Plasma Levels of Fetuin-A, Adipocyte Fatty Acid-Binding Protein and 8-Hydroxydeoxyguanosine in Patients with Metabolic Syndrome

Metabolik Sendromlu Hastalarda Plazma Fetuin-A, Adiposit-Yağ Asidi Bağlayıcı Protein ve 8-Hidroksideoksiguanozin Düzeyleri

Şölen HİMMETOĞLU,[a]
Oben ARI,[b]
Aysel YAKICI,[c]
Lale KOLDAŞ,[c]
Yıldız DİNÇER[d]

[a]Centro Laboratories,
[b]Clinic of Cardiology,
Bahçelievler Medicana Hospital,
Departments of
[c]Cardiology,
[d]Biochemistry,
İstanbul University
Cerrahpaşa Faculty of Medicine,
İstanbul

Geliş Tarihi/*Received:* 25.11.2013
Kabul Tarihi/*Accepted:* 25.11.2014

This paper has already been presented in the Sixth World Congress of Prevention of Diabetes, 8-11 April 2010, Dresden-Germany.

Yazışma Adresi/*Correspondence:*
Yıldız DİNÇER
İstanbul University
Cerrahpaşa Faculty of Medicine,
Department of Biochemistry, İstanbul,
TÜRKİYE/TURKEY
yldz.dincer @ gmail.com

ABSTRACT Objective: The aim of the present study was to investigate new markers for metabolic syndrome. Fetuin-A is a physiological inhibitor of insulin receptor and is associated with insulin resistance. Adipocyte fatty acid-binding protein regulates cellular lipid metabolism by carrying free fatty acids to various intracellular compartments. Recently antioxidant and insulinotropic roles of adipocyte fatty acid-binding protein were determined. 8-hydroxydeoxyguanosine is as a marker of oxidative DNA damage. In the present study, plasma levels of fetuin A, adipocyte fatty acid-binding protein and 8-hydroxydeoxyguanosine were determined in cases with metabolic syndrome. **Material and Methods:** A total of 60 cases with metabolic syndrome were included in the study. The control group was constituted by age-matched 20 healthy volunteers. Fasting blood samples were taken from cases, plasma levels of fetuin-A, adipocyte fatty acid-binding protein and 8-hydroxydeoxyguanosine were measured with competitive ELISA kits. **Results:** Plasma levels of 8- hydroxydeoxyguanosine and adipocyte fatty acid-binding protein levels were found to be significantly higher in the cases with metabolic syndrome in comparison to those in the control group. No significant difference was determined between study groups for fetuin A level. **Conclusion:** Plasma level of 8-hydroxydeoxyguanosine and adipocyte fatty acid-binding protein are at a high level in cases with metabolic syndrome. High 8- hydroxydeoxyguanosine level may be considered as an evidence for increased cancer risk in cases with metabolic syndrome. In addition, it was concluded that high adipocyte fatty acid-binding protein level may be related with hyperinsulinemia.

Key Words: Insulin resistance; metabolic syndrome X; Ahsg protein, mouse; fatty acid-binding proteins; 8-hydroxy-2'-deoxyguanosine

ÖZET Amaç: Bu çalışmada, metabolik sendrom için yeni belirteçler araştırmak amaçlanmıştır. Fetuin-A insülin reseptörünün fizyolojik inhibitörüdür ve insülin direnci ile ilişkilidir. Adiposit yağ asidi bağlayıcı protein serbest yağ asitlerini hücre içinde çeşitli kompartmanlara taşıyarak hücresel lipid metabolizmasını düzenler. Son yıllarda adiposit yağ asidi bağlayıcı proteinin antioksidan ve insülin salınımını uyarıcı etkileri olduğu belirlenmiştir. 8-hidroksideoksiguanozin, oksidatif DNA hasarı belirtecidir. Bu çalışmada, metabolik sendromlu olgularda plazma fetuin-A, adiposit yağ asidi bağlayıcı protein ve 8-hidroksideoksiguanozin düzeyleri belirlenmiştir. **Gereç ve Yöntemler:** Metabolik sendrom tanısı almış 60 hasta çalışma kapsamına alındı. Kontrol grubu, aynı yaş grubundaki 20 sağlıklı gönüllüden oluşturuldu. Hastalardan ve kontrollerden açlık kan örnekleri alınarak plazma fetuin-A, adiposit yağ asidi bağlayıcı protein ve 8-hidroksideoksiguanozin düzeyleri yarışmalı ELISA kitleri ile ölçüldü. **Bulgular:** Plazma 8-hidroksideoksiguanozin ve adiposit yağ asidi bağlayıcı protein düzeyleri hasta grubunda kontrol grubuna göre anlamlı olarak yüksek bulundu. Fetuin-A düzeyi bakımından çalışma grupları arasında anlamlı fark bulunmadı. **Sonuç:** Metabolik sendromlu olgularda plazma 8-hidroksideoksiguanozin ve adiposit yağ asidi bağlayıcı protein düzeyleri yüksektir. Yüksek 8-hidroksideoksiguanozin düzeyi metabolik sendrom vakalarında artmış olan kanser riski için bir kanıt olarak dikkate alınabilir. Ayrıca, yüksek adiposit yağ asidi bağlayıcı protein düzeyinin hiperinsülinemi ile ilişkili olabileceği sonucuna varıldı.

Anahtar Kelimeler: İnsülin direnci; metabolik sendrom X; Ahsg proteini, fare; yağ asidi-bağlayıcı proteinler; 8-hidroksi-2'-deoksiguanozin

doi: 10.5336/medsci.2013-38201

Turkiye Klinikleri J Med Sci 2015;35(1):1-7

Fetuin-A (alpha2-Heremans-Schmid glycoprotein) is a circulating multifunctional glycoprotein synthesized in liver. It is a calcification inhibitor and a negative acute-phase protein.[1,2] Fetuin-A is also physiological inhibitor of insulin receptor tyrosine kinase and its incerased levels are associated with insulin resistance in humans.[3,4] On the other hand, systemic oxidative stress is associated with insulin resistance.[5,6] Oxidative stress is a state in which production of reactive oxygen species (ROS) exceeds the capacity of antioxidant systems in the body. Attacks of ROS to DNA result in base oxidation. Major DNA oxidation product is 8-hydroxydeoxyguanosine (8-OHdG). 8-OHdG has a pro-mutagenic potential by mispairing with A residues, leading to an increased frequency of spontaneous G:C-T:A transversion. 8-OHdG residues on DNA are excised by constitutive enzymatic repair systems, appear in the blood, and are subsequently excreted in the urine. 8-OHdG level is a good biomarker for the measurement of endogenous oxidative DNA damage.[7]

Adipocyte fatty acid-binding protein (FABP4) is a member of the intracellular lipid-binding protein family and is predominantly expressed in adipose tissue. It is also expressed in macrophages. FABP4 regulates cellular lipid metabolism by carrying free fatty acids to various intracellular compartments, regulates expression of inflammatory cytokines, and modulates gene expression.[8] Recently, antioxidant and insulinotropic roles of FABP4 against ROS were revealed. FABP4 regulates glucose metabolism and regulates hepatic glucose production.[9-11]

Metabolic syndrome consists of a cluster of metabolic disorders such as abdominal obesity, insulin resistance, hypertension and hyperlipidemia. Oxidative stress is a contributory factor in the development of metabolic disorders. Increased oxidative stress in obesity and its impacts on metabolic syndrome have been well documented.[12,13] Although main functions of fetuin A and FABP4 are different, both of them have important effects in glucose metabolism, in addition both of them are related with oxidative state. Beyond conventional serum markers for metabolic syndrome, discovery of new markers which can be easily regulated by therapeutic approaches may be helpful for prevention of metabolic syndrome. The aim of the present study was to examine plasma levels of fetuin-A, FABP4 and 8-OHdG in cases with metabolic syndrome, and their relations with serum lipids, glucose, human serum C-reactive protein (HsCRP) and insulin resistance.

MATERIAL AND METHODS

Our study comprises 60 metabolic syndrome cases diagnosed in the Istanbul University, Cerrahpasa Medical Faculty Department of Cardiology. Cerrahpasa Medical Faculty Ethical Committee approval was taken in accordance with the principles of Declaration of Helsinki and informed consent was obtained from all subjects. Diagnosis criteria for metabolic syndrome were presence of abdominal obesity (waist circumference >102 cm for men and, >88 cm for women), fasting glucose concentration >100 mg/dl, blood pressure >130/80, high-density lipoprotein (HDL) <40 mg/dl for men and <50 mg/dl for women, and triglycerides >150 mg/dl. All cases underwent a standard questionnaire and physical examination. Cases with serious left ventricular hypertrophy, unstable coronary syndromes, history of acute coronary syndrom, decompanse heart failure, pulmonary embolism, thyroid disorder, acute and/or chronic inflammatory disease, renal disorder, infectious disease or those who were on drugs due to disease were excluded from the study. A total of 23 of the cases had been diagnosed with diabetes mellitus previously, they were being treated with metformin (850 mg/day) and glycemic control had been achieved. Diabetic cases who were being treated with insulin were excluded from the study. A total of 20 of the cases were diagnosed with hyperlipidemia and they were treated with fluvastatin (80 mg/day). A total of 20 of the cases were diagnosed with hypertension and they were used valsartan as angiotensin receptor blocker (ARB) (80/160 mg/day). A total of 20 of the cases were diagnosed with hypertension and hyperlipidemia and they were given both ARB and statin in the dosages mentioned above. Standard physical examination, electrocardiography, echocardiography, routine biochemical measurements were done and serum

levels of fetuin-A, FABP4 and 8-OHdG were measured in each patient. Insulin resistance was determined by homeostasis model assessment for insulin resistance (HOMA-IR).[12] It was calculated with this equation: HOMA-IR= Fasting serum glucose (mg/dl) x fasting insulin (μU/ml)/404. The control group was constituted by age-matched 20 healthy volunteers. None of the cases were currently smoker, were taking antioxidant supplement and any drug. Patient and control characteristics were shown in the Table 1.

Blood samples were collected into EDTA containing tubes, centrifuged at 1000 x g for 15 min, and immediately stored at -70°C as aliquot. Plasma levels of fetuin-A, FABP4 and 8-OHdG were measured with competitive ELISA kits from Assaypro, BioVendor, and Northwest, respectively. Serum levels of total cholesterol, HDL and low density lipoprotein (LDL), triglycerides, glucose, insulin, HsCRP were measured in routine analysis laboratory of the Cerrahpasa Medical Faculty Hospital.

STATISTICAL ANALYSIS

Characteristics and clinical data of the study groups and measured 8-OHdG, fetuin-A and FABP4 levels were given as median (min-max). Since data were not normally distributed, statistical analysis was performed by nonparametric tests. Data were analyzed by the Kruskall Wallis test. Mann-Whitney test was used for multiple comparisons between the groups. Sex variable was compared using the Chi-square test. Correlations between variables were examined by Spearman correlation coefficient. A value of $p<0.05$ was considered significant.

RESULTS

Serum levels of 8-OHdG (42 (7-83) ng/mL) and FABP4 (19 (8-43) ng/mL) were significantly higher in the cases with metabolic syndrome when compared to that of the controls (17 (3-45) ng/mL and 11 (5-26) ng/ml), respectively) ($p<0.001$ for both) but there was no significant difference for serum level of fetuin-A (Figure 1). The metabolic syndrome group was divided into subgroups according to aspects of disease. Group 1: hypertension and hyperlipidemia were present (n=20); Group 2: hyperlipidemia was present (n=20); Group 3: hypertension was present. Although fetuin-A level in the metabolic syndrome group was not significantly different from that of the control group, fetuin-A level in the cases with hypertension and hyperlipidemia (Group 1) was higher than those in the control

TABLE 1: Characteristics and clinical data of the study groups.

	Patient Group (n=60)	Control Group (n=20)
Gender		
Female	40	13
Male	20	7
Age (year)	54 (44-67)	52 (40-59)
BMI (kg/m^2)	30 (25-49)*	24 (21-30)
Waist circumference (cm)	97 (87-123)*	86 (82-95)
Fasting glucose (mg/dl)	122 (88-204)*	74 (66-100)
Serum insulin (μU/ml)	15 (7-56)*	4 (2-6)
HOMA	4 (12-19)*	1.0 (0.7-1.3)
HsCRP (mg/L)	5 (2-15)*	2 (1-3)
Total cholesterol (mg/dl)	236 (130-329)*	162 (143-183)
HDL (mg/dl)	35 (23-60)*	47 (38-66)
LDL (mg/dl)	148 (78-215)*	99 (82-122)
Triglyceride (mg/dl)	182 (55-792)*	103 (75-136)
Sys. blood pressure (mmHg)	150 (110-220)*	118 (92-139)
Dias. blood pressure (mmHg)	80 (60-110)*	75 (71-84)

* $p<0.001$ versus control group.

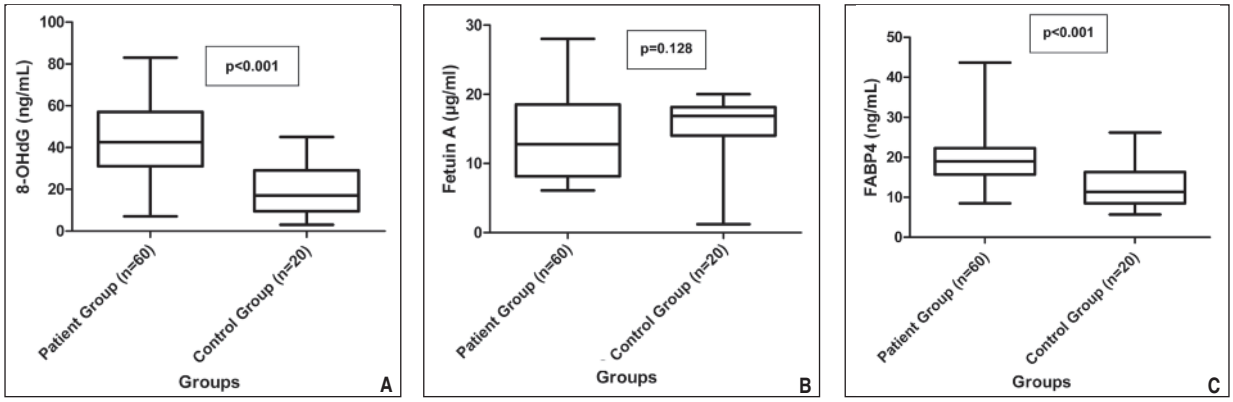

FIGURE 1: 8-hydroxydeoxyguanosine (8-OHdG) **A)**, fetuin-A **B)** and Adipocyte fatty acid-binding protein (FABP4) **C)** levels in the study groups.

group (p=0.010) and also higher than those in the cases with hypertension and subjects with hyperlipidemia (p=0.006 and p=0.008, respectively) (Table 2). Plasma level of 8-OHdG was higher in the cases with hypertension (Group 3) compared to those in both cases with hyperlipidemia (Group 2) and cases with hypertension and hyperlipidemia (Group 1) (p<0.001 for both), but no significant difference was determined for serum level of FABP4 between the subgroups (Table 2). Relations between serum lipids, glucose, HsCRP, insulin resistance, body mass index (BMI) and measured parameters were evaluated and following correlations were found in the metabolic syndrome group: 8-OHdG-fetuin-A (r=-0.492; p=0.004); 8-OHdG-insulin (r=-0.448; p=0.004); 8-OHdG-HDL (r=0.416; p=0.005); 8-OHdG-triglyceride (r=-0.288; p=0.036); 8-OHdG-HOMA-IR (r=-0.480; p=0.005); 8-OHdG-HsCRP (r=-0.370; p=0.004); fetuin A-systolic blood pressure (r=0.492; p=0.003); fetuin A-diastolic blood pressure (r=0.337; p=0.007); fetuin-A-insulin, (r=0.570; p=0.010); fetuin-A-fasting blood glucose, (r=0.272; p=0.047); fetuin-A-total cholesterol, (r=0.333; p=0.008); fetuin-A-LDL, (r=0.263; p=0.050); fetuin-A-triglyceride, (r=0.290; p=0.050); fetuin-A-HOMA IR, (r=0.591; p=0.003); fetuin-A-HsCRP, (r=0.422; p=0.005); FABP4-diastolic blood pressure (r=-0.225; p=0.050).

DISCUSSION

Systemic oxidative stress is associated with insulin resistance, visceral fat accumulation, and metabolic syndrome.[6,14] A membrane-bound NADH/NADPH oxidase is a major contributor of oxidative stress that is associated with these pathologies. In insulin-sensitive cells, upon insulin binding, ROS are produced by NADH/NADPH oxidase and relieve negative inhibition of the insulin signaling cascade by oxidizing negative regulators of the pathway. Thus ROS facilitate the transduction of the insulin signalling.[15] On the other hand, NADH/ NADPH oxidase can also generate ROS upon activation by proinflammatory cytokines and saturated fatty acids that interact with the toll-like receptor, both implicated in insulin resistance-associated conditions in adipocytes.[16] In addition, angiotensin II

TABLE 2: 8-OHdG, fetuin A and FABP4 levels in the patients with different aspects of metabolic syndrome.

	8-OHdG (ng/mL)	Fetuin A (µg/ml)	FABP4 (ng/ml)
Patients with hyperlipidemia (n=20)	44 (10-75)	9 (6-19b	20 (8-38)
Patients with hypertension (n=20)	56 (29-83)*	8 (6-16)	20 (10-43)
Patients with hyperlipidemia and hypertension (n=20)	31 (7-60)	19 (7-28)**	18 (11-21)
Controls (n=20)	17 (3-45)	16 (1-20)	11 (5-26)

* p<0.001 versus patients with hyperlipidemia, and versus patients with hyperlipidemia and hypertension; ** p<0.01 versus controls, versus patients with hyperlipidemia, and versus patients with hypertension.

which is a modulator of arterial blood pressure is a potent stimulator of both inflammation and oxidative stress via NADH/ NADPH oxidase.[17]

In a genetic rat model of metabolic syndrome, serum level of 8-OHdG was found to be gradually increased with the development of metabolic syndrome-like aspects in rats, which display abdominal obesity, hypertension, hyperglycemia, insulin-resistance, and hyperlipidemia.[18] Although various oxidative stress markers have been investigated[19,20], data demonstrating serum level of 8-OHdG in cases with metabolic syndrome are limited. Tokuda et al.[21] have reported that neither plasma nor urine 8-OHdG level was significantly different in patients with metabolic syndrome versus controls. In the present study, plasma level of 8-OHdG was found to be increased in the cases with metabolic syndrome, particularly in the cases with hypertension. Because of its mutagenic potential, 8-OHdG has been used in many studies not only as a biomarker for the measurement of endogenous oxidative DNA damage but also as a risk factor for cancer.[22] There is a considerable amount of evidence to indicate important role of metabolic syndrome in the etiology and progression of certain types of cancer. The association of metabolic syndrome with liver, pancreatic, gastric, colorectal, bladder, prostate, endometrial, cervical, and breast cancer have been reported.[23-25] In the present study, increased 8-OHdG level in the cases with metabolic syndrome may indicate the link between metabolic syndrome and cancer.

Animal studies showed that fetuin-A at a high concentration in serum inhibits insulin receptor tyrosine kinase activity.[26,27] In human studies higher fetuin-A levels were determined to be associated with insulin resistance[28,29] fatty liver,[30,31] and it was suggested as an independent risk factor for type 2 diabetes.[4,32,33] Serum fetuin-A was found to be independently associated with metabolic syndrome components including central obesity, high blood pressure, high blood glucose and high triglycerides by Xu et al.[34] Obese children with metabolic syndrome were shown to have a higher fetuin-A concentrations than obese children without metabolic syndrome and healthy controls.[35] Recently, higher fetuin-A level were determined to be associated with coexistence of elevated alanine aminotransferase and the metabolic syndrome in the general population.[36] In the present study, in contrast to previous studies, no significant difference was determined between study groups for serum level of fetuin-A. A limitation of this study was limited number of cases of the control group. The imbalance between case numbers of the study groups could have a negative impact on the statistical evaluation. However, fetuin-A level in the metabolic syndrome cases with hypertension and hyperlipidemia (Group 1) was higher than those in the controls. This gives rise to thought that togetherness of hypertension and hyperlipidemia is related to increased fetuin-A level. In agreement with this interpretation, serum fetuin-A level was found to be moderately correlated with blood pressure and slightly correlated with serum lipids. As interpreted by Ix et al.,[29] inhibition of the insulin receptor by fetuin-A may lead to increased lipolysis and efflux of free fatty acids from adipose tissue. This may, in turn, lead to increased production of very-low-density lipoprotein and finally increased serum lipids. Previous studies suggest that fetuin-A is a negative acute-phase reactant and its level falls during inflammation.[2] However some investigators have opposed this suggestion.[33] Inflammation was assessed by elevated HsCRP measurements in the present study. On the contrary to Ombrellino et al.[2] but in agreement with Ix et al.[29] and Hennige et al.,[33] we determined a positive correlation between serum levels of fetuin-A and HsCRP in the metabolic syndrome group.

Although FABP4 is a cytoplasmic protein, a significant portion of this protein is released from adipocytes and macrophages into blood. Xu et al.[37] have suggested that FABP4 is a circulating biomarker closely associated with obesity and components of the metabolic syndrome. Cohort studies showed that baseline serum FABP4 level is predictive for development of diabetes mellitus[38] and metabolic syndrome.[39] Recently, antioxidant[9] and insulinotropic[10] roles of FABP4 were shown. Oxidative stress was found to be increased in 3T3-L1 adipocytes in which FABP4 gene is silenced; using

the recombinant FABP4 it was shown that FABP4 itself functions as a ROS scavenger protein[9]. However, mechanism of its antioxidant effect has not been revealed yet. FABP4 potentiates hepatic glucose production and insulin secretion.[10,11] Lindsay et al.[10] recently demonstrated that in the presence of linoleate, FABP4 potentiates glucose-stimulated insulin secretion from β-cells in vitro and in vivo; and circulating FABP4 concentrations correlated with glucose-stimulated insulin secretion. In accordance with a previous study,[40] we determined high plasma level of FABP4 in the cases with metabolic syndrome. Insulin level and HOMA-IR were higher in the metabolic syndrome group in comparison to those in the control group. Hyperinsulinemia may be a result of high FABP4 level in these patients.

CONCLUSION

Our results show that plasma levels of 8-OHdG and FABP4 are at a high level in cases with metabolic syndrome. Because of its mutagenic property increased 8-OHdG level may be an evidence for increased cancer risk in these subjects. High FABP4 level may be related with high insulin level and insulin resistance . However, this was a preliminary study with limited number of cases of the control group. Further large scale studies are needed to confirm our findings and to elucidate underlying mechanisms.

Acknowledgement

This work was supported by the Research Fund of Istanbul University (Project no:UDP-5764/05022010).

REFERENCES

1. Suliman ME, García-López E, Anderstam B, Lindholm B, Stenvinkel P. Vascular calcification inhibitors in relation to cardiovascular disease with special emphasis on fetuin-A in chronic kidney disease. Adv Clin Chem 2008;46:217-62.
2. Ombrellino M, Wang H, Yang H, Zhang M, Vishnubhakat J, Frazier A, et al. Fetuin, a negative acute phase protein, attenuates TNF synthesis and the innate inflammatory response to carrageenan. Shock 2001;15(3): 181-5.
3. Kaushik SV, Plaisance EP, Kim T, Huang EY, Mahurin AJ, Grandjean PW, et al. Extended-release niacin decreases serum fetuin-A concentrations in individuals with metabolic syndrome. Diabetes Metab Res Rev 2009;25 (5):427-34.
4. Mori K, Emoto M, Yokoyama H, Araki T, Teramura M, Koyama H, et al. Association of serum fetuin-A with insulin resistance in type 2 diabetic and nondiabetic subjects. Diabetes Care 2006;29(2):468.
5. Houstis N, Rosen ED, Lander ES. Reactive oxygen species have a causal role in multiple forms of insulin resistance. Nature 2006;440 (7086):944-8.
6. Eriksson JW. Metabolic stress in insulin's target cells leads to ROS accumulation - a hypothetical common pathway causing insulin resistance. FEBS Lett 2007;581(19):3734-42.
7. Loft S, Poulsen HE. Cancer risk and oxidative DNA damage in man. J Mol Med (Berl) 1996;74(6):297-312.
8. Maeda K, Cao H, Kono K, Gorgun CZ, Furuhashi M, Uysal KT, et al. Adipocyte/ macrophage fatty acid binding proteins control integrated metabolic responses in obesity and diabetes. Cell Metab 2005;1(2): 107-19.
9. Kajimoto K, Minami Y, Harashima H. Cytoprotective role of the fatty acid binding protein 4 against oxidative and endoplasmic reticulum stress in 3T3-L1 adipocytes. FEBS Open Bio 2014;4:602-10.
10. Wu LE, Samocha-Bonet D, Whitworth PT, Fazakerley DJ, Turner N, Biden TJ, et al. Identification of fatty acid binding protein 4 as an adipokine that regulates insulin secretion during obesity. Mol Metab 2014;3(4):465-73.
11. Cao H, Sekiya M, Ertunc ME, Burak MF, Mayers JR, White A, et al. Adipocyte lipid chaperone AP2 is a secreted adipokine regulating hepatic glucose production. Cell Metab 2013;17(5):768-78.
12. Furukawa S, Fujita T, Shimabukuro M, Iwaki M, Yamada Y, Nakajima Y, et al. Increased oxidative stress in obesity and its impact on metabolic syndrome. J Clin Invest 2004; 114(12):1752-61.
13. Himmetoglu S, Teksoz S, Zengin K, Yesim T, Taskın M, Dincer Y. Serum levels of fetuin A and 8-hydroxydeoxyguanosine in morbidly obese subjects. Exp Clin Endocrinol Diabetes 2013;121(8):505-8.
14. Kunitomo M, Yamaguchi Y, Kagota S, Otsubo K. Beneficial effect of coenzyme Q10 on increased oxidative and nitrative stress and inflammation and individual metabolic components developing in a rat model of metabolic syndrome. J Pharmacol Sci 2008;107(2):128-37.
15. Goldstein BJ, Mahadev K, Wu X. Redox paradox: insulin action is facilitated by insulin-stimulated reactive oxygen species with multiple potential signaling targets. Diabetes 2005;54(2):311-21.
16. Bashan N, Kovsan J, Kachko I, Ovadia H, Rudich A. Positive and negative regulation of insulin signaling by reactive oxygen and nitrogen species. Physiol Rev 2009;89(1):27-71.
17. Weiss D, Sorescu D, Taylor WR. Angiotensin II and atherosclerosis. Am J Cardiol 2001;87(8A):25C-32C.
18. Yamaguchi Y, Yoshikawa N, Kagota S, Nakamura K, Haginaka J, Kunitomo M. Elevated circulating levels of markers of oxidative-nitrative stress and inflammation in a genetic rat model of metabolic syndrome. Nitric Oxide 2006;15(4):380-6.
19. Fujita K, Nishizawa H, Funahashi T, Shimomura I, Shimabukuro M. Systemic oxidative stress is associated with visceral fat accumulation and the metabolic syndrome. Circ J 2006;70(11):1437-42.
20. Lapointe A, Couillard C, Piché ME, Weisnagel SJ, Bergeron J, Nadeau A, et al. Circulating oxidized LDL is associated with parameters of the metabolic syndrome in postmenopausal women. Atherosclerosis 2007;191(2):362-8.

21. Tokuda F, Sando Y, Matsui H, Yokoyama T. N epsilon-(hexanoyl) lysine, a new oxidative stress marker, is increased in metabolic syndrome, but not in obstructive sleep apnea. Am J Med Sci 2009;338(2):127-33.
22. Valavanidis A, Vlachogianni T, Fiotakis C. 8-hydroxy-2' -deoxyguanosine (8-OHdG): A critical biomarker of oxidative stress and carcinogenesis. J Environ Sci Health C Environ Carcinog Ecotoxicol Rev 2009;27(2):120-39.
23. Esposito K, Chiodini P, Colao A, Lenzi A, Giugliano D. Metabolic syndrome and risk of cancer: a systematic review and meta-analysis. Diabetes Care 2012;35(11):2402-11.
24. Tilg H, Moschen AR. Mechanisms behind the link between obesity and gastrointestinal cancers. Best Pract Res Clin Gastroenterol 2014;28(4):599-610.
25. Zhang GM, Zhu Y, Ye DW. Metabolic syndrome and renal cell carcinoma. World J Surg Oncol 2014;12:236.
26. Rauth G, Pöschke O, Fink E, Eulitz M, Tippmer S, Kellerer M, et al. The nucleotide and partial amino acid sequences of rat fetuin. Identity with the natural tyrosine kinase inhibitor of the rat insulin receptor. Eur J Biochem 1992;204(2):523-9.
27. Srinivas PR, Wagner AS, Reddy LV, Deutsch DD, Leon MA, Goustin AS, et al. Serum alpha 2-HS-glycoprotein is an inhibitor of the human insulin receptor at the tyrosine kinase level. Mol Endocrinol 1993;7(11):1445-55.
28. Stefan N, Hennige AM, Staiger H, Machann J, Schick F, Kröber SM, et al. Alpha2-Heremans-Schmid glycoprotein/fetuin-A is associated with insulin resistance and fat accumulation in the liver in humans. Diabetes Care 2006;29 (4):853-7.
29. Ix JH, Shlipak MG, Brandenburg VM, Ali S, Ketteler M, Whooley MA. Association between human fetuin-A and the metabolic syndrome: data from the Heart and Soul Study. Circulation 2006;113(14):1760-7.
30. Reinehr T, Roth CL. Fetuin-A and its relation to metabolic syndrome and fatty liver disease in obese children before and after weight loss. J Clin Endocrinol Metab 2008;93(11):4479-85.
31. Ix JH, Wassel CL, Kanaya AM, Vittinghoff E, Johnson KC, Koster A, et al. Fetuin-A and incident diabetes mellitus in older persons. JAMA 2008;300(2):182-8.
32. Stefan N, Fritsche A, Weikert C, Boeing H, Joost HG, Häring HU, et al. Plasma fetuin-A levels and the risk of type 2 diabetes. Diabetes 2008;57(10):2762-7.
33. Hennige AM, Staiger H, Wicke C, Machicao F, Fritsche A, Häring HU, et al. Fetuin-A induces cytokine expression and suppresses adiponectin production. PLoS One 2008;3(3): e1765.
34. Xu Y, Xu M, Bi Y, Song A, Huang Y, Liu Y, et al. Serum fetuin-A is correlated with metabolic syndrome in middle-aged and elderly Chinese. Atherosclerosis 2011;216(1):180-6.
35. Ismail NA, Ragab S, El Dayem SM, Elbaky AA, Salah N, Hamed M, et al. Fetuin-A levels in obesity: differences in relation to metabolic syndrome and correlation with clinical and laboratory variables. Arch Med Sci 2012;8(5): 826-33.
36. Koch M, Jacobs G, Hampe J, Rosenstiel P, Krawczak M, Nöthlings U. Higher fetuin-A level is associated with coexistence of elevated alanine aminotransferase and the metabolic syndrome in the general population. Metab Syndr Relat Disord 2013;11(6):377-84.
37. Xu A, Wang Y, Xu JY, Stejskal D, Tam S, Zhang J, et al. Adipocyte fatty acid-binding protein is a plasma biomarker closely associated with obesity and metabolic syndrome. Clin Chem 2006;52(3):405-13.
38. Tso AW, Xu A, Sham PC, Wat NM, Wang Y, Fong CH, et al. Serum adipocyte fatty acid binding protein as a new biomarker predicting the development of type 2 diabetes: a 10-year prospective study in a Chinese cohort. Diabetes Care 2007;30(10):2667-72.
39. Xu A, Tso AW, Cheung BM, Wang Y, Wat NM, Fong CH, et al. Circulating adipocyte-fatty acid binding protein levels predict the development of the metabolic syndrome: a 5-year prospective study. Circulation 2007;115(12):1537-43.
40. Stejskal D, Karpisek M. Adipocyte fatty acid binding protein in a Caucasian population: a new marker of metabolic syndrome? Eur J Clin Invest 2006;36(9):621-5.