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CLINICAL STUDY

Serum levels of leptin, adiponectin, retinol binding protein 4 and leptin/adiponectin molar ratio as another possible marker of insulin resistance in obese

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Abstract: *Aim:* To determine the relationship between some adipokines and insulin resistance (IR) in obese. *Materials and methods:* 47 individuals were divided – 1. according to BMI to subgroups with normal weight, overweight and obesity, 2. according to IR HOMA index to subjects with IR (IR HOMA >1.88) and subjects without IR. Except the basic biochemical parameters, serum level of fasting insulin, leptin, retinol binding protein 4 (RBP4) (RIA), adiponectin (ELISA) and leptin/adiponectin (L/A) molar ratio were examined.
Results: 1. In correlation to BMI — subjects with BMI > 30 showed significantly higher level of insulin (16.1±4.1/5.9±4.1), leptin (51.9±26.4/14.7±14) and molar ratio L/A (3.1±1.8/0.48±0.2) ($p<0.01$) and significantly lower level of adiponectin (18.9±6.3/35.5±10.5) ($p<0.01$) in comparison to both other subgroups (normal weight, overweight). There was no statistically significant difference in RBP4 level between all subgroups, although the highest level of RBP4 was observed in subjects with BMI >30. In correlation to IR – subjects with IR showed significantly higher BMI (35.7±5.8/24.8±2.6), insulin (15.5±7.1/4.8±1.6), leptin (47.2±29.2/15.1±13), L/A molar ratio (2.7±0.3/0.5±0.1) ($p<0.01$), and RBP4 (561.6±152.5/450.9±101.7 ($p<0.05$) as well. In IR subjects, serum level of adiponectin was significantly lower in comparison to subjects without IR (19.8±6.3/32.2±0.8) ($p<0.01$).
Conclusion: Decreased level of adiponectin and increased level of leptin, RBP4 and leptin/adiponectin molar ratio in obese can be also considered as a marker of developing insulin resistance *(Tab. 2, Ref. 23)*. Full Text (Free, PDF) *www.bmj.sk.*
Key words: obesity, insulin resistance, leptin, adiponectin, L/A, RBP4.

Obesity is the most frequent metabolic disorder in the last years. As reported by WHO in developed countries there is one billion people with overweight (BMI >25 kg/m^2) and 300 million with obesity (BMI >30 kg/m^2). Obesity with accumulation of visceral and subcutaneous fat at the front of chest and abdomen represents metabolic and inflammatory stress for the organism (8). Obesity is often associated with insulin resistance (IR) and high risk of diabetes mellitus type 2 (DM2) development. High attention is increasingly focused on the question – by which mechanism does the obesity contribute to IR and DM2 development. Adipose tissue is an active tissue. Adipocytes produce many adipokines, growth factors and hormones that influence metabolic actions. The most studied are leptin, IL-6, TNF-alfa, resistin, adiponectin, angiotensin II and retinol binding protein 4 (RBP4). These adipokines possess both local and overall effects and act by autocrine, paracrine and endocrine mechanism (3, 19, 20). They influence appetite, energetic balance, immunity, insulin sensitivity, angiogenesis, blood pressure, lipid metabolism, homeostasis and may also play a role in cancer pathogenesis in obese (19). Their production depends on the volume and distribution of adipose tissue.

Increase of visceral fat results in higher release and subsequent storage of free fatty acids in insulin sensitive tissues. This leads to insulin resistance in skeletal muscles and liver. In addition, increased storage of FFA to pancreatic beta cells occurs. The effect of FFA on pancreatic beta cells is lipotoxic. Lipotoxicity is responsible for activation of intracellular signals, that result in apoptosis of pancreatic beta cells and consequently in reduction of their number. Both processes, IR and pancreatic beta cells damage, are in close connection to obesity and DM2 in obese (4, 18). Except that, increase of visceral fat mainly, causes dysregulation in production of adipokines (7). The production of insulin sensitising adipokines, represented by adiponectin, decreases the production of inflammatory adipokines. Inflammatory adipokines influence many functions negatively, namely appetite, energetic balance, immunity, insulin sensitivity, angiogenesis, blood pressure, lipid metabolism and homeostasis (8, 13, 19). In this way, overall effect of obesity on metabolism is mediated through adipokines and serum level of leptin, adiponectin, RBP4 reflects ectopic fat distribution in obese individuals (1, 4, 17).

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Tab. 1. BMI and results.

Parameters	BMI <25 n=14 x ±SD	BMI 25–30 n=12 x ± SD	BMI >30 n=21 x ± SD
Age (years)	51,2 ± 9,7	51,7 ± 10,4	45,5 ± 7,7
Cholesterol (mmol/l)	5,4 ± 0,8	5,0 ± 1,0	4,9 ± 1,0
TAG (mmol/l)	1,02 ± 0,5	0,97 ± 0,3	1,04 ± 0.5
Fasting plasma Glucose (mmol/l)	5,2 ± 0,8	6,6 ± 2,7	6,2 ± 2,4
Fasting Inzulin (μIU/ml)	5,9 ± 4,1	8,1 ± 5,3	16,1± 7,6**
IR HOMA	1,3 ± 0,9	2,2 ± 1,6	4,2 ± 1,9**
Systolic blood Pressure(mmHg)	121,6 ± 14,9	133,3 ± 20,9	144,5 ± 14,6**
Diastolic blood Pressure (mmHg)	76,6 ± 8,8	85,4 ± 15,4	93,1 ± 15,3**
Leptin (ng/ml)	14,7 ± 14	23,1 ± 16,6	51,9± 26,4**
Adiponectin (ng/ml)	31,5 ± 10,5	29,8 ± 11,2	18,9 ± 6,3**
L/A	0,48 ± 0,2	0,99 ± 0,9	3,1 ± 1,8**
RBP4 (ng/ml)	491,0 ± 86,6	489,0 ± 154,7	548,5 ± 162,4

IR HOMA – inzulin resistence indexes, RBP4 – retinol binding protein 4, TAG – triglycerides, L/A – leptin-to-adipomectin ratio, ** p<0.01

Tab. 2. Insulin resistance and results.

Parameters	IR detected n=27 x ±SD	IR undetected n=20 x ± SD	p
Age (years)	47,3 ± 9,4	50,7 ± 8,9	NS
BMI	35,7 ± 5,8	24,8 ± 2,6	P ≤ 0,01
Cholesterol (mmol/l)	4,9 ± 0,9	5,3 ± 0,7	NS
TAG (mmol/l)	1,0 ± 0,5	1,0 ± 0,3	NS
Fasting plasma Glucose (mmol/l)	6,7 ± 2,7	5,2 ± 0,7	P ≤ 0,01
Fasting Inzulin (μIU/ml)	15,5 ± 7,1	4,8 ± 1,6	P ≤ 0,01
IR HOMA	4,1 ± 1,8	1,1 ± 0,4	P ≤ 0,01
Systolic blood Pressure (mmHg)	144,1 ± 15,1	122,5 ± 16,5	P ≤ 0,01
Diastolic blood Pressure (mmHg)	92,9 ± 14,2	77,2 ± 11,6	P ≤ 0,01
Leptin (ng/ml)	47,2 ± 29,2	15,1 ± 13,0	P ≤ 0,01
Adiponectin (ng/ml)	19,8 ± 6,3	33,2 ± 10,8	P ≤ 0,01
L/A	2,7± 0,3	0,5 ± 0,1	P ≤ 0,01
RBP4 (ng/ml)	561,6 ± 152,5	450,9 ± 101,7	P ≤ 0,05

IR HOMA – inzulin resistence indexes, RBP4 – retinol binding protein 4, TAG – triglycerides, L/A – leptin-to-adipomectin ratio, BMI – (weight in kg / height in m^2)

Materials and methods

The study involved 47 individuals of similar age – 22 males (average age 49±8,9) and 25 females (average age 48.3±9.7). These were divided – according to BMI to 3 subgroups: 1) subjects with BMI <25. 2) BMI 25–30, and 3) BMI >30 – according to IR HOMA index (G0 x I0/22.5) to 2 subgroups: 1) subjects with insulin resistance (IR HOMA >1.88) and 2) subjects without insulin resistance.

In each subject blood samples were taken between 7 and 9 a.m. (venous blood from median cubital vein) and fasting glucose, cholesterol and triglycerides by standard biochemical method, insulin, leptin and RBP by RIA method and adiponectin by ELISA method were examined. Before the withdrawal in each individual blood pressure was measured with mercury barometer. From the obtained results leptin/adiponectin (L/A) molar ratio was estimated. The results were evaluated in correlation to BMI and in correlation to IR.

For statistical processing basic descriptive statistics and commercially available computer statistic programs were used. The hypothesis of equality of variances was tested by One-Way Analysis of Variance – ANOVA. For comparison between groups Tukey-Kramer method was applied. For testing the hypothesis of equality of two independent means unpaired T-test was used, or non-parametric Mann-Whitney test in case of statistically significant difference between scatters. Linear dependence itself verified regression and correlation analyses. Risk development IR in dependencies from adiponectin and leptin to adiponectin ratio examined Fisher scientific sample.

Results

From the obtained variables in correlation to BMI, subjects with BMI >30 showed significantly higher values of systolic and diastolic blood pressure, serum insulin level, IR HOMA, leptin and L/A molar ratio and significantly lower serum level of adiponectin (p≤0.01) than those with BMI <30. There was no statistical difference in serum levels of RBP4, however subject with BMI >30 showed the highest serum level of RBP4 (Tab. 1).

From the obtained variables in correlation to IR (Tab. 2), subjects with IR showed significantly higher fasting glucose, systolic and diastolic blood pressure, BMI, insulin, IR HOMA, leptin and L/A molar ratio and significantly lower level of adiponectin in comparison to subjects without IR (p≤0.01). Serum RBP4 level was also significantly higher in IR subjects (p≤0.05).

There was a positive correlation between serum leptin level and BMI (r=0.727, p≤0.01) and IR HOMA as well (r=0.685, p≤0.01), and negative correlation between adiponectin and BMI (r=-0.534, p<0.01), leptin (r=-0.471, p≤0.05), RBP4 (r=-0.483, p≤0.05), insulin (r=-0.423, p=0.08) and IR HOMA (r=-0.487,

p≤0.05). There was a positive correlation between L/A molar ratio and IR HOMA index as well (r=0.640, p≤0.01).

Subjects with low level of adiponectin showed 2.3x higher risk of IR development (p=0.34, OR 2.35, 95 % CI 0.607–10.049). Higher L/A molar ratio has increased relative risk of IR 1.05x (p=0.94, OR 1.05, CI 0.278–3.96).

Discussion

In obese subjects, the adipose tissue is infiltrated by macrophages, which produce inflammatory cytokines. The inflammation of adipose tissue is generally considered a main cause of peripheral insulin resistance development. Obesity, especially visceral one, is associated with increased production of inflammatory adipokines, represented by TNF-alfa, at the expense of protective adipokines, represented by adiponectin (2, 18). TNF--alfa is an inflammatory adipokine, which stimulates production of leptin, IL-6 and other inflammatory mediators. It is also a direct regulator of CRP production. It stimulates the lipolysis and its increased level is associated with hyperinsulinemia, IR in liver and muscles and with the increase of systolic blood pressure (21).

Adiponectin is a peptide inhibiting the food intake and positively affecting metabolic processes. It increases the transport of free fatty acids and their oxidation in muscles. It also decreases the amount of fat in muscle fibres. It affects the transfer of insulin signal both on receptor and post-receptor level. It increases insulin sensitivity, glucose utilization, inhibits gluconeogenesis and decreases glycemia. It inhibits differentiation of proadipocytes, regulating thus the growth of adipose tissue and decreasing the body weight (5, 16, 21).

Adiponectin affects anti-inflammatory processes in atherosclerosis (21). It is considered to be an anti-diabetic, anti-atherosclerotic and anti-inflammatory protein produced by adipocytes and a strong marker signaling the risk of diabetes development in obese (12, 13).

In accordance with findings of Norico Satoh 2004, we have proved, that increase of body weight is associated with decrease of adiponectin level, but also with increase of serum leptin level, higher L/A molar ratio and serum level of RBP4. RBP4 is a protein, also produced by adipocytes, which inhibits expression of GLUT4 transporter (a protein important for transmembrane glucose transport), decreases insulin signal, inhibits insulin effects, especially in skeletal muscles and supports gluconeogenesis in liver (16).

Serum levels of studied parameters were dependent not only on BMI, but in consistence with Graham et al (2006), Lee et al (2007), Takebayashi et al (2007) and Oda et al (2008), we observed higher level of glycemia, fasting insulinemia, leptin, L/A molar ratio and RBP4 and decreased level of adiponectin in obese subjects with IR.

In addition, decreased serum adiponectin level is an independent marker predisposing to development of hypertension (16), what was proven also in our study. Hypoadiponectinemia is associated with obesity, IR, changes in lipid profile, increase of inflammatory markers and higher risk of diabetes and cardiovascular diseases even in the youth (11, 22). Change of life style may positively influence serum level of adiponectin and thus decrease risk of diabetes development in obese, especially in young individuals (12).

Conclusion

Decreased level of adiponectin and increased level of leptin, RBP4 and L/A molar ratio in obese with abnormal fat distribution can be considered as a possible marker signaling the dysfunction of adipose tissue. The loss of body weight accompanied by changes in level of adipokines in obese, especially in young individuals, can contribute to decrease of IR development risk, as well as to impairment of metabolic syndrome with its related complications.

References

1. Anderwald C, Roden M. Adipotoxicity and the insulin resistance syndrome. Pediatr Endocrinol Rev 2004; 1: 310—319.

2. Bays HE, Abate N, Chandalia M. Adiposopathy: sick faty causes high blood sugar, high blood pressure, and dislipidemia. Future Cardiology 2005; 1: 39—59.

3. Carvalho E, Kotani K, Peroni OD, Kahn BB. Adipose-specific overexpression of GLUT4 reverses insulin resistance and diabetes in mice lacking GLUT4 selectively in muscle. Am J Physiol Endocrinol Metab 2005; 289: E 551—E 561.

4. Eldor R, Raz I. Lipotoxicity versus adipotoxicity. The deleterious effects of adipose tissue on beta cells in the pathogenesis of type 2 diabetes. Diabetes Res Clin Pract 2006; 74 S: S3—S8.

5. Gil-Campos M, Canete RR, Gil A. Adiponectin, the missing link in insulin resistance and obesity. Clin Nutr 2004; 23: 963—974.

6. Graham TE, Yang Q, Bluher M et al. Retinol-binding protein 4 and insulin resistance in lean, obese, and diabetic subjects. New Engl J Med 2006; 354: 2552—2563.

7. Cho YM, Youn BS, Lee H et al. Plasma retinol-binding protein-4 concentrations are eleved in human subjects with impaired glucose tolerance and type 2 diabetes. Diabetes Care 2006; 29: 2457—2461.

8. Lee YH, Pratley RE. The evolving role of inflammation in obesity and the metabolic syndrome. Curr Diab Rep 2005; 5: 70—75.

9. Lee DC, Lee JW, Im JA. Association of serum retinol binding protein 4 and insulin resistance in apparently healthy adolescents. Metabolism 2007; 56: 327—331.

10. Lihn AS, Pedersen SB, Richelsen B. Adiponectin: action, regulation and association to insulin sensitivity. Obes Rev 2005; 6: 13—21.

11. Maahs DM, Ogden LG, Kinney G L et al. Low plasma adiponectin levels predict progression of coronary artery clasification. Circulation 2005; 111: 747—753.

12. Mather KJ, Funahashi T, Matsuzawa Y et al. Adiponectin,change in adiponectin, and progression to diabetes in the diabets prevention program. Diabetes 2008; 57: 980—986.

13. Matsuzawa Y. Adiponectin: Identificatitoin, physiology and clinical relevance in matabolic and vascular disease. Atherosclerosis Supl 2005; 6: 7—14.

14. Norico Satoh, Mitsuhide Naruse, Takeshi Usui et al. Leptin-to-adiponectin ratio as a potential atherogenic index in obese type 2 diabetic patients. Diabetes Care 2004; 27: 2488—2490.

15. Oda N, Imamura S, Fujita T et al. The ratio of leptin to adiponectin can be used as an index of insulin resistance. Metab Clin Exper 2008; 57: 268—273.

16. Palomer X, Perez A, Blanco-Vaca F. Adiponectin: a new link between obesity, insulin resistance and cardiovascular disease. Med Clin (Barc) 2005; 124: 388—395.

17. Perseghin G, Lattuada G, De Gobelli F et al. Serum retinol- binding protein-4, leptin and adiponectin concentrations are related to ectopic fat accumulation. J Clin Endocrinol Metab 2007; 92: 4883—4888.

18. Redinger RN. The physiology of adiposity. J Ky Med Assoc 2008; 106: 53—62.

19. Ronti T, Lupattelli G, Mannarino E. The endocrine function of adipose tissue: An uptade. Clin Endocrinol 2006; 64: 355—365.

20. Soodini GR. Adiponectin and leptin in relation to insulin sensitivity. Metab Syndr Relat Disord 2004; 2: 114—123.

21. Sonnenberg GE, Krakower GR, Kissebah AH. A novel pathway to the manifestations of metabolic syndrome. Obes Res 2004; 12: 180—186.

22. Takebayashi K, Suetsugu M, Wakabayashi S et al. Retinol binding protein-4 levels and clinical features of type 2 diabetes patients. J Clin Endocrinol Metab 2007; 92: 2712—2719.

23. Trujillo ME, Scherer PE. Adiponectin — journey from an adipocyty secretory protein to biomarker of the metabolic syndrome. J Intern Med 2005; 257: 167—175.

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