 21 and 36 years. Obese women were referred from different centers to the National Research Centre obesity clinic between 2013 and 2014. Insulin resistance (IR) was estimated based on calculation of the homeostasis model assessment (HOMA) index for each patient. This was done using the formula: (fasting plasma insulin in IU/ml × fasting plasma glucose in mmol/l) / 22.5 [10,11].

Anthropometric parameters included body weight, height, mid upper arm circumference, and waist and hip circumferences have been measured. Skin fold thickness of biceps, triceps, subscapular, suprailliac and abdominal skin fold thickness were measured as well. All measurements were taken 3 times on the left side of the body and the mean of the 3 values was used. Body weight was measured to the nearest 0.1 kg and height was measured to the nearest 0.1 cm. Height was measured with the patients standing with their backs leaning against the stadiometer of the same scale.

Body mass index (BMI) was calculated as weight in kilograms divided by height in meters square (kg/m²). Mid upper arm circumference (MUAC) was measured using a flexible tape at the midpoint between the olecranon and acromial process on the upper right arm with the elbow flexed 90°. Waist circumference (WC) and hip circumference (HC) were measured in cm using a plastic, non-stretchable tape. WC was measured with light clothing at a level midway between the lower rib margin and the iliac crest standing and breathing normally. Hip circumference (HC) was measured at the level at the widest circumference over the buttocks (at the greater trochanter). Waist-to-hip ratio (WHR) was calculated. Skin fold thickness was measured to the nearest mm, except for low values (usually 5 mm or less) when it was taken to the nearest 0.5 mm. These readings were made at six sites on all subjects, at the biceps, triceps, subscapular and supra-illiac areas, using Holtain caliper (Ltd, Bryherian, Crymmych, Pembrokeshire). The subscapular skin fold was measured below the lower angle of the left scapula at a diagonal in the natural cleavage of the skin. Biceps skin fold thickness was measured at the level of the midpoint between the acromion (lateral edge of the acromion process) and the radius (proximal and lateral border of the radius bone) on the midline of the anterior surface of the arm, triceps skin fold thickness (vertical fold, midway between acromion, and olecranon processes on the posterior surface of the arm), and the position of the suprailliac skinfold was the diagonal fold just above the iliac crest even with the anterior axillary line, and abdominal skin fold was at 5 cm adjacent to the umbilicus to the right side. Subsequently, sum of skin folds were calculated. Anthropometric measurements were obtained according to standardized equipment and following the recommendations of the International Biological Program [12]. Systolic and diastolic blood pressures (SBP and DBP) were measured twice in the right arm in a sitting position after a 10 min rest period; using a mercury sphygmomanometer the average of the two measurements was used for analysis. Blood pressure was measured according to a standardized operating procedure using a calibrated sphygmomanometer and brachial inflation cuff (HEM-7200 M3, Omron Healthcare, Kyoto, Japan). Fat mass was measured by Tanita Body Composition Analyzer (SC-330).

Venous blood samples were collected by direct venipuncture after an overnight fast (minimum 12 h). Fasting plasma glucose and serum lipids (total cholesterol, high-density lipoprotein cholesterol (HDL-C) triglycerides (TG)) were measured by enzymatic colorimetric methods using a Hitachi auto analyzer 704 (Roche Diagnostics. Switzerland) [13]. Low density lipoprotein cholesterol (LDL-C) was calculated according to certain equation (LDL-C = Total cholesterol – Triglycerides/5 + HDL-C) [14]. Serum insulin concentration was analyzed by chemiluminescent immunoassay (Immulite2000, Siemens, Germany [15]). Insulin resistance was determined by the Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) calculated as the product of the fasting plasma insulin level (IU/mL) and the fasting plasma glucose level (mmol/L), divided by 22.5 [16]. Serum apelin and leptin levels were measured using commercially available enzyme-linked immunosassay (ELISA) kits (Phoenix Pharmaceuticals, Belmont, CA). The minimal detectable concentration was 0.17 ng/ml, the intra-assay error <5% and the inter-assay error <14%. Leptin was measured using ELISA kits from Linco Research Inc., St. Charles, MO, USA and Cayman Chemicals, Road Ann Harbor, MI, USA with a sensitivity of 0.125 ng/ml, intra-assay variation of 1.4-4.9% and inter assay variation of 1.3-8.6% for the Linco kit and a detection limit of 1 ng/ml and an intra- and inter-assay variation <9% for the Cayman kit.

2.1. Statistical analysis

Statistical presentation and analysis of the results were carried out using SPSS software version 17, spss Inc., Chicago, IL, USA. Statistical tests used chi-squared test, student’s ‘t’ test, analysis of variance, and tukey tests. General linear regression analysis was performed to identify associations between serum apelin levels and BMI and sum of skin folds and between leptin levels and WHR in obese women. Pearson’s correlation coefficient was used to test relation between serum apelin and leptin levels with metabolic biomarkers in obese women.
### 3. Results

The clinical and anthropometric features of the studied groups are presented in Table 1. The mean age of women in the control group (26.1 ± 3.2 year) was nearly similar to the mean age of the obese group (25.9 ± 2.3 year) and there was no significant difference between the two means. Obese women showed significant higher values of adiposity measures including BMI, MUAC, WC, HC, WHR, and body fat % compared to non-obese women of the same age ($P < 0.05$). Table 2 represents the biochemical features in the studied groups. Significant differences between the two groups of women were found in total cholesterol, triglycerides (TG), serum insulin serum concentrations and HOMA-IR with higher values in the obese women than in control one. Reduced High Density Lipoprotein cholesterol (HDL-C) was found in obese than in control one with statistical significant difference. Mean value of apelin serum concentration in obese women (369 ± 25 pg/ml) was higher compared to controls (272 ± 20 pg/ml). Also, serum leptin concentrations in obese women (26.3 ± 1.4 ng/ml) were higher compared to control subjects (12.18 ± 1.7 ng/ml). Table 3 shows correlation of apelin levels with metabolic parameters in obese women. Coefficients of correlation ($r$) and respective $P$-values showed significant positive correlation between apelin levels and total cholesterol, triglycerides, LDL-C, insulin and HOMA-IR levels and negative correlation with HDL-C in obese women. Similarly serum leptin showed significant positive correlations with SBP, DBP, total cholesterol, triglycerides, LDL-C, insulin and HOMA-IR levels and negative correlation with HDL-C in obese women.

Fig. 1 shows general linear regression analysis, illustrating significant correlation between serum leptin levels (ng/ml) and WHR in the obese group ($r = 0.769; p = 0.0001$). Fig. 2 shows the regression analysis between serum apelin levels and the BMI among the studied obese women, where, a significant positive correlation between apelin levels and the BMI ($P < 0.05$) has been detected. Moreover, regression analysis showed significant positive association between sum of skin folds and apelin level in obese women (Fig. 3).

### 4. Discussion

Obesity prevalence has been increasing seriously over the last 30 years [17]. Metabolic diseases such as insulin resistance, type 2 diabetes (T2D), hypertension, nonalcoholic fatty liver disease (NAFLD), polycystic ovarian diseases, and several types of cancer are caused by severe adiposity [18]. Adipose tissue hormones called adipokines such as leptin, and apelin have important role in obesity complications [19,20]. High plasma apelin has been reported by different authors in severe obesity and correlated with adiposity [21,22]. Moreover, leptin is positively correlated with insulin resistance, irrespective of body weight or adiposity, in normal and in diabetic patients [23]. Leptin plays a significant role in the pathophysiology of insulin resistance related to obesity. In obesity, leptin suppresses osteocalcin resulting in insulin resistance [24]. There is powerful relation between obesity and variations of insulin sensitivity status caused by apelin secretion by adipocytes [19]. It was reported that apelin inhibits secretion of insulin plasma systems [25,26].

In present study, we have shown that serum plasma apelin levels were positively correlated with BMI. This finding is similar to another study [27], suggesting a role of apelin in the pathogenesis of obesity. Also, other studies [21,22,28] concluded that apelin levels are significantly higher in obese people compared to control subjects, correlating positively with BMI. The regression analysis showed a significant positive association between subcutaneous fat accumulation and apelin level in obese women of the present study. Thus, obesity appears...
Figure 1  Correlation between serum leptin levels and WHR in obese women.

Figure 2  Correlation between serum apelin levels and BMI (kg/m²) in obese women.
to be an important factor determining plasma apelin concentration.

Moreover, we found that, in obese women, the significant higher levels of both plasma apelin and insulin could indicate that apelin homeostasis is impaired. It might also suggest that the high plasma insulin promote an increase in blood concentrations of apelin, as also suggested by another study [19].

Studies that have explored the relation between regional fat distribution and leptin level are limited [29,30]. In this study, plasma leptin concentrations in obese women showed higher values compared to control subjects and correlated significantly with WHR. So, there is a significant association between leptin concentrations and central obesity measured by waist-to-hip ratio. This is in accordance with a previous study who concluded that, in women leptin levels could remarkably be predicted from the central obesity [31]. However, another study suggested that leptin concentrations are not associated with WHR values and are dependent only on total fat and not on its distribution [32]. There is an inconsistency between the studies where other studies [31,7] showed significant correlations between leptin concentrations and fat distribution regardless of the overall obesity. In the present study, we observed that the waist circumference of obese was significantly higher than that of control women. This is in agreement with the study of previous authors [33]. They documented that the essential anthropometric factor that associated with IR is waist circumference. The authors concluded that excess intra-abdominal fat itself may have an essential impact on the pathogenesis of IR in obesity. Insulin directly regulates apelin production in adipocytes, demonstrating the potential link with obesity-associated variations of insulin sensitivity status [26].

In the present study, we have found that obese women had high serum insulin and leptin. These findings are in concordance with those authors [34], who found that serum insulin levels, and leptin levels increased significantly in obese patients compared with non-obese control individuals. Our findings are also supported by previous studies [35] who confirmed the association between hyperinsulinemia and obesity.

In the current study, it was observed that obese women had significantly higher serum triglycerides, LDL-C levels, and serum cholesterol levels when compared with control individuals. The same findings were observed by many authors [36–38].

5. Conclusions

Egyptian obese women exhibit higher leptin and apelin levels than controls. Serum apelin level is positively correlated abnormal metabolic parameters and with obesity indices. Also, serum leptin level is positively correlated with WHR. The present study suggests the significant role that mediated by adipokines for developing abnormal metabolic parameters among Egyptian obese women. These findings showed the significance of adiposity pattern and the importance of adipokines as biomarkers for adverse metabolic consequences of obesity. Early recognition of obesity pattern and long-term monitoring of both leptin and apelin are therefore necessary for prevention of obesity hazards.
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