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Insulin resistance, adipokines, and oxidative stress in nondiabetic, hypercholesterolemic patients: leptin as an 8-epi-prostaglandin $F_{2\alpha}$ determinant

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Abstract

Limited data are available on the association of insulin resistance, adipokines, and in vivo lipid peroxidation. We investigated the relationships between insulin resistance, adipokines (leptin, adiponectin, and resistin), and oxidative stress in nondiabetic, hypercholesterolemic patients. Seventy-six nondiabetic patients with hypercholesterolemia participated in this cross-sectional study. Fasting glucose and insulin concentrations were analyzed. Serum leptin, adiponectin, and resistin concentrations and urinary excretion of 8-epi-prostaglandin $F_{2\alpha}$ (8-epi-$PGF_{2\alpha}$) were determined using enzyme-linked immunosorbent assay. We divided all subjects into 3 groups, classified by the tertiles of homeostasis model assessment of insulin resistance (HOMA-IR) values, and clinical parameter comparisons were made among the 3 groups. The results showed that serum leptin ($P < .001$) and adiponectin levels ($P < .05$) were significantly different among the groups, although serum resistin was not different. Furthermore, the group with the highest HOMA-IR had a significantly higher urinary 8-epi-$PGF_{2\alpha}$ excretion than the group with the lowest HOMA-IR ($P = .017$). Circulating leptin was positively correlated with urinary 8-epi-$PGF_{2\alpha}$ ($r = 0.323$, $P < .01$) and HOMA-IR ($r = 0.524$, $P < .001$). Circulating adiponectin was negatively correlated with body mass index ($r = -0.252$, $P < .05$) and HOMA-IR ($r = -0.228$, $P < .05$). We could not find a relationship between circulating adiponectin or resistin and urinary 8-epi-$PGF_{2\alpha}$ excretion. Stepwise multiple linear regression analysis showed that leptin was associated with the urinary 8-epi-$PGF_{2\alpha}$ excretion after adjusting for age, sex, body mass index, blood lipids, and HOMA-IR ($P = .002$). In conclusion, our results show that more insulin-resistant state of nondiabetic, hypercholesterolemic patients is associated with decreased adiponectin and increased leptin and urinary 8-epi-$PGF_{2\alpha}$ levels, although no relationship with resistin was observed. Furthermore, serum leptin independently contributed to urinary 8-epi-$PGF_{2\alpha}$ excretion.

1. Introduction

Insulin resistance is one of the major characteristics of metabolic syndromes related to obesity and obesity-associated complications such as hypercholesterolemia, cardiovascular disease, and type 2 diabetes mellitus [1]. Emerging evidence has shown that insulin resistance is associated with increased oxidative stress, which may in turn contribute to endothelial dysfunction and an increased atherosclerotic risk [2]. In an animal experiment, hyperinsulinemia was found to enhance oxidative stress by reducing the rate of catalase synthesis [3]. In human studies, significant positive correlations between insulin resistance and oxidized circulating low-density lipoprotein particles and lipid peroxidation products in both nondiabetic [4] and type 2 diabetic individuals [5] were reported.

Adipokines, which regulate metabolic immunologic homeostasis [6], have been reported to be connected with insulin resistance and type 2 diabetes mellitus [7]. These proteins link obesity with obesity-associated complications. Of those adipokines, leptin has been shown to be strongly correlated with body mass index (BMI), fat accumulation, and insulin resistance [8,9]. On the contrary, adiponectin, one of the most abundant adipose tissue-specific adipokines, is

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Table 1
Characteristics and serum lipids and HOMA-IR of subjects according to tertiles of HOMA-IR groups

	Lowest HOMA-IR (n = 25)	Mid HOMA-IR (n = 26)	Highest HOMA-IR (n = 25)
HOMA-IR index	0.79 ± 0.19[a] (0.61-1.26)	1.59 ± 0.20[b] (1.27-1.98)	2.76 ± 0.98[c] (2.03-4.25)
Age (y)	59.0 ± 9.9	59.5 ± 8.3	58.0 ± 9.9
BMI (kg/m^2)	23.0 ± 3.3[a]	24.8 ± 2.1[b]	24.9 ± 2.6[b]
Male-female*	5:20	13:13	7:18
Postmenopausal (n)*	16	12	15
Triglyceride (mg/dL)	153.6 ± 67.7	160.7 ± 70.0	202.3 ± 102.5
Total cholesterol (mg/dL)	242.0 ± 30.9	225.5 ± 27.8	237.2 ± 22.9
LDL-C (mg/dL)	154.9 ± 25.7	145.5 ± 21.7	147.9 ± 15.3
HDL-C (mg/dL)	45.5 ± 15.1	40.0 ± 8.51	42.8 ± 9.6

Values are expressed as mean ± SD. Means with common superscripts indicate that the difference between means is not significant ($P < .05$).
* χ^2 Test.

reduced in obesity [10,11], insulin resistance, and type 2 diabetes mellitus [12], thus showing an insulin-sensitizing effect. Resistin is known to regulate glucose homeostasis and adipogenesis, and induce insulin resistance [13].

Given that obesity is a principal causative factor in metabolic syndrome development, including insulin resistance, it is plausible that there is an association between bioactive molecules released from adipose tissue and oxidative stress induced from, at least in part, insulin resistance. Information on associations among insulin resistance, adipokines, and in vivo lipid peroxidation is limited, especially in hypercholesterolemic subjects. Therefore, we investigated these relationships and examined a role for adipokines in vivo lipid peroxidation in nondiabetic, hypercholesterolemic patients.

2. Materials and methods

2.1. Subjects

Seventy-six hypercholesterolemic patients (20-75 years old, 25 males and 51 females; low-density lipoprotein cholesterol [LDL-C] ≥130mg/dL at screening) participated in the present study. Subjects that were taking hypolipidemic medication, antioxidative vitamins, or who had been diagnosed with type 2 diabetes mellitus were excluded. Body weight and height were measured and BMI was calculated. Venous blood samples were collected from the forearm in EDTA-treated and plain tubes after a fasting period. The tubes were immediately covered with aluminum foil and placed on ice until they arrived at the analytical laboratory, where they were stored at −70°C. All patients gave written informed consent, and the institutional review board at the Yonsei University Medical Center approved the study protocol.

2.2. Serum lipid profiles

Serum cholesterol, LDL-C, and high-density lipoprotein cholesterol (HDL-C) were measured with commercially available kits (Choongwae, Seoul, Korea) by enzymatic methods. Serum triglyceride levels were analyzed using a total glycerol test kit (Roche, Basel, Switzerland). All determinants were done on a Hitachi 747 autoanalyzer (Hitachi, Tokyo, Japan).

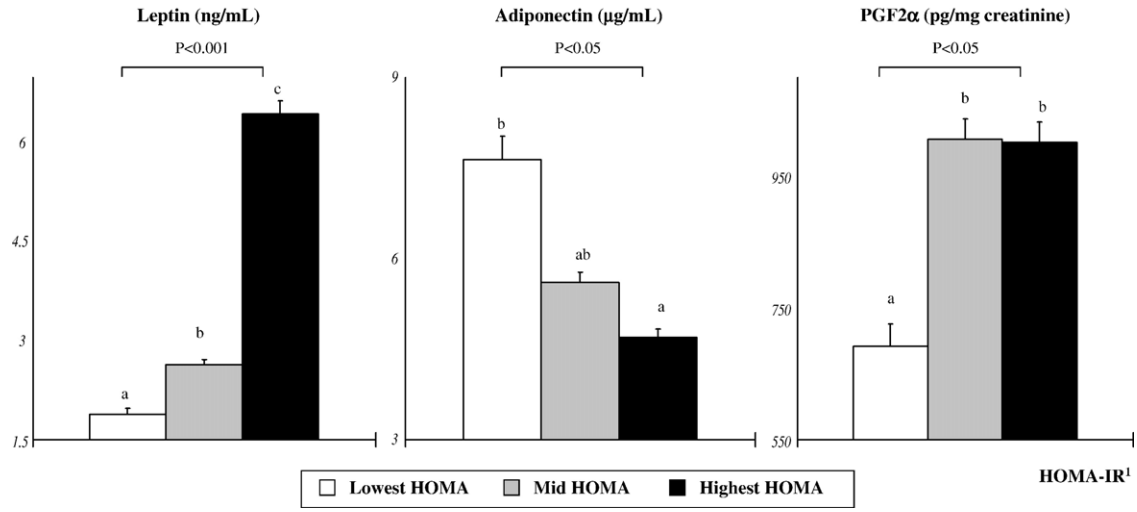

Fig. 1. Serum leptin, adiponectin, and urinary excretion of 8-epi-$PGF_{2\alpha}$ according to HOMA-IR groups. Values are expressed as mean ± SD. Means with common superscripts indicate that the difference between means is not significant ($P < .05$). [1]HOMA IR = fasting serum insulin (μU/mL) × fasting serum glucose (mmol/L)/22.5.

Table 2
Stepwise multiple regression analysis to identify factors influencing urinary $PGF_{2\alpha}$

Dependent variable	Model	Independent variable	Adjusted β coefficients	P	R	P
$PGF_{2\alpha}$	1 Step	Leptin[a]	.323	.008	0.323	.008
	2 Step	Leptin[a]	.361	.002	0.442	.001
		Age	.305	.009		

Independent variables include age, sex, BMI, waist circumference, alcohol intake, smoking, blood lipid, and leptin.
[a] Log-transformed.

2.3. Serum glucose, insulin, and homeostasis model assessment

Fasting serum glucose concentrations were measured by the glucose oxidase method using a Beckman Glucose Analyzer (Beckman Instruments, Irvine, CA). Insulin was measured by radioimmunoassays with commercial kits from Immuno Nucleo (Stillwater, MN). We calculated the homeostasis model assessment of insulin resistance (HOMA-IR) using the equation: HOMA-IR = fasting insulin (μU/mL) × glucose (mmol/L)/22.5 [14].

2.4. Urine 8-epi-$PGF_{2\alpha}$

A fasting urine sample was collected with 1% butylated hydroxytoluene before blood collection. 8-Epi-prostaglandin $F_{2\alpha}$(8-epi-$PGF_{2\alpha}$) was measured using an enzyme immunoassay (Bioxytech-Isoprostane Assay kit, OXIS International, Portland, OR). The extent of the resultant reaction was determined by the A_{650} read on a Victor^2 (Perkin Elmer Life Sciences, Turka, Finland). Urinary creatinine was measured by the alkaline picrate (Jaffe) reaction [15], and urinary 8-epi-PGF_2 concentrations were expressed as picogram per milligram of creatinine.

2.5. Adiponectin, leptin, and resistin analysis

Serum resistin (Human Resistin Enzyme-Linked Immunosorbent Assay Kit, R&D Systems, Minneapolis, MN) and adiponectin levels (Human Adiponectin Enzyme-Linked Immunosorbent Assay Kit, Biovendor, BRNO, Czech Republic) were measured by enzyme immunoassay. The resultant color reaction was read with a Victor^2 measuring A_{450}. A Packard Cobra II 5005 γ-Counter with Human Leptin Radioimmunoassay Kit (Linco, Research, St Charles, MO) was used to measure leptin concentrations.

2.6. Statistical analysis

The SPSS 12.0 software package (SPSS, Chicago, IL) was used for statistical analysis. Data are presented as mean ± SD. Each variable was examined for normal distribution, and abnormally distributed variables were log-transformed. We used the Pearson correlation coefficient to evaluate relationships between variables. Differences in variables among the tertiles of HOMA-IR were evaluated using 1-way analysis of variance with Bonferroni multiple tests and χ^2 test. A stepwise multiple regression analysis was used to determine the contributing factors to urinary 8-epi-$PGF_{2\alpha}$ excretion. We considered the following independent variables: age, sex, BMI, blood lipids (total cholesterol, triglyceride, HDL-C, and LDL-C), HOMA-IR, and leptin levels. P values of less than .05 were considered statistically significant.

3. Results

Mean age and LDL-C levels of the 76 nondiabetic, hypercholesterolemic patients were 59.2 ± 9.3 years and 149.4 ± 21.5 mg/dL, respectively. The 76 subjects were classified into 3 groups based on HOMA-IR values. Age, sex ratio, and postmenopausal status did not differ among the groups (Table 1).

Fig. 1 presents the 3 adipokine (adiponectin, leptin, and resistin) levels and urinary 8-epi-$PGF_{2\alpha}$ excretion as a measure of in vivo lipid peroxidation among the 3 groups. There were significant differences in serum levels of leptin ($P < .001$) and adiponectin ($P < .05$) among the 3 groups, and no resistin difference (9.22 ± 4.71 ng/mL for the lowest HOMA-IR group vs 9.64 ± 5.46 ng/mL for highest HOMA-IR group, $P > .05$). The results showed that significantly higher levels of leptin and lower levels of adiponectin were observed in highest HOMA-IR group than in the lowest HOMA-IR group. Furthermore, urinary 8-epi-$PGF_{2\alpha}$ excretion was significantly higher in the highest HOMA-IR group than in the lowest HOMA-IR group ($P = .017$).

We examined the relationship between adipokine levels and body mass index, blood lipids, urinary 8-epi-$PGF_{2\alpha}$ levels, and HOMA-IR. Circulating leptin was positively correlated with BMI ($r = 0.314$, $P < .01$), urinary 8-epi-$PGF_{2\alpha}$ ($r = 0.323$, $P < .01$), and HOMA-IR ($r = 0.524$, $P < .001$). Circulating adiponectin was negatively correlated with BMI ($r = -0.252$, $P < .05$) and HOMA-IR ($r = -0.228$, $P < .05$). On the other hand, we found no relationship between circulating adiponectin and urinary 8-epi-$PGF_{2\alpha}$ excretion. Serum resistin levels tended to correlate with BMI ($P = .06$); however, no relationship was observed between resistin and HOMA-IR or urinary 8-epi-$PGF_{2\alpha}$ excretion.

Stepwise multiple linear regression analysis was performed to further investigate the role of leptin as an in vivo lipid peroxidation marker. This test showed that leptin was associated with urinary 8-epi-$PGF_{2\alpha}$ excretion (Table 2). In addition, all these effects remained statistically significant after adjusting for age, sex, body mass index, blood lipids, and HOMA-IR ($P = .002$).

4. Discussion

Adipokines, such as adiponectin, leptin, and resistin, profoundly influence insulin sensitivity and glucose metabolism

[16]. They have also been suggested to provide a molecular link between obesity and insulin resistance. Adiponectin has been reported to improve insulin sensitivity by increasing fat oxidation, resulting in reduced circulating fatty acid levels and reduced intracellular triglyceride contents in liver and skeletal muscle [17]. Leptin appears to act as both an insulin sensitizer and a contributor to the insulin-resistant phenotype [18]; therefore, it is unclear whether insulin resistance can be related directly to leptin. However, there is general agreement that the expression and secretion of leptin are increased in obesity, insulin resistance, and that a strong correlation exists between adiposity and plasma leptin concentrations [19,20]. In the present study, we observed the decreases in adiponectin and increases in leptin levels in more insulin-resistant state of nondiabetic, hypercholesterolemic patients. These results are consistent with the previous human studies demonstrating the negative correlation between adiponectin and insulin resistance [19] and positive correlation between plasma leptin and insulin resistance [12], which results in metabolic syndrome.

Resistin has been reported to reduce glucose tolerance and is up-regulated in both genetic and diet-induced obesity [21]. Rajala et al [22] reported that resistin induced severe hepatic insulin resistance but not peripheral insulin resistance, suggesting that resistin has a much more complex role in insulin sensitivity. It has been reported that resistin does not correlated to obesity and insulin resistance [23,24], although Silha et al [25] showed a significant association between resistin and HOMA-IR. In the present study, we found no significant relationship between resistin and insulin resistance; therefore, it must be weaker than the leptin or adiponectin relationships. In addition, the resistin expression levels in adipocytes are lower than leptin and adiponectin levels [21], so resistin seems to be less important in linking obesity with insulin resistance. Several studies have reported the relationship between insulin resistance and oxidative stress. Talior et al [26] recently showed that reactive oxygen species production is significantly increased in adipocytes from insulin-resistant mice fed a high-fat diet. On the other hand, Furukawa et al [27] reported that an increase in adipose tissue in oxidative stress is an early instigator of obesity-associated metabolic syndrome. There is also evidence that oxidative stress increases insulin resistance by impairing insulin receptor signaling [28]. In human studies, Urakawa et al [29] showed that obesity was important for increased oxidative stress, which triggered insulin resistance. In our study, we measured urinary 8-epi-$PGF_{2\alpha}$ excretion as measured by marker for in vivo systemic oxidative stress. F_2-isoprostanes are products of free radical–catalyzed lipid peroxidation of arachidonic acid [30]. They are found esterified to phospholipids, released by phospholipases into the plasma, and subsequently removed via the kidney [31]. Elevated F_2-isoprostane concentrations in plasma and urine have been found to be associated with oxidative stress [32,33]. Our result showed that individuals with the highest HOMA-IR showed increased urinary 8-epi-$PGF_{2\alpha}$ excretion compared with individuals with the lowest HOMA-IR, providing additional evidence relating insulin resistance and oxidative stress.

With regard to relationships between adipokines and lipid peroxidation, our results showed that urinary 8-epi-$PGF_{2\alpha}$ positively correlated with the circulating leptin, although no significant association was seen with circulating adiponectin and resistin. Several studies have reported on the association between leptin and oxidative stress. Leptin was found to increase the production of reactive oxygen species followed by an increase in endothelial cell fatty acid oxidation [34]. In an animal experiment, leptin administration induced a decrease in plasma paraoxonase 1 activity and an increase in rat oxidative stress [35]. Limited data are available on the relationship between increased oxidative stress and adiponectin or resistin. Recently, Nakanishi et al [36] reported on the capability of adiponectin to protect against oxidative stress in Japanese Americans, a result not found in our study. In the present study, we excluded the patients diagnosed with type 2 diabetes mellitus because diabetes is commonly associated with insulin resistance and oxidative stress. In this setting, we found a significant association between leptin and an in vivo lipid peroxidation marker, which was not shown in adiponectin or resistin. These results indicate that the leptin can be more potently associated with oxidative stress than the other adipokines.

In conclusion, our results show that insulin-resistant state of nondiabetic, hypercholesterolemia is associated with the reduced adiponectin and increased leptin and urinary 8-epi-$PGF_{2\alpha}$ excretion, whereas no relationship with resistin was observed. Furthermore, serum leptin independently contributed to urinary 8-epi-$PGF_{2\alpha}$ excretion. This study helps to provide an understanding of the mechanisms linking insulin resistance, adipokines, and oxidative stress, which lead to atherosclerosis and cardiovascular disease.

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References

[1] Mizuno T, Matsui H, Imamura A, Numaguchi Y, Sakai K, Murohara T, et al. Insulin resistance increases circulating malondialdehyde-modified LDL and impairs endothelial function in healthy young men. Int J Cardiol 2004;97:455-61.

[2] Carantoni M, Abbasi F, Warmerdam F, Klebanov M, Wang PW, Chen YDI, et al. Relationship between insulin resistance and partially oxidized LDL particles in healthy, nondiabetic volunteers. Arterioscler Thromb Vasc Biol 1998;18:762-7.

[3] Xu L, Badr MZ. Enhanced potential for oxidative stress in hyperinsulinemic rats imbalance between hepatic peroxisomal hydrogen peroxide production and decomposition due to hyperinsulinemia. Horm Metab Res 1999;31:278-82.

[4] Chen NG, Azhar S, Abbasi F, Carantoni M, Reaven GM. The relationship between plasma glucose and insulin response to oral

glucose, LDL oxidation, and soluble intercellular adhesion molecule–1 in healthy volunteers. Atherosclerosis 2000;152:203-8.
[5] Nourooz-Zadeh J, Tajaddini-Sarmadi J, McCarthy S, Betteridge J, Wolff SP. Elevated levels of authentic plasma hydroperoxides in NIDDM. Diabetes 1995;44:1054-8.
[6] Ahima RS. Central actions of adipocyte hormones. Trends Endocrinol Metab 2005;16:307-13.
[7] Chandran M, Ciaraldi T, Philips SA, Henry RR. Adiponectin: more than just another fat cell hormone? Diabetes Care 2003;26:2442-50.
[8] de Courten M, Zimmet P, Hodge A, Collins V, Nicolson M, Staten M, et al. Hyperleptinaemia: the missing link in the metabolic syndrome? Diabet Med 1997;14:200-8.
[9] Zimmet P, Hodge A, Nicolson M, Staten M, de Courten M, Moore J, et al. Serum leptin concentration, obesity and insulin resistance in Western cross sectional study. Br Med J 1996;313:965-9.
[10] Okamoto Y, Kihara S, Ouchi N, Nishida M, Arita Y, Kumada M, et al. Adiponectin reduces atherosclerosis in apolipoprotein E–deficient mice. Circulation 2002;106:2767-70.
[11] Berg AH, Combs TP, Du X, Brownlee M, Scherer PE. The adipocyte-secreted protein Acrp30 enhances hepatic insulin action. Nat Med 2001;7:947-53.
[12] Xydakis AM, Case CC, Jones PH, Hoogeveen RC, Liu MY, Smith SO, et al. Adiponectin, inflammation, and the expression of the metabolic syndrome in obese individuals: the impact of rapid weight loss through caloric restriction. J Clin Endocrinol Metab 2004;89:2697-2703.
[13] Rea R, Donnelly R. Resistin: an adipocyte-derived hormone. Has it a role in diabetes and obesity? Diabetes Obes Metab 2004;6:163-70.
[14] Mathews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28:412-9.
[15] Campins-Falco P, Sevillano-Cabeza A, Llobat-Estelles M. Kinetic and chemometric studies of the determination of creatinine using the Jaffe reaction. Part 2. Application to human serum samples: kinetic behaviour and chemometric evaluation of the determination. Analyst 1989; 114:603-7.
[16] Fasshauer M, Paschke R. Regulation of adipocytokines and insulin resistance. Diabetologia 2003;46:1594-603.
[17] Saltiel AR. New perspectives into the molecular pathogenesis and treatment of type 2 diabetes. Cell 2001;104:517-29.
[18] Ceddia RB, Koistinen HA, Zierath JR, Sweeney G. Analysis of paradoxical observations on the association between leptin and insulin resistance. FASEB J 2002;16:1163-76.
[19] Staiger H, Tschritter O, Machann J, Thamer C, Fritsche A, Maerker E, et al. Relationship of serum adiponectin and leptin concentrations with body fat distribution in humans. Obes Res 2003;11:368-72.
[20] Tufano A, Marzo P, Enrini R, Morricone L, Caviezel F, Ambrosi B. Anthropometric, hormonal and biochemical differences in lean and obese women before and after menopause. J Endocrinol Invest 2004; 27:648-53.
[21] Kougias P, Chai H, Lin PH, Yao Q, Lumsden AB, Chen C. Effects of adipocyte-derived cytokines on endothelial functions: implication of vascular disease. J Surg Res 2005;126:121-9.
[22] Rajala MW, Obici S, Scherer PE, Rossetti L. Adipose-derived resistin and gut-derived resistin-like molecule—beta selectively impair insulin action on glucose production. J Clin Invest 2003;111: 225-230.
[23] Heilbronn LK, Rood J, Janderova L, Albu JB, Kelley DE, Ravussin E, et al. Relationship between serum resistin concentrations and insulin resistance in nonobese, obese, and obese diabetic subjects. J Clin Endocrinol Metab 2004;89:1844-8.
[24] Vozarova de Courten B, Degawa-Yamauchi M, Considine RV, Tataranni PA. High serum resistin is associated with an increase in adiposity but not a worsening of insulin resistance in Pima Indians. Diabetes 2004;53:1279-84.
[25] Silha JV, Lrsek M, Skrha JV, Sucharda P, Nyomba BLG, Murphu LJ. Plasma resistin, adiponectin and leptin levels in lean and obese subjects: correlatiom with insulin resistance. Eur J Endocrinol 2003; 149:331-5.
[26] Talior I, Tennenbaum T, Kuroki T, Eldar-Finkelman H. PKC-delta–dependent activation of oxidative stress in adipocytes of obese and insulin-resistant mice: role for NADPH oxidase. Am J Physiol Endocrinol Metab 2005;288:E405-11.
[27] 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;114:1752-61.
[28] Ogihara1 T, Asano1 T, Katagiri H, Sakoda H, Anai M, Shojima1 N, et al. Oxidative stress induces insulin resistance by activating the nuclear factor–B pathway and disrupting normal subcellular distribution of phosphatidylinositol 3-kinase. Diabetologia 2004;47:794-805.
[29] Urakawa H, Katsuki A, Sumida Y, Gabazza EC, Murashima S, Morioka K, et al. Oxidative stress is associated with adiposity and insulin resistance in men. J Clin Endocrinol Metab 2003;88:4673-6.
[30] Basu S. Isoprostanes: novel bioactive products of lipid peroxidation. Free Radic Res 2004;38:105-22.
[31] Nourooz-Zadeh J, Cooper MB, Ziegler D, Betteridge DJ. Urinary 8-epi-$PGF_{2\alpha}$ and its endogenous β-oxidation products (2,3-dinor and 2,3-dinor-5,6-dihydro) as biomarkers of total body oxidative stress. Biochem Biophys Res Commun 2005;330:731-6.
[32] Nourooz-Zadeh J, Smith CC, Betteridge DJ. Measures of oxidative stress in heterozygous familial hypercholesterolaemia. Atherosclerosis 2001;156:435-41.
[33] Sampson MJ, Gopaul N, Davies IR, Hughes DA, Carrier MJ. Plasma F2 isoprostanes: direct evidence of increased free radical damage during acute hyperglycemia in type 2 diabetes. Diabetes Care 2002; 25:537-41.
[34] Bouloumie A, Marumo T, Lafontan M, Busse R. Leptin induces oxidative stress in human endothelial cells. FASEB J 1999;13:1231-8.
[35] Beltowski J, Wojcicka G, Jamroz A. Leptin decreases plasma paraoxonase 1 (PON1) activity and induces oxidative stress: the possible novel mechanism for proatherogenic effect of chronic hyperleptinemia. Atherosclerosis 2003;170:21-9.
[36] Nakanishi S, Yamane K, Kamei N, Nojima H, Okubo M, Kohno N. A protective effect of adiponectin against oxidative stress in Japanese Americans: the association between adiponectin or leptin and urinary isoprostane. Metabolism 2005;54:194-9.