The Relationship Between Retinol/Retinol Binding Protein 4 ratio, resistin and inflammation in non diabetic obese Indonesian men

Anna Meiliana,[1,2] Gatot S. Lawrence,[1] lhamjaya Patellongi,[1] Andi Wijaya,[1,2] Suryani As'ad.[1]

[1] Doctoral/Master Program in Biomedical Science-Clinical Chemistry, Faculty of Medicine, Hassanuddin University, Makassar, Indonesia

[2] Prodia Clinical Laboratory, Jakarta, Indonesia

Abstrak

Tujuan *Penelitian ini bertujuan untuk menilai korelasi antara rasio retinol/RBP4 dan resistin dengan inflamasi (diwakili oleh hsCRP) pada pria obese non-diabetes di Indonesia.*

Metode *Penelitian dilakukan secara potong lintang pada 125 subjek. Parameter yang diukur adalah retinol, RBP4, resistin, dan hsCRP. Uji korelasi dilakukan antara retinol, RBP4, resistin, hsCRP dan rasio Retinol/RBP4. Rasio retinol/RBP4 dibagi menjadi dua kelompok yaitu rasio tinggi (>0,9) dan rasio rendah (≤0,9). Cut off hsCRP ditentukan: <1 mg/l menandakan risiko inflamasi rendah, 1-3 mg/l risiko inflamasi sedang, dan 3-10 mg/l risiko inflamasi tinggi (terkait dengan risiko PJK). Kemudian dilakukan uji tabulasi silang untuk melihat kecenderungan tingkat inflamasi pada subjek yang dapat digambarkan oleh rasio retinol/RBP4 dan resistin.*

Hasil *Retinol ditemukan berkorelasi kuat dengan RBP4 (r=0,53; p<0,01) dan resistin. Ditemukan korelasi positif tidak bermakna antara resistin dan rasio retinol/RBP4 terhadap hsCRP, Pada kelompok rasio tinggi, ditemukan 17,6% subjek dengan risiko inflamasi rendah, 26,4% risiko inflamasi sedang dan 20,8% risiko inflamasi tinggi. Pada kelompok dengan rasio rendah ditemukan 8% subjek dengan risiko inflamasi rendah, 20% risiko inflamasi sedang dan 7,2% risiko inflamasi tinggi. Kombinasi antara rasio retinol/RBP4 dan resistin menunjukkan 12% dari jumlah subjek kelompok "rasio tinggi dan resistin rendah" memiliki risiko inflamasi rendah, dan 8% memiliki risiko inflamasi tinggi, sementara pada kelompok "rasio rendah resistin tinggi" ditemukan 3,2% subjek memiliki risiko inflamasi rendah dan 13,6% subjek memiliki risiko inflamasi tinggi.*

Kesimpulan *Kombinasi parameter rasio retinol/RBP4 dengan resistin memberikan gambaran yang lebih baik mengenai risiko inflamasi pada subjek obese non-diabetes dibandingkan dengan parameter rasio saja.* ***(Med J Indones 2010; 19:57-63)***

Abstract

Aim To verify the correlation between Retinol/RBP4 Ratio, and resistin with inflammation (represented by hsCRP) in non-diabetic obese Indonesian men

Methods This was a cross sectional study using 125 subjects. Measured parameters were retinol, RBP4, resistin, and hsCRP. Correlation between retinol, RBP4, resistin, hsCRP and Retinol/RBP4 Ratio was calculated. Cut off point of hsCRP were classiied as follows: <1 mg/l for low risk of inflammation, 1-3 mg/l for moderate risk, and 3-10 mg/l for high risk (according to CVD risk). The Retinol/RBP4 ratio was dichotomized into high (>0.9) and low ratio (≤0.9). The cross tabulation test was performed to predict the inflammation trends described by Retinol/RBP4 Ratio and resistin.

Results Retinol was found strongly correlated with RBP4 and resistin (r=0.53; p<0.01). A positive but not significant correlation was found between resistin and Retinol/RBP4 Ratio with hsCRP. In high ratio group, 17.6% subjects were found with low risk inflammation, 26.4% with moderate risk, and 20.8% with high risk, in low ratio group, 8% subjects were low risk inflammation, 20% moderate risk, and 7.2% high risk. Combination between ratio and resistin showed that in "high ratio and low resistin" group, 12% subjects have low risk of inflammation and 8% have high risk. Meanwhile in "low ratio and high resistin" group, 3.2% subjects were found having low risk and 13.6% high risk of inflammation.

Conclusions Combination between Retinol/RBP4 Ratio and resistin showed better description about the inflammation risk in non-diabetic obese subjects compare to the ratio itself. ***(Med J Indones 2010; 19:57-63)***

Key words: *Retinol, RBP4, resistin, hsCRP, obesity, inflammation*

Correspondence email to: anna_m@prodia.co.id

Obesity is a multi-factorial disorder, developed as a result of genetic and environmental changes, along with lack of physical activity resulting an imbalance in energy homeostasis, and accumulation of excess energy as fat. It is increasing at an alarming rate even in developing countries.[1]

Many studies stated that obesity is a low grade chronic inflammation. In recent years, White Adipose Tissue (WAT) has been considered as an endocrine organ because of its capacity to secrete hormones and cytokines. Thus, adipose tissue is not only known for its capacity to store the excess of dietary energy in the form of TG, but also is now recognized as a fundamental participant in the control of energy metabolism by secreting many proteins called adipocytokines such as resistin, Retinol Binding Protein 4 (RBP4), Tumor Necrosis Factor a (TNF-a), Interleukin 6 (IL-6), leptin, vaspin, visfatin, omentin, chemerin, apelin, etc.[2-4]

C-reactive protein (CRP) is a protein found in the blood, the level of which rises in response to inflammation (an acute-phase protein). CRP is synthesized by the liver in response to factors released by fat cells (adipocytes). It is a member of the pentraxin family of proteins. CRP rises up to 50,000-fold in acute inflammation, such as infection. It rises above normal limits within 6 hours, and peaks at 48 hours. It has a constant half-life, and therefore its level is mainly determined by the rate of production (and hence the severity of the precipitating cause). The high-sensitivity CRP (hs-CRP) test measures low levels of CRP using laser nephelometry. The test gives results in 30 minutes with a sensitivity down to 0.04 mg/L. Normal concentration in healthy human serum is usually lower than 10 mg/L.[5,6]

Retinol Binding Protein 4 (RBP4) is considered to be the primary carrier of retinol to the tissues. Some studies showed the correlation between RBP4 and inflammation but some others failed.[7] RBP4 secretion in the liver is strongly influenced by abundance of retinol. In the fasted state, vitamin A circulates primarily as retinol bound to RBP (*holo*-RBP) in approximately a 1:1 molar ratio, and the free RBP is termed as *apo*-RBP.[8] This gives a suggestion that not all RBP4 have the inflammatory properties, but the free / *apo*-RBP do, represented by ratio retinol to RBP4.

Vitamin A, an important micronutrient with a wide biological functions such as vision, embryonic development, immune function, etc, stored in majority in the liver but also found in adipose tissues together with small amounts of retinyl esters.[9,10] The carboxylic acid form of vitamin A negatively affects preadipocyte survival.[11] Serum retinol concentration is commonly used to assess Vitamin A status in human because the gold standard procedure, biopsy, is not feasible.[7]

Resistin first found as the bridge between obesity and insulin resistance in mice by Steppan et al.[12] The next study by Bokarewa et al showed that in human, resistin has a different properties. In their study resistin secreted mainly in monocytes and macrophages showed an inflammatory properties by upregulated TNF-a and IL-6 expression, and by a positive loopback mechanism increasing its own expression.[13] Resistin is induced during differentiation of 3T3-L1 preadipocytes, suggesting it also serve as a feedback signal to restrict adipose tissue expansion. Recently it is showed that retinol could affect resistin expression, suggested via a PPAR-g pathway.[8]

This study was performed, to assess the correlation between Retinol/RBP4 ratio and Resistin with inflammation in Non Diabetic Obese Indonesian Men.

METHODS

Study Design

This was a crossectional study using 125 subjects, correlating Retinol/RBP4 ratio and resistin, and various metabolic profiles to hsCRP which represents inflammation status. Subjects with concentration of hsCRP <1 are classified as having low risk of inflammation, hsCRP 1–3 showed moderate risk inflammation and hsCRP concentration 3–10 showed high risk inflammation. Data collection was commenced from March–June 2009. The study proposal has been approved by the Health Research Ethics Committee of the Faculty of Medicine, University of Hasanuddin, Makassar.

Subjects

Subjects recruited was employees of PT Showa Indonesia and PT Posmi Cikarang Indonesia, who met the inclusion criteria: those who apparently healthy, male, aged 30 – 60 years old, with waist circumferences ≥ 90 cm, willing to participate in this study, and agree to follow the study protocol. Each subject was provided with explanation about the study, and signed informed consent prior to the commencement of the study.

Subjects consuming anti-inflammatory drugs, statins or thiazolidinedione for the last 3 weeks were excluded from the study. Dabetic subjects with fasting plasma glucose > 126 mg/dl, had liver dysfunction, kidney dysfunction, asthma, and acute inflammation (hsCRP > 10 mg/dl) were also excluded.

Anthropometric Measurement

Body weight (BW) was measured in kilograms to the nearest 0.1 kg, with light clothes on, using a beam scale Tanita (Tanita, Japan). Height (Ht) was measured in centimeters to the nearest 0.1 cm, in standing position with socks and shoes removed, using a microtois (stature meter). Waist circumference was measured in centimeters to the nearest 0.1 cm, using a flexible non-elastic tape made by Roche (Roche, Switzerland). The Waist Circumferences is measured at the part of the trunk located midway between the lower costal margin (bottom of lower rib) and the iliac crest (top of pelvic bone) while the person is standing, with feet about 25-30 cm apart. The measurer should stand beside the individual and fit the tape snugly, without compressing any underlying soft tissues. The circumference should be measured to the nearest 0.5 cm, at the end of a normal expiration.

Biochemical Assessment

After a 12 hours fasting, blood samples were collected in the morning between 07:00 to 10:00 am. Plasma and serum were separated immediately by centrifugation and aliquots were frozen at -20^0C for subsequent batched analysis for Retinol, RBP4, Resistin, and *high-sensitivity* C-reactive protein (*hs*CRP). Other biochemical marker were assessed soon after plasma separation.

Serum triglycerides, total cholesterol (TC), low and high-density lipoprotein cholesterol (LDL-C and HDL -C), Fasting Plasma Glucose, creatinine, alanine amino transferase (ALT) and aspartate amino transferase (AST) levels were assayed by the Prodia Clinical Laboratory's routine chemistry procedures. Serum AST, ALT and creatinine were determined using the International Federation of Clinical Chemistry (IFCC) method and reagents manufactured by Roche (Mannheim, Germany). The Standard automated glucose oxidase method was used to determined glucose, reagents manufactured by Roche (Mannheim, Germany). Homogeneous method was used to determine HDL-C, using reagents manufactured by Daiichi® Tokyo-Japan. The phosphate glycerol oxidase-peroxidase anti-peroxidase (GPO-PAP) method was used to determine triglycerides, using reagent manufactured by Roche (Mannheim, Germany).

Fasting plasma Retinol was measured using a commercially available HPLC kit from Immundiagnostik AG, Bensheim. Serum RBP4 was determined using an enzyme-linked immunoassay (ELISA) kit from Adipogen, Inc. Seoul, Korea. Resistin determined using ELISA kit from R&D Systems, Inc. Minneapolis, USA.

Retinol/RBP4 ratio was assessed by dividing the retinol concentration (mM) with RBP4 (mM). High ratio was defined as ratio > 0.9 and low ratio was defined as ratio ≤ 0.9 (16).

Data Analysis

Data analysis was done using SPSS 16.0 statistical analysis software for Windows (SPSS Inc., Chicago, IL, USA). Distributions of continuous variables were assessed for normality using the Kolmogorov-Smirnov. For continuous variables with normal distributions, such as anthropometric and biochemical measures, descriptive statistics were presented as mean ± SD. Associations between continuous variables with approximately normal distributions were described using Spearman's correlation coefficients. All tests were two-sided and considered significant at $p < 0.05$. Cross-tabulation test was performed to compare the inflammation incident between groups with different stages of ratio.

RESULTS

Table 1 showed general description of subjects participated in this study. Subjects' age from 30 –53 years old, with WC between 91–125 cm. All subjects are non diabetic men with FPG < 126 mg/dl. Subjects' AST and ALT are less than 2 times of normal concentration, and bilirubin concentration less than 1.2 mg/dl, showing that they have no liver dysfunction. Creatinin concentration less than 1.6 mg/dl indicating that subjects have no kidney impairment.

Table 2 showed that retinol has a strong correlation to RBP4 and resistin. A univariate linear regression analysis with RBP as the dependent parameter showed a tight correlation between RBP and retinol ($r = 0.53$; $p = 0.001$).

Table 1. Subjects' Basic Clinical and Biochemical Characteristics

Variables	Min (n = 125)	Max (n = 125)	Means±SD
Age (years)	30	53	38.18 ± 5.44
Clinical Variables			
WC (cm)	91	125	97.5 ± 5.71
Height (cm)	156	185	166.36 ± 5.31
Weight (Kg)	66	120	80.32 ± 8.02
SBP (mmHg)	90	150	114.36 ± 13.15
DBP (mmHg)	60	110	76 ± 10.16
Biochemical Variables			
Bilirubin (mg/dl)	0.07	0.42	0.23 ± 0.06
AST (U/l)	17	60	29.86 ± 9.17
ALT (U/l)	11	90	39.3 ± 16.29
Creatinin (mg/dl)	0.6	1.4	1.09 ± 0.15
FPG (mg/dl)	70	119	89.94 ± 8.69
LDL (mg/dl)	39	313	139.24 ± 37.62
Triglyceride (mg/dl)	42	1312	192.34 ± 133.33
HDL (mg/dl)	29	71	44.38 ± 7.73
HOMA IR	0.52	16.64	2.21 ± 1.99
hsCRP (mg/l)	0.24	9.12	2.32 ± 1.82
Retinol (µmol/l)	0.59	6.14	2.67 ± 1.01
RBP4 (µmol/l)	1.23	5.25	2.58 ± 0.73
Resistin (ng/ml)	0.67	23.38	8.96 ± 5.30
Retinol/RBP4 Ratio	0.22	2.38	1.05 ± 0.35

Description: WC = Waist Circumference; SBP = Systolic Blood Pressure; DBP = Diastolic Blood Pressure; AST = Aspartate Aminotransferase; ALT = Alanin Aminotransferase ; FPG = Fasting Plasma Glucose; HDL-C= High Density Lipoprotein Cholesterol; LDL-C = Low Density Lipoprotein Cholesterol; HOMA IR = Homeostasis Model Assessment of Insulin Resistance; RBP4 = Retinol Binding Protein 4; hsCRP = high sensitivity C-reactive protein.

Table 2. Correlation between Retinol, RBP4, Resistin and hsCRP

	R	***p***
Retinol vs		
RBP4	0.53**	0.00
Resistin	-0.2*	0.03
hsCRP vs		
Retinol	0.00	0.99
RBP4	-0.05	0.59
Resistin	0.14	0.13
Retinol/RBP4 Ratio	0.04	0.68

Description: *= significant at $p<0.05$; ** = significany at $p<0.001$.

There was a positive but not significant correlation found between the parameters (resistin and the Retinol/RBP4 Ratio) to hsCRP, meanwhile retinol showed no correlation at all to hsCRP, and RBP4 showed an unconsistent correlation. Crosstabulation graph (figure 3) also didn't give much information about the effect of Retinol/RBP4 Ratio to hsCRP. This figure showed that in high ratio group, it was found 17.6% subjects with low risk inflammation, 26.4% subjects with moderate risk inflammation, and 20.8% subjects with high risk inflammation, meanwhile in low ratio group it was found 8% subjects with low risk inflammation, 20% subjects with moderate risk inflammation, and 7.2% subjects with high risk inflammation.

When the ratio was combined with resistin (figure 4), it showed a clear trends, where the subjects with low risk inflammation decrease from 12% in "high ratio and low resistin" group into 3.2% in "low ratio and high resistin" group, meanwhile the number of subjects with high risk inflammation increase from 8% in the first group into 13.6% in the other group. This showed us that using this combination parameter could give us better information about the inflammation status in obese subjects.

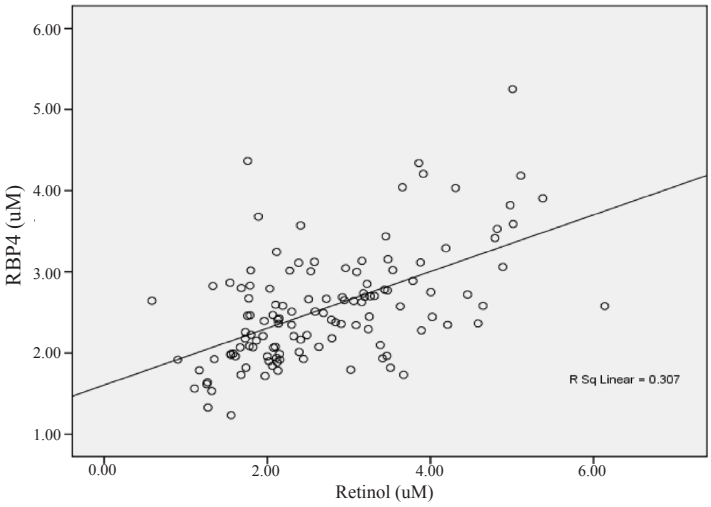

Figure 1. Correlations Between Retinol and RBP4 Concentration

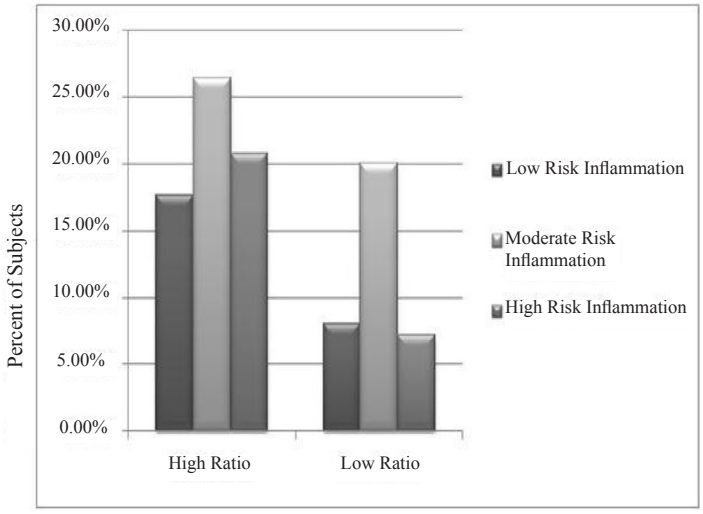

Figure 2. Crosstabulation between Retinol/RBP4 Ratio to hsCRP

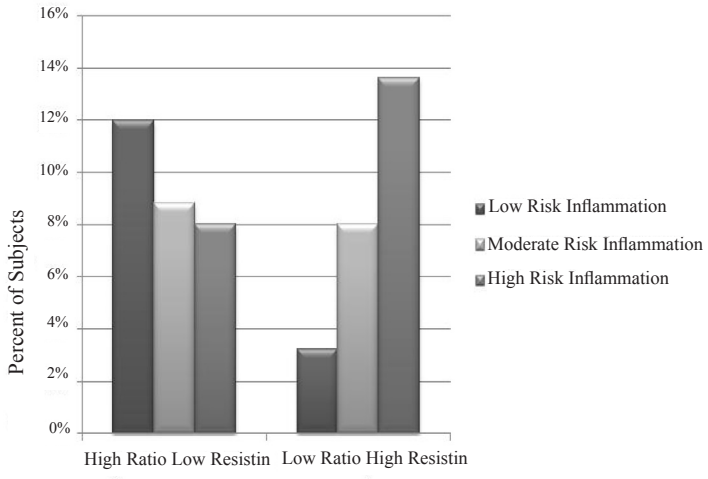

Figure 3. Crosstabulation between combination Retinol/RBP4 Ratio and Resistin to hsCRP

DISCUSSION

This study was designed to verify the correlation between Retinol/RBP4 Ratio, and resistin with inflammation (represented by hsCRP) in non-diabetic obese Indonesian men. We did the investigation only in men to clear the hormonal bias.[14] Only a few studies investigate about using serum retinol as a covariate association analysis involving RBP, and our study was expected to provide more understanding about this problem.

In this study, RBP4 was found to be strongly correlated with retinol. A univariate linear regression analysis with RBP as the dependent parameter showed a tight correlation between RBP and retinol ($r = 0.53$; $p = 0.001$). Retinol only induced the secretion of RBP4 from the liver. This RBP4 then will bind retinol in about 1:1 molar ratio, called *holo*-RBP. The elevation of RBP4 in obese subjects may be explained as the greater amounts of RBP4 in circulation derived from adipose tissue. Perhaps there was not enough retinol to bind RBP4 in adipose tissue so therefore *apo*-RBP4 is released into the plasma.[15,16]

Retinol was found to be negatively correlated with resistin in this study. Retinol was known to affect resistin expression by several pathways: First, PPAR-g, which is likely to mediate the inhibitory effect of TZDs on the resistin gene, regulates transcription as heterodimer with Rexinoid Receptors (RXR), and the PPAR:RXR, unlike other RXR-containing heterodimers, can be activated by ligands of both partners. Second, resistin gene expression is induced by CCAAT/enhancer-binding protein-a (C/EBP-a) and Retinoid Acid, the acid from of vitamin A is known to inhibit the transcriptional activity of C/EBP transcription factors.[11]

By assessing the Retinol/RBP4 Ratio, we were provided with a better information than the RBP4 alone. This ratio represented the RBP4 which didn't bind with retinol. It was suggested that the *apo*-RBP4 were in a free state or binded with an unknown ligand. We divide the ratio groups based on the study by Mills et al into high ratio (which is ratio > 0.9) and low ratio (ratio ≤ 0.9).[11]

Their study showed that utilization of retinol/RBP4 could help to isolates such influences on RBP4 regulation. In non obese adults, they found the ratio is 0.9 ± 0.22, significantly higher compare to diabetic obese adults (0.73 ± 0.13). They didn't make any observation in non-diabetic obese adults however. Our study showed the ratio for non-diabetic obese Indonesian men is 1.05 ± 0.35.

In figure 3, it was showed that either in high or low ratio groups, the inflammation risk trends was similar. Combining the ratio with resistin gave us a better description about the inflammation risk in subjects. These give a suggestion, the ratio could give us good information about inflammation and insulin resistant risk in normal subjects,[11] but in obese subjects, combining this ratio and resistin gave us more reliable information.

In, conclusion, our study showed that the combination between Retinol/RBP4 Ratio and resistin showed better description about the inflammation risk in non-diabetic obese subjects compared to the ratio itself, where the

subjects with low risk inflammation decrease from 12% in "high ratio and low resistin" group into 3.2% in "low ratio and high resistin" group, meanwhile the number of subjects with high risk inflammation increase from 8% in the first group into 13.6% in the other group.

Acknowledgment

This study was fully funded by the Prodia Foundation for Research and Training. We are indebted to the technical staff of the Prodia Clinical Laboratory, Clinical Trial and Research and Development Departments for their valuable assistance in undertaking specimen collection in the field and biochemical assessments.

REFERENCES

1. Huang TTK, McCrory MA. Dairy intake, obesity and metabolic health in children and adolescents: knowledge and gaps. *Nutr Rev* 2005;63:71– 80.
2. Va´zquez-Vela MEF, Torres N, Tovar A. White adipose tissue as endocrine organ and its role in obesity. *Arc Med Res* 2008; 39: 715 – 28.
3. Wozniak SE, Gee LL, Wachtel MS, Frezza EE. Adipose tissue: the new endocrine organ? A review article. *Dig Dis Sci*. 2009; 54: 1847 – 56.
4. Rasouli N, Kern PA. Adipocytokines and the metabolic complications of obesity. *J Clin Endocrinol Metab* 2008; 93: S64–S73.
5. Pepys MB, Hirschfield GM. C-reactive protein: a critical update. *J Clin Invest* 2003; 111: 1805 – 12.
6. Clyne B, Olshaker JS. The C-reactive protein. *J Emerg Med* 1999; 17: 1019-25.
7. Felipe F, Bonet ML, Ribot J, Palou A. Modulation of resistin expression by retinoic acid and vitamin a status. *Diabetes* 2004; 53: 882 – 9.
8. Tanumihardjo SA. Assesing vitamin A status: past, present and future. *J Nutr* 2004; 134: 290S – 293S.
9. Villarroya F, Giralt M, Iglesias R. Retinoids and adipose tissues: metabolism, cell differentiation and gene expression. *Int J Obes Relat Metab Disord* 1999; 23: 1– 6.
10. Bonet ML, Ribot J, Felipe F, Palou A. Vitamin A and the regulation of fat reserves. *Cell Mol Life Sci* 2003; 60: 1311–21.
11. Felipe F, Bonet ML, Ribot J, Palou A. Modulation of resistin expression by retinoic acid and vitamin a status. *Diabetes* 2004; 53: 882 – 9.
12. Steppan CM, Bailey ST, Bhat S, Brown EJ, Banerjee RR, Wright CM, et al. The hormone resistin links obesity to diabetes. *Nature* 2001; 409: 292 – 3.
13. Bokarewa M, Nagaev I, Dahlberg L, Smith U, Tarkowski A. Resistin, an adipokine with potent proinflammatory properties. *J Immunol* 2005; 174: 5789 – 95.
14. Asimakopoulos B, Milousis A, Gioka T, Kabouromiti G, Gianisslis G, Troussa A, et al. Serum pattern of circulating adipokines throughout the physiological menstrual cycle. *Endocr J* 2009; 56: 425 – 33.
15. Graham TE, Yang Q, Blüher M, Hammarstedt A, Ciaraldi TP, Henry RR, et al. Retinol-binding protein 4 and insulin resistance in lean, obese, and diabetic subjects. *N Engl J Med* 2006; 354: 2552 – 63.
16. Mills JP, Furr HC, Tanumihardjo SA. Retinol to retinol-binding protein (RBP) is low in obese adults due to elevated apo-RBP. *Exp Biol Med* (Maywood) 2008; 233: 1255 – 61.