RESEARCH ARTICLE

Waist Circumference was Positively Correlated with Chemerin, Retinol-Binding Protein 4 and hsCRP

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Abstract

BACKGROUND: Central obesity is associated with various chronic metabolic disorders characterized by abnormal cytokine production, increased acute phase reactants, and activation of inflammatory signaling pathways. This study was aimed to investigate the association of waist circumference, chemerin, and retinol binding protein (RBP)-4 with inflammation in men with central obesity.

METHODS: The research was conducted with a cross-sectional design involving 68 centrally obese male subjects aged 30 to 60 years old, with waist circumference (WC) > 90 cm. All subjects fulfilled the inclusion and exclusion criteria. Anthropometric parameters, fasting glucose, creatinine, SGOT, SGPT, and hsCRP were measured. Serum concentrations of chemerin and RBP4 were measured by ELISA.

RESULTS: The trend lines showed that chemerin, RBP4, and hsCRP increased with WC. Pearson correlation test showed a positively significant correlation between WC and hsCRP ($r = 0.242$, $p < 0.05$); and also between chemerin and hsCRP ($r = 0.244$, $p < 0.05$) and RBP4 ($r = 0.321$, $p < 0.01$). Subjects were stratified into four groups based on their chemerin and RBP4 levels (high chemerin/high RBP4, high chemerin/low RBP4, low chemerin/high RBP4, or low chemerin/low RBP4). Subjects who were in the high chemerin/low RBP4 group were more likely to have high level of inflammation (47.6%), but subjects with high chemerin/high RBP4 showed low level of inflammation (42.9%) as compared with the other three groups.

CONCLUSIONS: We concluded that increased WC was correlated with elevated levels of chemerin, RBP4, and hsCRP. High chemerin was correlated with increased level of RBP4 as well as with high level of inflammation.

KEYWORDS: waist circumference, chemerin, RBP4, hsCRP, inflammation

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Introduction

Obesity, the most common nutritional disorder in industrialized countries, is associated with increased mortality and morbidity of cardiovascular disease (CVD) (1). Report of the National Association of Health Research in 2007 mentions the prevalence of obesity in the population aged ≥ 15 years of Indonesian people was 10.3%, with the proportion of 13.9% in males and 23.8% in females, while the prevalence of central obesity was 18.8% (2). Increased prevalence of obesity was closely related to the prevalence of metabolic syndrome (MetS). The prevalence of MetS among a number of Asian populations appears to be between 10 to 30%. The risk of developing type 2 diabetes (T2DM) is 10 times higher among subjects with MetS than among healthy subjects,

and 3 to 10 times more likely to develop cardiovascular disease. The prevalence of metabolic dearrangements is associated with abdominal adiposity that s to high risk of morbidity and mortality (3).

Visceral or central obesity is characterized by the accumulation of adipose tissue in the abdominal cavity is associated with the incidence of insulin resistance (IR) that can develop into T2DM (4). Central obesity is determined by the measurement of waist circumference (WC), and Asian men with WC above 90 cm are categorized as having central obesity (5). The early symptom of impaired function of adipose tissue is altered adipokine serum concentration that may contribute to the development of obesity-associated disorders. The study of Bahceci has also found a positive correlation of adipocyte size with tumor necrosis factor α (TNF-α), interleukin 6 (IL-6), and hsCRP (6).

Chemerin is a recently identified adipokine that is highly expressed in the liver and adipose tissue and is associated with adiposity, insulin resistance, MetS risk factors, and nonalcoholic fatty liver (7,8). Chemerin is thought to regulate adipogenesis and metabolic homeostasis in murine and human adipocytes. Additionally, chemerin modulates the innate immune system through its binding to the orphan G-protein coupled receptor, chemokine-like receptor 1 (CMKLR1, ChemR23) and modulates chemotaxis of immature dendritic cells and macrophages (9-11). Recent studies have associated chemerin with several inflammatory markers in obesity and T2DM (12, 13). However, the role of chemerin in the association of inflammation with obesity-related diseases in overweight and obese Asian people has not been much studied.

RBP4 is produced by hepatocytes but it is also secreted by human adipose tissue and expressed in mature adipocytes (the expression in stroma vascular fraction of adipose tissue being negligible) (14). Until recently, the function of RBP4 was thought to be in the delivery of retinol to tissues, but more evidence has shown that RBP4 is associated with obesity-related disorders and IR (15-18). Previous studies have shown that expression of chemerin and CMKLR1 may be induced by retinoic acid. Yang Q has shown that RBP4 is highly expressed in adipose tissue, and this expression increases with obesity (19). Because RBP4 is the major circulating transporter of retinoic acid, elevated RBP4 levels associated with obesity may lead to increased delivery of retinoic acid to adipose tissue in obese animals, and to subsequent up-regulation of chemerin and CMKLR1 expression (7). Therefore, in this study we aimed to investigate the interaction between chemerin and RBP4 in centrally obese men to assess the role of chemerin and RBP4 in adipose tissue. In this study we examined the associations between increased waist circumference and secretion profile of adipokines, especially chemerin and RBP4. We also investigated how this adipokine interaction was associated with the level of inflammation. For this, we used cross-sectional data from community-dwelling participants without clinical diabetes.

Methods

SUBJECTS

Participants were recruited from communities in Surabaya, Bandung, and Jakarta. The sample population compromised centrally obese men aged 30 to 60 years. WC above 90 cm was determined by measuring the waist between the lower rib and iliac crest. Information on medical history, present condition, and drugs were obtained by interview. Other variables e.g. smoking and alcohol habits and medication were investigated by individual interviews using a structured questionnaire. Subjects with fasting glucose ≥ 126 mg/dL or GFR < 60 mL/minute or SGOT /SGPT of more than twice the normal value or hsCRP ≥ 10 mg/L, were excluded. This study was approved by the Ethics Committee of Hasanuddin University Faculty of Medicine (registered number: UH12010011), and written informed consent was obtained from each subject.

BLOOD COLLECTION AND BIOCHEMICAL ANALYSIS

Blood samples were collected in the morning after over-night fasting. Venous blood was collected, centrifuged, and the separated serum was frozen immediately at -20°C. Serum levels of fasting glucose, SGOT, SGPT, creatinine, and hsCRP were assayed using autoanalyzer Cobas (Roche Diagnostics). Circulating chemerin and RBP4 levels were measured using a commercially available enzyme-linked immunosorbent assay (ELISA, BioVendor and Quantikine, respectively).

STATISTICAL ANALYSIS

Statistical analysis was performed using SPSS for Windows version 15.0 (USA, SPSS Inc.). All values were expressed as mean ± standard deviation (SD), unless otherwise specified. Pearson correlation coefficients were calculated to evaluate the relationships between serum chemerin, RBP4, and hsCRP. Hypothesis testing was two-tailed at a significance level of 0.05.

Results

Clinical characteristics of study subjects

Table 1 shows the anthropometric and metabolic characteristics of the participants. The subjects comprised 68 central obese men aged 43.00 ± 6.62 (range, 30-60) years and the mean WC was 100.80 ± 6.51 (range, 91-114) cm. Scatter graph of adipokines vs WC shows chemerin, RBP4, and hsCRP increase with WC which are almost similar in shape (Figure 1).

Relations between WC, chemerin, RBP4, and inflammation

Pearson correlation test showed a positively significant correlation between serum chemerin and hsCRP ($r = 0.244, p < 0.05$) and RBP4 ($r = 0.321, p < 0.01$). Significant correlation was also found between WC and hsCRP ($r = 0.242, p < 0.05$).

After confirming that there was a statistically significant interaction between chemerin and RBP4, the study subjects were stratified into four groups based on their median levels of chemerin and RBP4: 1) low chemerin/low RBP4, 2) low chemerin/high RBP4, 3) high chemerin/low RBP4, or 4) high chemerin/high RBP4, for further analyses. Based on our initial analyses, we anticipated that participants with high levels of chemerin and RBP4 would show high level of inflammation. However, the result showed high inflammation level was predominant in the subjects with high chemerin/low RBP4 (47.6%) compared to the other three groups (Figure 2). In addition, subjects with high chemerin/high RBP4 showed low inflammation level (42.9%).

Table 1. Clinical and biochemical characteristics of the study subjects

Parameter	Min	Max (n = 68)	Median	Mean ± SD
Age	30.00	60.00	43.00	43.00 ± 7.00
Body Mass Index (kg/m²)	23.94	36.85	28.88	29.38 ± 3.07
Waist Circumference (cm)	91.00	114.00	100.00	101.00 ± 6.75
SBP (mmHg)	105.00	187.00	127.00	128.00 ± 14.63
DBP (mmHg)	65.00	126.00	80.00	84.00 ± 11.76
SGOT (U/L)	12.00	50.00	22.00	23.00 ± 6.49
SGPT (U/L)	7.00	80.00	25.00	30.00 ± 15.80
Fasting Glucose (mg/dL)	76.00	109.00	92.00	91.50 ± 8.35
GFR (ml/min/1.73 m2)	60.66	132.21	87.56	87.10 ± 15.20
hsCRP (mg/L)	0.15	9.93	1.66	2.04 ± 1.84
Adiponektin (µg/mL)	1.14	13.38	3.45	3.72 ± 1.77
Chemerin (ng/mL)	206.36	676.78	409.4	417.09 ± 95.79
RBP4 (µg/mL)	22.29	99.79	48.67	53.32 ± 17.20

Data are given as mean ± standard deviation, minimum, maximum, and median. The Kolmogorov-Smirnov test was used to test the normally distributed variables. SBP: systolic blood pressure, DBP: diastolic blood pressure, SGOT : Serum Glutamic Oxaloacetic Transferase, SGPT : Serum Glutamic Piruvic Transferase, hsCRP: high-sensitivity C-reactive protein, RBP4 : Retinol Binding Protein 4.

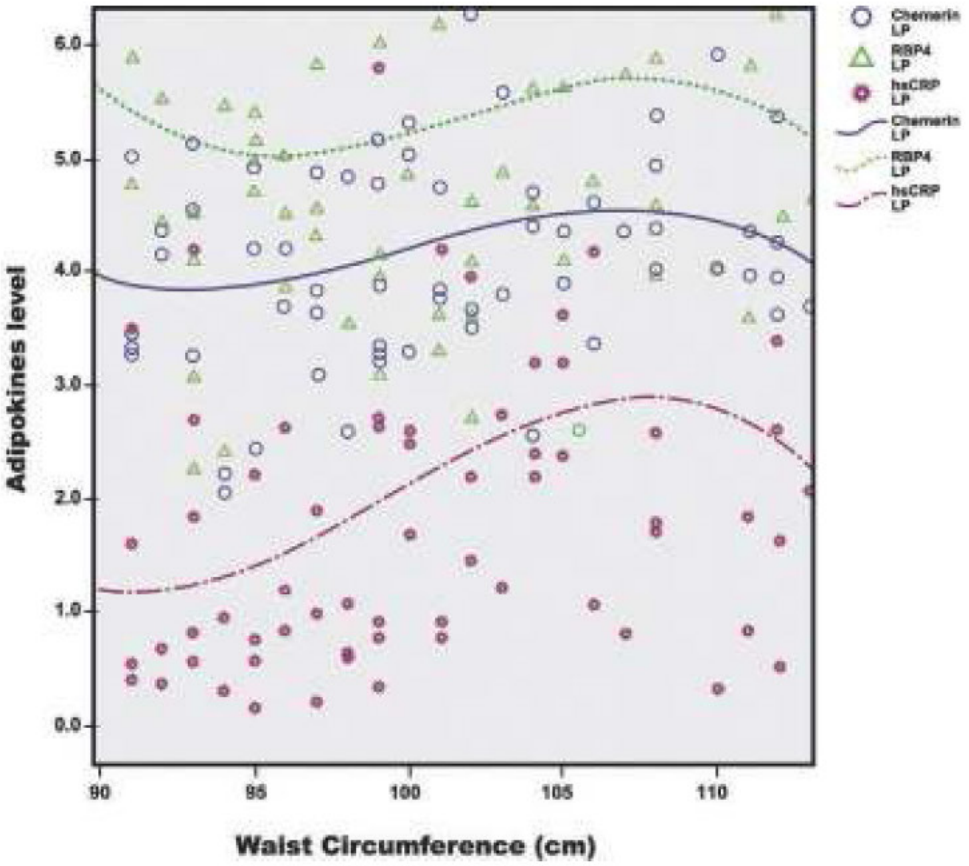

Figure 1. Scatter graph of chemerin, RBP4 and hsCRP versus WC. The graph expresses similarity of shape between chemerin, RBP4 and hsCRP. The trend line of mean data also shows increased values with increased WC.

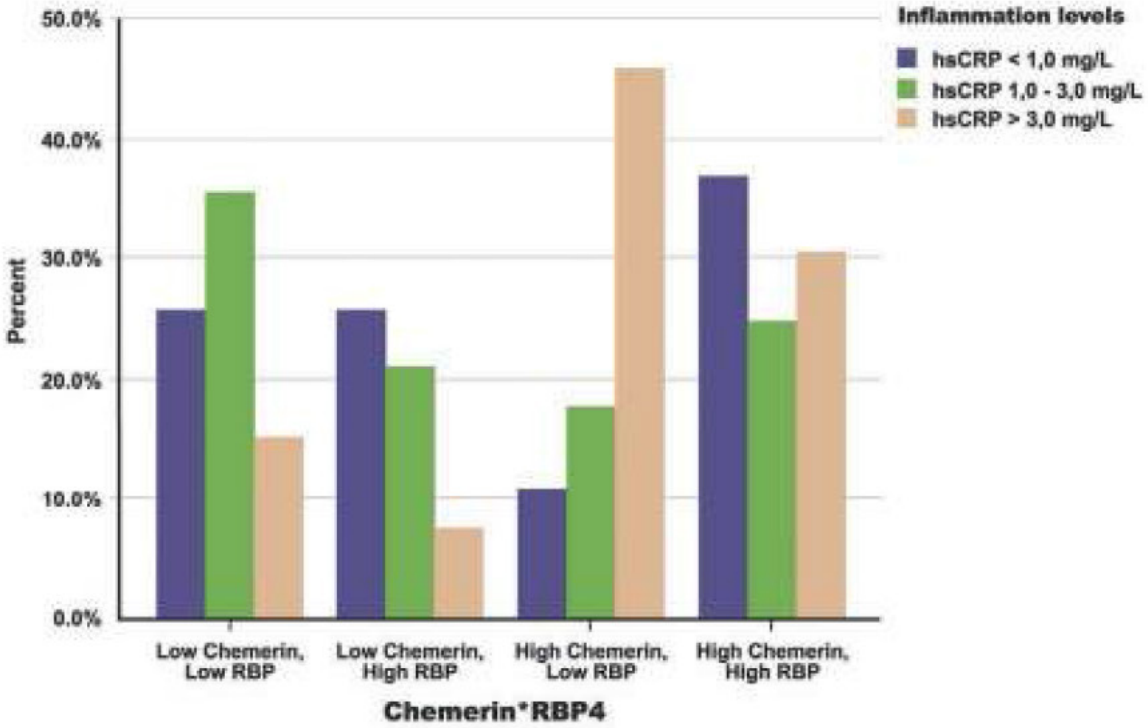

Figure 2. Cross tabulation of level of adipokines versus level of inflammation. The levels of inflammation were divided into three groups: low level of inflammation (hsCRP < 1 mg/L), medium level of inflammation (hsCRP 1 – 3 mg/L), and high level of inflammation (hsCRP > 3 mg/L).

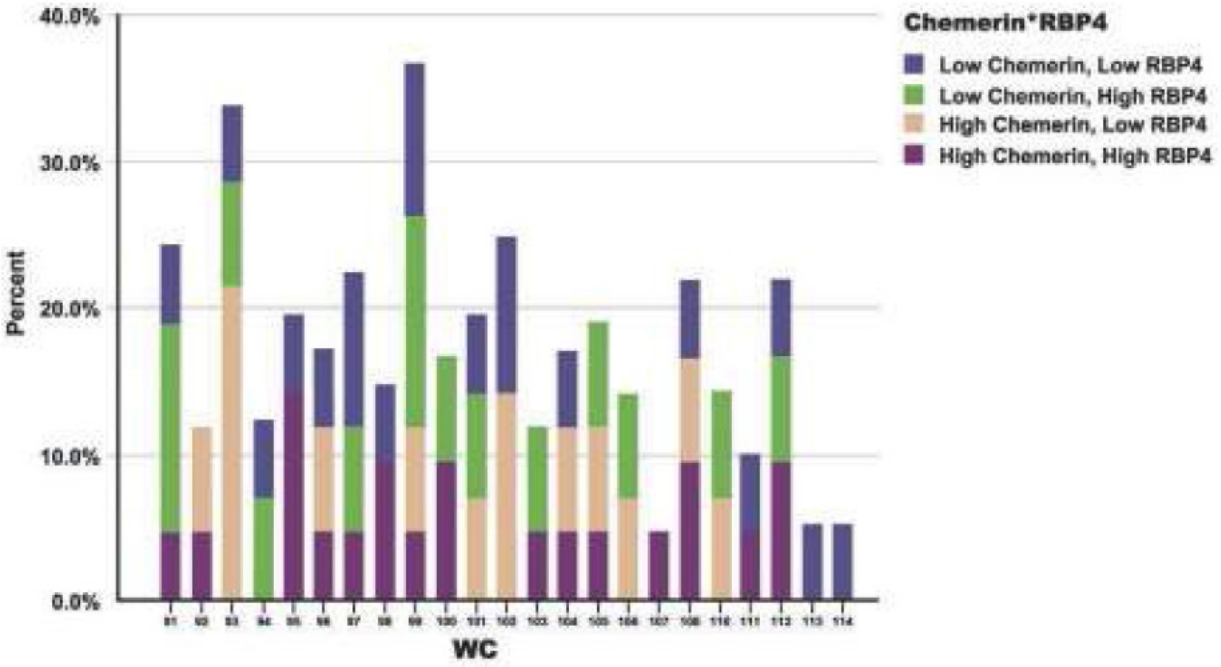

Figure 3. Stacked graph of levels of adipokines versus WC.

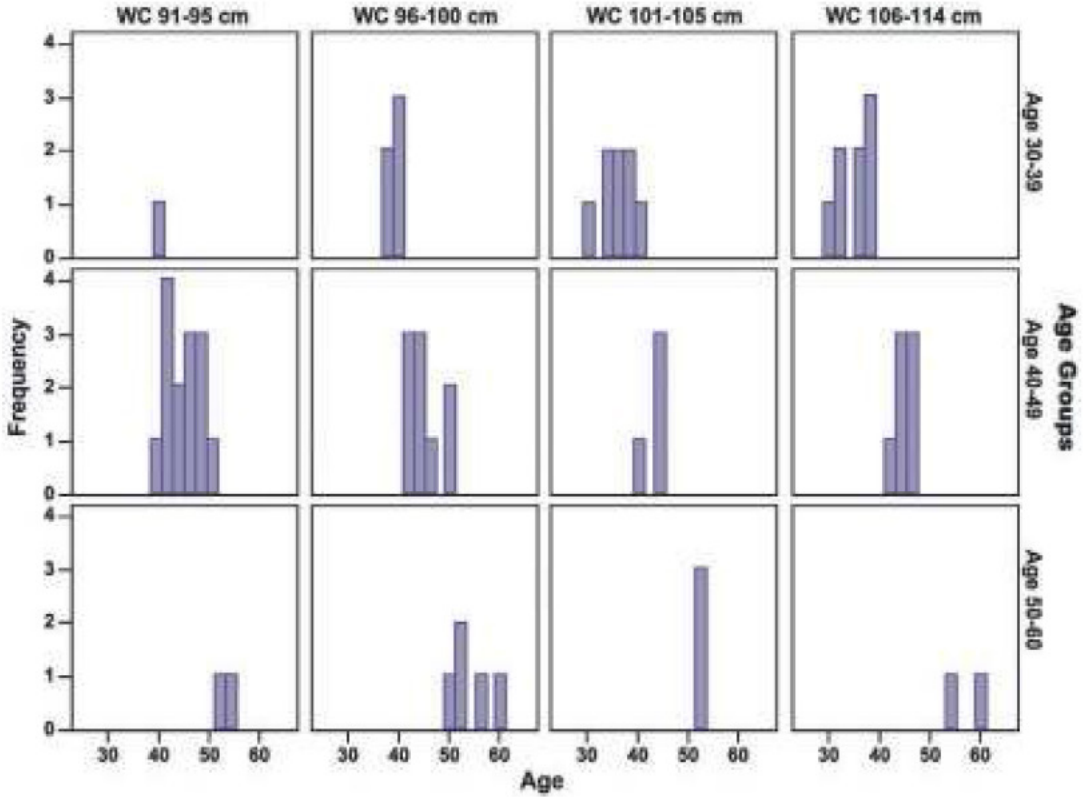

Figure 4. Histogram of age versus WC (divided by quartile). Our data show subjects with WC >100 cm are dominantly young men, so they have low level of chemerin and inflammation.

The stacked graph between WC vs interaction of chemerin and RBP4 shows subjects with high chemerin/ high RBP4 was predominant in those with WC of 95 cm, but spread evenly on all other WC values (Figure 3). This result supports our indication that elevation of chemerin and RBP4 is caused by increased WC. For further investigation, we made a histogram of quartile of WC vs age groups, and the graph showed subjects with WC 95 cm were predominant in the age 40 to 49 years (Figure 4).

Discussion

Increased prevalence of overweight and obesity throughout the world has become a serious problem. As we know, obesity is caused by energy imbalance, meaning more calories are consumed than expended. Increase in caloric consumption comprises increased size of meals, consumption of sugar-sweetened beverages, and refined carbohydrates. Sedentary lifestyle reduces daily energy expenditure such as television watching with remote control, work that discourages much walking, less physical education or activity, and more sedentary tasks. Other determinants of energy imbalance may include decreased sleep, infectious agents such as adenovirus-36, consumption of trans-fat or fast food, perinatal exposures, and differences in macronutrient quality (e.g., lower vs higher glycemic-load carbohydrates) that might alter metabolism or appetite. Energy imbalance leads to storage of excess calories in adipose tissue, which cause both hypertrophy and hyperplasia of adipocytes (20).

Hypertrophy and hyperplasia of adipocytes are associated with intracellular abnormalities of adipocyte function, particularly endoplasmic reticulum and mitochondrial stress, resulting in the production of adipokines and inflammatory mediators (20). Previous studies have shown that increased WC would elevate CRP level. In the present study, we examined positive correlation between WC and hsCRP ($r = 0.242$, $p < 0.05$) which proved that central obesity or increased WC were linked to inflammation, whereas hsCRP was a marker for low grade inflammation as well as for atherosclerosis, obesity, hyperglycemia and hypertension (21). In addition, we found that WC were positively correlated with SGPT ($r = 0.386$, $p < 0.01$) which proved the occurrence of metabolic deterioration was caused by increased WC.

CRP is produced mostly by hepatocytes that increase when triggered by elevated plasma concentrations of interleukin-6 produced mainly by macrophages and adipocytes (22). Excess of calories in obesity will change physical adipocyte cell (hypertrophy) and the surrounding area of adipocyte. Hypertrophied adipocytes begin to secrete low levels of TNFα, which can stimulate pre-adipocytes to produce monocyte chemoattractant protein-1 (MCP-1) (23). *In vitro* studies suggest that TNFα also stimulate hyperplasia in a paracrine function (24). Proliferating pre-adipocytes have the ability to be phagocytotic, but this function decreases when proliferation is stopped. Macrophages can also take up and store lipids, and adipocytes and macrophages share a number of important genes and markers like fatty acid transporters (i.e. aP2/fatty acid binding protein (FABP)-4) and the transcription factor peroxisome proliferator activated receptor (PPAR)γ (25). When activated, both are able to express IL-6 and TNFα, and adipocytes are able to express macrophage markers. In addition, macrophages are observed more frequently in human, and expression of MCP-1 is greater in visceral fat than in subcutaneous fat, and is correlated with waist circumference and possibly insulin resistance (26).

Chemerin is secreted by adipocytes as prochemerin (18 kDa) and transformed into an active protein in the presence of proteolytic enzymes, which can act as activator or inhibitor proteins (27). At the beginning of inflammation in adipose tissue, serine protease derived C1s and Cathepsin G protein in adipose tissue was involved, so chemerin would cause chemotaxis of macrophages to adipose tissue. Furthermore, chemerin is also associated with adipogenesis, where studies show that chemerin and its receptor (CMKLR1) are expressed in adipose tissue *Psammomys obesus* (especially white adipose tissue) and increased levels of chemerin and CMKLR1 occurs in the process of differentiation of pre-adipocytes into adipocytes (7,10). Recent evidence suggests that inflammatory cytokines may play a role in chemerin secretion from adipose tissue and TNF-α produces a time-dependent increase in serum total chemerin and bioactive chemerin *in vivo* (28).

In obese subjects serum RBP4 increases by 2 to 3 fold (29, 30). In the present study, we also found elevated levels of RBP4 2 to 3 times higher than normal levels (data not shown). In addition, 40-50% from the total cell number of adipose tissue in obesity was macrophages and has a positively significant correlation between expression mRNA RBP4 and infiltrating macrophages, so that RBP4 secreted by macrophages also have biological effects on adipose tissue (16). We suggest that positively significant correlation we found between chemerin and RBP4 is associated with this activation of macrophages in adipose tissue.

The increase of fat mass causes increased intracellular lipids, hypertrophy (increased adipocyte size), hyperplasia (increased numbers of adipocytes), and finally angiogenesis. Some animal experiments suggest that adipocyte hyperplasia may occur later than hypertrophy and be associated with greater severity and less reversibility of metabolic consequences, but these potential differences are not well established, so further research is needed to elucidate the relative importance of adipocyte hypertrophy *vs* hyperplasia in humans (31). Some evidence suggests that adipocyte hyperplasia, or adipogenesis, occurs throughout life, both in response to normal cell turnover as well as in response to excess calorie intake (24). In our study, high levels of chemerin were found almost in all subjects with WC of 91 cm to 112 cm (Figure 3). We think it depends on the duration of obesity. According to the data above, we suspect that the process of hypertrophy and hyperplasia that occur in adipose tissue may vary between individual subjects. When we combine our graph (Figure 3) with histogram (Figure 4), we found that high chemerin/high RBP4 occurred predominantly in subjects with WC 95 cm and in the age 40-49 years. So, we think that the duration of obesity in the subjects aged 40-49 years was long enough to cause hyperplacia of adipocytes to occur, that is pre-adipocytes cells begin to differentiate into mature adipocytes that are able to secrete other pro-inflammatory cytokines. Increased levels of chemerin will speed up the process of differentiation, and also influence elevated levels of RBP4-induced adipocytes differentiation (7, 32).

From the overall results of this study, we suggest that chemerin has a role in adipocyte hyperplasia that might occur later than does hypertrophy. Adipose tissue is highly dynamic in maintaining the excess of nutrition in accordance with homeostasis of the body. At the beginning of excess of fat, adipocytes have the ability to enlarge (hypertrophy) in modulating the metabolic activity. Hypertrophied adipocytes cause low grade inflammation that attracts macrophage infiltration, which then activates chemerin secreted by mature adipocytes. Chemerin induces the differentiation of pre-adipocytes into mature adipocytes (hyperplasia) and influenced elevated levels of RBP4. The newly formed adipocytes will act as a metabolic buffer that can absorb excess fat from the circulation. However, if obesity continues, new adipocyte will continue to enlarge and attract macrophages to the adipose tissue, causing an increased level of inflammatory markers. But as we know, only approximately 10% of fat cells are renewed annually at all adult ages and levels of body mass index (33). The condition when adipose tissue is not able anymore to form new adipocytes cells required for storage of excess fat will be underlying to the development of T2DM (34).

This research was conducted with cross-sectional study because this design allows using the general population, not just hospitalized patients. In addition, this design is relatively simple, giving a quick results because the limitation of research time and that it can examine many variables at a time. Cross-sectional design does not have the risk of having lost samples to follow-up (drop out), and can be used for further research, such as cohort studies. However, this design has some weaknesses: 1) it can't determine cause and effect because data collection is carried out at one same time, 2) it requires a considerable number of subjects, especially if many variables are studied, and 3) it can't give an idea of prognosis, incidence and cause of the disease. To minimize these weaknesses, screening was done through the inclusion and exclusion criteria as well as by studying the medical history and lifestyle of patients by means of a questionnaire. The subjects were also restricted to men to avoid bias due to the secretion of adipokines that was influenced by the hormone estrogen in women (35). Further studies using methods that can distinguish chemerin variants are needed to define the exact relationship between chemerin and other inflammatory cytokines. This new approach might prove to be a valuable prognostic tool that would complement existing strategies in pharmacologic and lifestyle interventions for treatment of obesity-associated diseases.

Conclusion

In conclusion, we have demonstrated that increase in waist circumference was correlated positively with elevated levels of chemerin, RBP4, and hsCRP. Elevated chemerin level correlated with increased levels of RBP4, which might be induced during adipocytes differentiation process, as well as with high levels of inflammation.

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