Serum Apelin-12 Concentration in Saudi Obese Middle-Aged Men

Rowyda Nawwaf Al-Harithy (PhD)

Department of Biochemistry, King Abdul-Aziz University, Jeddah, Saudi Arabia
dr.alharithy@gmail.com, ralharithy@kau.edu.sa

Abstract: Apelin-12 is one of the most potent forms of apelin, an adipocytokine that might contribute to the decrease in body weight. The aim of this project was to compare the level of serum apelin-12 in obese and non-obese middle-aged men and to assess the relationship of serum apelin-12 concentration with anthropometric parameters and lipid profile. In this cross-sectional study, 150 obese men (body mass index (BMI) $\geq 30 kg/m^2$) of age range between 35-56 years were selected. For the purpose of comparison, 140 non-obese age-matched men (BMI $\leq 25 kg/m^2$) were chosen as a control group. Anthropometric parameters were measured and peripheral blood samples were obtained for biochemical assays. Serum apelin levels were determined by enzyme-linked immunosorbent assay (ELISA). The data showed that serum apelin-12 levels were not significantly (P=0.48) different between the obese and the non-obese men. The serum apelin-12 levels did not correlate with the anthropometric parameters and the lipid profile in the two groups. The finding of this research suggests that serum apelin-12 concentration is not involved in the pathological mechanism of obesity in middle-aged men.
[Rowyda Nawwaf Al-Harithy. **Serum Apelin-12 Concentration in Saudi Obese Middle-Aged Men.** *Life Sci J* 2014;11(10):1113-1117]. (ISSN:1097-8135). http://www.lifesciencesite.com. 167

Key words: Apelin-12 level, obese, middle-age men, Saudi.

1. Introduction

Adipose tissue is vital for the human life. Its excessive accumulation determines the development of obesity and the obesity-related diseases, including type II diabetes, atherosclerosis and its complications, osteoarthritis, rheumatoid arthritis, and cancer (Schelbert, 2009). Although the causes and mechanisms involved in the diseases that are linked to obesity are not fully understood, imbalance in the production of adipokines contributes to the development of metabolic disorders (Castan-Laurell et al., 2005; Lago et al., 2007; Lago et al., 2009; Wozniak et al., 2009; Jung and Choi, 2014).

Apelin is a biologically active peptide that has been identified in 1998 as a member of the adipokines (Tatemoto et al., 1998). The peptide was isolated from bovine stomach extracts and apelin mRNA is highly expressed in various tissues, including adipose tissue (Habata et al., 1999; O'Carroll et al., 2000; Kawamata et al., 2001; Kasai et al., 2004; Xie et al., 2006; Shirasuna et al., 2008). Therefore, its tissue distribution suggests that apelin signaling is involved in a broad range of physiological functions. A group of researchers very recently have identified plasma apelin as a novel biomarker for predicting type II diabetes in men (Ma et al., 2014). In regard to the up-regulation of apelin, insulin is considered one of the main regulators of apelin production in adipocytes (Boucher et al., 2005). The apelin levels are reported to increase in association with insulin resistance and hyperinsulinemia (Boucher et al., 2005; Bełtowski, 2006). Other factors positively regulate apelin expression in adipocytes are tumor necrosis factor alpha (TNF-α) and lipopolysaccharide (Daviaud et al., 2006; Geurts et al., 2011). On the other hand, apelin expression was found to participate in regulation of blood pressure, cardiac contractility, fluid balance and stimulation of adrenocorticotropic hormone (ACTH) release by the pituitary (Szokodi et al., 2002; Taheri et al., 2002; Reaux-Le Goazigo et al., 2007; Zhu et al., 2013). In humans, apelin is encoded by the apelin gene (*APLN*) that is located on chromosome Xq25–26.1 and encodes a prepropeptide of size 77-amino acid that is processed into isoforms of varying lengths (Tatemoto et al., 1998; Lee et al., 2000; De Mota et al., 2004; Miettinen et al., 2007; Azizi et al., 2008). To date, 46 fragments of active apelin peptides have been identified from apelin-55 to apelin-12 (Mesmin et al., 2011).

The apelin pre-proprotein peptide, apelin-12, is one of the most potent C-terminal fragments of the polypeptide that possesses a high affinity to apelin receptor (*APLNR*) (Langelaan and Rainey, 2009). It has been shown that apelin-12 is involved in lowering blood pressure via a nitric oxide-dependent mechanism, and proposed to be involved in the pathogenesis of childhood atopic asthma (Tatemoto et al., 2001; Machura et al., 2013a). It can serves as an excellent indicator to distinguish children with atopic dermatitis from those without disease (Machura et al., 2013b). It is also involved in the upregulation of cardiac antioxidant defense systems and attenuation of lipid peroxidation (Pisarenko et al., 2014). Tasci and his group have also found that plasma apelin-12 was lower in patients with elevated LDL-C (Tasci et al., 2007).

The studies cited above indicate the importance of apelin-12. Nowadays there are an increasing number of studies assessing apelin in an attempt to clarify its physiological role in obesity. Despite the notable interest, there are still many unknowns, and, in some cases, opposite results have been described. In the literature, the knowledge about the role of apelin-12 in the pathological mechanism of obesity is not sufficient enough in middle-aged men. Unfortunately, very few data exist on differences in plasma concentrations of apelin-12 between obese and non-obese middle-aged men. In the present study, the aim was looking for potential differences in serum apelin-12 concentrations between obese and non-obese middle-aged men. Additionally, the relationship of serum apelin-12 concentrations with anthropometric parameters and lipid profile was assessed.

2. Material and methods

Subjects

In this cross-sectional study, 150 randomly selected middle age obese men (body mass index (BMI) $\geq$ 30kg/m^2) were enrolled. For the purpose of comparison of the serum apelin levels, 140 non-obese age-matched men (BMI $\leq$ 25kg/m^2) were selected as a control group. Both groups were chosen from subjects who visited King Abdul Aziz Hospital; Jeddah, for annual health examinations. All volunteers were of Saudi origins. All subjects underwent complete physical examinations and routine biochemical analysis of blood. None of the volunteers had been on any medication treatment or symptoms of a recent illness. All the volunteers had no history of any clinically significant medical condition. Subjects with diabetes, high blood pressure, and endocrine disorders were excluded. All participants gave their informed consent before enrollment in the study. This study approved by the Ethic Committee of the King Abdul-Aziz University (KAU).

Anthropometric 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. The BMI was calculated as weight (kg) divided by height in meters squared (m^2). 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.

Measurement of serum apelin-12 level

Blood samples were collected after overnight fasting for more than 12 hours. Serum apelin-12 concentrations were determined by using enzyme-linked immunosorbent assay (ELISA) specific for apelin-12 (Human Apelin-12 ELISA Kit. Phoenix Pharmaceuticals, Belmont, CA) Intra- and inter-assay CVs were 5%, and 14%, respectively. Duplicate measurements were performed in a single experiment. The apelin-12 concentration was evaluated based on standard curve carried out for serial dilution available in kit standards. Absorbance was measured using spectrophotometer (µQuant, Microplate Reader, Bio-Tek, USA) at 450nm wavelengths.

Determination of lipid profile

All lipid profile parameters were calculated automatically colorimetric test on Dimension Clinical chemistry system (Model: Dimension Xpand Plus, Dade Behring, serial number: 2004080721, Germany).

Table I. Physical and chemical characteristics of the obese and the non-obese middle-aged men

Variables	Obese group (BMI$\geq$ 30 kg/m^2) N=150	Non-obese group (BMI$\leq$ 25kg/m^2) N=140	*P*
Age (year)	48.3±9.7	46.0±7.8	0.51
Height (cm)	168.5±6.2	166.2±5.4	0.69
Weight (kg)	98.9±8.3	65.1±6.2	0.0001**
BMI (kg/m^2)	35.1±3.2	22.1±2.3	0.0001**
WC (cm)	77.1±31.5	67.2±27.7	0.37
HC (cm)	85.5±35.4	65.5±31.3	0.25
WHR	0.90±0.9	1.03±0.9	0.29
Cholesterol (mg/dl)	176.8±40.4	168.2±28.1	0.51
Triglyceride (mg/dl)	203.1±98.6	118.6±62.7	0.15
HDL (mg/dl)	43.1±8.1	43.6±5.8	0.57
LDL (mg/dl)	95.4±31.2	104.1±22.5	0.37
Total lipid (mg/dl)	7.19±0.5	7.18±0.4	0.56
Apelin (ng/ml)	0.61±0.6	0.39±0.2	0.48

Statistical analysis

All values are presented as mean± SD. Comparisons between groups were made using Mann-Whitney test. Correlations were analyzed by Spearman's rank correlation coefficient. For all analysis, P-value < 0.05 was considered as statistically significant. All statistical analyses were carried out using the SPSS for Windows V16.0 (SPSS Inc., Chicago, IL, USA).

Mann Whitney U test was used for the analysis.

Values were represented as the mean ± standard deviation.

WHR: waist-to- hip ratio; BMI: body mass index; WC: waist circumference; HC: hip circumference; HDL: High-density lipoprotein; LDL: Low-density lipoprotein.

**P: Highly significant.

3. Results

Characteristics of the 150 obese males and the 140 control subjects are presented in Table 1. Both groups were similar in age (P=0.51), height (P= 0.69), waist circumference (WC) (P=0.37), hip circumference (HC) (P=0.25), and waist-to-hip ratio (WHR) (P=0.29). They were significantly different in weight (P= 0.0001) and BMI (P=0.0001). There was no significant difference (P= 0.48) in the mean value of apelin-12 levels between the two groups (Table 1). Correlation between serum apelin-12 to the anthropometric and lipid profile parameters of the obese and the non-obese middle-aged men showed no correlation with any of the anthropometric parameters and the lipid profile.

4. Discussion

The relationship between the serum apelin-12 and obesity could be an important determinant of the pathophysiological mechanism involved in the metabolic syndrome. The present study analyzed the concentration of apelin-12 in obese and non-obese Saudi middle-aged men. The aim was to test the hypothesis that circulating apelin-12 is involved in the pathological mechanism of obesity in middle-aged men. In contrast to the hypothesis, there was no significant difference in the mean value of apelin-12 levels between the obese and the non-obese men.

Apelin has been proposed as a novel beneficial adipokine that is related to insulin resistance, cardiovascular risk factors, and obesity. Therefore, apelin-12 expected to be related to body adiposity. Although apelin has been linked to obesity in few studies (Boucher et al., 2005; Heinonen et al., 2005; Li et al., 2006; Erdem et al., 2008; Soriguer et al., 2009), data were controversial and the different forms for apelin present in the circulation were not identified in most of the analysis. Up to date, data about the behavior of apelin-12 in obese middle-aged men are limited. Only few studies evaluating apelin-12 in children and obese adults with obesity related diseases were conducted. Heinonen and his group investigated basal plasma levels of apelin in morbid obese patients (BMI=48+/-1 kg/m2n=32) undergoing gastric banding surgery and observed significantly higher serum apelin concentrations compared with normal-weight subjects (Heinonen et al., 2005). Boucher and his team investigated the influence of obesity and hyperinsulinemia in plasma apelin levels in eight moderately obese and nine-age matched male controls. They reported higher plasma apelin and insulin levels in obese adult males and suggested that the rise in plasma insulin could promote an increase in blood concentrations of apelin (Boucher et al., 2005). Subsequent work recruited a small number (54) of mixed-gender (16 type II Diabetes, 26 obese non-diabeteic, and 12 non-obese controls) and found no evidence of a relationship between apelin and obesity (Soriguer et al., 2009). They demonstrated that obesity is not the main determinant of the rise in apelin levels. They suggested that the association between apelin levels, glucose concentrations and insulin sensitivity presents a point that apelin might play a role in the pathogenesis of diabetes. The data of the present study is in an agreement with the above results, in regard to the fact that obesity is not the cause of the rise in apelin levels. It is possible that the obese group in the present study might not have developed the metabolic consequences of obesity and they were metabolically healthy obese. Therefore, there was no significant difference in the mean value of apelin-12 levels between the obese and the non-obese men. The present study supports the suggestion that obesity is not the cause of the rise in apelin levels and that the concentration of apelin-12 is not involved in the pathological mechanism of obesity in middle-aged men.

Apelin levels in adults showed an association with all of the features of the metabolic syndrome, including obesity (Boucher et al., 2005; Heinonen et al., 2005), diabetes (Li et al., 2006; Erdem et al., 2008), and insulin resistance (Glassford et al., 2007; Karbek et al., 2014). Although the current study revealed that the levels of apelin-12 did not correlate significantly with anthropometric parameters in the non-obese and the obese middle-aged men, the result of the present work confirms lack of relationship between serum apelin-12 levels and obesity. Other finding of the current study involved lipid profile. In the two groups, serum apelin-12 level did not correlate with the lipid profile, as shown in other studies. A group searched plasma apelin levels in patients with elevated low-density lipoprotein (LDL)-cholesterol having no additional disorder. Thirty-three patients with hypercholesterolemia and 50 age-, sex-, and body mass index-matched healthy controls were evaluated for their apelin plasma. It was found that apelin is decreased in non-obese, non-diabetic and normotensive patients with elevated LDL-cholesterol (Tasci et al., 2007). Angelova et al., work revealed significant correlation of apelin and LDL-C in men with metabolic syndrome (Angelova et al., 2014). The results of the above studies indicate that the change to apelin level was due to abnormal lipid profile. In this study, there was no change in the level of apelin-12 in

the obese group. There was no relationship of serum apelin-12 level to the anthropometric parameter and the lipid profile. The gained meaning is that there is no relationship between apelin-12 and obesity in metabolically healthy middle-aged men. The present study has some limitations. The number of the subjects studied was relatively small and the study was a cross-sectional and not prospective cohort study.

5. Conclusion

The finding of no significant difference in the mean value of apelin-12 levels between the obese and the non-obese men suggests that the concentration of apelin-12 is not involved in the pathological mechanism of obesity in middle-aged men. In addition, the study revealed that the levels of apelin-12 did not correlate significantly with the anthropometric parameters and the lipid profile. These results confirm the lack of the relationship between serum apelin-12 levels and obesity in the obese middle-aged men. Further prospective study is needed to clarify the role of apelin-12 in the pathological mechanism of obesity.

Conflict of interest statement

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Correspondence to:
Dr. Rowyda Nawwaf Al-Harithy
Molecular Geneticist
Associate Professor
P. O. Box 40288 Jeddah 21499,
Kingdom of Saudi Arabia.
Fax: 00966-12-6393640
Emails: ralharithy@kau.edu.sa
dr.alharithy@gmail.com

References

1. Angelova P, Kamenov Z, Tsakova A. Apelin and Testosterone Levels in Men with Metabolic Syndrome. Open Journal of Endocrine and Metabolic Diseases 2014; 4:35-43.
2. Azizi M, Iturrioz X, Blanchard A, Peyrard S, De Mota N, Chartrel N, et al. Reciprocal regulation of plasma apelin and vasopressin by osmotic stimuli. J Am Soc Nephrol 2008; 19:1015–24.
3. Bełtowski J. Apelin and visfatin: unique "beneficial" adipokines upregulated in obesity? Med Sci Monit 2006; 12(6): RA112-19.
4. Boucher J, Masri B, Daviaud D, Gesta S, Guigné C, Mazzucotelli A, et al. Apelin, a newly identified adipokine up-regulated by insulin and obesity. Endocrinology 2005; 146(4): 1764–71.
5. Castan-Laurell I, Boucher J, Dray C, Daviaud D, Guigné C, Valet P. Apelin, a novel adipokine overproduced in obesity: friend or foe? Mol Cell Endocrinol 2005; 245 (1-2): 7–9.
6. Daviaud D, Boucher J, Gesta S, Dray C, Guigne C, Quilliot D, et al. TNFalpha up-regulates apelin expression in human and mouse adipose tissue. FASEB J 2006; 20(9): 1528–30.
7. De Mota N, Reaux-Le Goazigo A, El Messari S, Chartrel N, Roesch D, Dujardin C, et al.Apelin, a potent diuretic neuropeptide counteracting vasopressin actions through inhibition of vasopressin neuron activity and vasopressin release. Proc Natl Acad Sci USA 2004; 101:10464–69.
8. Erdem G, Dogru T, Tasci I, Sonmez A, Tapan S. Low plasma apelin levels in newly diagnosed type 2 diabetes mellitus. Exp Clin Endocrinol Diabetes 2008; 116(5): 289–92.
9. Geurts L, Lazarevic V, Derrien M, Everard A, Van Roye M, Knauf C, et al. Altered gut microbiota and endocannabinoid system tone in obese and diabetic leptin-resistant mice: impact on apelin regulation in adipose tissue. Front Microbiol 2011; 2: 149.
10. Glassford AJ, Yue P, Sheikh AY, Chun HJ, Zarafshar S, Chan DA, et al. HIF-1 regulates hypoxia- and insulin-induced expression of apelin in adipocytes. Am J Physiol Endocrinol Metab 2007; 293: E1590–6.
11. Habata Y, Fujii R, Hosoya M, Fukusumi S, Kawamata Y, Hinuma S, et al. Apelin, the natural ligand of the orphan receptor APJ, is abundantly secreted in the colostrum. Biochim Biophy Acta 1999; 1452(1):25–35.
12. Heinonen MV, Purhonen AK, Miettinen P, Pääkkönen M, Pirinen E, Alhava E, et al. Apelin, orexin-A and leptin plasma levels in morbid obesity and effect of gastric banding. Regul Pept 2005; 130(1-2): 7–13.
13. Jung UJ, Choi MS. 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 2014, 15, 6184-6223.
14. Karbek B, Bozkurt NC, Topaloglu O, Aslan MS, Gungunes A, Cakal E, et al. Relationship of vaspin and apelin levels with insulin resistance and atherosclerosis in metabolic syndrome. Minerva Endocrinol 2014; 39(2):99-105.
15. Kasai A, Shintani N, Oda M, Kakuda M, Hashimoto H, Matsuda T, et al. Apelin is a novel angiogenic factor in retinal endothelial cells. Biochem Biophys Res Commun 2004; 325(2): 395–400.
16. Kawamata Y, Habata Y, Fukusumi S, Hosoya M, Fujii R, Hinuma S, et al. Molecular properties of apelin: tissue distribution and receptor binding. Biochim Biophys Acta 2001; 1538(2-3): 162–71.

17. Lago F, Dieguez C, Gómez-Reino J, Gualillo O. Adipokines as emerging mediators of immune response and inflammation. Nat Clin Pract Rheumatol 2007; 3(12):716–24.
18. Lago F, Gómez R, Gómez-Reino JJ, Dieguez C, Gualillo O. Adipokines as novel modulators of lipid metabolism. Trends Biochem Sci 2009; 34(10): 500–10.
19. Langelaan DN, Rainey JK. Headgroup-dependent membrane catalysis of apelin-receptor interactions is likely. J Phys Chem 2009; B113: 10465–10471.
20. Lee DK, Cheng R, Nguyen T, Fan T, Kariyawasam AP, Liu Y, et al. Characterization of apelin, the ligand for the APJ receptor. J Neurochem 2000; 74(1): 34–41.
21. Li L, Yang G, Li Q, Tang Y, Yang M, Yang H, et al. Changes and relations of circulating visfatin, apelin, and resistin levels in normal, impaired glucose tolerance, and type 2 diabetic subjects. Exp Clin Endocrinol Diabetes 2006; 114(10): 544-548.
22. Ma WY, Yu TY, Wei JN, Hung CS, Lin MS, Liao YJ, et al. Plasma apelin: A novel biomarker for predicting diabetes. Clin Chim Acta 2014; 435:18-23.
23. Machura E, Ziora K, Ziora D, Swietochowska E, Krakowczyk H, Halkiewicz F, et al. Serum apelin-12 level is elevated in schoolchildren with atopic asthma. Respir Med 2013a; 107(2): 196–201.
24. Machura E, Szczepanska M, Ziora K, Ziora D, Swietochowska E, Barc-Czarnecka M, et al. Evaluation of adipokines: apelin, visfatin, and resistin in children with atopic dermatitis. Mediators Inflamm 2013b; 2013: Article ID 760691.
25. Mesmin C, Fenaille F, Becher F, Tabet JC, Ezan E. Identification and characterization of apelin peptides in bovine colostrum and milk by liquid chromatography-mass spectrometry. J Proteome Res 2011; 10(11): 5222–5231.
26. Miettinen KH, Magga J, Vuolteenaho O, Vanninen EJ, Punnonen KR, Ylitalo K, et al. Utility of plasma apelin and other indices of cardiac dysfunction in the clinical assessment of patients with dilated cardiomyopathy. Regul Pept 2007; 140:178–84.
27. O'Carroll AM, Selby TL, Palkovits M, Lolait SJ. Distribution of mRNA encoding B78/apj, the rat homologue of the human APJ receptor, and its endogenous ligand apelin in brain and peripheral tissues. Biochim Biophy Acta 2000; 1492 (1): 72–80.
28. Pisarenko OI, Lankin VZ, Konovalova GG, Serebryakova LI, Shulzhenko VS, Timoshin AA, et al. Apelin-12 and its structural analog enhance antioxidant defense in experimental myocardial ischemia and reperfusion. Mol Cell Biochem 2014; 391(1-2): 241-50.
29. Reaux-Le Goazigo A, Alvear-Perez R, Zizzari P, Epelbaum J, Bluet-Pajot MT, Llorens-Cortes C. Cellular localization of apelin and its receptor in the anterior pituitary: evidence for a direct stimulatory action of apelin on ACTH release. Am J Physiol Endocrinol Metab 2007; 292(1): E7–15.
30. Schelbert KB. Comorbidities of obesity. Prim Care 2009; 36(2): 271–85.
31. Shirasuna K, Shimizu T, Sayama K, Asahi T, Sasaki M, Berisha B, et al. Expression and localization of apelin and its receptor APJ in the bovine corpus luteum during the estrous cycle and prostaglandin F2alpha-induced luteolysis. Reproduction 2008; 135(4): 519–25.
32. Soriguer F, Garrido-Sanchez L, Garcia-Serrano S, Garcia-Almeida JM, Garcia-Arnes J, Tinahones FJ, et al. Apelin levels are increased in morbidly obese subjects with type 2 diabetes mellitus. Obes Surg 2009; 19(11): 1574-80.
33. Szokodi I, Tavi P, Földes G, Voutilainen-Myllylä S, Iives M, Tokola H, et al. Apelin, the novel endogenous ligand of the orphan receptor APJ, regulates cardiac contractility. Circ Res 2002; 91(5): 434–44.
34. Taheri S, Murphy K, Cohen M, Sujkovic E, Kennedy A, Dhillo W, et al. The effects of centrally administered apelin-13 on food intake, water intake and pituitary hormone release in rats. Biochem Biophys Res Commun 2002; 291(5): 1208–12.
35. Tasci I, Dogru T, Naharci I, Erdem G, Yilmaz MI, Sonmez A, et al. Plasma apelin is lower in patients with elevated LDL-cholesterol. Exp Clin Endocrinol Diabetes 2007; 115:428–32.
36. Tatemoto K, Hosoya M, Habata Y, Fujii R, Kakegawa T, Zou MX, et al. Isolation and characterization of a novel endogenous peptide ligand for the human APJ receptor. Biochem Biophys Res Commun 1998; 251(2): 471–76.
37. Tatemoto K, Takayama K, Zou MX, Kumaki I, Zhang W, Kumano K, et al. The novel peptide apelin lowers blood pressure via a nitric oxide-dependent mechanism. Regulatory Peptides 2001; 99:87–92.
38. Wozniak SE, Gee LL, Wachtel MS, Frezza EE. Adipose tissue: the new endocrine organ? a review article. Dig Dis Sci 2009; 54(9): 1847–56.
39. Xie H, Tang SY, Cui RR, Huang J, Ren XH, Yuan LQ, et al. Apelin and its receptor are expressed in human osteoblasts. Regul Pept 2006; 134(2-3): 118-25.
40. Zhu P, Huang F, Lin F, Yuan Y, Chen F, Li Q. Plasma apelin levels, blood pressure and cardiovascular risk factors in a coastal Chinese population. Ann Med 2013; 45(7): 494-8.

9/28/2014