KMJ

THE KUWAIT MEDICAL JOURNAL

Ref. AKA/KCB-2/x005

Date October 18, 2005

Dr. Rowyda N. Al-Harithy·
Assistant Professor
Department of Biochemistry
P.O. Box 40288
Jeddah 21499
KINGDOM OF SAUDI ARABIA

Sub.: **Acceptance of Manuscript for Publication**

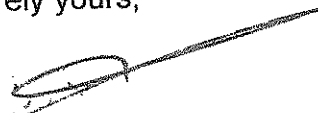**Re:** **Manuscript No. KMJ-034-005**

Dear Dr. Al-Harithy,

I am pleased to inform you that your manuscript titled **"Strong Association between serum levels of Leptin and Resistin in Non-Diabetic Saudi Adult Women"** has been accepted for publication.

It will be published under the section **'Original Article'** in one of the forthcoming issues of KMJ.

If need be, we will send you a copy of the galley proof which is to be returned to us within a day at the time of its publication.

We thank you once again for your support and cooperation.

Sincerely yours,

Dr. Adel Khader Ayed
Editor

Strong association between serum levels of leptin and resistin in non-diabetic Saudi adult women

Rowyda . N . Al-Harithy*

Department of Biochemistry, King Abdul-Aziz University, Jeddah, Saudi Arabia.

Running title: Leptin, resistin, waist, hip and Saudi women

*Correspondence: Dr. Rowyda N. Al-Harithy, Assistant professor, Biochemistry Department, P.O.Box 40288 Jeddah 21499, Kingdom of Saudi Arabia.

(Fax. 966-2-6393640. Mobile. 966-55444238. E-mail: ralharithy@yahoo.com)

Abstract

Objective: To evaluate the relationship between serum leptin and resistin concentrations in non-diabetic Saudi adult women.

Methods: The present study included 104 (42 obese and 62 non-obese) healthy non-diabetic Saudi adult women. The volunteers covered a range of age between 20 to 40 years and were recruited from among employees and students at King Abdul-Aziz University (Jeddah). Serum concentrations of leptin and resistin were measured after overnight fasting. Because of proven menstrual fluctuations, leptin levels were determined by collecting blood samples on day 3, 10, 17, and 24 from the beginning of menstruation. Anthropometrics measurements and fasting glucose levels were taken at the time of the collection.

Results: The obese subjects had significantly higher serum leptin (5.28 vs. 3.56ng/ml; $P=0.012$) and resistin (16.06 vs. 12.95ng/ml; $P=0.0001$) levels than non-obese subjects. The Bivariate correlation analysis between leptin and resistin were highly significant in the obese group ($r=0.784$, $P=0.002$). The findings from the above correlation analysis were further explored using Stepwise Multiple Linear regression analysis with resistin concentrations as the dependent variable; and leptin, waist, hip, height, and weight as the independent variables. The analysis demonstrated that the determinant of resistin concentration is leptin ($r=0.784$, $P=0.002$) in obese women.

Conclusion: The present study confirms the existence of a strong correlation between leptin and resistin also confirms the influence of waist circumference on resistin concentration in the serum of healthy adult Saudi women.

2

Introduction

Obesity has become a major issue because of its links to hypertension, dyslipidemia, type II diabetes and insulin resistance syndrome (1-3). Data emerging over the past several years have shown a worldwide increase in the number of obese people (4-6). In Saudi Arabia the prevalence of obesity among female and, to a lesser extent, among male adults has reached epidemic proportions (7, 8). The mechanisms by which some individuals protect themselves against body weight gain remain poorly understood. Most researchers agree that energy balance and, hence, body weight are regulated phenomena.

The fact that adipose tissue is not only an inert storage depot, but rather a tissue actively secreting proteins, may indicate new factors for regulating whole body metabolism and homeostasis (9,10). The endocrine function of adipose tissue was conclusively established by the discovery of leptin in 1994 (11) and that influenced a conceptual shift leading to increased interest in other adipocyte products as signaling molecules. Resistin is another adipose-derived hormone that was discovered in 2001 by the group associated with Dr. Mitchell A.Lazar from the University of Pennsylvania School of Medicine (12). The two adipocytokines, leptin and resistin, are detectable in human serum and their circulating levels were found to be elevated in proportion to the degree of obesity (13,14).

Leptin, the product of the obese (ob) gene, first gained widespread attention as a satiety signal. Initial studies revealed that the hormone binds to receptors in the hypothalamus and influence the expression of several neuropeptides that regulate energy intake, energy expenditure and food intake. Since then, scientists have demonstrated that leptin's role is not confined to suppressing appetite. Leptin is a master regulator of the human hormone

system. Leptin concentrations were found to be about four-fold higher in obese human than in normal lean subjects (15, 16) and higher in women than in men at a given level of body mass index (BMI) (17, 18). More interesting findings in the studies of women are the significant fluctuations in leptin levels during the mensutrual cycle (19, 20).

Resistin is a 12.5 kDa cystiene-rich peptide that is secreted from adipocytes and present in the circulation (12, 21). In human, studies revealed that serum resistin levels are positively correlated with changes in BMI, body fat, glucose and insulin in obese individuals (22, 23). However, and in contrast with the above-mentioned observations, other studies have cast some doubts on those relationships (24, 25). There are continued uncertainties about the possible relationship between serum concentrations of resistin and obesity. Although resisin seems to be one of promising parameters that could help to elucidate the pathophysiology of the origin of peripheral insulin resistance and its association with obesity, the relevance of resistin and its physiological role in humans remain unknown.

With regard to the fact that the interplay between leptin and resistin is still poorly studied, the aim of the present study was to evaluate the relationship between leptin and resistin in non-diabetic Saudi adult women that are well-matched with respect to age and BMI.

4

Subjects and Methods:

The present study included 104 (42 obese and 62 non-obese) healthy non-diabetic Saudi adult women with regular menstrual cycles (28 ± 1 days), whose characteristics are shown in Table 1. The volunteers covered a range of age between 20 to 40 years and were recruited from among employees and students at King Abdul-Aziz University (Jeddah). Based on a medical history and a fasting blood profile, these subjects were healthy. Non had evidence of diabetes (fasting glucose <5.5mmol/L), and non were taking any medication. All participants gave their informed consent before enrollment in the study. Serum concentrations of leptin, resistin, and glucose were measured after overnight fasting. Because of proven menstrual fluctuations, leptin levels were determined by collecting blood samples on day 3, 10, 17, and 24 from the beginning of menstruation. For resistin, blood samples were drawn only once. Serum leptin (ng/ml) was evaluated in duplicate runs using a commercial Direct ELISA Human leptin kit according to the procedure provided by the company (Diagnostics Biochem Canada Inc). Serum resistin concentration (ng/ml) was evaluated in duplicate using a resistin enzyme immunoassay (ELISA) kit (Phoenix Pharmaceuticals, Inc. USA). The resistin standards used in the assay were 0.05, 0.5, 5, 50, and 500ng/ml. The sensitivity of the assay was 0.79ng/ml, and the intra-assay coefficient of variation was 5%. Blood glucose was measured using One Touch System (Johnson & Johnson, USA).

Anthropometry

Anthropometric measurements, including height, weight, waist (WC) and hip circumferences (HC) were performed as described (26). Body mass index (BMI) was calculated as weight (kg) divided by height 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.

Statistical analyses

Statistics were performed using SPSS for Windows version 10. Descriptive statistics were presented as mean values ± S.D. Correlations were performed using Pearson's correlation coefficient. The Independent-Sample T Test was used to compare means. Stepwise Multiple Regression analysis was also performed to establish the independent associations between variables. A P value of less than 0.05 was considered statistically significant. The values presented in this paper are 2-tailed.

Results:

The obese subjects had significantly higher serum leptin (5.28 vs. 3.56ng/ml; P=0.012) and resistin (16.06 vs. 12.95ng/ml; P=0.0001) levels than non-obese subjects (Table 1). Additionally, the obese subjects had a significant higher body mass index (32.87 vs. 24.84kg/m^2; P=0.0001), waist (94.77 vs. 78.31 cm; P=0.0001), and hip circumferences (117.46 vs. 98.42 cm; P=0.0001) than non-obese women. The analysis also showed that the mean fasting glucose level for obese and lean subjects (3.96 vs.3.85mmol/l) is within the normal non-diabetic range

Pearson Bivariate correlation analyses were performed to assess relationships between serum resistin, leptin, and all other measured parameters in each group (obese and non-obese). In considering the obese group, serum Leptin levels showed a significant correlation with resistin (r=0.784, P=0.002), BMI (r=0.810, P=0.001), weight(r=0.713, P=0.006), and hip circumference (r=0.630, P=0.021). No correlation with WHR, height, waist, and glucose levels was observed. In the same group resistin showed a significant correlation with leptin, BMI (r=0.876, P=0.0001), waist (r=0.509, P= 0.049), and weight (r=0.682, P=0.010). No correlation was observed with WHR, weight, hip, and glucose (Table 2). The correlation between leptin and resistin was no longer significant after adjusting for BMI, indicating that this association is due to confounding by BMI. Further adjustment for waist, hip, and WHR did not alter the significant of the correlation between leptin and resistin (Table 3).

In lean subjects, Leptin showed a correlation with BMI (r=0.457P=0.010), hip (r=0.379, P-0.035), and weight (r=0.501, P=0.004). Resistin showed a correlation with waist

(r=0.422, P=0.018), BMI (r=0.447, P=0.012), and WHR (r=0.414, P=0.021). No correlation was shown between leptin and resistin in the lean group (r=0.249, P=0.176).

The findings from the above Bivariate correlation analysis were further explored using Stepwise Multiple Linear regression analysis with resistin concentrations as the dependent variable; and leptin, waist, hip, height, and weight were used as independent variables. The analysis demonstrated that leptin is a major determinant of serum resistin concentrations (r=0.784, P=0.002) in obese women (Table 4).

Discussion:

This study reports for the first time the relationship of leptin and resistin in adult obese Saudi women. There was a highly significant correlation between leptin and resistin concentrations. In addition to this observation, there was a high percentage of prediction for resistin.

It has been discovered over the past few years that adipocytes synthesize and release a variety of peptide and non-peptide compounds that allow a crosstalk of adipose tissue with other organs as well as within the adipose tissue. Although, leptin and resistin are biological active molecules that have been shown to increase in obese subjects and are proposed to link obesity with diabetes, their relationship and interplay is still poorly understood.

A previous study examined 124 well controlled type II diabetic individuals, with clinical signs of inflammatory disease of the respiratory tract and healthy volunteers in order to compare resistin levels between the three groups. Although, resitin concentration in the serum of the patients with severe inflammatory disease was higher than in healthy population or in the well controlled subjects with type II diabetes, no significant correlations between leptin and resistin were found. In healthy individuals, a strong correlation was found between the concentrations of leptin and resistin. With regard to this fact, the authers assume that in healthy individuals there exists a metabolic regulation in which both factors are involved (27). The data of the present study confirms the results of the previous study and suggests an existence of cross-talk regulation between leptin and resistin levels.

Another finding of this study is that leptin circulating levels showed a significant correlation to BMI and hip measurements but not to waist circumference, whereas resistin levels showed a significant relationship to BMI and waist circumference and did not relate to hip measurements. Previous studies reported that the subcutaneous fat tissues is the major site of leptin production and resistin is expressed at higher levels in visceral rather than subcutaneous fat depots in humans (28, 29). In addition, Kok *et al* indicated that waist circumference is a valid index of visceral fat accumulation and can therefore be used as an indicator of health risks associated with visceral obesity (30). Thus, the present data adds to a growing body of evidence that indicates that in human resistin is highly expressed in human visceral fat compartments and is more strongly influenced by waist measurements. This study also suggests that hip circumference is a valid index of subcutaneous fat accumulation and can be used as an indicator of leptin level in healthy population.

Among the strengths of the current study is the result of the Stepwise Multiple Linear regression analysis with resistin as the dependent value, which excluded body mass index in the statistical model, which determined that leptin was the significant predictor of resistin level within this cohort. Leptin explained 78% of the resistin variance in healthy Saudi women. This finding may be in part explained by the fact that both hormones are produced almost exclusively by adipocytes. Another suggestion is that leptin production from subcutaneous adipose tissue might contribute to the development of visceral fat which in consequence, produces resistin. This indicates that in human subjects, resistin is associated more closely with leptin .

Conclusion :

This study confirmed the following:

1. Existence of a strong correlation between leptin and resistin concentrations in the serum of healthy adult Saudi women.

2. Influence of waist circumference on resistin concentration.

This study suggested the following:

1. A direct or indirect crosstalk between resistin and leptin.

2. Influence of hip circumferences in leptin levels

2. Leptin plays a role in predicting resistin levels in healthy adult Saudi women.

Research into the interplay role of resistin and leptin in human biology is still in its initial stage. More information regarding the interaction of adipose hormones in human health and disease is needed to be study.

References

1. freedman DS, Dietz WH, Srinivasan SR, Berenson GS. The relation of overweight to cardiovascular risk factors among children and adolescents: the Bogalusa Heart Study. Pediatrics 1999; 103:1175-1182

2. Sinha R, Fisch G, Teague B, Tamborlane WV, Bsnysd B, Allen K, Savoye M, Rieger V, Taksali S, Barbetta G, Sherwin RS, Caprio S. Prevalence of impaired glucose tolerance among children and adolescents with marked obesity. N Engl J Med 2002; 346:802-810.

3. Roth L, Qiang X, Marban SL, Redelt H, Lowell BC. The obesity pandemic: where have we been and where are we going? Obes Res 2004 Nov; 2:88S-101S.

4. Filozof C, Gonzalez C, Sereday M, Mazza C, Braguinsky J. Obesity prevalence and trends in Latin-American countries. Obes Rev 2001; 2:99-106.

5. Ogden CL, Flegal KM, Carroll MD, Johnson CL. Prevalence and trends in overweight among US children and adolescents, 1999-2000. JAMA 2002; 288:1728-1732

6. Wang Y, Monteiro C, Popkin BM. Trends of obesity and underweight in older children and adolescents in the United States, Brazil, China, and Russia. Am J Clin Nutr 2002; 75:971-977

7. Al-Nuaim AR, Al-Rubeaan K, Al-Mazrou Y, Al-Attas O, Al-Daghari N, Khoja T. High prevalence of overweight and obesity in Saudi Arabia. Int J Obes Relat Metab Disord. 1996 Jun; 20:547-52.

8. El-Hazmi MA, Warsy AS. Prevalence of overweight and obesity in diabetic and non-diabetic Saudis. East Mediterr Health J. 2000 Mar-May; 6: 276-282.

9. Havel PJ. Control of energy homeostasis and insulin action by adipocyte hormones: leptin, acylation stimulating protein, and adiponectin. Curr Opin Lipidol 2002; 13:51-59.

10. Steppan CM, Lazar MA. Resistin and obesity-associated insulin resistance. Trends Endocrinol Metab. 2002; 13:18-23.

11. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. Nature 1994; 372:425-432.

12. Steppan CM, Brown EJ, Wright CM, Bhat S, Banerjee RR, Dai CY, Enders GH, Silberg DG, Wen X, Wu GD, Lazer MA. A family of tissue-specific resistin-like molecules. Proc Natl Acad Sci U S A 2001; 98; 502-506.

13. Degawa-Yamauchi M, Bovenkerk JE, Juliar BE, Watson W, Kerr K, Jones R, Zhu Q, Considine RV. Serum resistin (FIZZ3) protein is increased in obese humans. J Clin Endocrinol Metab. 2003; 88:5452-5455.

14. Yannakoulia M, Yiannakouris N, Bluher S, Matalas A, Klimis-Zacas D, Mantzoros CS. Body fat mass and macronutrient intake in relation to circulating soluble leptin receptor, free leptin index, adiponectin, and resistin concentrations in healthy humans. J Clin Endocrinol Metab. 2003; 88:1730-1736.

15. McConway MG, Johnson D, Kelly A, Griffin D, Smith J, Wallace AM. Differences in circulating concentrations of total, free and bound leptin relate to gender and body composition in adult humans. Ann Clin Biochem. 2000; 37:717-723

16. Al-Harithy RN. Relationship of leptin concentration to gender, body mass index and age in Saudi Adults. SMJ. 2004; 25:10086-1090.

17. Haffner SM, Stem MP, Miettinen H, Wei M, Gingerich RL,. Leptin concentrations in diabetic and non-diabetic Mexican Americans. Diabetes. 1996; 45:822-824.

18. Saad MF, Damani S, Gingerich RL, Riad-Gabriel MG, Khan A, Boyadjian R, Jinagouda SD, el-Tawil K, Rude RK, Kamdar V. Sexual dimorphism in plasma leptin concentration. J Clin Endocrinol Metab. 1997; 82: 579-584.

19. Riad-Gabriel MG, Jinagouda SD, Sharma A, Boyadjian R, Saad MF. Changes in plasma leptin during the menstrual cycle. Eur J Endocrinol 1998; 139:528-531.

20. Teirmaa T, Luukkaa V, Rouru J, Koulu M, Huupponen R. Correlation between circulating leptin and luteinizing hormone during the menstrual cycle in normal-weight women. Eur J Endocrinol 1998; 139:190-194.

21. Bing C, Gomez-Ambrosi J, Zabalegui N, Williams G, Trayhum P. Resistin and RELM-α gene expression in white adipose tissue of lactating mice. Biochem Biophys Res Commun 2002; 296: 458-462.

22. Azuma K, Katsukawa F, Oguchi S, Murata M, Yamazaki H, Shimada A, Saruta T. Correlation between serum resistin level and adiposity in obese individuals. Obes Res 2003;11: 997-1001.

23. McTeman PG, McTeman CL, Chetty R, Jenner K, Fisher FM, Lauer MN, Crocker J, Barnett AH, Kumar S. Increased resistin gene and protein expression in human abdominal adipose tissue. J Clin Endocrinol Metab 2002; 87: 2407.

24. Savage DB, Sewter CP, Klenk ES, Segal DG, Vidal-Puig A, Considine RV, O'Rahilly S. Resistin/fizz3 expression in relation to obesity and peroxisome proliferators-activated receptor-γ action in humans. Diabetes 2001; 50: 2199-2202.

25. Lee JH, Chan JL, Yiannakouris N, Kontogianni M, Estrada E, Seip R, Orlova C, Mantzoros CS. Circulating resistin levels are not associated with obesity of insulin resistance in humans and are not regulated by fasting or leptin administration: cross-sectional and interventional studies in normal, insulin-resistant, and diabetic subjects. J Clin Endocrinol Metab 2003; 88: 4848-4856.

26. Huang KC, Lin WY, Lee LT, Chen CY, Lo H, Hsia HH, Liu IL, Shau WY, Lin RS. Four anthropometric indices and cardiovascular risk factors in Taiwan. Int J Obes Relat Metab Disord 2002; 26: 1060-1068.

27. Stejskal D, Adamovska S, Bartek J, Jurakova R, Proskova J. Resistin-concentrations in persons with type2 diabetes mellitus and in individuals

with acute inflammatory disease. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub Papers 2003; 147: 63-69.

28. McTeman, PG, McTernan CL, Chetty R, Jenner K, Fisher FM, Lauer MN, Crocker J, Barnet AH, Kumar S. Increased resistin gene and protein expression in human abdominal adipose tissue. J. Clin. Endocriol. Metab 2002; 87:2407-2413.

29. Park KG, Park KS, Kim MJ, Kim HS, Suh YS, Ahn JD, Park KK, Chang YC, Lee IK. Relationship between serum adiponectin and leptin concentrations and body fat distribution. Diabetes Res Clin Pract 2004; 63:135-142.

30. Kok P, Seidell JC, Meinders AE. The value and limitations of the body mass index (BMI) in the assessment of the health risks of overweight and obesity. Ned Tijdschr Geneeskd 2004; 148: 2379-2382.

Table1: Description of study subjects.

Parameters	Non-obese (n=62)	Obese (n=42)	P -value
BMI (kg/m2)	24.84 ± 3.2	32.66 ± 2.67	0.0001
WHR	0.79 ± 0.05	0.81 ± 0.04	NS
Weight (kg)	61.32 ± 7.77	83.50 ± 9.49	0.0001
Height (cm)	158.13 ± 6.16	159.86 ± 6.11	NS
Waist (cm)	78.31± 7.03	94.29 ± 5.85	0.0001
Hip (cm)	98.42 ± 5.13	116.93 ± 5.72	0.0001
Glucose (mmol/l)	3.85 ± 0.61	4.04 ± 0.59	NS
Leptin (ng/ml	3.56 ± 1.65	5.08 ± 2.09	0.012
Resistin (ng/ml)	12.95 ± 3.16	16.21 ± 2.19	0.001

Values were represented as the mean ± SD; the data were statistically analyzed using Independent-Sample T Test. P-value ≤ 0.05 was used as a criterion of significance. P-value ≤ 0.01 was used as a criterion of high significance. Abbreviations: BMI, body mass index; WHR, waist-to-hip ratio; NS, not significant.

Table 2: Correlation coefficients of leptin, resistin, and different variables in obese subjects (n=42).

Varibles	Resistin	Leptin
BMI (kg/m^2)	0.876***	0.810***
WHR	0.259	0.024
Weight (kg)	0.682**	0.713**
Height (cm)	0.019	0.128
Waist (cm)	0.509*	0.422
Hip (cm)	0.424	0.630*
Glucose (mmol/l)	0.003	0.066
Resistin (ng/ml)		0.784**
Leptin (ng/ml)	0.784**	

Statistics were computed by Pearson's correlations
WHR: waist to hip ratio; BMI: body mass index.
*$P<0.05$, **$P<0.01$, ***$P,0.001$

Table 3: Correlation between leptin and resistin after adjusting
for BMI, waist, hip, and WHR in obese subjects.

Variables	R	P
BMI (kg/m²)	0.0134	0.965
Waist (cm)	0.527	0.046
Hip (cm)	0.589	0.034
WHR	0.634	0.020

WHR: waist to hip ratio; BMI: body mass index.

Table 4: Stepwise Multiple Linear regression analysis showing regression coefficients ($\beta\pm$ s.e) with resistin concentration as dependent variable and listed variables as independent variables for non-obese and obese non-diabetic women.

Independent variables	Non-obese (n=62)	Obese (n=42)
Leptin (ng/ml)	0.093 ± 0.587	0.784 ± 0.002**
Waist (cm)	0.461 ± 0.008**	0.217 ± 0.316
Hip (cm)	- 0.042 ± 0.859	- 0.116 ± 0.653
Weight (kg)	- 0.034 ± 0.863	0.249 ± 0.376
Height (cm)	- 0.179 ± 0.330	- 0.083 ± 0.682

*P<0.05, **P<0.01, ***P<0.001