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Involvement of low adiponectin levels in impaired glucose tolerance

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Abstract

To investigate the association between serum adiponectin levels and 2-hour post–75-g oral glucose load glycemia, we conducted 75-g oral glucose tolerance tests in 50 subjects who had been diagnosed as having impaired glucose tolerance (IGT) within the prior 3 years. When adjusted for age, body mass index, and sex, serum adiponectin levels in the IGT and diabetes mellitus groups were significantly lower than those in the normal glucose tolerance and impaired fasting glucose groups ($P < .0001$). To determine which variables had significant impacts on 2-hour post–oral glucose glycemia, we performed multiple regression analyses. In stepwise analysis, serum adiponectin levels showed the highest F value (F = 6.43), suggesting the adiponectin level to be an independent predictor of 2-hour post–oral glucose glycemia. Thus, our clinical data suggest the involvement of low adiponectin levels in IGT and diabetes mellitus. To further assess this possibility, we prepared mice fed a high-fat diet for 2 months as an IGT model. Afterward, we compared the 2-hour postglucose glycemia in the IGT mice overexpressing recombinant adiponectin with that in control IGT mice. Mice overexpressing adiponectin showed significantly blunted 2-hour postglucose glycemia (5.66 ± 0.39 mmol/L) as compared with control mice (8.52 ± 0.67 mmol/L), whereas fasting glycemia was not significantly altered by adiponectin overexpression. Taken together, our results reveal the plasma glucose level in response to a glucose load to be negatively associated with serum adiponectin levels, suggesting low adiponectin levels to be one of the predictors of abnormal glucose homeostasis in IGT.

1. Introduction

In recent years, adipocytes have been recognized to secrete a variety of proteins such as tumor necrosis factor (TNF) α, plasminogen activator inhibitor–1, leptin, resistin, and adiponectin/ACRP30 [1-5]. These proteins are termed *adipocytokines* and are likely to be involved in the development of insulin resistance [6-8]. Among them, adiponectin is an insulin-sensitizing hormone, which is exclusively expressed in adipose tissues. A negative correlation (r value = −0.66 for men, −0.48 for women) was observed between plasma adiponectin levels and body mass index (BMI) [9]. Reduced concentrations of this hormone appear to be related to the pathophysiology of insulin resistance and atherosclerosis [6,10]. Screening for mutations in the adiponectin gene, for example, revealed patients carrying an I164T missense mutation to have markedly decreased plasma levels of adiponectin, as well as atherosclerotic vascular diseases [11]. Furthermore, as direct evidence, several previous reports demonstrated administration of adiponectin to decrease the plasma glucose levels of normal mice [12-14]. Adiponectin-KO mice exhibited insulin resistance when fed a high-fat diet [6,15,16]. These observations indicate that adiponectin is an insulin-sensitizing adipocytokine that prevents the development of type 2 diabetes mellitus and cardiovascular disease (CVD) in obesity.

In this study, we investigated the relationship between 2-hour post–75-g oral glucose glycemia and serum circulating adipocytokine levels (adiponectin, leptin, TNFα) in patients with impaired glucose tolerance (IGT) and demonstrated the adiponectin level to be an independent predictor of 2-hour post–oral glucose glycemia. Moreover, we further assessed the involvement of adiponectin in plasma glucose levels in response to the glucose load, using high-fat diet–fed mice overexpressing recombinant adiponectin via adenovirus-mediated transfection. Our findings indicate low

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adiponectin levels to be involved in the mechanism of abnormal glucose homeostasis in glucose-intolerant individuals.

2. Patients and methods

2.1. Design and patients

We conducted 75-g oral glucose tolerance test (OGTT) in 50 Japanese patients 35 to 74 years of age who had been diagnosed as having IGT within the prior 3 years. They were later admitted to our hospital (Saitama Medical University Hospital), underwent blood examinations, and were given exercise and diet therapy instructions to prevent IGT from progressing to type 2 diabetes mellitus. Patients whose latest hemoglobin A_{1C} (HbA_{1C}) was between 5.3% and 6.5% were recruited. None had been treated with antidiabetic oral hypoglycemic agents in the past. None of the subjects had evidence of CVD based on physical examination and electrocardiographic findings. Patients with impaired hepatic function (serum aspartate aminotransferase/alanine aminotransferase >40), poor renal function (serum creatinine >1.5), or severe obesity (BMI >30) were excluded. Study subjects provided written informed consent to undergo 75-g OGTT. For the purpose of this study, patients were classified into categories of abnormal glucose homeostasis. The glucose homeostasis categories were defined based on the American Diabetes Association criteria, as previously described [17]. Using the modified criteria, we defined *diabetes mellitus* (DM) as having a fasting plasma glucose (FPG) ≥126 mg/dL or 2-hour postglucose glycemia ≥200 mg/dL, *normal glucose tolerance* (NGT) as having an FPG level <110 mg/dL and 2-hour postglucose glycemia <140 mg/dL, *impaired fasting glucose* (IFG) as having an FPG level ≥110 mg/dL but <126 and 2-hour postglucose glycemia <140 mg/dL, and *IGT* as having an FPG level <126 mg/dL and 2-hour postglucose glycemia ≥140 mg/dL but <200 mg/dL.

2.2. Laboratory data

Fasting plasma glucose, HbA_{1C}, immunoreactive insulin, total cholesterol, triglyceride (TG), and high-density lipoprotein (HDL) cholesterol concentrations were measured using standard laboratory techniques. Body weights and blood pressures were also measured at the same time as OGTT. Hormone concentrations were determined using commercially available kits; that is, serum adiponectin was measured by enzyme-linked immunosorbent assay (Otsuka Pharmaceutical, Tokyo, Japan), serum leptin by radioimmunoassay (Linco Research, St Charles, MO), and serum TNFα by enzyme immunoassay (Golden Bridge Internal, Lynnwood, WA).

2.3. Animals

Nine-week–old male mice (C57bl/KsJ, n = 14) were purchased from Clea Japan (Osaka, Japan). After a 2- to 3-day acclimatization period, the mice were maintained on a 12-hour:12-hour light/dark cycle, were fed the indicated high-fat diet [18] ad libitum, and had unlimited access to water. Two months later, all mice were divided into a LacZ-transferred group (L group) and an adiponectin-transferred group (A group), and adenovirus-mediated gene transfer was performed. Before the glucose challenge, the animals were fasted for 8 hours. Intraperitoneal glucose tolerance tests (ipGTTs) using 2 mg/g body weight were conducted as previously described [19]. Plasma insulin was measured with an insulin immunoassay (Shibayagi, Gunma, Japan).

2.4. Transient expression of adenovirus-mediated adiponectin and Western blot analysis

Recombinant adenovirus containing full-length murine adiponectin complementary DNA was prepared as reported previously [20]. Adenovirus expressing LacZ was used as a control vector. Mice were treated with recombinant adenovirus containing LacZ or murine adiponectin complementary DNA by systemic injection into the tail vein. Three days after virus infection, we confirmed increased serum adiponectin levels by Western blot analysis. Serum samples from mice were directly solubilized in Laemmli sample buffer and electrophoresed through a 10% sodium dodecyl sulfate–polyacrylamide gel followed by electrotransfer onto a nitrocellulose membrane. Commercially available antimurine adiponectin antibody (CHEMICON International, Temecula, CA) was used for the first 2-hour incubation at room temperature. After blotting with the antibody, detection was performed using an ECL chemiluminescent kit (Amersham Pharmacia Biotech, Little Chalfont, United Kingdom) according to the manufacturer's instructions. Quantitations were performed using a Molecular Imager (Bio-Rad Laboratories, Hercules, CA).

2.5. Statistical analysis

Data are presented as means ± SEs. Log transformation of continuous variables was used when needed to satisfy distributional requirements for parametric tests. Differences in clinical characteristics were assessed by the unpaired Student t test, and a P value less than .05 was considered statistically significant. Subsequently, analysis of covariance (ANCOVA) was used to test between-group differences, adjusting for covariates. Multiple regression analysis was used to assess the relations of multiple factors to 2-hour post–oral glucose glycemia, adjusting for covariates and using a stepwise model. Variables were excluded from the stepwise analysis if the F value was less than 4.0. The extended Fisher exact test was used to examine the significance of the association between 2 variables in a 2 × 2 contingency table. Statistical analyses were performed using Stat View software (Version 5.01; SAS Institute, Cary, NC).

3. Results

Characteristics of 4 categories, divided according to the results of 75-g OGTT, are also presented in Table 1. The

Table 1
Characteristics of the 4 glucose metabolism categories

	NGT	IFG	IGT	DM
Age (y)	55 ± 4	59 ± 4	60 ± 4	61 ± 2
BMI (kg/m^2)	24.0 ± 0.9	23.0 ± 0.5	24.8 ± 1.2	25.7 ± 1.5
HbA_{1C} (%)	5.4 ± 0.1	5.7 ± 0.3	5.9 ± 0.1	6.1 ± 0.2*
FPG (mg/dL)	92 ± 2	114 ± 2*	110 ± 4*	116 ± 6*
Fasting IRI (μU/mL)	6.3 ± 0.9	6.4 ± 1.1	9.0 ± 1.9	9.3 ± 2.8
TG (mg/dL)	114 ± 12	162 ± 49	146 ± 28	131 ± 18
T-Chol (mg/dL)	213 ± 6	212 ± 10	216 ± 9	212 ± 9
HDL cholesterol (mg/dL)	61 ± 4	61 ± 7	52 ± 4	55 ± 4
Adiponectin (μg/mL)	9.6 ± 2.0	9.2 ± 3.7	6.0 ± 0.9**	6.3 ± 1.0**
Leptin (ng/mL)	10.3 ± 2.4	9.8 ± 0.8	9.4 ± 2.7	8.0 ± 1.8
TNFα (pg/mL)	6.0 ± 1.0	5.6 ± 0.8	6.2 ± 1.7	6.0 ± 0.8

Data are means ± SE. Normal glucose tolerance: n = 15, male-female = 3/12; IFG: n = 5, male-female = 3/2); IGT: n = 13, male-female = 7/6; DM: n = 17, male-female = *** 12/5. IRI indicates immunoreactive insulin; T-Chol, total cholesterol.

* Significant difference ($P < .05$) relative to NGT by unpaired t test.

** Significant difference ($P < .05$) relative to NGT and IFG by unpaired t test.

*** Significant difference ($P < .05$) relative to NGT by Fisher exact test.

male-female ratio in the DM group was significantly higher and FPG in the NGT group was significantly lower than those in other groups. Fig. 1 shows the serum adiponectin levels of the 4 groups. When adjusted for age, BMI, and sex, serum adiponectin levels in the IGT and DM groups were significantly lower than those in the NGT group ($P < .0001$). Serum adiponectin levels in the IFG group were comparable with those in the NGT group and were also significantly higher than those in the IGT and DM groups. Afterward, we subdivided the study population at the borderline plasma glucose value of 140 mg/dL for 2-hour post–oral glucose loading and conducted a multivariate ANCOVA adjusting for potential confounders (sex, BMI, age, HbA_{1C}). In this analysis, only adiponectin was markedly lower with 2-hour post–oral glucose hyperglycemia (plasma glucose >140 mg/dL); that is, changes in other adipocytokines did not reach statistical significance (Table 2). In addition, to determine which variables had significant impacts on 2-hour post–oral

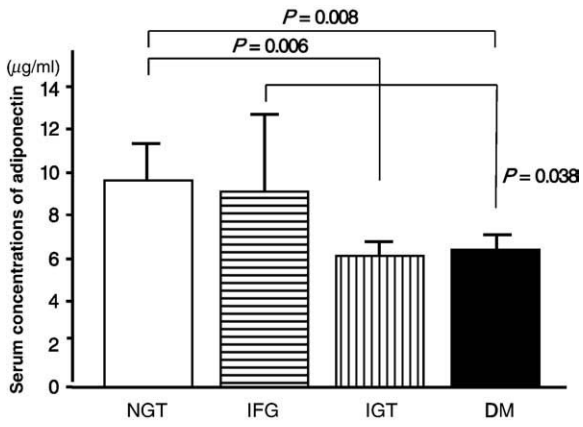


Fig. 1. Serum adiponectin levels in NGT (n = 15), IFG (n = 5), IGT (n = 13), and DM (n = 17) subjects. P adjusted for sex, BMI, and age by ANCOVA.

Table 2
Adipocytokine concentrations at plasma glucose levels greater than and less than 140 mg/dL

	Plasma glucose (120 min)		Adjusted P
	<140 mg/dL (n = 19)	≥140 mg/dL (n = 31)	
Adiponectin (μg/mL)	9.5 ± 6.8	6.2 ± 2.5	<.0001
Leptin (ng/mL)	9.7 ± 9.0	9.0 ± 6.7	.53
TNFα (ng/mL)	5.9 ± 3.7	5.9 ± 4.0	.99

Data are means ± SE. P adjusted for sex, BMI, age, and HbA_{1C} by ANCOVA.

glucose glycemia, we performed univariate and multiple regression analyses (Table 3). In univariate analysis, male sex ($P = .02$) and a lower adiponectin level ($P = .03$) were significant values predicting 2-hour post–oral glucose hyperglycemia. In stepwise analysis, serum adiponectin levels showed the highest F value (F = 6.43) among sex, age, BMI, serum TG, total cholesterol, HDL cholesterol, adiponectin, TNFα, and leptin, suggesting the adiponectin level to be an independent predictor of 2-hour post–oral glucose hyperglycemia among these variables.

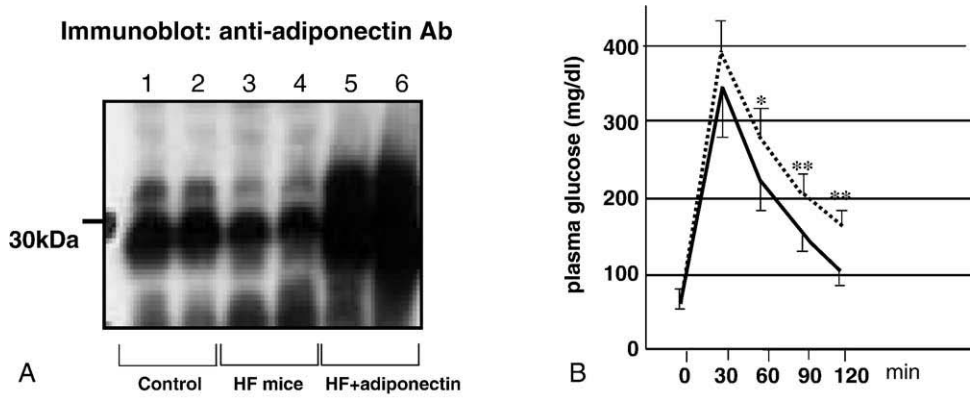
In the experiments using mice fed the high-fat diet, we confirmed increased serum adiponectin levels (2.5-fold) in the A group by immunoblot analysis (Fig. 2A) 3 days after virus infection. Fig. 2B shows the results of ipGTT. Body weights did not differ between the 2 groups (30.6 ± 1.0 g, L group; 31.1 ± 1.2 g, A group) in the experiments involving glucose challenge tests. The FPG levels at the time of ipGTT were 4.22 ± 0.30 mmol/L (L group) and 3.89 ± 0.34 mmol/L (A group), not significantly different. The 2-hour postglucose glycemia in A group (5.66 + 0.39 mmol/L) was significantly blunted as compared with that in L group (8.52 ± 0.67 mmol/L), whereas fasting glycemia was not significantly altered by adiponectin overexpression.

4. Discussion

To our knowledge, the present study is the first to demonstrate low adiponectin levels in IGT subjects diag-

Table 3
Relations of plasma glucose at 120 minutes (OGTT) to variables

Variables	Simple correlation	Partial correlation	P value for univariate analysis	F value for multiple regression (stepwise analysis)
Sex	−0.33	−0.32	.02	4.25
Age	0.10	0.26	.10	2.40
BMI	0.25	0.26	.08	2.67
TG	0.16	0.21	.27	1.65
T-Chol	0.11	0.20	.44	1.80
HDL cholesterol	0.26	0.26	.11	2.75
Adiponectin	−0.32	−0.33	.03	6.43
Leptin	−0.05	−0.14	.76	0.07
TNFα	−0.16	−0.17	.28	1.33



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	Fasting Insulin (10^{-10}mol/l)	Insulin (10^{-10}mol/l) (2h post glucose load)
HF mice	0.72±0.17	1.93±0.38
HF mice +adiponectin	0.67±0.19	0.96±0.31

Fig. 2. Adiponectin-mediated regulation of glucose disposal assessed by ipGTT. A, Immunoblotting analysis of serum adiponectin levels. Representative data from 2 of the 7 mice in each group are presented. Lanes 1 and 2: normal chow diet; lanes 3 and 4: L group, high-fat diet–fed mice injected with adenovirus expressing LacZ; lanes 5 and 6: A group, high-fat diet–fed mice injected with adenovirus expressing recombinant adiponectin. B, Glucose curve under ipGTT. Solid line: A group (n = 7); dotted line: L group (n = 7). * $P < .05$ and ** $P < .01$ compared with L group.

nosed by OGTT. As a previous study showed lower serum adiponectin values in obese than in normal subjects and also that a low adiponectin level is linked to insulin resistance [9], we performed ANCOVA, adjusting for BMI, to exclude the possibility that the association of adiponectin with 2-hour postglucose glycemia is dependent on its relationship with obesity. Moreover, in our data, BMI was not significantly associated with 2-hour postglucose glycemia ($P = .08$, Table 3), whereas BMI correlated negatively with serum adiponectin levels. These results suggest that the adiponectin concentration is more closely related to plasma glucose levels in response to a glucose load than to obesity, which is consistent with previous results [21]. In addition, a low adiponectin level is also one of the risk factors for coronary diseases [22,23]. Thus, based on these data, the increased risk for CVD observed in IGT patients [24,25] is due, at least in part, to lower-than-normal levels of adiponectin.

Intensive studies have elucidated the physiologic mechanism by which adiponectin improves insulin sensitivity. In the liver, adiponectin enhances insulin-induced suppression of hepatic glucose output, which is attenuated in insulin-resistant states [12,13]. Adiponectin reduces TG contents of skeletal muscles via fatty acid oxidation, which thereby ameliorates insulin resistance caused by tissue TG accumulation [26]. These effects were exerted via adiponectin-induced activation of adenosine monophosphate–activated protein kinase, peroxisome proliferator–activated receptor α, and p38 mitogen–activated protein kinase [27].

In our study, post–glucose loading hyperglycemia was greatly improved by adiponectin overexpression, which is consistent with previous reports [15,28]. On the other hand, fasting glucose levels did not differ significantly between the A and L groups, whereas fasting glucose levels of A group mice tended to be decreased as compared with those of L group mice. These results demonstrate circulating adiponectin to play a crucial role in regulating 2-hour postglucose glycemia, but not fasting glycemia. As the serum insulin levels in A group mice were lower than those in L group mice (Fig. 2C), the glucose-lowering effect of adiponectin is attributable to improved insulin resistance, rather than stimulation of insulin secretion. Although one previous study found that adiponectin did not affect fasting glucose levels [29], our results are not consistent with those of most other studies, which demonstrated adiponectin to lower fasting glucose levels [12-15]. We can explain this discrepancy as follows: as compared with fasting glucose levels in mice fed a normal chow diet, the increase in mice fed a high-fat diet was so small that adiponectin-mediated suppression of fasting glucose levels was undetectable.

We demonstrated 2-hour postglucose hyperglycemia to negatively correlate with serum adiponectin levels in our clinical study and that transient overexpression of recombinant adiponectin largely ameliorated postglucose hyperglycemia in high-fat–fed mice. These results suggest low adiponectin levels to be associated with IGT, which in turn could be involved in the development of atherosclerosis.

References

[1] Friedman JM, Halaas JL. Leptin and the regulation of body weight in mammals. Nature 1998;395:763-70.

[2] Hotamisligil GS. The roles of TNF*a* and TNF receptors in obesity and insulin resistance. J Intern Med 1999;245:621-5.

[3] Maeda K, Okubo K, Shimomura I, Funahashi T, Matsuzawa Y, Matsubara 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.

[4] Shimomura I, Funahashi T, Takahashi M, Maeda K, Kotani K, Nakamura T, et al. Enhanced expression of PAI-1 in visceral fat; possible contributor to vascular disease in obesity. Nat Med 1996;2: 800-3.

[5] Steppan CM, Bailey ST, Bhat S, Brown EJ, Banerjee RR, Wright CM, et al. The hormone resistin links obesity to diabetes. Nature 2001;409:307-12.

[6] Maeda N, Shimomura I, Kishida K, Nishizawa H, Matsuda M, Nagaretani H, et al. Diet-induced insulin resistance in mice lacking adiponectin/ACRP30. Nat Med 2002;8:731-7.

[7] Uysal KT, Wiesbrock SM, Marino MW, Hotamisligil GS. Protection from obesity-induced insulin resistance in mice lacking TNF-alpha function. Nature 1997;389:610-4.

[8] Banerjee RR, Rangwala SM, Shapiro JS, Rich AS, Rhoades B, Qi Y, et al. Regulation of fasted blood glucose by resistin. Science 2004; 303:1195-8.

[9] Arita Y, Ouchi N, Takahashi M, Maeda K, Miyagawa J, Hotta K, et al. Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. Biochem Biophys Res Commun 1999;247:79-83.

[10] Ouchi N, Kihara S, Arita Y, Okamoto Y, Maeda K, Kurihara H, et al. Adiponectin, an adipocyte-derived plasma protein, inhibits endothelial NF-kappa B signaling through a cAMP-dependent pathway. Circulation 2000;102:1296-301.

[11] 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.

[12] 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.

[13] Combs TP, Berg AH, Obici S, Scherer PE, Rossetti L. Endogenous glucose production is inhibited by the adipose-derived protein Acrp30. J Clin Invest 2001;108:1875-81.

[14] Fruebis J, Tsao TS, Javorschi S, Ebbets-Reed, Erickson MR, Yen FT, et al. Proteolytic cleavage product of 30-kDa adipocyte complement-related protein increases fatty acid oxidation in muscle and causes weight loss in mice. Proc Natl Acad Sci U S A 2001;98:2005-10.

[15] Kubota N, Terauchi Y, Yamauchi T, Kubota T, Moroi M, Matsui J, et al. Disruption of adiponectin causes insulin resistance and neointimal formation. J Biol Chem 2002;277:25863-6.

[16] Yamauchi T, Kamon J, Waki H, Imai Y, Shimozawa N, Hioki N, et al. Globular adiponectin protected ob/ob mice from diabetes and ApoE-deficient mice from atherosclerosis. J Biol Chem 2003;278:2461-8.

[17] Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diabetes Care 1997;20:1183-97.

[18] Anai M, Funaki M, Ogihara T, Kanda A, Onishi Y, Sakoda H, et al. Enhanced insulin-stimulated activation of phosphatidylinositol 3-kinase in the liver of high fat fed rats. Diabetes 1999;48:158-69.

[19] Shimomura I, Hammer RE, Richardson JA, Ikemoto S, Bashmakov Y, Goldstein JL, et al. Insulin resistance and diabetes mellitus in transgenic mice expressing nuclear SREBP-1c in adipose tissue: model for congenital generalized lipodystrophy. Genes Dev 1998;12: 3182-94.

[20] Inukai K, Nakashima Y, Watanabe M, Takata N, Sawa T, Kurihara S, et al. Regulation of adiponectin receptor gene expression in diabetic mice. Am J Physiol Endocrinol Metab 2005;288:E876-82.

[21] Abbasi F, Chu JW, Lamendola C, McLaughlin T, Hayden J, Reaven GM, et al. Discrimination between obesity and insulin resistance in the relationship with adiponectin. Diabetes 2004;53:585-90.

[22] Kawano T, Saito T, Yasu T, Saito T, Nakamura T, Namai K, et al. Close association of hypoadiponectinemia with arteriosclerosis obliterans and ischemic heart disease. Metabolism 2005;54:653-6.

[23] Hashimoto N, Kanda J, Nakamura T, Horie A, Kurosawa H, Hashimoto T, et al. Association of hypoadiponectinemia in men with early onset of coronary heart disease and multiple coronary artery stenosis. Metabolism 55:1653-1657.

[24] Tominaga M, Eguchi H, Manaka H, Igarashi K, Kato T, Sekikawa A. Impaired glucose tolerance is a risk factor for cardiovascular disease, but not impaired fasting glucose: the Funagata Diabetes Study. Diabetes Care 1999;22:920-4.

[25] The DECODE Study Group, European Diabetes Epidemiology Group. Glucose tolerance and mortality: comparison of WHO and American Diabetes Association diagnostic criteria. Lancet 1999;354:617-21.

[26] Yamauchi T, Kamon J, Waki H, Terauchi Y, Kubota N, Hara K, et al. The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. Nat Med 2001;7:941-6.

[27] Yoon MJ, Lee GY, Chung JJ, Ahn YH, Hong SH, Kim JB. Adiponectin increases fatty acid oxidation in skeletal muscle cells by sequential activation of AMP-activated protein kinase, p38 mitogen–activated protein kinase and peroxisome proliferator–activated receptor a. Diabetes 2006;55:2562-70.

[28] Shklyaev S, Aslanidi G, Tennant M, Prima V, Kohlbrenner E, Kroutov V, et al. Sustained peripheral expression of transgene adiponectin offsets the development of diet-induced obesity in rats. Proc Natl Acad Sci U S A 2003;100:14217-22.

[29] Satoh H, Nguyen MTA, Trujillo M, Imamura T, Usui I, Scherer PE, et al. Adenovirus-mediated adiponectin expression augments skeletal muscle insulin sensitivity in male Wistar Rats. Diabetes 2005;54: 1302-13.