ARTICLE

The Relationship between Retinol-Binding Protein-4 and Cardiometabolic Risk Factors in Obese Patients with Type 2 Diabetes Mellitus

Naglaa K Idriss[1], Ahmed Abdel-Galeel[2], Salwa R Dimitry[2], Eman A Abdel Aal[2]

[1]Department of Medical Biochemistry, Faculty of Medicine, Assuit University, Assuit, Egypt.
[2]Department of Cardiology, Faculty of Medicine, Assuit University, Assuit, Egypt.

Corresponding author: Dr. Naglaa K Idriss Email: naglaaidriss@hotmail.com
Published: 22 February 2015
Ibnosina J Med BS 2015;7(1):17-26
Received: 14 September 2014
Accepted: 14 December 2014
This article is available from: http://www.ijmbs.org

Abstract

Background: Retinol-binding protein-4 (RBP4) is an adipocyte-secreted hormone considered to link obesity with cardiovascular complications. The oxidative stress has been implicated in the pathophysiology of obesity. We evaluated serum RBP4 and plasma total thiols (TT) in generalized obesity (GO) and abdominal obesity (AO) in relationship to classical cardiovascular risk factors. Glycated haemoglobin (HbA1c%), C-reactive protein (CRP) and lipid profile were also evaluated. Patients and Methods: Sixty obese patients were recruited [30 abdominally obese (AO) patients, (15 male and 15 female, mean (SD), 49.5 (5.5) years) were measured by waist circumference (WC > 102 cm for men or > 88 cm for women) and waist/hip ratio (WC divided by that of the hips of > 0.9 for men and > 0.85 for women)] and [30 generalized obese (GO) patients (22 male and 8 female; mean (SD); 42.5 (8) years) were measured by body mass index (BMI). [BMI ≥ 30.0-34.9 kg/m^2, with normal WC] compared to 20 healthy subjects (14 males and 6 females; mean age (SD); 36.60 (5.97) years). Results: AO had significantly higher circulating RBP4 levels in comparison to GO ($p< 0.05$). Total thiols levels were significantly lower in AO compared to GO patients ($p< 0.05$). CRP significantly elevated in AO compared to GO patients ($p<0.05$). Total serum cholesterol, triglycerides and Hb1Ac% increased with BMI, WC and waist/hip ratio (WHR). Conclusion: The study reveals that RBP4 is autonomously related to visceral fat accumulations and cardiovascular diseases. The study also reveals the beneficial effect of TT against obesity and cardiovascular disease and the potential clinical applicability of RBP4 and total thiols in cardiovascular diseases.

Key words: Retinol binding protein-4 abdominal obesity, generalized obesity, cardiovascular disease.

Introduction

Obesity is associated with increase in the risk of type 2

diabetes mellitus (T2DM), cardiovascular disease (CVD), stroke, hypertension, and cancer (1). Obesity is a widespread state characterized by the extreme accumulation and storage of fat in adipose tissue. It is a main cause of insulin resistance, which is linked to the development of metabolic syndrome and T2DM. These pathologies are related to the development and progression of atherosclerosis, one of the leading causes of morbidity and mortality in industrialized countries (2-4).

Framingham Heart Study found that obese individuals are at increased risk to develop heart failure compared to non-obese individuals (2). There are cross relations between the CVD risk and increased waist circumference and it is now accepted that accumulated visceral fat depot acts as a large endocrine gland that becomes inflamed, secreting adipokines that may produce an insulin resistance and proinflammatory state, which increases cardiovascular disease risk (4,5). Dyslipidemia is a recognized risk factor for atherosclerosis and is frequently associated with insulin resistance and, therefore, with obesity (3). The two major quantitative lipid abnormalities in these patients are increased triglyceride levels and low high-density lipoprotein (HDL) levels. As these factors are related with an elevated proportion of small and dense low-density lipoprotein particles (sdLDL), atherogenic dyslipidemia is diagnosed (6). This condition has emerged as an important marker of the increased cardiovascular disease risk observed in patients with obesity, MetS, insulin resistance and T2DM (6,7). Retinol-binding protein-4 (RBP4) is one of the fatty acid binding proteins adipokines that has been correlated significantly with both obesity and cardiovascular disease (7,8). RBP4 secreted primarily by the liver and adipocytes, is a recently recognized adipokine apparently linked to obesity and its comorbidities in humans, especially insulin resistance, T2DM dysregulation and may be hepatic dysfunction (9), renal dysfunction (10), lipid metabolism and inflammation (11). Relationships between RBP4 and other traditional and non-traditional risk factors for CVD, such as inflammatory factors and oxidative stress have been confirmed in larger populations (12). In addition, growing evidence suggests that RBP4 plays a role in lipid metabolism to an even greater extent than insulin resistance. In fact, many human studies have found a strong relationship between RBP4 and triglycerides, and possible association with insulin resistance (13,14). Adiponectin is an adipose specific hormone that has insulin sensitizing properties, anti-inflammatory properties and is protective against obesity related disorders (13). Oxidative stress appears to play a major role in the development of CVD (15). Oxidative stress has major role in the development of major obesity-related complications such as CVD and diabetes (16). Several inflammatory biomarkers such as homocysteine and CRP are involved in the production of oxygen radicals in vessel wall promoting atherosclerotic disease causing oxidative vascular damage and conversely, antioxidants such as (vitamin C&E, β-carotene and thiol containing compounds) have protective effects against CVD development (15). Glutathione is the most intracellular thiol that has a role in many cellular functions including the antioxidant defense against CVD and obesity (17). CRP is a downstream marker of inflammation that has multiple effects, including complement binding, augmentation of expression of adhesions molecules, decreased expression of the vasodilator endothelial nitric oxide synthase, may stimulate the expression of the thrombotic factor PAI-1 and may induce oxidative stress (18). Thus, in order to gain additional impending into the relation between RBP4, and plasma Total thiols (TT) in generalized obesity (GO) and abdominal obesity (AO) and lipid metabolism. The following aspects were explored: 1) the potential association of serum RBP4 levels with glycated hemoglobin (HbA1c%); 2) metabolic parameters as independent predictors of RBP4; and 3) the correlation between RBP4 and C- reactive protein (CRP) and lipid profile in both types of obesity

.

Patients and Methods

Study design

This prospective, hospital-based and case-controlled study was conducted in Obesity Clinic, Coronary Care Unit, and Cardiac Department at Assuit University Hospital, Egypt, between December 2013 and May 2014. Procedures involving human subjects were approved by the Ethics Committee of Assuit University Hospital. Written informed consent was obtained from all participants. A cohort of 60 patients was recruited. Thirty generalized obese (GO) patients (22 males and 8 females) measured by BMI ≥ 30-34.9 kg/m^2, with normal waist circumference (< 102 cm for men or < 88 cm for women), mean age 42.77 ± 8.24. Thirty abdominally obese (AO) patients (15 males and 15 females) measured by waist circumference (> 102 cm for men or > 88 cm for women) and waist-hip ratio circumference (the circumference of the waist divided by that of the hips of > 0.9 for men and > 0.85 for women) (19) with BMI ≥ 30-34.9 kg/m^2, mean age 49.63 ± 5.41. Twenty healthy subjects (control) (14 males and 6 females) with normal body mass index 18.5-24.9 kg/m^2, with normal waist circumference

Table 1. Demographic data in abdominal obesity and generalized obesity.

Groups	Abdominal obesity		Generalized obesity		Healthy control		P-value
Data	No. 30	%	No. 30	%	No. 20	%	
Gender :Male/Female	15/15	50/50	22/8	73.3/26.7	14/6	70.0/30	< 0.1
Age (years)							< 0.05*
Mean ± SD	49.63 ± 5.41		42.77 ± 8.24		36.60 ± 5.97		
Range	35 – 55		30 – 55		30 – 50		
Smoking: Smoker / Nonsmoker	8/22	26.7.63.3	5/25	16.7/83.3	0/14	0/100	< 0.34
Physical activity: Active / Non-active:	5/25	16.7/83.3	15/15	50/50	20/0	100/0	< 0.05*
Family history of premature CAD: males / females/ both	5/2/0	16.7/6.7/0	6/4/2	20/13.3/6.7	0/0/0	0/0/0	< 0.33

Data were presented by ANOVA and chi-square test. There was statistical significant difference between 2 study population groups (AO) and (GO) for age and physical activity. Positive was considred positive in male first degree relative before age 55 and in females first degree relative before age 65.
**Significant Statistical difference between (AO) & (GO).*

Table 2. Plasma levels of all indices in abdominal obesity and generalized obesity. Data are presented as mean(SD).

Groups.	Abdominal obesity	Generalized obese	Healthy control	P-value
RBP-4 (ug/ml)	34.08 (7.72)a	15.72 (10.89)b	3.05(0.63)	<0.05*
TT (mmol/L)	0.56 (0.21)	1.16 (0.29)	1.08 (0.24)	<0.05*
CRP (mg/l)	24.20 (25.48)a	14.00 (14.38)b	1.20 (0.04)	<0.05*
HbA1c (%)	8.79 (2.89)	6.27 (2.25)	3.85 (0.73)d	<0.05*

*RBP-4: Retinol binding protein-4, TT: Total thiol, CRP: C-reactive protein, HB A1c: Hemoglobin A1c. Kruskall-Wallis test, all P<0.05, AOa group has significantly higher levels of RBP4 and CRP compared to GOb. AO group has significantly lower serum levels of TT compared to GO (p<0.05). AO has significantly higher levels of HbA1c compared to GO (p<0.05). *Significant Statistical difference between (AO) & (GO).*

(< 102 cm for men or < 88 cm for women), mean age (36.6±6.0). Exclusion criteria were; presence of fluid retention such as ascites and lower limb edema, abdominal organomegaly and pregnancy or lactation, recent change of oral contraceptive formulation, severe disease, history of cardiovascular disease or chronic inflammatory disease, pharmacological treatment for diabetes-antidiabetic agents or insulin-, secondary obesity (hypothyroidism, Cushing's syndrome), and pharmacological treatment for dyslipidemia or hypertension.

Table 3. Anthropometric measurements in abdominal obesity and generalized obesity. Data are expressed as mean (SD).

Groups	Abdominal obesity	Generalized obesity	Healthy control	P-value
Weight (kg)	88.63(8.26)	82.20 (6.34)	61.05 (6.68)	< 0.05*
Height (cm)	167.67 (6.88)	163.70 (7.11)	168.40 (8.85)	< 0.05*
BMI (kg/m^2)	31.49 (1.38)	30.68 (0.77)	21.49 (1.32)	< 0.05*
Waist circumference (cm)	114.60 (6.04)	93.93 (6.30)	92.50 (5.92)	< 0.05*
Hip circumference (cm)	122.70 (6.52)	110.70 (6.69)	111.00 (4.28)	< 0.05*
Waist/hip ratio (males)	0.95(0.02)	0.86 (0.02)	0.86 (0.02)	< 0.05*
Waist/hip ratio females)	0.92(0.04)	0.81 (0.03)	0.77 (0.03)	< 0.05*

Data were presented by ANOVA and T-test. There was significant difference between 2 study population groups (AO) and (GO) for weight, height, BMI, waist circumference, hip circumference and waist/hip ratio. *Significant Statistical difference between (AO) and (GO).

Table 4. Laboratory data of glucose, lipid metabolism and kidney function in abdominal obesity and generalized obesity. Values are expressed as mean(SD).

Groups	Abdominal obesity	Generalized obese	Healthy control	P-value
FBG (mg/dl)	130.20 (32.28)	104.17 (34.42)	80.90 (5.57)	< 0.05*
2-HPPBS (mg/dl)	188.10 (45.75)	159.40 (46.91)	121.85 (7.67)	< 0.05*
Total cholesterol (mg/dl)	178.57 (31.28)	176.03 (17.96)	157.20 (21.90)	<0.70
Triglyceride (mg/dl)	128.00 (26.39)	124.90 (21.32)	107.50 (13.27)	<0.61
HDL-C (mg/dl)	58.70 (16.96)	65.87 (10.80)	72.65 (8.15)	<0.1
LDL-C (mg/dl)	96.73 (31.36)	87.40 (25.28)	66.55 (12.34)	<0.20
Serum creatinine (mg/dl)	0.99 (0.74)	0.91 (0.38)	0.81 (0.13)	<0.61

*FBG: Fasting blood glucose, 2-HPPBS: 2-hour post prandial blood sugar, HDL: High density lipoprotein-cholesterol, LDL: Low density lipoprotein-cholesterol. Data were presented by ANOVA, Mann-whitney and T- test. There was statistical significant difference between 2 study population groups (AO) and (GO) for FBG and 2-HPPBG. *Significant Statistical difference between (AO) and (GO).*

Clinical assessments

Generalized obese and abdominally obese groups were compared for the current cardiovascular risk factors (diabetes mellitus (DM), hypertension (HTN), dyslipidemia, smoking, family history of premature coronary artery disease, physical inactivity, peripheral arterial disease (PAD), coronary artery disease (CAD), chronic kidney disease (CKD) and inflammatory markers such as CRP, Retinol binding protein 4 and total thiol) and were matched for age and sex. Clinical history of DM, HTN, CAD, CKD,

Table 5. Clinical history of diabetes, hypertension, coronary artery disease, peripheral artery disease and chronic kidney disease in abdominal obesity and generalized obesity.

Groups	Abdominal obesity		Generalized obese		Healthy control		P-value
Data	Number	Percent	Number	Percent	Number	Percent	
Diabetic Status:							
Non Diabetic	6	20.0	19	63.3	20	100.0	<0.05*
Type 1 DM	5	16.7	2	6.7	0	0.0	
Type 2 DM	19	63.3	9	30.0	0	0.0	
Hypertension:							<0.05*
Hypertensive	10	33.3	4	13.3	0	0.0	
Normotensive	20	66.7	26	86.7	20	100.0	
Coronary artery disease:							<0.63
AMI	7	23.3	5	16.7	0	0.0	
Unstable angina	13	43.3	10	33.3	0	0.0	
MI and Unstable angina	2	6.7	3	10.0	0	0.0	
Normal	8	26.7	12	40.0	20	100.0	
Peripheral artery disease:							<1.00
Yes	1	3.3	1	3.3	0	0.0	
No	29	96.7	29	96.7	20	100.0	
Chronic Kidney Disease:							<0.11
None	26	86.7	30	100.0	20	100.0	
Proteinuria & Hematuria	2	6.7	0	0.0	0	0.0	
Regular dialysis treatment	2	6.7	0	0.0	0	0.0	

*DM: Diabetes mellitus, AMI: Acute Myocardial infarction, PAD: Peripheral arterial disease, CKD: Chronic kidney disease. Data were presented by Chi-square test. There was significant difference between (AO) and (GO) for DM and HTN. *Significant Statistical difference between (AO) and (GO).*

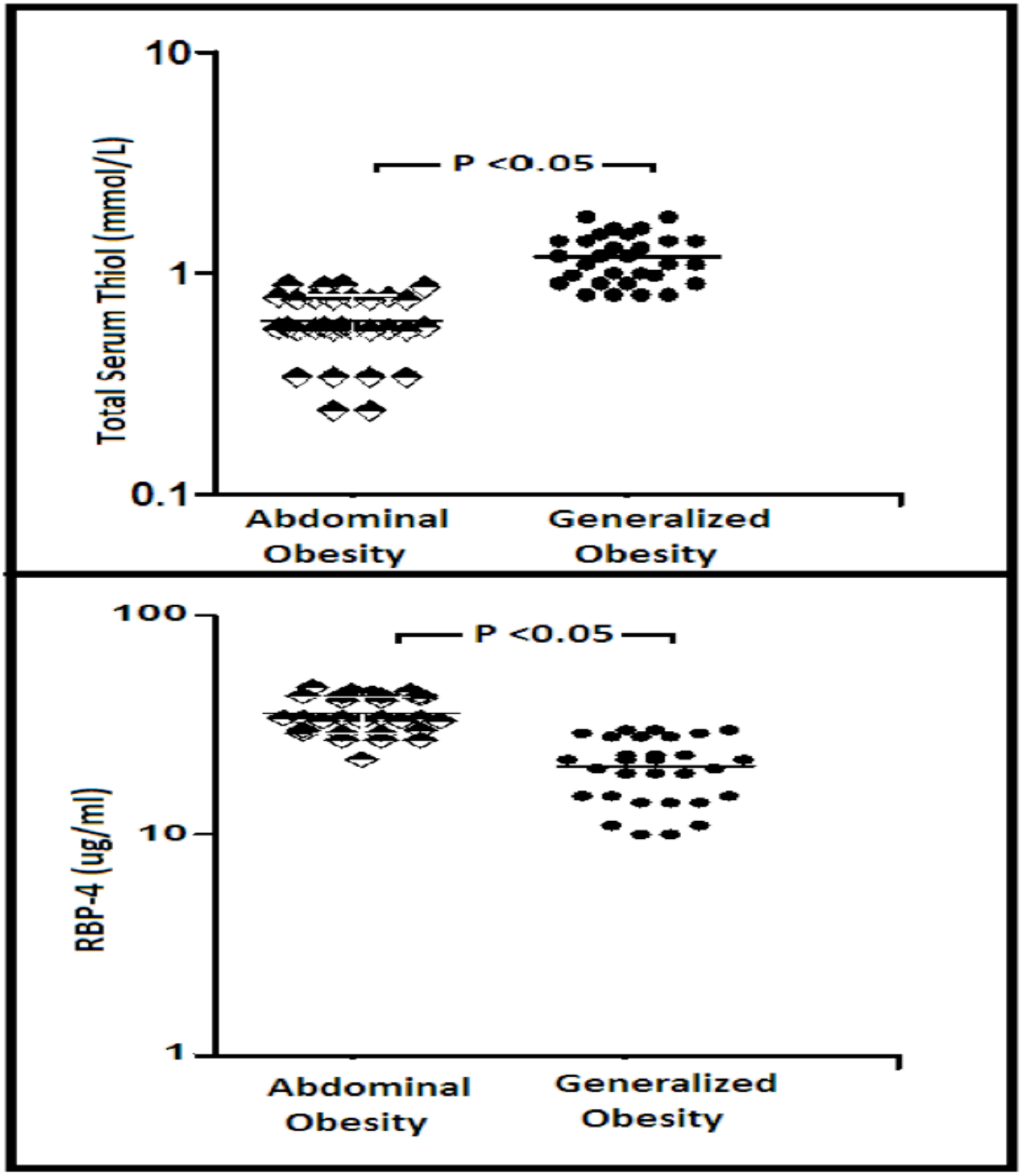

Figure 1. Levels of total plasma thiols (upper) and serum RBO-4 (lower) in patients with abdominal and generalized obesity.

PAD, physical activity, smoking and family history of premature CAD were done. Clinical examination of heart rate, blood pressure, peripheral arterial pulsations, cardiac examination and Electrocardiogram (ECG) were done.

Laboratory investigation

Venous blood was drained from ante-cubital vein without using tourniquet via a narrow bore needle syringe. Venous blood samples were collected into serum separator tube for serum RBP4 and into EDTA tubes for plasma total thiol measurement after 8-hour fasting. Centrifugation at 3,000 rpm (1,000 xg) for 20 min at 4°C. All aliquots were stored at -70°C up to patch analysis of biomarkers. Serum RBP-4 levels were measured by enzyme-linked immunosorbent assays (ELISA) using commercial kits (R&D Systems, Abingdon, Oxfordshire, UK). Plasma levels of TT were measured by colorimetric methods (20). Inter- and intra-assay coefficients of variation for all assays were < 5% and <10% respectively. Fasting blood glucose (FBG) (8-hour fasting), two-hour postprandial blood sugar (2-h PPBS) and glycated hemoglobin (HbA1C) and C-reactive protein (CRP) were measured. Serum fasting lipid profile (12-hour fasting) (LDL-C, HDL-C, total cholesterol and triglyceride). Serum creatinine were measured using Hitachi 911 automated analyzer at Assuit University Hospital Laboratory.

Statistical analysis

Following application of the Shapiro-Wilkes test to determine a normal distribution, non-categorical data distributed normally are expressed as mean (standard deviation) and data distributed non-normally are expressed as median (inter quartile range). Categorical data are analyzed by the Chi-squared test. Continuously variable data are analyzed by ANOVA or the Kruskal-Wallis test. Tukey's post-hoc test was used to determine differences between groups. Correlations were required by Spearman's rank method. A probability of less than 0.05 was considered as statistically significant. Analyses were done using SPSS version 16.

Results

The demographic data of patients and HCs participated in our study are presented in Table 1. Differences in research indices are presented in Table 1 and Figure 1 & 2. Abdominal obesity had significantly higher circulating RBP-4 in compared to GO ($p< 0.05$) (Table 2, Figure 2) and healthy subjects (14 male and 6 female; aged (mean±SD) 36.6±6.0 years; $p< 0.05$). Total thiols levels had significantly lower in AO patients compared to GO ($p< 0.05$) (Table 2, Figure 1). Plasma levels of FBG, 2-HPPBS and plasma HbA1c were increased significantly in AO ($p< 0.05$) (Table 2 & 3). CRP levels were significantly higher in AO ($p< 0.05$) (Table 2). Positive correlations (Spearman's rho) were evident between RBP-4 and plasma levels of HbA1c% ($r =0.371$, $p< 0.05$) in AO and in GO ($r= 0.567$, $p< 0.05$). The levels of total cholesterol, triglyceride and LDL-C were higher in AO than in GO but without significant statistical difference (Table 4). There was significant statistical difference between AO and GO for DM, HTN, physical inactivity, weight, height, BMI, WC, hip circumference and waist/hip ratio ($P< 0.05$) (Tables 3 & 5).

Discussion

The current study revealed serum levels of RBP4 in obese people with cardiovascular disease. Abdominally obese individuals have higher concentrations of RBP4 than generalized obese and also demonstrated that plasma levels of HbA1c were significantly advanced in individuals with higher serum levels of RBP4 compared to individuals with low serum levels of RBP4. This is in agreement with previous reports that RBP4 concentrations is linked with distribution of body fat rather than body weight per se since it's more highly correlated with waist/hip ratio or visceral fat than with BMI (12). In addition, plasma concentrations of RBP4 were have been shown to be significantly higher in subjects with impaired glucose tolerance (IGT) or T2DM than in subjects with normal glucose tolerance (21). Circulating RBP4 levels were found to be increased in obese subjects with T2DM (7) and they correlated with the severity of insulin resistance in non-diabetic participants with family history of T2DM (22). However, other workers reported that circulating RBP4 levels were similar in obese, overweight and lean women (23). Thus, RBP4 may be a convenient marker not only for T2DM but also as an indicator for adiposity.

In the present study, we used BMI, WC and W/H ratio circumference as measures for AO & GO to investigate the relationship between adiposity and cardiovascular risk factors (DM, HTN, physical inactivity, smoking, coronary artery disease, chronic kidney disease, peripheral arterial disease and family history of premature CAD) in both men and women as suggested that BMI and WC are the most widely used parameters for determining obesity because they are easily obtained (23). Our results stated that HTN and DM are more prevalent among individuals with abdominal obesity highlighting the relationship between

abdominal obesity and cardiovascular risk factors and this fact stands in males and females. A strong correlations between generalized obesity and either high blood pressure and diabetes mellitus (24). There were strong associations between coronary artery disease (CAD) and obesity irrespective to its type and sex difference. Large case-control study found that WHR was more strongly associated with CAD than with BMI in both men and women (25). It was proposed that obesity represents a major risk factor for atherosclerosis, in which systemic obesity-related inflammation is believed to be the main culprit (26).
There were inverse relations between physical activity and both types of obesity either abdominal or generalized in this study, since physical inactivity was higher among abdominally obese than generalized obese individuals. This concurred with previous reports that decreased physical activity may lead to obesity and obese persons are usually physically less active (27) and that a large body weight and fat mass can be reduced by increasing the short term and long term level of physical activity (28,29).

Our study also showed that there was no positive relationship between smoking and both types of obesity either abdominal obesity or generalized obesity as non-smokers were higher in both groups of patients than smokers. This is in agreement with Hart et al who found a negative correlations between obesity and smoking (30), but not with others who reported smokers and more ex-smokers among obese individuals (24).

Peripheral ischemic arterial disease was not affected by the type of obesity similar to previous reports of an increase in the risk of ischemic vascular disease among obese or overweight individuals, which indicates that the risk of a cardiovascular event may not be associated with high BMI (24).

The lipogram provided the molecular evidence in our study. We established that all lipid parameters were abnormally higher in abdominally obese individuals compared to generalized obese individuals except for HDL-C which was abnormally lower in AO than in GO. However, smaller differences in hypercholesterolemia for the different levels of obesity based on BMI and abdominal obesity were found by others (24). There were consistent changes in HDL-C and triglycerides with obesity, since obesity seems to be associated with lower HDL-C and increased triglycerides in people of all ethnic groups but less consistent change on LDL-C concentrations has been found (31).

CRP was found to be higher in our abdominal obesity group than generalized obesity group in women and men. Bochud et al. reported that CRP gene expression was positively increased with high BMI, WC and fat mass in women, whereas no such evidence was found in men (32). It is well noted that CRP is an established CV risk marker and carry predictive power for coronary events and patients with elevated basal levels of CRP are at an increased risk of diabetes, hypertension and cardiovascular disease (33). CRP is associated with insulin resistance and this increases risk of DM, obesity and cardiovascular risk and possible synergistic effect of obesity, insulin resistance and DM on the chronic low level inflammation may play role in atherosclerosis pathogenesis and so, CRP may be a novel therapeutic target (34). Our results showed an inverse relation between plasma total thiol (TT) and obesity either abdominal or generalized. Abdominally obese persons had lower concentrations of TT than generalized obese persons. This concurred with previous reports that obesity diminishes antioxidant defense by altering the activity of cytochrome P-450 and reducing the antioxidant enzymes such as catalase, glutathione peroxidase, glutathione reductase (35). Oxidative stress was showed to have a key role in cardiovascular disease development (15) and oxidative stress levels were elevated in human obesity, and these levels are modifiable with various lifestyle modifications and surgical interventions (36).

The major limitation of the present study was the cross-sectional propose, which prohibited us from concluding a cause-effect relationship of RBP4 with CAD. Even though we did not be familiar with distinctions between AO and GO, the cross-sectional design of our study did not allow us to assess the relationship of RBP4 with either AMI occurrence or long-term clinical outcomes. Since the preponderance of patients with classical cardiovascular risk factors (e.g. diabetes, dyslipidemia, hypertension etc.) were previously treated, we cannot decree out the believable effects of pharmaceutical agents (e.g. statins) on RBP4, leading to underestimation of its predictive power. Another important limitation was the considerable differences in some biochemical parameters between CAD and non-CAD groups, which might have affected RBP4 fluctuations. Finally, as our control group sample comprised of patients with cardiovascular risk factors, we couldn't extrapolate our conclusions to healthy subjects.

In conclusion, plasma RBP4 considered to be an important

indicator of intra-abdominal adipose mass and insulin-glucose homeostasis regulation. This suggests a potential role for RBP4 as a convenient marker not only for T2DM but also for cardiovascular risk. Obesity creates the oxidant conditions of for diseases such as diabetes, heart disease, hypertension and CVD. Oxidative stress in obesity is a systemic problem that must be corrected either by improving antioxidant defenses through fat volume reduction, exercise and dietary modification, or a combination of the three.

References

1. Flegal KM, Kit BK, Graubard BI. Association of All-cause mortality with overweight and obesity using standard body mass index categories. JAMA 2013;309(1):71-82.
2. Kenchaiah S, Evans JC, Levy D, Wilson PWF, Benjamin EJ, Larson MG, et al. Obesity and the risk of heart failure. N Engl J Med 2002;347(5):305-13.
3. Katzmarzyk OT, Mire E, Bouchard C. Abdominal obesity and mortality: The Pennington Center Longitudinal Study. Nutr Diabetes 2012;2(8):e42-e45.
4. Van Dam RM and Hu FB. Lipocalins and insulin resistance: etiological role of retinol-binding protein 4 and lipocalin-2? Clin Chem 2007;53(1):5-7.
5. Apovian CM and Gokce N. Obesity and cardiovascular disease. Circulation 2012;125:1178-82.
6. Kotnik P, Fischer-Posovszky P, Wsbitsch M. RBP4-a controversial adipokine. Eur J Endocrinology 2011;156(5):703-11.
7. Yang Q, Graham TE, Mody N, Preitner F, Peroni OD, Zabolotny JM, et al. Serum retinol binding protein 4 contributes to insulin resistance in obesity and type 2 diabetes. Nature 2005;436(7049):356-62.
8. Yamaaki N, Yagi K, Kobayashi J, Nohara A, Ito N, Asano A, et al. Impact of serum retinol binding protein 4 levels on regulation of remnant like particles triglycerides in type 2 diabetes mellitus. J Diabetes Res 2013;2013:1-5.
9. Bahr MJ, Boeker KH, Manns MP and Tietge UJ. Decreased hepatic RBP4 secretion is correlated with reduced hepatic glucose production but is not associated with insulin resistance in patients with liver cirrhosis. Clin Endocrinol (Oxf) 2008;70(1):60-5.
10. Mody N, Graham TE, Tsuji Y, Yang Q and Kahn BB. Decreased clearance of serum retinol-binding protein and elevated levels of transthyretin in insulin resistant ob/ob mice. Am J Physiol Endocrinol Metab 208;294(4): E785-E793.
11. Balagopal P, Graham TE, Kahn BB, Altomare A, Funanage V and George D. Reduction of elevated serum retinol binding protein in obese children by lifestyle intervention: association with subclinical inflammation. J Clin Endocrinol Metab 2007;92(5):1971-4.
12. Lee JW, Im JA, Lee HR, Shim JY, Youn BS, Lee DC. Visceral adiposity is associated with serum retinol binding protein-4 levels in healthy women. Obesity (Silver Spring) 2007;15(9):2225-32.
13. Balagopal P, Ferranti SD, Cook S, Daniels SR, Gidding SS, Hayman LL, et al. Non-traditional risk factors and biomarkers for cardiovascular disease: Mechanistic, research, and clinical considerations for youth: A scientific statement from the American Heart Association. Circulation 2011;123(23):2749-69.
14. Vergès B, Guiu B, Cercueil JP, Duvillard L, Robin I, Buffier P, et al. Retinol-binding protein 4 is an independent factor associated with triglycerides and a determinant of very low-density lipoprotein-apolipoprotein B100 catabolism in type 2 diabetes mellitus. Arterioscler Thromb Vasc Biol 2012;32:3050–7
15. Shimizu H, Kiyohara Y, Kato I, Kitazono T, Tanizaki Y, Kubo M, et al. Relationship between plasma glutathione levels and cardiovascular disease in a defined population: The Hisayama Study. Stroke 2004;35(9):2072-7.
16. Higdon JV; Frei B. Obesity and oxidative stress: a direct link to CVD? Arterioscler Thromb Vascular Biol 2003;23:365-7.
17. Chatterjee A. Reduced glutathione: A radio protector or a modulator of DNA repair activity? Nutrients 2013;5(1):525-42.
18. Devaraj S, Singh U, Jialal I. The evolving role of C-reactive protein in atherothrombosis. Clin Chem 2009;55(2):229-38.
19. Yusuf S, Hawken S, Ounpuu S, Bautista l, Franzosi MG, Commerford P, et al. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. Lancet 2004;364(9438):937-52.
20. Ellman GL. Tissue sulfhydryl groups. Arch Biochem Biophys 1959;82(1):70-7.
21. Cho YM, Youn BS, Lee H, Lee N, Min SS, Kwak SH, et al. Plasma retinol-binding protein-4 concentrations are elevated in human subjects with impaired glucose tolerance and type 2 diabetes. Diabetes Care 2006;29(11):2457-61.
22. Koch A, Weiskirchen R, Sanson E, Zimmermann HW, Voigt S, Dückers H, et al. Circulating retinol binding

protein 4 in critically ill patients before specific treatment: prognostic impact and correlation with organ function, metabolism and inflammation, Critical Care 2010;14(5):R179-90.
23. Janke J, Engeli S, Boschmann M, Adams F, Böhnke J, Luft FC, et al. Retinol binding protein 4 in human obesity. Diabetes 2006;55(10):2805-10.
24. García SE, Alonso TV, Alonso JL, Sanz RA, Muñoz SL, Sanz JC, et al. Obesity in Castile and Leon, Spain: Epidemiology and association with other cardiovascular risk factors. Revista Espanola de Cardiologia 2011;64(1):63-6.
25. Yusuf S, Hawken S, Ounpuu S, Bautista l, Franzosi MG, Commerford P, et al. Obesity and the risk of myocardial infarction in 27000 participants from 52 countries: a case-control study. Lancet 2005;366(9438):1640-9.
26. Rocha VZ, Libby P. Obesity, inflammation and atherosclerosis. Nat Rev Cardiol 2009;6(6):399-409.
27. Petersen L, Schnohr P and Sorensen TI. Longitudinal study of the long-term relation between physical activity and obesity in adults. Int J Obes Relat Metab Disord 2004;28(1):105-12.
28. Donnelly JE, Hill JO, Jacobsen DJ, Potteiger J, Sullivan DK, Johnson SL, et al. Effects of a 16-month randomized controlled exercise trial on body weight and composition in young, overweight men and women: the Midwest Exercise Trial. Arch Intern Med 2003;163(11):1343-50.
29. Jakicic JM, Marcus BH, Gallagher KI, Napolitano M; Lang W. Effect of exercise duration and intensity on weight loss in overweight, sedentary women: a randomized trial. JAMA 2003;290(10):1323-30.
30. Hart CL, Gruer L and Watt GCM. Cause specific mortality, social position, and obesity among women who had never smoked: 28 year cohort study. BMJ 2011;342(d3785):1-14.
31. Howard BV, Ruotolo G; Robbins DC. Obesity and dyslipidemia. Endocrinol Metab Clin North Am 2003;32(4):855-67.
32. Bochud M, Marquant F, Marques-Vidal PM, Vollenweider P, Beckmann JS, Mooser V, et al. Association between C-reactive protein 1 and adiposity in women. J Clin Endocrinol Metab 2009 94(10): 3969-77.
33. Dehghan A, Kardys I, Maat MP, Uitterlinden AG, Sijbrands EJ, Bootsma AH, et al. Genetic variation, C-reactive protein levels and incidence of diabetes. Diabetes 2007;56(3):872-8.
34. Veliga-Asimi Z. C reactive protein in obese PCOS women and the effect of metformin therapy. Bosn J Basic Med Sci 2007;7(1):90-3.
35. Cazzola R, Rondanelli M, Russo-Volpe S, Ferrari E, Cestaro B. Decreased membrane fluidity and altered susceptibility to peroxidation and lipid composition in overweight and obese female erythrocytes. J Lipid Res 2004;45(10):1846-51.
36. Vincent HK, Taylor AG. Biomarkers and potential mechanisms of obesity-induced oxidant stress in humans. Int J Obes (Lond). 2006;30(3):400-18.