THE HIGH PLASMA RETINOL BINDING PROTEIN 4 LEVEL AS A RISK FACTOR CONSEQUENTLY OF TYPE 2 DIABETES MELLITUS OF ABDOMINAL OBESITY

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ABSTACT

Abdominal obesity (Ab-Ob) related to cardiometabolic risk, that is risk factor constellation for succeeded cardiovasculer disease and type 2 Diabetes Mellitus (DM). That factors such as atherogenic dislipidemia, hypertension, hyperglycemia, protrombotic state, and proinflammation state. Type 2 DM characterised by insulin resistance (IR). Plasma levels of retinol binding protein 4 (RBP4) that is secreted by adipocytes are increased in insulin resistance (IR) state. Experiment in mice suggest that elevated RBP4 level cause IR. Although the underlying mechanism is not clearly understood, RBP4 considered play importance role consequently of type 2 DM in Ab-Ob.

This research was carried out to determine the role of high plasma RBP4 level as a risk factor consequently of type 2 DM in Ab-Ob. The research was conducted by cross sectional analytic in 81 patients with Ab-Ob and case control study with matching on 33 patients with Ab-Ob type 2 DM as cases and 33 patients with Ab-Ob non type 2 DM as control. The plasma of TNF-α, sTNFR1, and RBP4 levels was measured by ELISA. IR status of the patients was determined by HOMA-IR, whereas the β-cell function was determined by HOMA-B. Ab-Ob was defined by using criteria for Asian peoples (male WC ≥ 90 cm; female WC ≥ 80 cm). The result of 81 patients with Ab-Ob showed that both plasma of TNF-α and sTNFR1 levels were significant positive correlated with plasma RBP4 level (coeficient correlation r = 0,294; p = 0,008 dan r = 0,458; p = <0,001 respectively). In addition, the plasma of RBP4 level significantly positive correlation with HOMA-IR (r = 0,450; p = 0,000) and significantly negative correlation with HOMA-B (r = -0,564; p = <0,001). In the matched case-control study, it was shown that mean plasma of RBP4 level of type 2 DM group (76,08 ± 16,84 µg/ml) statistically higher than that without type 2 DM group (41,13 ± 14,75 µg/ml) (p = <0,001). The odds ratio higher plasma of RBP4 level was 5,426 (CI 95%; 1,343 – 21, 928) statistically significant for increases risk type 2 DM (p = < 0,05). It has been proven that RBP4 was a dominant and consisten risk factor (66.9%, p = < 0.001) which influenced the incidence of type 2 DM in Ab-Ob.

It can be concluded that high plasma of RBP4 level have a greater risk to suffered from type 2 DM compared to low plasma of RBP4 in Ab-Ob. The high plasma of RBP4 level is most dominant and consistent risk factor consequently of type 2 DM. These mechanism could behind the association between high plasma of RBP4 level and type 2 DM.

Key word: Abdominal obesity, RBP4, HOMA-IR, HOMA-B and type 2 DM.

INTRODUCTION

Abdominal obesity (Ab-Ob) is strongly related with the pathogenesis of IR and type 2 DM. Adipose tissue may be viewed as an endocrine organ that secreted many types of adipokines (such as free fatty acid, tumor necrosis factor-α, interleukin-6, and adiponectin) that modulate the action of insulin, protrombosis, and protrombosis.[1,2,3] Moreover, RBP4, a new fat-derived adipokin that specifically binds to retinol, has recently been reported to provide a link between obesity and IR.[4] RBP4 was discovered while trying to indentify the substance responsible for regulating insulin sensitivity in mice either lacking or overexpression GLUT4 in adipose tissue.[5,6] It is regulated reciprocally in adipose tissue of mice overexpressing or lacking GLUT4. Circulating RBP4 level were reported to be raised in several different mouse models of obesity and IR.[4] Increasing the circulating levels of RBP4 leads to glucose intolerance, whereas knock out of the RBP4 gene increases insulin sensitivity.[4] Study on human, serum RBP4 level correlated with magnitude of IR in subjects with obesity, impaired glucose tolerance, or type 2 DM, and in non obese, non diabetic subjects with strong family history of type 2 DM.[7] Study in Ab-Ob patients have been reported thats plasma of RBP4 level correlated with RBP4 mRNA,[8] and inflammation in adipose tissue.[9]

Accordingly to the above findings, the pathobiological mechanism associated with the high plasma of RBP4 level in IR state and type 2 DM has not been studied. The plasma of RBP4 level is assumed to increase in Ab-Ob and linked to inflammation of adipose tissue and β-cell function. Therefore, it is importance to carry out a study to know high plasma of RBP4 level as risk factor type DM event in Ab-Ob.

MATERIAL AND METHODS

The study was conducted in two stages. First, a cross sectional analytic study was used to find out the relationship between plasma of TNF-α, soluble of tumor necrosis receptor (sTNFR)1 and RBP4 level, and plasma of RBP4 level with β-cell function. The second stage, a matching case control study was

performed to know relationship between high plasma of RBP4 level with consequently of type 2 DM in Ab-Ob. The accessible population was all Ab-Ob patients who visited Sanjiwani Hospital. The study subjects were patients with Ab-Ob (intended samples) selected from the accessible population that met the inclusion and exclusion by using *consecutive sampling technique*. The actual study subjects were patients with Ab-Ob who had confirmed to involve in this study by signing and informed consent. The criteria for Ab-Ob using the value waist circumference for men ≥ 90 cm and for women ≥ 80 cm. Exclusion criteria of study were: patients with coronarry heart disease, acute or chronic renal failure, acute or chronic liver malfunction, infection, malignancy, family hystori of type 2 DM, taking drugs such as insulin, oral hypoglycemic, anti-inflammatory, anti-hypertensive, and hipolipidemia since at least last two weeks and patient who did not consent to involve in the study. Patients who finally consent to joint the study were informed about the study aims.

Blood samples were obtained from each subject after 8-hour overnight fast from an antecubital vein into Vacuteiner tubes. Fasting and 2 hours post prandial serum glucose, total cholesterol, trygliceride (TG), low-density lipoprotein (LDL)-cholesterol, and high-density lipoprotein (HDL)-cholesterol levels were measured by enzymatic procedures using an autoanalyzer (Hitachi 912, Roche Diagnostic GmbH). Fasting insulin was measured by chemiluminescence immunoassay (Immulite 2000). Plasma sTNFR1, TNF-α , and RBP4 levels were measured by an enzym immunoassay kit (R&D Systems, Inc., Minneapolis, United State of America). IR was estimated by homeostasis model assessement of IR (HOMA-IR).[10] β-cell function was estimated by homeostasis model assessement of β-cell function (HOMA-B).[11]

All collected data were first assessed before taken for analysis. The normality test of *Kosmogorov-Smirnov* was done to asses wether the data were normally distributed or not. Descriptive statistics was used to illustrate patients characteristics. Patient characteristics were compared among the two groups using one-way ANOVA. Pearson's correlation coefficients were calculated to evaluate the relationship between plasma of RBP4 levels and several risk factors in this

study. *Forward conditional* logistic regression analyses was used to asses *Odd's Ratio* (OR) between the high plasma of RBP4 level and consequently of type 2 DM with control confounding variables (*adjusted odd's ratio*). Path analyses models were also used to analyze the influence of risk factors that cause direct and indirect occurrence of type 2 DM. The above statistic analyses used $p < 0.05$ as the significance standard, by means of satistic software *SPSS for windows version 15.0.*

RESULTS

A total 81 out of 104 of Ab-Ob patients were met the requirement as sample in this study, 37 were men (46%) and 44 were women (54%). Distribution of patient proportion suffering from type 2 DM was 40.7%, while dislipidemia and hypertension were 76.5% and 49.4% respectively. Most of the obtained data were not normally distributed by using *Kosmogorov-Smirnov* test at 5% significance level, except for height and blood HDL-cholesterol level. By using bivariate correlation, age, fasting blood sugar level, plasma of TNF-α , sTNFR1 levels, and HOMA-IR were significantly positive correlated; whereas HOMA-B was significantly negative correlated with plasma of RBP4 level (Table 1).

As many as 33 pairs of Ob-Ab patients with type 2 DM (as cases) and without type 2 DM (as control) from earlier cross-sectional study sample were used to determine the association between plasma of RBP4 level and type 2 DM. Selection for control group was done by paired case based on age (Matching). From the data shown in Table 2, there are several variables were significantly different between case and control groups. There are several variables that potentially have confounding effects on the relationship between plasma RBP4 level and type 2 DM status, which may affect the difference between case and control characteristics. It then analysed by using *forward conditional* logistic regression analysis to evaluate the association of RBP4 as a risk factor in type 2 DM status. Table 3 represents regression analysis result that Ob-Ab patients with high plasma RBP4 level (≥ cuttoff) was likely 5.426 times to have type 2 DM compare to the patients who had plasma RBP4 under cuttoff (95% CI: 1.343 –

21.928). It implies that high plasma of RBP4 levels increase the risk of type 2 DM in the Ab-Ob patients.

Table 1. Correlation between plasma of RBP4 level and various metabolic variables in Ab-Ob patients

Variable	Plasma of RBP4 levels	
	r	p
Age	0,240	0,031
Waist circumference (cm)	0,053	0,637
Blood pressure (mmHg):		
- systolic	0,045	0,692
- diastolic	0,099	0,380
Fasting blood glucose (mg/dl)	0,639	<0,001
- Cholesterol total (mg/dl)	0,180	0,109
- HDL – cholesterol (mg/dl)	0,008	0,943
- LDL – cholesterol (mg/dl)	0,172	0,124
- Tryglicerid (mg/dl)	0,182	0,104
Colesterol total/HDL ratio	0,125	0,265
TNF-α (pg/ml)	0,294	0,008
*s*TNF R1 (ng/ml)	0,418	<0,001
HOMA-IR	0,458	<0,001
HOMA-B	-0,564	<0,001

In this research type 2 DM was dependent variable, while TNFα, sTNFR1, HOMA-IR, HOMA-B, and RBP4 were risk variables. However, only HOMA-IR directly influenced consequently of type 2 DM, whereas other variables influenced indirectly through other risk variables. The result of analysis model of risk factors for type 2 DM shows of RBP4 level was the most dominant and consistent risk factor (66.9%, $p < 0.001$) on the consequently of type 2 DM in the patients with Ab-Ob. RBP4 concentration influenced both directly and indirectly through negative effects on HOMA-B and a positive on HOMA-IR (Figure 1).

Table 2. Characteristic between Ab-Ob patients with type 2 DM (as cases) and without type 2 DM (as control)

Characteristics	Cases (n = 33)	Control (n = 33)	p
Age (year)	50,94 ± 6,15	50,76 ± 6,15	0,136
Weight (kg)	88,44 ± 13,10	87,44 ± 12,24	0,789
Height (cm)	159,98 ± 7,77	160,98 ± 6,80	0,592
Waist circumferences (cm)	103,92 ± 8,06	103,89 ± 9,02	0,990
Systolic BP (mmHg)	142,94 ± 24,62	137,21 ± 25,02	0,409
Diastolic BP (mmHg)	83,12 ± 11,65	78,96 ± 13,73	0,236
Hypertesion	51,52%	12,12%	<0,001
Fasting blood sugar (mg/dl)	197,76 ± 59,04	96,33 ± 9,63	<0,001
2 hours PP blood sugar (mg/dl)	303,97 ± 77,28	119,91 ± 23,87	<0,001
Total cholesterol (mg/dl)	221,61 ± 35,911	212,64 ± 39,65	0,273
Hypercholesterolemia (mg/dl)	72,73%	54,55%	0,110
HDL - cholesterol (mg/dl)	46,42 ± 8,44	45,67 ± 6,56	0,613
HDL-hypocholesterolemia	33,33%	36,36%	0,768
LDL - cholesterol (mg/dl)	146,24 ± 33,38	132,73 ± 28,56	0,071
LDL-hypercholesterolemia	63,60%	54,50%	0,309
Tryglicerid (mg/dl)	169,97 ± 65,65	164,42 ± 78,64	0,739
Hypertrigliceridemia	57,58%	54,55%	0,786
Total cholesterol / HDL ratio	6,53 ± 8,63	4,73 ± 1,05	0,247
Insulin (μIU/ml)	7,85 ± 3,59	7,69 ± 5,13	0,877
HOMA-IR	3,70 ± 2,18	1,84 ± 1,33	<0,001
HOMA-B (%)	22,87 ± 13,99	75,69 ± 43,33	<0,001
RBP4 (μg/ml)	76,08 ± 16,84	41,13 ± 14,75	<0,001
TNF-α (pg/ml)	15,22 ± 4,48	11,18 ± 5,25	0,004
sTNFR1 (ng/ml)	5,88 ± 2,75	4,15 ± 1,54	0,005

Table 3. Multiple logistic regression analysis risk factors consequently of type 2 DM

Variables		*Adjusted OR*	CI 95%	p
1. Model 1	a. High HOMA-IR	6,923	(2,243 – 21,367)	0,001
	b. Constant	0,070		0,001
2. Model 2	a. High RBP4	5,858	(1,514 – 22,673)	0,010
	b. High HOMA-IR	6,758	(2,042 – 22,370)	0,003
	c. Constant	0,008		0,000
3. Model 3	a. High RBP4	5,426	(1,343 – 21,928)	0,018
	b. High HOMA-IR	5,129	(1,475 – 17,834)	0,010
	c. High sTNFR1	3,712	(1,122 – 12,281)	0,032
	d. Constant	0,002		0,000

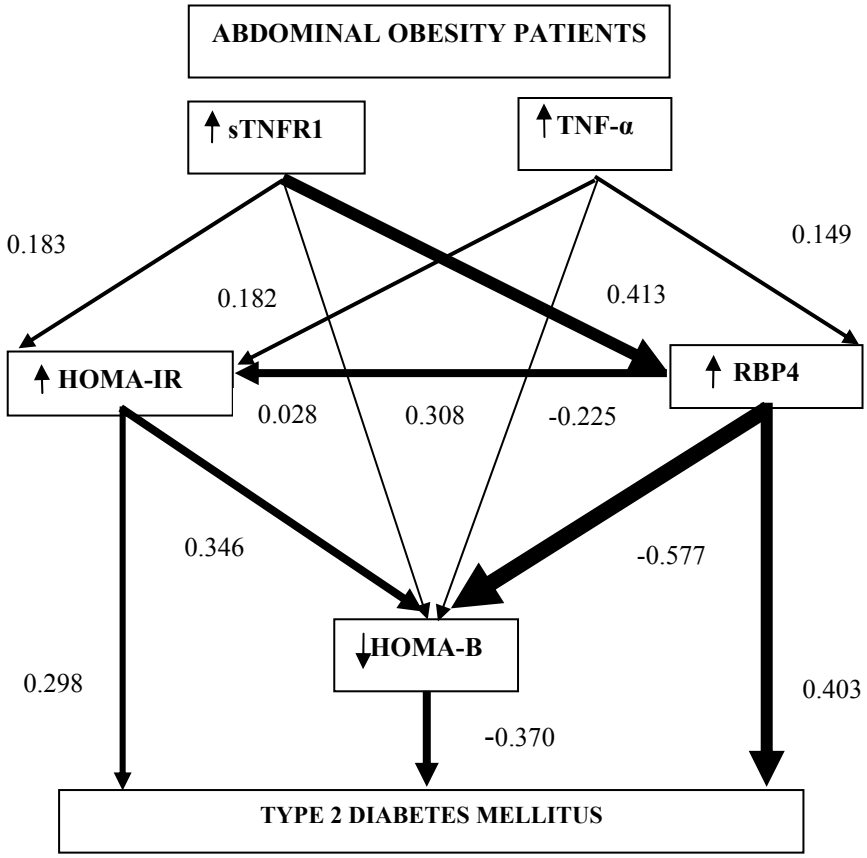

Figure 1 Summary of the study

DISCUSSION

Tumor Necrosis Factor-α and *Retinol Binding Protein 4*

TNF-α will activate adipokin expression change which involved in occurence of IR and type 2 DM in Ab-Ob patients through inflammatory signaling.[7] The increase of RBP4 expression resulted from adiposa tissue and liver is influenced by TNF-α.[9] We found a positive correlation between TNF-α concentration and plasma RBP4. Therefore, there is an indication that Ob-Ab is correlated with the increase of adipose tissue inflammatory response which is positively associated to the increased concentration of plasma RBP4.

Concentration of RBP4 in blood circulation is influenced by the quantity of abdominal fat. When the abdominal fat is decreased, it then leads to the decrease of RBP4 concentration.[12,30] Other researchers have reported the

concentration of circulating RBP4 was not associated with RBP4 expression in adipose tissue.[13] This difference can be explained due to the different definition that were used in size criteria for Ab-Ob.

Soluble Tumor Necrosis Factor Receptor 1, Retinol Binding Protein 4 and Insulin Resistance

Examination of TNF-α system activity based on TNF-α receptor concentration will be more reliable, considering that this protein is easy to be recognized in the plasma and further, illustrate the degree of TNF- α system activity.[14] sTNFR1 is a major mediator of TNF-α activity in the pathophysiology of IR and type 2 DM.[15,16] Activation of MAP4K4 by TNF-α through TNFR1 would reduce the expression of PPARγ and GLUT4 adiposit.[17, 18] The decrease in GLUT4 expression would interfere the glucose transport in adipose tissue, which then stimulates the increased expression of RBP4.[19] In this study, we found a significant positive correlation of sTNFR1 concentration with high plasma RBP4. The increase of RBP4 expression in Ab-Ob is correlated with the occurrence of RI in adipose tissue. The increase of this plasma RBP4 is then going to cause disruption in signaling skeletal muscle insulin and accompanied with stimulation of liver glucose production.[9] In Ab-Ob patients, the high plasma RBP4 concentration correlated with the degree of IR (measured by using the HOMA-IR).

Thus, it seems that there is a positive link between inflammation with the increased expression of RBP4, which is associated with the occurrence of IR.

Retinol Binding Protein 4 and Beta Cell Secretion Function

A significant negative correlation between high plasma RBP4 concentration with beta cell secretion function in Ab-Ob patients was found in this study (measured by using the HOMA-B). This correlation can be the basic background for the association between the increased concentration of RBP4 and the incidence of type 2 DM.

Pathophysiologically, retinol has correlation with beta cell function. RBP4 circulation forms bond with transtiretin (TTR) protein, which is a functional

component of the increase of beta cell secretion.[20] In the circulation, TTR binds strongly to RBP4. This can prevent TTR effect on beta cell secretion.[21] Obtained data above implies that Ab-Ob patients could not adapt the low level of insulin sensitivity in the decreased insulin secretion.

Retinol Binding Protein 4 and type 2 diabetes mellitus

Plasma RBP4 concentration of type 2 DM was statistically higher than non-DM type 2 in this research. The high plasma of RBP4 level in type 2 DM may be related to the degree of obesity and RI.[22] In this study, the Ab-Ob patients with high plasma of RBP4 level have 5 times greater risk to get type 2 diabetes mellitus compared to Ab-Ob patients with low plasma of RBP4 level ($p = <0.05$).

First report was made in this study regarding high plasma RBP4 level as a risk factor for the incidence of Ab-Ob type 2 diabetes. There are several previous studies that reported a significant correlation of plasma levelof RBP4 with the incidence of type 2 diabetes. However, these studies did not report how much of the RBP4 level influences the consequently of type 2 DM.[23,24]

Risk Factors Effects on the Incidence of type 2 DM

Type 2 diabetes mellitus is a group of metabolic disorders are complex and heterogeneous. This type of DM resulted from IR and reduced secretion of cell beta combination.[25] The failure of beta cell was caused by low concentration of adiponectin and high concentration of TNF-α. Indeed, these different concentrations are associated with the increase of diabetes risk, which is also related to insulin sensitivity and pancreatic function.[26] In this study, plasma TNF-α concentration could not be proven as a risk factor of the type 2 diabetes incidence. This may be due to TNF-α is more active as autocrine / paracrine in the tissue. It is suspected that TNF-α endocrine effect is mediated by sTNFR1, which results in decreased expression of PPARγ and GLUT4 adipose.[23] The declined GLUT4 expression causes IR in adipocytes, and then stimulate the RBP4 expression.[4]This process is likely occurred in Ab-Ob, where we got the level of

sTNFR1 plasma affected the incidence of type 2 diabetes indirectly through RBP4 and HOMA-IR.

RBP4 injection in normal mice will lead to IR, while the paralyzed rat have an increase of RBP4 in insulin sensitivity.[4] This study proved that high plasma RBP4 level gives a positive influence on HOMA-IR. On the other hand, this protein was proved to give negative impact on HOMA-B. Thus, it seems that negative influence of RBP4 to HOMA-B is not associated with IR.[27] IR state is an important risk factor for type 2 DM, although it is not the central pathological process of the disease. Beta cell dysfunction and apoptosis are important risk factors, which is likely that RBP4 correlated with IR and beta cell function.[28,29] This study has statistically and significantly proved that high plasma of RBP4 level is the most dominant and consistent risk factor, which affects directly or indirectly the consequently of type 2 DM, through the HOMA-IR and HOMA-B.

CONCLUSION

In conclusion, high plasma of RBP4 level was significantly proven as a new risk factor type 2 DM. In accord with relationship between risk factor, plasma of RBP4 as a dominant and consistent risk factor influences consequently of type 2 DM in the Ab-Ob.

REFERENCES

1. Rajala, M.W., Scherer, P.E. 2003. Minireview: The Adipocyte-At the Crossroads of Energy Homeostasis, Inflammation, and Atherosclerosis. Endocrinology, 114(9): 3765-3773.

2. Ronti, T., Lupattelli, G., Mannarino, E. 2006. The endocrine function of adiposa tissue: an update. Clin Endocrinol, 64: 355-365

3. Bastard, J.P., Maachi, M., Lagathu, C., Caron, M., Vidal, H., Capeau, J., Feve, B. 2006. Recent advances in the relationship between obesity, inflammation, and insulin resistance. Eur Cytokine Netw, 17: 4-12.

4. Yang, Q., Graham, T.E., Mody, N., Preitner, F., Peroni, O.D., Quadro, J.L., Kahn, B.B. 2005. Serum retinol binding protein 4 contributes to insuline resistance in obesity and type 2 diabetes. Nature, 436: 356-362.

5. Abel, E.D., Peroni. O., Kim. J.K., Kim, Y.B. 2001. Adipose-selective targeting of GLUT4 gene impair insulin action in muscle and liver. Nature, 409: 729-733.

6. Shepherd, P.R., Kahn, B.B. 1999. Glucose transporters and insulin action- implications for insulin resistance and diabetes mellitus. N Engl J Med, 341: 248-257.

7. Graham, T.E, Yang, Q., Blüher, M., Hammarstedt, A., Ciaraldi, T.P., Henry, R.R., Wason, C.J., Oberbach, A., Jansson, P.A., Smith, U., Kahn, B.B. 2006. Retinol-Binding Protein 4 and Insuline Resistance in Lean, Obese, and Diabetic Subjects. N Engl J Med, 354: 2552-2563.

8. Klöting, N., Graham, T.E., Berndt, J., Kralisch, S., Kovacs, P., Wason,C.J., Fasshauer, M., Schön, M.R., Stumvoll. M., Blüher, M., Kahn, B.B. 2007. Serum retinol-binding protein is more highly expressed in visceral than in subcutaneus adipose tissue and is marker of intra-abdominal fat mass. Cell Metab., 6: 79-87.

9. Yao-Borengasser, A.W., Varma,V., Bodles, A.M., Rasouli, N., Phanavanh., Lee, M.J., Starks, T., Kern, L.M., Spencer III, H.J., Rashidi, A.A., McGehee, R.E., Fried, S.K., Kern ,P.A. 2007. Retinol Binding Protein 4 Expression in Humans: Relationship to insulin resistance, Inflammation, and response to Pioglitazone. J Clin Endocrinol Metab, 92: 2590-2597.

10. Matthews, D.R., Hosker, J.P., Rudenski, A.S., Naylor, B.A., Treacher, D.F., Turner, R.C. 1985. Homeostasis model assesment: Insulin resistance and beta-cell function from plasma glucose and insulin concentration in man. Diabetologia, 28: 412-419.

11. Haffner, S.M., Gonzalez, C., Miettinen, H., Kennedy, E., Stern, M.P. 1996. A Prospective Analysis of the HOMA Model. The Mexico City Diabetes Study. Diabetes care, 19: 1138-1141.

12. Lee, J-W., Im, J-A., Lee, H-R., Shim, J-Y., Youn, B-S., Lee, D-C. 2007. Visceral Adiposity Is Associated with Serum Retinol Binding Protein-4 in Healthy Women. Obesity, 15: 2225-2232.

13. Janke, J., Engeli, S., Boschmann, M., Adams, F., Böhnke, J., Luft, F.C., Sharma, A.M., Jordan, J. 2006. Retinol-binding protein 4 in human obesity. Diabetes, 55: 2805-2810.

14. Zahorska-Markiewicz, B., Janowska, J., Olzanecka-Glinianowicz, M., Zurakowski, A. 2000. Serum concentrations of TNF-α and soluble TNF-α receptors in obesity. Int J Obes, 24: 1392-1395.

15. Uysal, K.T., Weisbrock, S.M., Hotamisligil, G.S. 1998. Fuctional Analysis of Tumor Necrosis Factor (TNF) Receptors in TNF-α-Mediated Insulin Resistance in Genetic Obesity. Endocrinology, 139: 4832-4838.

16. Sethi, J.K., Xu, H., Uysal, K.T., Wiesbrock, S.M., Scheja, L., Hotamisligil, G.S. 2000. Characterisation of receptor-spesific TNF-alpha functions in adipocyte cell lines lacking type 1 and 2 TNF-α receptors. FEBS Lett., 469: 77-82.

17. Sugnami, T., Tanimoto-Koyama, K., Nishida, J, Itoh, M., Yuan, X., Mizuarai, S., Kotani, H., Yamaoka, S., Aoe, S., Kamei, Y., Ogawa, Y. 2007. Role of the Toll-like receptor 4/NF-kappaB pathway in saturated fatty acid-induced inflammatory changes in the interaction between adipocytes and macrophage. Arterioscler Thromb Vasc Biol, 27: 84-89.

18. Tesz, G., Guilherme, A., Guntur, K.V.P., Hubbard, A,C., Tang, X., Chawla, A., Czech, M.P. 2007. Tumor Necrosis Factor α (TNFα) Stimulates Map4k4 Expression trough TNFα Receptor 1 Signaling to c-Jun And Activating Transcription Factor 2. J Biol Chem, 282: 19302-19312.

19. Guilherme, A., Virbasius, J.V., Puri, V., Czech. P. 2008. Adipocyte dysfunctions linking obesity to insulin resistance and type 2 diabetes. Mol Cel Biol, 9: 367-377.

20. Refai, E., Dekki, N., Yang, S-N., Imreh, G., Cabrera, O., Yu, L., Yang, G., Norgren, S., Rössner, S.M., Inverardi, L., Ricordi, C., Olivecrona, G., Anderson, M., Jörnvall., Berggren, J-O., Juntti-Berggren, L. 2005. Transthyretin constitutes a functional component in pancreatic β-cell stimulus-secretion coupling. PNAS, 47: 17020-17025.

21. Broch, M., Vedrell, J., Ricart, W., Richart, C., Fernandez-Real, J.M. 2007. Circulating retinol-Binding Protein-4, Insulin Sensitivity, Insulin Secretion, and Insulin Disposition Index in Obese and Nonobese Subjects. Diabetes Care, 30: 1802-1806.

22. Graham, T.E., Kahn, B.B. 2008. Tissue-spesific alterations of glucose transport and molecular mechanism of intertissue communication in obesity and type 2 diabetes. Horm Metab Res, 39: 717-721.

23. Cho, Y.M., Youn, B.S., Lee, H., Lee, N., Min, S.S., Kwak, S.H., Lee, H.K., Park, K.S. 2006. Plasma Retinol-Binding Protein-4

Concentrations Are Elevated in Human Subjects With Impaired Glucose Tolerance and Type 2 Diabetes. Diabetes Care, 29: 2457-2461.

24. Takebayashi, K., Suetsugu, M., Wakabayashi, S., Aso, Y., Inukai, T. 2007. Retinol Binding Protein-4 Levels and Clinical Features of Type 2 Diabetes Patients. J Clin Endocrinol Metab, 92: 2712-2719.

25. Hajer, G.R., van Haeften, T.W., Visseren, F.L.J. 2008. Adipose tissue dysfunction in obesity, diabetes, and vascular diseases. Eur Heart J, 29: 2959-2971.

26. Prentki, M., Nolan, C.J. 2006. Islet beta cell failure in type 2 diabetes. J Clin Invest, 116: 1802-1812.

27. Graham, T.E., Wason, C.J., Blüher, M., Kahn, B.B. 2007. Shortcomings in methodology complicate measurements of serum retinol binding protein (RBP4) in insulin-resistant human subjects. Diabetologia, 50: 814-823.

28. Kahn, S.E., Hull, R.L., Utzschneider, K.M. 2006. Mechanisms linking obesity to insulin resistance and type 2 diabetes. Nature, 444: 840-846.

29. Esteve, E., Ricart, W., Fernandez-Real, J.M. 2009. Adipocytokines and Insulin Resistance. Daibetes Care, 32: S362-S367.

30. Tschoner, A., Sturm, W., Engl, J., Kaser, S., Laimer, M., Laimer, E., Weiss, H., Patsch, J.R., Ebenbichler, C.F. 2008. Retinol Binding Protein 4, Visceral Fat, and the Metabolic Syndrome: Effects of Weight Loss. Obesity, 16: 2439-2444.