Journal of Genetic Engineering and Biotechnology (2017) **15**, 423–429

HOSTED BY

ELSEVIER

Academy of Scientific Research & Technology and National Research Center, Egypt

Journal of Genetic Engineering and Biotechnology

www.elsevier.com/locate/jgeb

ORIGINAL ARTICLE

Serum apelin levels and metabolic risk markers in obese women

Moushira Zaki [a,*], Sanaa Kamal [a], Wafaa Ezzat [b], Naglaa Hassan [a], Walaa Yousef [a], Hanaa Ryad [a], Ramy Mohamed [a], Eman Youness [c], Walaa Basha [a], Yasser Elhosary [b]

[a] *Biological Anthropology Department, Medical Research Division, Giza, Egypt*
[b] *Internal Medicine Department, Medical Research Division, Giza, Egypt*
[c] *Medical Biochemistry Department, Medical Research Division, National Research Centre, Giza, Egypt*

Received 22 November 2016; revised 6 April 2017; accepted 27 May 2017
Available online 9 June 2017

KEYWORDS

Obese;
Women;
Serum apelin;
HOMA-IR

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.

1. Introduction

An excess of body fat leads to obesity, which is a chronic and complex disease. The increased prevalence of obesity in adults, adolescent and children make it one of the principal public health problems [1]. Several obesity-related comorbidities are the results of an increase in the incidence of obesity and caused by adipose tissue hormones called adipokines [2]. Adipokines family includes leptin, adiponectin, and resistin and apelin has been added to that family. [3]. Adipokines participate actively in metabolic functions [4]. A relation has been established between Apelin and obesity. Glucose and lipid

* Corresponding author.
E-mail address: moushiraz@yahoo.com (M. Zaki).
Peer review under responsibility of National Research Center, Egypt.

http://dx.doi.org/10.1016/j.jgeb.2017.05.002

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 ethicscommittee 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, suprailiac 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/m^2). Mid upper arm circumference (MUAC) was measured using a flexible tape at the midway 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-iliac areas, using Holtain caliper (Ltd, Bryberian, 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 suprailiac 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 immunoassay (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).

Table 1 Descriptive clinical data and anthropometric parameters of obese and non-obese women.

Features	Non-obese	Obese	P
Age (years)	26.1 ± 3.2	25.9 ± 2.3	0.26
BMI (kg/m^2)	21.12 ± 2.8	36.2 ± 6.9	0.001
SBP (mmHg)	116.2 ± 17.2	150.3 ± 14.3	0.04
DBP (mmHg)	66.6 ± 9.0	74 ± 11.3	0.8
MUAC (cm)	26.5 ± 2.4	36.9 ± 5.3	0.001
WC (cm)	74.39 ± 7.7	103.1 ± 15.5	0.004
HC (cm)	95.33 ± 8.5	123.59 ± 13.8	0.008
WHR	0.76 ± 0.09	0.91 ± 0.07	0.02
Sum SF (mm)	89.19 ± 26.4	156.6 ± 31.9	0.08
Body fat%	11.7 ± 4.9	35.5 ± 14.2	0.001

BMI, Body Mass Index; Sum SF, sum of skin folds; SBP, Systolic blood pressure; DBP, Diastolic blood pressure; WC, waist circumference; HC, Hip Circumference; WHR, waist–hip ratio; MUAC, mid upper arm circumference.

Table 2 Biochemical features of the studied women.

Parameters	Non-obese	Obese	P
Glucose (mg/dl)	85 ± 11.2	98.5 ± 57.2	0.3
Total cholesterol (mg/dl)	187.36 ± 47.07	206.68 ± 50.09	0.04
Triglycerides (mg/dl)	105.06 ± 47.02	149.09 ± 83.21	0.001
HDL-C (mg/dl)	48.1 ± 5.9	46.9 ± 15.4	0.01
LDL-C (mg/dl)	121.6 ± 23.5	144.4 ± 13.5	0.1
Insulin (μU/mL)	8.2 ± 1.4	30.3 ± 15.66	0.001
HOMA-IR	2.44 ± 1.99	5.67 ± 1.08	0.03
Leptin (ng/ml)	12.1 ± 1.7	26.3 ± 1.4	0.04
Apelin (pg/ml)	272 ± 20	369 ± 25	0.02

HOMA-IR, Homeostatic Model Assessment for Insulin Resistance; HDL-C, High Density Lipoprotein Cholesterol; LDL-C, Low Density Lipoprotein Cholesterol.

Table 3 Correlation of serum apelin and leptin levels with metabolic variables in the obese women.

	Apelin (pg/ml)		Leptin (ng/ml)	
	r	P	r	P
Glucose (mg/dl)	0.053	0.54	0.053	0.54
SBP (mmHg)	0.14	0.44	0.44	0.04
DBP (mmHg)	0.12	0.24	0.42	0.02
Total cholesterol (mg/dl)	0.45	0.02	0.55	0.01
Triglycerides (mg/dl)	0.56	0.01	0.54	0.02
HDL-C (mg/dl)	−0.34	0.05	−0.33	0.05
LDL-C (mg/dl)	0.66	0.01	0.56	0.02
Insulin (μU/mL)	0.75	0.02	0.45	0.04
HOMA-IR	0.86	0.01	0.66	0.02

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 an 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

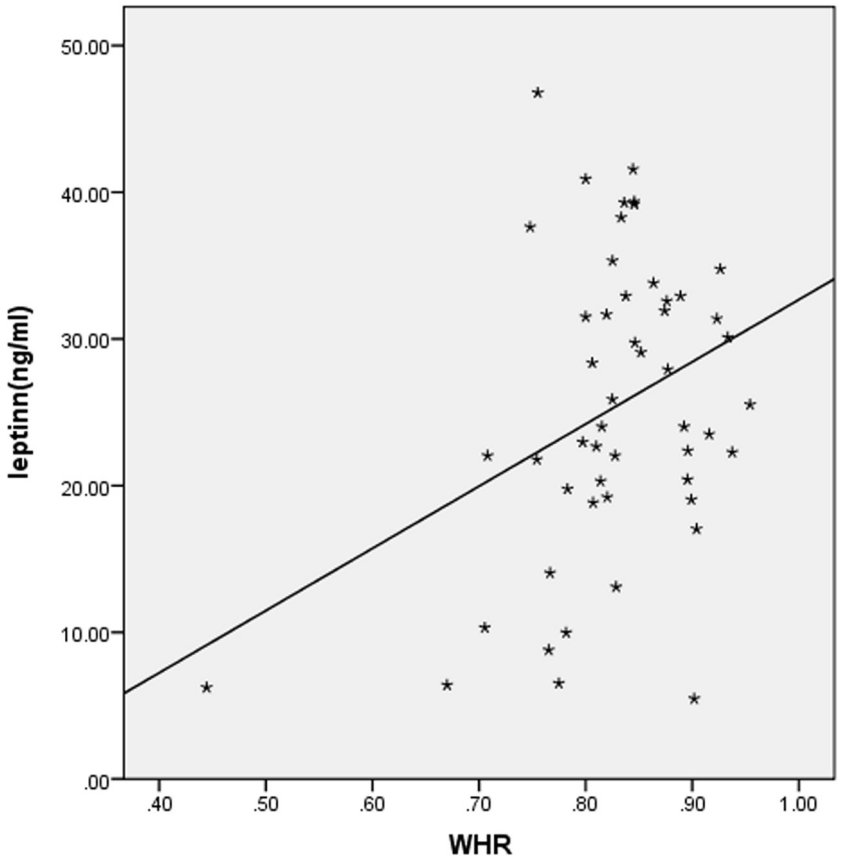

Figure 1 Correlation between serum leptin levels and WHR in obese women.

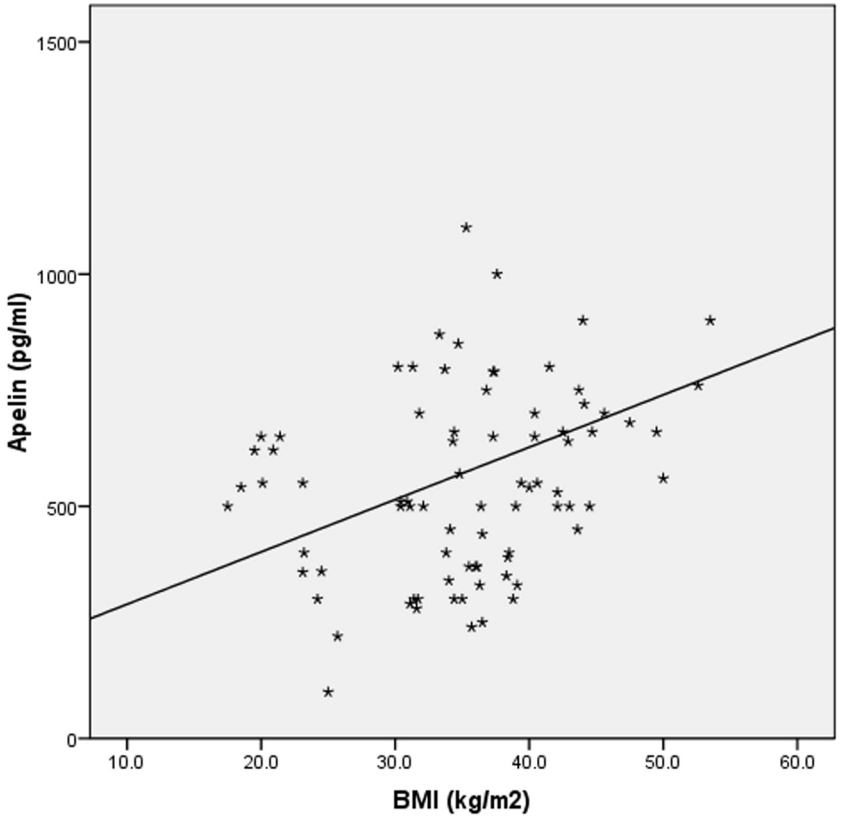

Figure 2 Correlation between serum apelin levels and BMI (kg/m^2) in obese women.

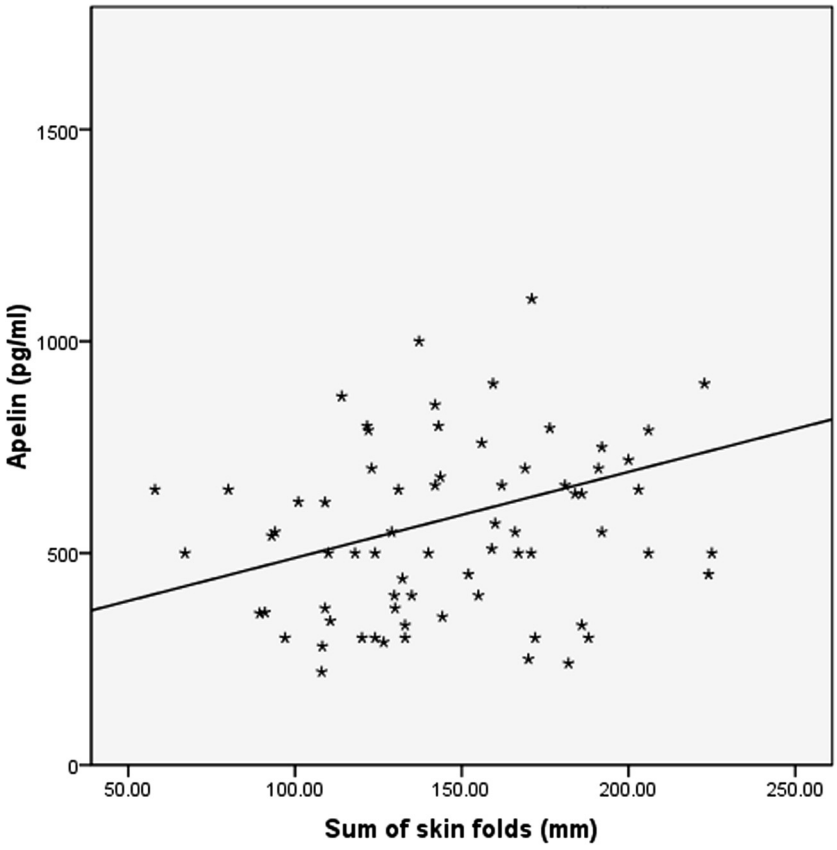

Figure 3 Correlation between serum apelin levels and sum of skin folds (mm) in obese women.

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 adipocytokines 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.

References

[1] M. Bennasar-Veny, A.A. Lopez-Gonzalez, P. Tauler, M.L. Cespedes, T. Vicente-Herrero, A. Yañez, et al, Body adiposity index and cardiovascular health risk factors in caucasians: a comparison with the body mass index and others, PLoS One 8 (5) (2013) e63999.

[2] U.J. Jung, M.-S. Choi, Obesity and its metabolic complications: the role of adipokines and the relationship between obesity, inflammation, insulin resistance, dyslipidemia and nonalcoholic fatty liver disease, Int. J. Mol. Sci. 15 (4) (2014) 6184–6223.

[3] X. Sun, X. Wu, Y. Zhou, X. Yu, W. Zhang, Evaluation of apelin and insulin resistance in patients with PCOS and therapeutic effect of drospirenone-ethinylestradiol plus metformin, Med. Sci. Monitor Int. Med. J. Exp. Clin. Res. 21 (2015) 2547–2552.

[4] A. AL-Suhaimi, A. Shehzad, Leptin, resistin and visfatin: the missing link between endocrine metabolic disorders and immunity, Euro. J. Med. Res. 18 (2013) 12.

[5] I. Castan-Laurell, C. Dray, C. Attané, T. Duparc, C. Knauf, P. Valet, Apelin, diabetes, and obesity, Endocrine 40 (1) (2011 Aug) 1–9.

[6] I. Castan-Laurell, J. Boucher, C. Dray, D. Daviaud, C. Guigné, P. Valet, Apelin, a novel adipokine over-produced in obesity: friend or foe?, Mol Cell Endocrinol. 245 (1–2) (2005) 7–9.

[7] C.E. Ruhl, J. Everhart, Leptin concentrations in the United States: relations with demographic and anthropometric measures, Am. J. Clin. Nutr. 74 (3) (2001 Sep) 295–301.

[8] E.S. Arnardottir, G. Maislin, N. Jackson, R.J. Schwab, B. Benediktsdottir, K. Teff, T. Gislason, The role of obesity, different fat compartments and sleep apnea severity in circulating leptin levels: the Icelandic Sleep Apnea Cohort study, Int. J. Obesity 37 (6) (2013) 835–842.

[9] M.Y. Al Maskari, A.A. Alnaqdy, Correlation between serum leptin levels, body mass index and obesity in omanis, Sultan Qaboos Univ. Med. J. 6 (2) (2006) 27–31.

[10] P. Gayoso-Diz, A. Otero-Gonzaíez, M.X. Rodriguez-Alvarez, F. Gude, C. Cadarso-Suarez, F. García, et al, IR index (HOMA-IR) levels in a general adult population: curves percentile bygender and age. The EPIRCE study, Diabetes Res. Clin. Pract. 94 (2011) 146–155.

[11] D.R. Matthews, J.P. Hosker, A.S. Rudenski, B.A. Naylor, D.F. Treacher, R.C. Turner, Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man, Diabetologia 28 (1985) 412–419.

[12] J. Hiernaux, J.M. Tanner, Growth and physical studies, in: J.S. Weiner, S.A. Lourie (Eds.), Human Biology: A Guide to Field Methods Oxford. U.K: IBP, Oxford, U.K., 1969 (IBP. London, Blackwell Scientific Publications).

[13] V. Hirschler, K. Karin Oestreicher, G. Maccallini, C. Aranda, Relationship between obesity and metabolic syndrome among Argentinean elementaryschool children, Clin. Biochem. 43 (2010) 435–441.

[14] W.T. Friedewald, R.I. Levy, D.S. Fredrickson, Estimation of the concentration of low-density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge, Clin. Chem. 18 (6) (1972).

[15] C.H. Chu, J.K. Lee, H.M. Keng, M.J. Chuang, C.C. Lu, M.C. Wang, et al, Hyperthyroidism is associated with higher plasma endothelin-1 concentrations, Exp. Biol. Med. 231 (2006) 1040–1043 (PubMed.).

[16] R. Matthews, J.P. Hosker, A.S. Rudenski AS, Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man, Diabetologia 28 (1985) 412–419.

[17] World Health Organization, Obesity and Overweight. <http://www.who.int/mediacentre/factsheets/fs311/en/> (accessed on 5 May 2013).

[18] J. Gómez-Ambrosi, J. Salvador, C. Silva, C. Pastor, F. Rotellar, M.J. Gil, J.A. Cienfuegos, G. Frühbeck, Increased cardiovascular risk markers in obesity are associated with body adiposity: role of leptin, Thromb. Haemost. 95 (6) (2006 Jun) 991–996.

[19] J. Boucher, B. Masri, D. Daviaud, S. Gesta, C. Guigné, A. Mazzucotelli, et al, Apelin, a newly identified adipokine up-regulated by insulin and obesity, Endocrinology 146 (2005) 1764–1771.

[20] D. Garcia-Diaz, J. Campion, F. Milagro, et al, Adiposity dependent apelin gene expression: relationships with oxidative and inflammation markers, Mol. Cell. Biochem. 305 (2007) 87–94.

[21] K. Higuchi, T. Masaki, K. Gotoh, S. Chiba, I. Katsuragi, K. Tanaka, T. Kakuma, H. Yoshimatsu, Apelin, an APJ receptor ligand, regulates body adiposity and favors the messenger ribonucleic acid expression of uncoupling proteins in mice, Endocrinology 148 (2007) 2690–2697.

[22] Bruce C. Frier, Deon B. Williams, David C. Wright, Mitochondrial content the effects of apelin treatment on skeletal muscle, Am. J. Physiol. Regul. Integr. Comp. Physio. 297 (2009) R1761–R1768.

[23] S. Fischer, M. Hanefeld, S.M. Haffner, C. Fusch, U. Schwanebeck, C. Köhler, et al, Insulin-resistant patients with type 2 diabetes mellitus have higher serum leptin levels independently of body fat mass, Acta Diabetol. 39 (2002) 105–110.

[24] G. Paz-Filho, C. Mastronardi, M.-L. Wong, J. Licinio, Leptin therapy, insulin sensitivity, and glucose homeostasis, Indian J. Endocrinol. Metabol. 16 (Suppl 3) (2012) S549–S555.

[25] W.M. Sorhede, C. Magnusson, B. Ahren, The apj receptor is expressed in pancreatic islets and its ligand, apelin, inhibits insulin secretion in mice, Regul. Pept. 131 (2005) 12–17.

[26] S.N. Assaad, A.A. El-Aghoury, E.M. El-Sharkawy, E.Z. Azzam, M.A. Salah, Study of serum apelin and its relation to obesity-associated hypertension, Egypt J. Obes. Diabetes Endocrinol. 1 (2015) 28–35.

[27] S. Sheibani, P. Hanachi, M.A. Refahiat, Effect of aerobic exercise on serum concentration of apelin, TNFα and insulin in obese women, Iran. J. Basic Med. Sci. 15 (6) (2012) 1196–1201.

[28] M.V. Heinonen, A.K. Purhonen, P. Miettinen, M. Pääkkönen, E. Pirinen, E. Alhava, et al, Apelin, orexin-A and leptin plasma levels in morbid obesity and effect of gastric banding, Regul. Pept. 130 (2005) 7–13.

[29] F.B. Hu, C. Chen, B. Wang, M.J. Stampfer, X. Xu, Leptin concentrations in relation to overall adiposity, fat distribution, and blood pressure in a rural Chinese population, Int. J. Obes. 25 (2001) 121–125.

[30] A. Minocci, G. Savia, R. Lucantoni, M.E. Berselli, M. Tagliaferri, G. Calo, et al, Leptin plasma concentrations are dependent on body fat distribution in obese patients, Int. J. Obes. Relat. Metab. Disord. 24 (9) (2000) 1139–1144.

[31] M.T. Guagnano, E. Ballone, V. Colagrande, V.R. Della, M.R. Manigrasso, D. Merlitti, et al, Large waist circumference and risk of hypertension, Int. J. Obes. Relat. Metab. Disord. 25 (2001) 1360–1364.

[32] S.R. Mahadik, Association between adipocytokines and insulin resistance in Indian hypertensive patients, Indian Heart J. 64 (2012) 35–39.

[33] N.K. Yadav, C. Thanpari, M.K. Shrewastwa, R.K. Mittal, Comparison of lipid profile in type-2 obese diabetics and obese non-diabetic individuals. A Hospital Based Study from Western Nepal. Kathmandu Univ Med J (KUMJ), vol. 10, 2012, pp. 44-47.

[34] J.S. Thakur, S. Bisht, Blood Lipid Profile of Obese and Non-obese Sedentary College Men, VSRD-TNTJ, vol. 1, 2010, pp. 26–29.
[35] A. Nagila, M. Bhatt, B. Poudel, P. Mahato, D. Gurung, S. Prajapati, et al, Thyroid stimulating hormone and its correlation with lipid profile in the obese Nepalese population, J. Clin. Diagn. Res. 2 (2008) 932–937.
[36] P. Samatha, M. Venkateswarlu, Prabodh V. Siva, Lipid profile levels in type 2 diabetes mellitus from the tribal population of Adilabad in Andhra Pradesh, India, J. Clin. Diagn. Res. 6 (2012) 590–592.
[37] E.S. Idogun, E.I. Unuigbe, P.S. Ogunro, O.T. Akinola, A.A. Famodu, Assessment of the serum lipids in Nigerians with type 2 diabetes mellitus complications, Pak. J. Med. Sci. (Part 1) 23 (2007) 708–712.
[38] J.P. Després, I. Lemieux, Abdominal obesity and metabolic syndrome, Nature 444 (2006) 881–887.