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Serum level of the adiponectin and adiponectin I164T polymorphism in hypertensive adolescents

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= Abstract =

Purpose : Adiponectin is a molecule that plays an important role in the metabolic syndrome. In addition, its concentration is known to be decreased in obesity, type 2 diabetes, and coronary artery disease. Although a relationship between hypertension and serum adiponectin concentrations has been reported by several authors, such findings continue to be debated. We investigated whether hypoadiponectinemia is related to hypertension in adolescents and studied the associated genetic polymorphism.

Methods : Forty hypertensive adolescents (Age 16-17 years old) and twenty normotensive matched subjects were included. Serum adipo-nectin, insulin, renin, aldosterone and angiotensin converting enzyme (ACE) levels were compared. Their carotid intima-media thickness (cIMT) and pulse wave velocity (PWV) were measured. Polymorphisms of the adiponectin I164T gene were investigated using polymerase chain reaction (PCR).

Results : The hypertensive adolescents had significantly greater cIMT and PWV. In addition, the serum aldosterone, renin and insulin levels were significantly higher in the hypertensive group. The plasma concentrations of adiponectin did not differ significantly between the two groups. TC genotype was not found in our study subjects; they all had the TT genotype of the adiponectine gene.

Conclusion : The results of our study showed that adiponectin levels were not significantly different in adolescents with hypertension. There was no distinctive genetic polymorphism observed in this group of patients. Further large scale studies are needed to clarify the association between genetic variations and adiponectin in hypertensive adolescents. (Korean J Pediatr 2009;52:187-193)

Key Words : Adiponectin, Hypertension, Adolescent

Introduction

Adiponectin is a 247 amino acid peptide, predominantly produced by adipocytes from white adipose tissue that accounts for about 0.05% of total serum proteins. Its concentration ranges from 5 to 30 ug/mL[1-5]. It consists of an N-terminal collagenous and a C-terminal globular domain, and shares homologous subunits with the complement factor C1q[1, 3].

In population-based human studies, adiponectin has been shown to be inversely correlated with the body mass index (BMI), insulin resistance, triglyceride levels, C-reactive protein (CRP) and diabetes risk, and positively associated with high-density lipoprotein cholesterol (HDL-c) levels[6-10]. Adiponectin suppresses almost all of the processes involved in atherosclerotic vascular change, including the expression of adhesion molecules in vascular endothelial cells[11, 12] and the formation of foam cells in vivo[13]. In addition, it exhibits anti-atherosclerotic activity in vivo[14]. Recent studies have shown that hypoadiponectinemia is related to insulin resistance in essential hypertension and that the renin angiotensin system (RAS) blockade increases adiponectin concentrations with improvement in insulin sensitivity[15] although this finding is not confirmed in all studies.

In addition to environmental factors, genetic factors also contribute to the pathophysiology of these disorders and these factors are associated with the serum adiponectin levels[16, 17]. The adiponectin gene maps to chromosome 3q27. The I164T polymorphism (T→C substitution at nucleotide

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517 leading to an amino acid substitution from isoleucine to threonine at position 164 is said to be more frequent in type 2 diabetes, cardiovascular diseases and in individuals with hypoadiponectinemia[16, 18].

To date, there is no clear explanation of the underlying genetics of adiponectin levels and blood pressure. The purpose of this study was to investigate the effect of serum adiponectin on blood pressure and analyze the polymorphism of the adiponectin gene among hypertensive adolescents. In addition, we attempted to confirm the hypothesis that hypoadiponectinemia is related to insulin resistance.

Materials and methods

1. Patients

Forty hypertensive patients were included in the study group. Twenty normotensive subjects were enrolled as the control group. The participants ages were between 16 and 17. We included hypertensive adolescents with a systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg.

The hypertensive adolescents were boys and girls from middle and high school who were identified during routine health check up. All subjects provided written informed consent.

2. Anthropometric data

All hypertensive students were sent to the pediatric clinic. Their elevated blood pressure was confirmed by averaging three blood pressure measurements taken after five minutes of rest with an oscillometric monitor. Our patients had never been diagnosed or treated for hypertension before. Height and body weight were measured from which the BMI and obesity index were calculated.

3. Intima-media thickness of the common carotid artery

Measurements of the carotid artery were obtained by a real-time B-mode ultrasound imager (iU22, intelligent Ultrasound System; Philips, Amsterdam, Netherlands) using a 12.5 MHz probe. The IMT and lumen diameter were measured in the same arterial segment. The patients were the in supine position for 30 minutes before the measurements were made. The following equations were used to calculate the carotid artery compliance and elasticity.

Intimal medial thickness (IMT, mm)

Systolic diameter (sD, mm)

Diastolic diameter (dD, mm)

ΔP: pulse pressure

Lumen cross-sectional area = $\pi dD^2/4$

Wall cross-sectional area = π (dD/2+ IMT)^2 - π (dD/2)^2

Cross-sectional compliance = $\pi(sD^2-dD^2)/4\Delta P$ ($mm^2.mmHg-1$)

Cross-sectional distensibility = $(sD^2-dD^2)/(dD^2\Delta P)$($mmHg^{-1}.10^{-2}$)

4. Pulse wave velocity (PWV) and ankle brachial index (ABI)

Brachial-ankle PWV (BaPWV) and ABI were measured using a VP-1000 (Colin Co., Komaki, Japan). The volume plethysmographic technique was used, PWV, ABI (the ratio of systolic blood pressure in the ankle to that in the brachial artery), blood pressure of the extremities, electrocardiography and heart sounds were obtained simultaneously and automatically. Cuffs were wrapped on both the arms and the ankles, and electrocardiogram electrodes were placed at the left sternal border. As the pulse wave contours in the four extremities were recorded, the cuffs inflated and deflated automatically. The cuffs were attached to the plethysmographic sensor that determined the volume pulse form. The blood pressure was measured from the oscillometric pressure sensor. The BaPWV was determined by the pulse transit time and the distance between these two segments. The distance of each segment was calculated automatically, based on the height of the subjects. All measurements were made during regular sinus rhythm.

5. Serum adiponectin, renin, insulin, aldosterone and ACE levels

Venous blood was drawn from all patients and control subjects after overnight fasting. The samples were stored at -70℃ for subsequent assay. The plasma concentration of adiponectin was evaluated by radioimmuno assay (Human Adiponectin 125 tubes RIA kit. Linco Research, Inc. St. Charles, Missouri 63304, USA).

The plasma insulin, renin and aldosterone concentrations were measured by radioimmuno assay and the ACE concentration by ELISA.

6. Determination of the adiponectin I164T genotype

To determine the adiponectin I164T genotype, genomic DNA was amplified by PCR. Five minutes of denaturation at 94℃ was followed by 35 cycles of 1 minute denaturation at 94℃, 1 minute at 60℃ and 1 minute at 72℃. After cleavage with 10U of restriction enzyme Bcc I (New England Biolab,

Beverly, MA, USA), the PCR products were detected on a 2% agarose gel. The polymorphisms were determined by sequencing. The following PCR primers were used for I164T. 5-CCC ATT CGC TTT ACC AAG ATC-3 and 5-GAA GAA AGC CTG TGA AGG TG-3. The PCR products were purified using the QIAquick PCR purification kit (Qiagen). The purified PCR products were sequenced using the ABI3100 genetic analyzer (Applied Biosystems) (Fig. 1).

7. Statistical analysis

All statistical analyses were performed with the SPSS/PC software package (SPSS version 11.0) program. Descriptive statistics are presented as means and standard deviations. The univariate analyses for group comparisons of continuous variables were performed using the student t-test.

The correlations among continuous variables were determined using the Pearson correlation coefficient. A P value less than 0.05 was considered as statistically significant.

Results

1. Anthropometric data

The weight was significantly higher in the hypertensive group compared to the normotensive group (77.2±14.4 kg vs. 54.4±15.5 kg). The BMI was significantly higher in hypertensive group than in the normotensive group (26.6±4.2 kg/m^2 vs. 21.2±3.0 kg/m^2). The obesity index was also higher in hypertensive group compared to the normotensive group (123.3±19.4% vs. 104.6±14.0%). The height was not significantly different in comparisons between the groups (Table 1).

2. Blood pressure

The systolic (149.5±7.9 mmHg vs. 115.9±15.9 mmHg, P<0.05) and diastolic blood pressures (81.1±8.1 mmHg vs. 72.6±10.5 mmHg, P<0.05) were significantly different in comparisons between the two groups (Table 2).

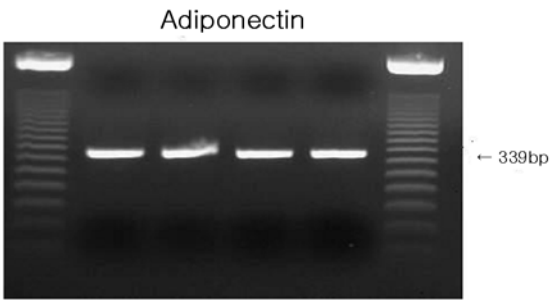

Fig. 1. Adiponectin I164T gene expression in hypertensive adolescents. The RT PCR resulted in a 339 bp band.

3. Serum aldosterone, renin, insulin, ACE and adiponectin levels

Serum aldosterone (147.2±83.1 ug/mL vs. 80.3±50.1 ug/mL), renin (4.2±3.1 ug/mL vs. 2.6±3.4 ug/mL) and insulin (25.4±13.8 ug/mL vs. 7.9±2.9 ug/mL) levels were significantly higher in the hypertensive group (P<0.05, Table 3). However, no significant difference was observed for the ACE and adioponectin levels. For the hypertensive group, the serum adiponectin level was inversely correlated with insulin (r=0.31, P<0.05, Fig. 2).

4. Adiponectin I164T polymorphism in the hypertensive group

The RT PCR resulted in a 339 bp band (Fig. 3). The TC genotype was not found in our study subjects; they all had

Table 1. Anthropometric Data of Study Group

Parameters	Hypertensive group (n=40)	Control group (n=20)
Height (cm)	169.9±7.9	158.1±16.1
Weight (kg)	77.2±14.4*	54.4±15.5
BMI (kg/m^2)	26.6±4.2*	21.2±3.0
Obesity index (%)	123.3±19.4*	104.6±14.0

Abbreviations : BMI, body mass index; BP, blood pressure
*Significantly different (P<0.05) from control group

Table 2. Comparison of Blood Pressure in Hypertensive Group and Control Group

Blood Pressure (mmHg)	Hypertensive group	Control group
Systolic BP	149.5±7.9*	115.9±15.9
Diastolic BP	81.1±8.1*	72.6±10.5

Abbreviation : BP, blood pressure
*Significantly different (P<0.05) from control group

Table 3. Comparison of Aldosterone, Renin, Insulin, ACE, and Adiponectin Levels in Hypertensive Group vs Control Group

Parameters	Hypertensive group (n=40)	Control group (n=20)
Aldosterone (ug/mL)	147.2±83.1*	80.3±50.1
Renin (ng/mL/hr)	4.2±3.1*	2.6±3.4
Insulin (uIU/mL)	25.4±13.8*	7.9±2.9
ACE (U/L)	51.6±30.9	45.4±18.5
Adiponectin (ug/mL)	5.3±2.8	5.1±1.3

Abbreviation : ACE, angiotensin converting enzyme
*Significantly different (P<0.05) from control group

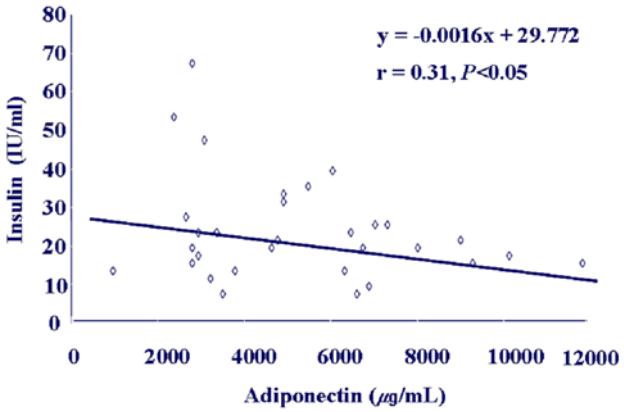

Fig. 2. Linear correlation between serum adiponectin and insulin level in hypertensive group.

Table 4. Frequency of PCR Primers for Adiponectin I164T Gene Polymorphism in Hypertensive Group

TT No (%)	TC No (%)	Total No (%)
40 (100)	0 (0)	40 (100)

Abbreviation : PCR, polymerase chain reaction

the TT genotype of the adiponectin gene (Table 4).

5. Geometric analysis of the common carotid artery in hypertensive group vs control group

The hypertensive adolescents had significantly greater values for the carotid IMT than the normotensive subjects (0.58±0.14 mm vs. 0.5±0.01 mm) (P<0.05). The systolic (8.00±0.78 mm) and diastolic (7.00±0.81 mm) diameter for the hypertensive patients were also larger than those of the normotensive patients (7.00±0.57 mm vs 6.00±0.63 mm). However, the cross sectional compliance and cross sectional distensibility did not show any statistical difference (Table 5).

6. Pulse wave velocity and ankle-brachial index

The right heart brachial-ankle pulse wave velocity (RhbaPWV) and left hbaPWV of the hypertensive group were 726.5±56.4 cm/sec and 713.7±136.6 cm/sec respectively. Only the RhbaPWV was significantly increased compared to the normal controls (P<0.05). Similarly, the right ABI in the hypertensive group was significantly lower than the normotensive group (99.8±8.2 vs 104.8±7.9). However, there was no significant difference in the left ABI (Table 6).

Table 5. Geometric Analysis of the Common Carotid Artery in Hypertensive Group vs Control Group

Parameters	Hypertension (n=40)	Control group (n=20)
Carotid IMT (mm)	0.58±0.14*	0.5±0.01
Systolic diameter (mm)	8.00±0.78*	7.00±0.57
Diastolic diameter (mm)	7.00±0.81*	6.00±0.63
Cross sectional compliance (mm^2/mmHg)	0.17±0.04	0.24±0.08
Cross sectional distensibility ($mmHg^{-1}/10^{-2}$)	0.0157±0.002	0.0250±0.004

Abbreviation : IMT, intima media thickness
*P<0.05 significantly different from control group

Table 6. Pulse Wave Velocity and Ankle-Brachial Index in Hypertensive Group vs Control Group

Parameters	Hypertensive group (n=40)	Control group (n=20)
RhbaPWV (cm/sec)	726.5±56.4*	690.6±56.0
LhbaPWV (cm/sec)	713.7±136.6	702.6±51.5
R-ABI	99.8±8.2*	104.8±7.9
L-ABI	98.0±8.5	104.2±7.1

Abbreviations : baPWV, brachial ankle pulse wave velocity; ABI, ankle-brachial index
*P<0.05 significantly different from control group

Discussion

As mentioned earlier, prior studies have demonstrated that hypoadiponectinemia is related to insulin resistance in patients with essential hypertension and RAS blockade increases adiponectin concentrations with improvement in insulin sensitivity[15].

The diagnosis of hypertension in children is difficult because normal blood pressure levels vary with age, gender and height. Children and adolescents with essential hypertension are a unique group to investigate the pathogenesis of hypertension. Unlike adults, they have not been exposed to risk factors such as diabetes, alcohol or cigarette smoking. Over the past 20 years, the prevalence of childhood obesity has increased tremendously, and at least 30% of obese children are hypertensive[19]. In the present study, weight, BMI and the obesity index were significantly higher in the hypertensive patients. In addition, the carotid IMT which is an established marker for early, preclinical atherosclerosis was higher in the hypertensive subjects. The PWV is used as an index of atherosclerosis and arterial compliance. Our patients in the hypertensive group had higher PWV measurements.

Increased renal sodium reabsorption and blood volume expansion are central features to the development of hypertension associated with obesity in both experimental models and in humans[20]. The augmented sympathetic nervous system activity, activation of the RAS, and higher intrarenal pressure associated with abdominal obesity are especially important[20].

Adiponectin is a hormone exclusively produced and secreted by adipocytes. Adioponectin has demonstrated antiatherogenic, insulin sensitizing, and anti-inflammatory properties. Plasma adiponectin has been shown to be inversely correlated with the BMI and visceral adiposity[5] and is increased with weight loss[21, 22]. Hypoadiponectinemia may be an important risk factor for coronary heart disease[23]. Lower adiponectin concentrations have been associated with hypertension in some studies. Yamamoto et al.[24] and Adamczak et al.[25] reported that the serum adiponectin level is negatively correlated with blood pressure. However, in another study conducted by Yang et al.[26] there was no significant relationship between the adiponectin level and hypertension. Mallamaci et al.[27] found that adiponectin was higher in hypertensive patients than in normotensive patients; they explained that this finding might be due to the expression of a counter-regulatory response aimed at mitigating the endothelial damage and cardiovascular risk associated with high arterial pressure.

In the present study, there was no significant difference in the adiponectin levels between the hypertensive group and the normotensive group. The adiponectin level was 5.3 ±2.8 ug/mL in the hypertensive group and 5.1±1.3 ug/mL in the normotensive group,.

Along with adiponectin, other biochemical markers related to hypertension were measured. Renin and angiotensin levels were significantly higher in the hypertensive group; all of the study patients were diagnosed with essential hypertension. Interestingly, the insulin and adiponectin levels had a negative correlation. Hyperinsulinemia is thought to link obesity and hypertension. Obesity is associated with fasting hyperinsulinemia and a greater insulin response to glucose overload, which occurs to compensate for peripheral insulin resistance[20]. Despite these facts, the role of hyperinsulinemia in obesity-hypertension continues to be debated. Epidemiological evidence has shown that the association between hyperinsulinemia and blood pressure is not consistent. In theory a relationship between hypoadiponectinemia and hypertension would make sense, but our data did not support a relationship.

We examined whether an adiponectin I164T polymorphism was associated with hypertension in the adolescents. None of our study subjects had the TC genotype. This finding may be due to the fact that there were no significant differences in the adiponectin levels in comparisons between the study and the control groups or perhaps it was due to the small number of subjects involved in the study. The genetic investigation revealed that subjects with the I164T polymorphism (T-to-C substitution at nucleotide 517 leading to amino acid substitution from isoleucine to threonine at position 1[64]) more frequently had diabetes and had lower concentrations of adiponectin. The I164T polymorphism of the adiponectin gene was significantly higher in Japanese patients with type II diabetes or coronary artery disease[28, 29]. However, a recent study reported that the I164T polymorphism was not found in obese French Caucasian patients with the type 2 diabetes[30]. Such findings suggest that ethnic differences play a role in the genetic variations identified. A larger population study is required to elucidate these differences.

This study was designed as a cross-sectional investigation with a limited number of subjects. Further study with a prospective design and a larger sample of subjects is needed to confirm our findings. In addition, several important determinants of plasma adiponectin levels, such as waist circumference and ethnic background, were not considered in the anlayses. The fact that age may play a role in determining the adiponectin level should also be taken into consideration. Furthermore, we could not determine the duration of hypertension which may be an important factor to consider. However, to date there is limited information on essential hypertension in adolescents and our study results add to the understanding of this condition.

In conclusion, the result of the present study showed that adiponectin levels were not significantly related to hypertension in adolescents. In addition, no genetic association was found with I164T polymorphisms. Further study is needed to elucidate the effects of the serum adiponectin on blood pressure and to analyze adiponectin polymorphisms among hypertensive adolescents.

한 글 요 약

고혈압 청소년에서의 혈청 adiponectin치와 adiponectin I164T 유전자다형성

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목 적 : Adiponectin은 대사 증후군에서 중요한 역할을 하는 물질이며, 비만, 2형 당뇨, 관동맥 질환 등에서 그 수치가 감소하는 것으로 알려져 있다. 고혈압과 혈청 adiponectin치의 관계는 여러 저자에 의하여 발표되었으나 아직 논란의 여지가 남아있는 상태이다. 우리는 낮은 adiponectin치가 청소년 고혈압과 어떤 관계가 있는지, 또 영향을 끼치는 기전이 유전적인지 혹은 생물학적인 것인지 알고자 유전자 다형성 분석을 하였다.

방 법 : 16에서 17세 사이의 40명의 고혈압 청소년과 20명의 대조군 청소년을 대상으로 각 군의 혈청 adiponectin, insulin, renin, aldosterone, ACE를 측정하여 비교하였다. cIMT와 PWV도 측정하였다. PCR법으로 adiponectin I164T 유전자 다형성을 조사하였다.

결 과 : 고혈압 청소년군에서 cIMT와 PWV가 의미있게 높았다. 혈청 insulin, renin, aldosterone치도 고혈압군에서 의미있게 높았다. 두 군간에 adiponectin치는 의미있는 차이를 보이지 않았다. 본 연구에 참여한 청소년에서는 TC genotype은 발견되지 않았고 모두 TT genotype을 보였다.

결 론 : 결론적으로 adiponectin치는 고혈압 청소년 군에서나 정상군에서나 차이가 없었다. 두 군에서 유전적인 다형성도 보이지 않았다. 더 큰 규모의 연구가 필요할 것으로 생각된다.

References

1) Scherer PE, William S, Fogliano M, Baldini G, Lodish HF. A novel serum protein similar to C1q, produced exclusively in adipocytes. J Biol Chem 1995;270:26746-9.
2) Maeda K, Okubo K, Shimomura I, Funahashi T, Matsuzawa Y, Matzubara K. cDNA cloning and expression of a novel adipose specific collagen-like factor, apM1(adipose most abundant gene transcript 1). Biochem Biophys Res Commun 1996;221:286-9.
3) Hu E, Liang P, Spiegelman BM. AdipoQ is a novel adipose-specific gene dysregulated in obesity. J Biol Chem 1996;271:10679-703.
4) Nakano Y, Tobe T, Choi-Miura NH, Mazda T, Tomita M. Isolation and characterization of GBP28, a novel gelatin-binding protein purified from human plasma. J Biochem 1996;120:803-12.
5) Arita Y, Kihara S, Ouchi N, Takahashi M, Maeda K, Miyagawa J, et al. Paradoxical decrease of an adipocyte specific protein, adiponectin, in obesity. Biochem Biophys Res Commun 1999;257:79-83.
6) Cnop M, Havel PJ, Utzschneider KM, Carr DB, Sinha MK, Boyko EJ, et al. Relationship of adiponectin to body fat distribution, insulin sensitivity and plasma lipoproteins: evidence for independent roles of age and sex. Diabetologia 2003;46:459-69.
7) Kazumi T, Kawaguchi A, Sakai K, Hirano T, Yoshino G. Young men with high normal blood pressure have lower serum adiponectin, smaller LDL size, and higher elevated heart rate than those with optimal blood pressure. Diabetes Care 2002;25:971-6.
8) Matsubara M, Maruoka S, Katayose S. Decreased plasma adiponectin concentrations in women with dyslipidemia. J Clin Endocrinol Metab 2002;87:2764-9.
9) Spranger J, Kroke A, Mohlig M, Bergmann MM, Ristow M, Boeing H, et al. Adiponectin and protection against type 2 diabetes mellitus. Lancet 2003;361:226-8.
10) Linsay RS, Funahashi T, Hanson RL, Matsuzawa Y, Tanaka S, Tataranni PA, et al. Adiponectin and development of type 2 diabetes in the Pima Indian population. Lancet 2002; 360: 57-8.
11) Ouchi N, Kihara S, Arita Y, Maeda K, Kuriyama H, Okamoto Y, et al. Novel modulator for endothelial adhesion molecules: Adipocyte-derived plasma protein adiponectin. Circulation 1999;100:2473-6.
12) Ouchi N, Kihara S, Arita Y, Maeda K, Kuriyama H, Okamoto Y, et al. Adiponectin, an adipocyte-derived plasma protein, inhibits endothelial NF-kappaB signaling through a cAMP-dependent pathway. Circulation 2000;102:1296-301.
13) Arita Y, Kihara S, Ouchi N, Maeda K, Kuriyama H, Okamoto Y, et al. Adipocyte-derived plasma protein adiponectin acts as a platelet derived growth factor-BB-binding protein and regulates growth factor-induced common postreceptor signal in vascular smooth muscle cell. Circulation 2002;105:2893-8.
14) Okamoto Y, Arita Y, Nishida M, Muraguchi M, Ouchi N, Takahashi M, et al. An adipocyte-derived plasma protein, adiponectin, adheres to injured vascular walls. Horm Metab Res 2000;32:47-50.
15) Furuhashi M, Ura N, Higashiura K, Murakami H, Tanaka M, Moniwa N, et al. Blockade of the renin-angiotensin system increases adiponectin concentrations in patients with essential hypertension. Hypertension 2003;42:76-81.
16) Ohashi K, Ouchi N, Kihara S, Funahashi T, Nakamura T, Sumitsuji S, et al. Adiponectin I164T mutation is associated with the metabolic syndrome and coronary artery disease. J Am Coll Cardiol 2004;43:1195-200.
17) Hulver MW, Saleh O, MacDonald KG, Pories WJ, Barakat HA. Ethnic differences in adiponectin levels. Metabolism 2004;53:1-3.
18) Kondo H, Shimomura I, Matzuzawa Y, Kumada M, Takahashi M, Matsuda M, et al. Association of adiponectin mutation with type 2 diabetes: a candidate gene for the insulin resistance syndrome. Diabetes 2002;51:2325-8.
19) Ogden CL, Flegal KM, Caroll MD, Johnson CL. Prevalence and trends in overweight among US children and adolescent, 1999-2000 JAMA 2002;288:1728-32.
20) Francischetti EA, Genelhu VA. Obesity-hypertension: an ongoing pandemic. Int J Clin Pract 2007;61:269-80.

21) Hotta K, Funahashi T, Arita Y, Takahashi M, Matsuda M, Okamoto Y, et al. Plasma concentrations of novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. Arterioscler Thromb Vasc Biol 2000;20:1595-9.
22) Yang WS, Lee WJ, Funahashi T, Tanaka S, Matsuzawa Y, Chao CL, et al. Weight reduction increases plasma levels of an adipose-derived, anti-inflammatory protein, adiponectin. J Clin Endocrinol Metab 2001;86:3815-9.
23) Kumada M, Kihara S, Sumitsuji S, Kawamoto T, Matsumoto S, Ouchi N, et al. Association of hypoadiponectinemia with coronary artery disease in men. Arterioscler Thromb Vasc Biol 2003;23:85-9.
24) Yamamoto Y, Hiroshi H, Saito I, Tomita M, Taniyama M, Matsubara K, et al. Correlation of the adipocyte-derived protein adiponectin with insulin resistance index and serum high-density lipoprotein-cholesterol, independent of body mass index, in the Japaneses population. Clin Sci 2002;103: 137-42.
25) Adamczak M, Wiecek A, Funahashi T, Chudek J, Kokot F, Matsuzawa Y. Decreased plasma adiponectin concentration in patients with essential hypertension. Am J Hypertens 2003;16:72-5.
26) Yang WS, Lee WJ, Funahashi T, Tanaka S, Matsuzawa Y, Chao CL, et al. Plasma adiponectin levels in overweight and obese Asians. Obes Res 2002;10:1104-10.
27) Mallamaci F, Zoccali C, Cuzzola F, Tripepi G, Cutrupi S, Parlongo S, et al. Adiponectin in essential hypertension. J Nephrology 2002;15:507-11.
28) Kondo H, Shimomura I, Matsukawa Y, Kumada M, Takahashi M, Matsuda M, et al. Association of adiponectin mutation with type 2 diabetes: a candidate gene for the insulin resistance syndrome. Diabetes 2002;51:2325-8.
29) Ohashi K, Ouchi N, Kihara S, Funahashi T, Nakamura T, Sumitsuji S, et al. Adiponectin I164T mutation is associated with the metabolic syndrome. J Am Coll Cardiol 2004;43: 1195-200.
30) Vasseur F, Helbecque N, Dina C, Lobbens S, Delannoy V, Gaget S, et al. Single-nucleotide polymorphism haplotypes in the both proximal promoter and exon 3 of the AMP1 gene modulate adipocyte-secreted adiponectin hormone levels and contribute to the genetic risk for type 2 diabetes in French Caucasians. Hum Mol Genet 2002;11:2607-14.