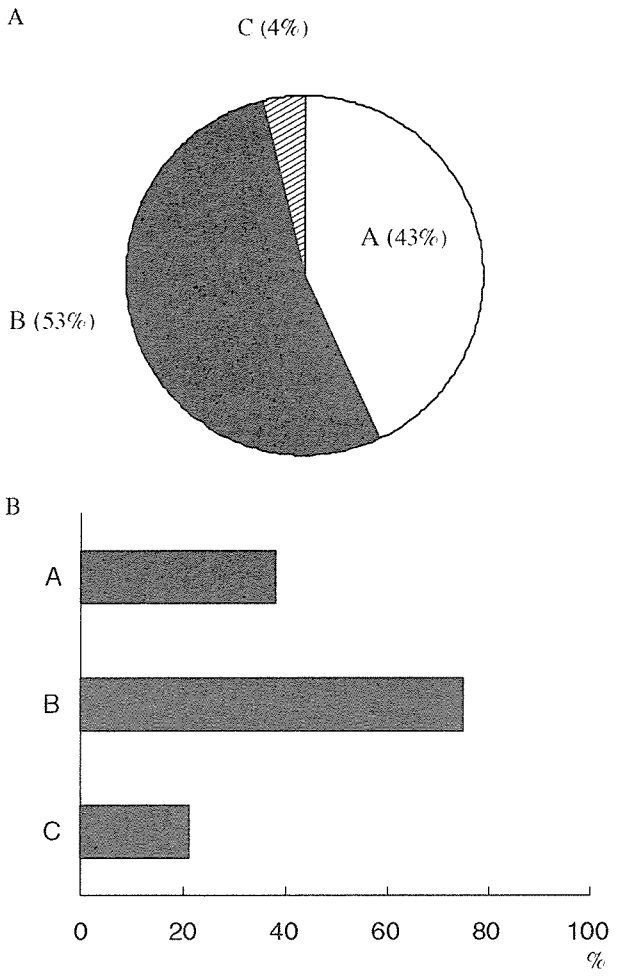

Figure 3 The survey of private practitioners in Kyoto Prefecture. (A) What do you think about acceptance of patients referred from the university hospital? A. I will accept patients referred from the university hospital. B. I will consider acceptance of patients depending on the case. C. I will not accept patients referred from the university hospital. (B) How patients should be followed after referral to private practices? A. Only private practitioners follow referred patients. B. Patients attend the university hospital clinic two or three times a year in addition to routine follow-up by private practitioners. C. Patients attend the university hospital clinic for specific disease and attend private practitioner's office for management of general medical problems.

practitioner training.[5] Therefore, information about their specialty is necessary on referral of patients to private practitioners. In this survey, 98% of the respondents replied their specialty, which was useful information for building a database.

The responses to the question regarding the role of the university hospital were interesting, showing that physicians working at the university hospital are groping for the role. Undoubtedly, the biggest advantage of the university hospital is the expertise of many specialists in all areas of medicine. Thus, the majority of

Table 3 The rate of private practitioners providing in-home medical service

1. In-home medical examination at the patient's request	61.8%
2. Regular home visit medical service	47.0%
Management of patients undergoing	
3. home oxygen therapy	14.9%
4. intravenous hyperalimentation	13.5%
5. tracheotomy	9.7%
6. mechanical ventilation	5.5%
7. Management of pressure sores	25.5%
8. Management of stoma	14.2%
9. End of life care	12.5%

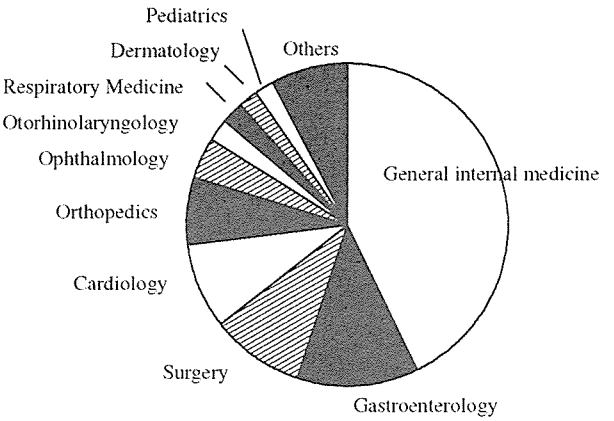

Figure 4 Specialties of private practitioners providing in-home medical service.

respondents replied that the university hospital should focus on advanced and specialist medical service. However, this idea would lead to a hospital offering relatively small areas of medical service. Some respondents indicated that the university hospital should be a general hospital accepting patients with all kinds of diseases and providing high quality service in all general areas of medicine. The former view clearly defines a role of the university hospital as an advanced medical center, but may limit the function of the hospital. On the other hand, the latter view emphasizes a role in the local medical service, but the difference between the university hospital and district general hospitals is unclear. Education of medical students and residents, residency training in family practice, lifelong medical education programs and promotion of collaboration between district general hospitals would also be an important role for the university hospital from the latter viewpoint. It would be a challenge for the university hospital to cope with these two roles.

In this survey, we found that many private practitioners provide advanced in-home medical services

including management of patients undergoing mechanical ventilation or intravenous hyperalimentation, and that some specialists other than internists including ophthalmologists, otorhinolaryngologists and dermatologists also offer in-home medical services. It would be possible that a team of private practitioners could manage medically complex patients requiring both general and specialist care at home. Furthermore, a role of social service departments at university hospitals is increasingly important in facilitating postdischarge referrals and the use of in-home medical services provided by private practitioners.

Interestingly, similar opinions were provided by physicians at the university hospital and private practitioners regarding desirable collaboration and problems to be solved. Both proposed a collaboration model in which physicians at the university hospital examine and diagnose patients referred by private practitioners and follow them in collaboration with private practitioners after treatment is provided and patients become stable. Moreover, improvement of the emergency medical service, promotion of interchange of ideas and information between physicians at the university hospital and private practitioners and the development of a lifelong medical education program were raised as future challenges by both groups of physicians. These results indicated that physicians at the university hospital and private practitioners share a view on desirable collaboration and problems, and that solving these problems would be a key to the successful collaboration.

Careful and comprehensive assessment is required for older patients when they are discharged from hospitals and are referred to private practices. Caplan *et al.* reported that comprehensive geriatric assessment (CGA) and multidisciplinary intervention improved health outcomes of older people at risk of deteriorating health and admission to hospitals.[6] CGA was also effective for targeted outpatients in terms of slowing functional decline.[7] Therefore, geriatricians and health care professional teams at university and district general hospitals should play a role in assessing older patients at risk, creating a comprehensive plan of care and communicating it to the person's physician.[8] Geriatricians, skilled at balancing the benefits and burdens of therapy and managing geriatric syndromes, should coordinate care with other health-care professionals to ensure that their patients' needs are met. Since physicians who provide the majority of health care for older people are not geriatricians, it is also important for geriatricians to teach physicians and other health-care professionals how to provide the best care for older persons.[8]

In the United Kingdom, medical service is provided by the National Health Service (NHS), in which almost everyone is registered with a general practice.[9,10] About 80% of contacts with the NHS take place in general practice and referral from general practitioners is required to have specialist services.[9,10] In this system, general practitioners play a role as primary care physicians delivering the full range of general medical services as well as gate keepers to refer patients to specialists. Although there are several problems including wide variations of the quality provided in general practice, routinely booked appointments for general practitioners, long waiting times for elective care and limited choice for patients,[9,10] the system of registration of patients with general practice may be worthy of consideration in Japan.

The World Health Organization (WHO) ranked France first in overall health system performance among the 191 countries surveyed in its World Health Report 2000.[11] The health care systems in France and Japan share common principles; universal coverage and free access.[12] These two countries also face common problems including growing elderly populations and increasing health care budgets. The Organization for Economic Cooperation and Development (OECD) suggested a reform plan for the health care system in France focusing on introduction of diagnosis-related payment in hospitals, monitoring for flagrant overuse of specialist service and frequent changes of home doctors, and the development of preventive care programs.[12]

Health promotion is defined as the process of enabling people to increase control over and to improve their health.[13] Self care, community participation and healthy environments are key mechanisms intrinsic to health promotion.[14,15] Self care is a process whereby people make decisions and actions about their own health and community participation focuses on the bottom-up approach in planning and implementation of health development programs.[14,15] Private practitioners in collaboration with university and district general hospitals should play a role in health promotion movement, providing educational programs and helping plan and implement health promotions and disease control programs in the community.

In conclusion, we propose that both hospital staff and patients should realize that physicians at the university hospital are not home doctors. We should encourage patients to use home doctors and it is important to construct a system in which patients are supported by primary care provided by home doctors and specialist service provided at the university hospital. Improvement of out-of-hour service at the university hospital and promotion of routine collaboration between the university hospital and private practices would further enhance bilateral referral of patients. Promotion of collaboration with other health-care professionals in the local medical community[16] and the development of lifelong medical education programs and family medicine residency training programs[5] are also important. Overcoming the problems and obstacles highlighted in this study and constructing collaboration models will be a

key for university hospitals to fully contribute to establishing a quality medical system.

Acknowledgments

This study was supported by the Research Grant for Longevity Sciences (15C-3) from the Ministry of Health, Labour and Welfare of Japan.

References

1 Ikegami N, Campbell JC. Health care reform in Japan: the virtues of muddling through. *Health Aff (Millwood)* 1999; **18**: 56–75.
2 World Health Organization. Health situation in Japan. [Cited 26 Sep 2006.] Available from URL: http://www.wpro.who.int/countries/jpn/health_situation.htm
3 Ikegami N, Campbell JC. Japan's health care system: containing costs and attempting reform. *Health Aff (Millwood)* 2004; **23**: 26–36.
4 Cabinet Office, Government of Japan. Structural reform of the Japanese economy: basic policies for macroeconomic management (Prepared by Cabinet Office). [Cited 26 Sep 2006.] Available from URL: http://www.kantei.go.jp/foreign/policy/2001/structural/0626keizaizaisei_e.html
5 Murai M, Kitamura K, Fetters MD. Lessons learned in developing family medicine residency training programs in Japan. *BMC Med Educ* 2005; **5**: 33.
6 Caplan GA, Williams AJ, Daly B, Abraham K. A randomized controlled trial of comprehensive geriatric assessment and multidisciplinary intervention after discharge of elderly from the emergency department – the DEED II study. *J Am Geriatr Soc* 2004; **52**: 1417–1423.
7 Boult C, Boult LB, Morishita L, Dowd B, Kane RL, Urdangarin CF. A randomized clinical trial of outpatient geriatric evaluation and management. *J Am Geriatr Soc* 2001; **49**: 351–359.
8 American Geriatrics Society Core Writing Group of the Task Force on the Future of Geriatric Medicine. Caring for older Americans: the future of geriatric medicine. *J Am Geriatr Soc* 2005; **53**: S245–S256.
9 Marshall M, Wilson T. Competition in general practice. *BMJ* 2005; **331**: 1196–1199.
10 Oliver A. The English National Health Service: 1979–2005. *Health Econ* 2005; **14**: S75–S99.
11 World Health Organization. World Health Organization assesses the world's health systems. [Cited 26 Sep 2006.] Available from URL: http://www.who.int/inf-pr-2000/en/pr2000-44.html
12 Lenain Patrick. OECD Observer: Sante to the French health system. [Cited 26 Sep 2006.] Available from URL: http://www.oecdobserver.org/news/fullstory.php/aid/356/Sant%E9_to_the_French_health_system_.html
13 Ottawa Charter for Health Promotion. Ottawa Charter for Health Promotion. First International Conference on Health Promotion. [Cited 26 Sep 2006.] Available from URL: http://www.who.int/hpr/NPH/docs/ottawa_charter_hp.pdf
14 Anderson JM. Empowering patients: issues and strategies. *Soc Sci Med* 1996; **43**: 697–705.
15 Bhuyan KK. Health promotion through self-care and community participation: elements of a proposed programme in the developing countries. *BMC Public Health* 2004; **4**: 11.
16 Tanaka M. Multidisciplinary team approach for elderly patients. *Geriat Gerontol Int* 2003; **3**: 69–72.

0021-972X/06/$15.00/0
Printed in U.S.A.

The Journal of Clinical Endocrinology & Metabolism 91(4):1600–1603

doi: 10.1210/jc.2005-2271

BRIEF REPORT

Lower Total Fasting Plasma Adiponectin Concentrations Are Associated with Higher Metabolic Rates

Nicola Pannacciulli, Joy C. Bunt, Emilio Ortega, Tohru Funahashi, Arline D. Salbe, Clifton Bogardus, and Jonathan Krakoff

Obesity and Diabetes Clinical Research Section (N.P., J.C.B., E.O., A.D.S., C.B., J.K.), Phoenix Epidemiology and Clinical Research Branch, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Department of Health and Human Services, Phoenix, Arizona 85016; and Department of Medicine and Molecular Science (T.F.), Osaka University Graduate School of Medicine, Suita, Osaka 565-0871 Japan

Context: The possible role of adiponectin, a protein uniquely produced by the adipose tissue and significantly reduced in obesity and other insulin-resistant states, in the regulation of energy expenditure (EE) is still poorly understood.

Objective: The objective of the study was to investigate the relationship between total fasting plasma adiponectin concentrations and the various components of EE measured in a metabolic chamber in Pima Indians and to test whether body fat distribution may have a role in this association.

Design: This was a cross-sectional study.

Setting: The study was an inpatient clinical research unit.

Participants: Sixty nondiabetic Pima Indians (45 males and 15 females), aged 18–45 yr, spanning a wide range of adiposity (body mass index 19.6–46.2 kg/m^2) participated in the study.

Main Outcome Measures: Total fasting plasma adiponectin concentrations, EE (24-h respiratory chamber), insulin sensitivity (euglycemic-hyperisulinemic clamp), body composition (dual-energy x-ray absorptiometry), and body fat distribution (waist to thigh ratio) were the main outcome measures.

Results: Total fasting plasma adiponectin concentrations are negatively associated with sleep EE adjusted for sex, age, fat-free mass, and fat mass. This correlation is still significant, although attenuated, after inclusion of insulin-stimulated glucose disposal among the regressors and further attenuated when adjusted also for waist to thigh ratio.

Conclusions: The decrease in total fasting plasma adiponectin concentrations that accompanies fat accumulation may be a mechanism to prevent further weight gain by decreasing insulin sensitivity and increasing energy expenditure. (***J Clin Endocrinol Metab* 91: 1600–1603, 2006**)

PLASMA CONCENTRATIONS OF adiponectin are significantly reduced in obese and diabetic mice and humans as well as patients with hypertension and cardiovascular diseases (1). Conversely, weight loss is associated with an increase in adiponectin concentrations in some (2) but not all (3) studies. Total fasting plasma adiponectin concentrations are lower in Pima Indians, a population with a high prevalence of obesity and type 2 diabetes, compared with Caucasians, although this ethnic difference is no longer significant after adjusting for insulin sensitivity (4).

The role of adiponectin in energy balance is yet to be established. Animal studies have provided contradictory results, indicating both a positive (5) and negative (6) effect of adiponectin on energy expenditure (EE). Human studies have shown a negative association between resting metabolic rate (RMR) measured by the ventilated hood technique and plasma adiponectin in both male and female Caucasians (7). A previous study of Pima Indians and Caucasians did not show a significant association between 24-h EE, as measured in a metabolic chamber, and total fasting plasma adiponectin concentrations but did not assess the various components of daily metabolic rate (8).

Central fat is a stronger determinant of plasma adiponectin concentrations than overall adiposity (4). Central fat accumulation is associated with higher EE, an observation also found in Pima Indians (9). Therefore, we hypothesized that body fat distribution may mediate the relationship between adiponectin and metabolic rates.

The aims of this study were to investigate the relationship between total fasting plasma adiponectin concentrations and the different components of daily EE in a group of Pima Indian men and women spanning a wide range of adiposity and to test whether body fat distribution may have a role in this association. EE was measured in a respiratory chamber, which provides an accurate measure of a person's 24-h EE.

Subjects and Methods

Subjects

Sixty nondiabetic Pima Indians [45 male and 15 females, aged 28 ± 7 yr (range 18–45)] from the Gila River Indian Community were in-

First Published Online January 31, 2006

Abbreviations: EE, Energy expenditure; M, insulin-stimulated glucose disposal; min-max, minimum-maximum; RMR, resting metabolic rate; SLEEP, sleep EE; SPA, spontaneous physical activity; WTR, waist to thigh ratio.

cluded in the study. The analysis focused on only those subjects, part of a larger study of metabolic predictors of type 2 diabetes in Pima Indians and Caucasians, for whom both insulin-stimulated glucose disposal and EE along with total fasting plasma adiponectin concentrations were available. Before participation, volunteers were fully informed of the nature and purpose of the study, and written informed consent was obtained. The experimental protocol was approved by the Institutional Review Board of the National Institute of Diabetes and Digestive and Kidney Diseases and the Tribal Council of the Gila River Indian Community. All subjects were found to be free of disease according to physical examination, medical history, and laboratory testing. On admission to the metabolic ward, subjects were placed on a standard weight maintenance diet (20, 30, and 50% of daily calories provided as protein, fat, and carbohydrate, respectively) for 3 d before testing. Glucose tolerance was assessed by a 75-g oral glucose tolerance test according to the criteria of the World Health Organization. Only nondiabetic subjects were studied. Body composition was measured by dual-energy x-ray absorptiometry (DPX-L, Lunar Corp., Madison, WI).

EE

EE was measured in a respiratory chamber. Volunteers entered the chamber at 0745 h after an overnight fast and remained there for 23½ h. Meals were provided at 0800, 1130, 1600, and 1900 h (an evening snack). The rate of EE was measured continuously, calculated for each 15-min interval, and then extrapolated to 24 h (24-h EE). Spontaneous physical activity (SPA) was detected by radar sensors and expressed as percentage of time over the 24-h period in which activity was detected. Sleep EE (SLEEP) was defined as the average EE of all 15-min periods between 2330 and 0500 h during which SPA was less than 1.5%. SLEEP is an estimate of the basal metabolism necessary to support life. Physical activity-related EE was calculated by multiplying the mean SPA values by the slope of the regression line of 24-h EE *vs.* SPA.

Two-step hyperinsulinemic-euglycemic glucose clamp

Insulin-stimulated glucose disposal (M) was assessed at physiological and supraphysiological insulin concentrations using a two-step hyperinsulinemic-euglycemic glucose clamp, as previously described (10).

Analytical measurements

Blood samples were drawn after an overnight fast, at least 3 full days after subjects had consumed a weight-maintenance diet on the metabolic ward. Plasma glucose concentrations were measured using the glucose oxidase method (Beckman Instruments Inc., Fullerton, CA). Plasma insulin concentrations were measured with an automated RIA (Concept 4, ICN Biochemicals, Costa Mesa, CA). Plasma adiponectin concentrations were measured with a validated sandwich ELISA using an adiponectin-specific antibody (intra- and interassay coefficients of variation were 3.3 and 7.4%, respectively), as previously reported (4).

Data analysis

All statistical analyses were performed using software of the SAS Institute (SAS version 8.2; Cary, NC). Throughout the text, the data are expressed as means ± SD. Relationships between total fasting plasma adiponectin concentrations and the other study variables were assessed using Spearman correlation coefficients. Linear regression analysis was used to model the effects of adiponectin on the EE parameters as well as the effects of selected anthropometric and metabolic variables on total fasting plasma adiponectin concentrations.

Results

Total fasting plasma adiponectin concentrations [median (minimum-maximum [min-max]): 6.7 (3.1–15.3) μg/ml] were negatively associated with body mass index (mean ± SD: 31.4 ± 6 kg/m^2; r = −0.36, P = 0.004), percent body fat (mean ± SD: 30 ± 7%; r = −0.31, P = 0.02), waist circumference (mean ± SD: 104 ± 14 cm; r = −0.33, P = 0.01), waist to thigh ratio (WTR; mean ± SD: 1.6 ± 0.1; r = −0.36, P = 0.004), fasting concentrations of glucose (mean ± SD: 84 ± 10 mg/dl; r = −0.34, P = 0.007), fasting concentrations of insulin (median [min-max]: 40 (7–108) μIU/ml; r = −0.44, P = 0.0004), and SLEEP (mean ± SD: 1634 ± 249 kcal per 24 h; r = −0.42, P = 0.0009, Fig. 1) but not 24-h EE (mean ± SD: 2338 ± 359 kcal per 24 h; r = −0.25, P = 0.06) and positively correlated with M [median (min-max): 2.2 (1.7–6.9) mg/kg estimated metabolic body size^{-1}/min^{-1}; r = 0.49, P < 0.0001]. M and WTR, but not percent body fat, were significant independent determinants of total fasting plasma adiponectin concentrations, explaining a total of 40% of the variance in this measure (Table 1).

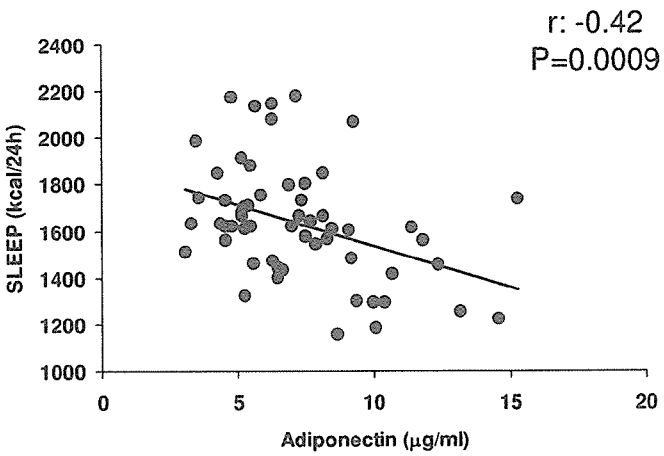

FIG. 1. Spearman correlation between total fasting plasma adiponectin concentrations and SLEEP.

We have previously reported in a larger sample (9) that SLEEP is determined by fat-free mass, fat mass, sex, and age (Table 2, model 1 in this study population). Total fasting plasma adiponectin concentrations were negatively associated with SLEEP after adjustment for sex, age, fat-free mass, and fat mass using general linear modeling and explained an additional 3% of the variance of SLEEP (Table 2, model 2). The correlation between total fasting plasma adiponectin concentrations and SLEEP was still significant, although attenuated, after inclusion of M among the regressors (Table 2, model 3). This association was further attenuated when adjusted also for WTR (Table 2, model 4).

No independent associations were found between total fasting plasma adiponectin concentrations and 24-h EE or physical activity-related EE.

TABLE 1. Determinants of total fasting plasma adiponectin concentrations[a]

Variable	β	P
Sex (male)	0.05	0.35
Age (yr)	0.01	0.33
Body fat (%)	0.01	0.52
WTR	−0.36	0.02
Fasting glucose (mg/dl)	−0.01	0.07
Fasting insulin (μIU/ml)	−0.08	0.47
Insulin-stimulated glucose disposal (mg/kg·EMBS^{-1}·min^{-1})	0.39	0.03

EMBS, Estimated metabolic body size.
[a] Using multiple linear regression modeling.

TABLE 2. Determinants of SLEEP[a]

Variable	Model 1 ($P = 0.0001$, $R^2 = 0.80$)		Model 2 ($P < 0.0001$, $R^2 = 0.83$)		Model 3 ($P < 0.0001$, $R^2 = 0.83$)		Model 4 ($P < 0.0001$, $R^2 = 0.83$)	
	β	P	β	P	β	P	β	P
Sex (male)	0.01	0.78	0.01	0.68	0.01	0.67	0.01	0.72
Age (yr)	−0.01	0.85	−0.01	0.80	−0.01	0.72	−0.01	0.69
Fat-free mass	0.01	<0.0001	0.01	<0.0001	0.01	<0.0001	0.01	<0.0001
Fat mass	0.01	0.06	0.01	0.12	0.01	0.16	0.01	0.16
Adiponectin			−0.01	0.01	−0.01	0.03	−0.07	0.06
M					−0.01	0.44	−0.01	0.45
WTR							0.01	0.77

[a] Using multiple linear regression modeling.

Discussion

Adiponectin is an adipocyte-specific protein that circulates in the bloodstream at concentrations 100- to 1000-fold higher than those of other hormones and cytokines, and, in contrast to all other adipocytokines, plasma concentrations of adiponectin are markedly decreased in obesity, type 2 diabetes, and coronary artery disease (1). In this study, and similar to a recent report in Caucasians using the ventilated hood technique to measure RMR (7), total fasting plasma adiponectin concentrations were negatively associated with sleep energy expenditure in Pima Indians, independent of sex, age, and body composition.

Adiponectin circulates as lower-molecular-weight hexamers and larger multimers of high molecular weight (11). The percentage of high-molecular-weight adiponectin is a better predictor of insulin sensitivity under thiazolidinedione treatment (12) and glucose intolerance than total adiponectin (13). Therefore, total plasma adiponectin concentrations may not represent as accurate an estimate of the physiological effects of this protein as the ratio between its isoforms.

This cross-sectional analysis did not allow us to determine whether energy expenditure affects adiponectin concentrations or whether adiponectin lowers metabolic rate. In their study of Caucasians, however, Ruige *et al.* (7) proposed that higher plasma adiponectin concentrations occur as a defense against comorbidities of obesity in subjects with lower RMR. On the other hand, adiponectin might lower EE as well. A negative association between adiponectin and other adipocytokines known to stimulate thermogenesis, such as leptin and TNFα, has been reported (14). Moreover, adiponectin inhibits the activation of signal transducer and activator of transcription-3, which is central to the effects of leptin on EE (15).

Adiponectin is a unique adipocytokine in that its plasma concentrations are markedly decreased in obesity, leading to a reduction in insulin sensitivity (4). Obesity-associated insulin resistance may represent a mechanism to counteract further expansion of body fat and therefore limit obesity, despite the drawback of an increased risk of developing type 2 diabetes. The decrease in plasma adiponectin concentrations with weight gain may also help to prevent fat accumulation by allowing for a higher EE. In fact, lower total fasting plasma adiponectin concentrations were associated with less weight gain in a prospective study of Pima Indians (16).

Eventually reduced concentrations of adiponectin may help to limit obesity but, at the same time, impair insulin sensitivity, thus leading to glucose intolerance and type 2 diabetes. The function of low plasma adiponectin as an independent predictor of type 2 diabetes has previously been established (17). Interestingly, SLEEP was higher in Pima Indians with either type 2 diabetes or impaired glucose tolerance, compared with normal glucose-tolerant subjects (18). Furthermore, a progressive increase in resting EE and insulin-induced thermogenesis paralleled the deterioration of glucose tolerance from normal glucose tolerance to impaired glucose tolerance to type 2 diabetes in that study (18). Determinants of higher EE in glucose intolerance include hepatic glucose production, insulin-mediated glucose disposal, and fasting concentrations of insulin and free fatty acids (18). Lower total fasting plasma adiponectin concentrations are associated with higher hepatic glucose production in Pima Indians (19). Therefore, low concentration of circulating adiponectin may explain the higher EE observed in glucose intolerance.

The present study also demonstrated that total fasting plasma adiponectin concentrations in Pima Indians are more closely related to body fat distribution than to overall adiposity, as previously reported (4). The role of central fat in reducing adiponectin concentration may offer insight into the negative association between this adipocytokine and sleep EE. In fact, a positive correlation between central fat accumulation and EE has been established in previous studies of both Caucasians (20) and Pima Indians (9), and further adjustment of the association between adiponectin and adjusted sleep EE for WTR in the present study attenuated the strength of the correlation.

In conclusion, there is a negative association between total adiponectin and EE in Pima Indians that may be mediated, in part, by body fat distribution. We speculate that the decreased total adiponectin concentration associated with overweight and especially with central fat accumulation may represent a mechanism inhibiting further weight gain by affecting both insulin sensitivity (decrease) and EE (increase).

Acknowledgments

We thank the members of the Gila River Indian Community for their participation in this study. We also thank the nursing and dietary staffs, physician assistants, and lab technicians of the clinical research center for their valuable assistance.

Received October 19, 2005. Accepted January 19, 2006.

Address all correspondence and requests for reprints to: Nicola Pannacciulli, M.D., Ph.D., Obesity and Diabetes Clinical Research Section,

Phoenix Epidemiology and Clinical Research Branch, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Department of Health and Human Services, 4212 North 16th Street, Phoenix, Arizona 85016. E-mail: nicolap@mail.nih.gov.

This work was supported by the Intramural Research Program of the National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases.

All the authors have nothing to declare.

References

1. **Matsuzawa Y** 2005 Adiponectin: identification, physiology and clinical relevance in metabolic and vascular disease. Atheroscler Suppl 6:7–14
2. **Yang WS, Lee WJ, Funahashi T, Tanaka S, Matsuzawa Y, Chao CL, Chen CL, Tai TY, Chuang LM** 2001 Weight reduction increases plasma levels of an adipose-derived anti-inflammatory protein, adiponectin. J Clin Endocrinol Metab 86:3815–3819
3. **Xydakis AM, Case CC, Jones PH, Hoogeveen RC, Liu MY, Smith EO, Nelson KW, Ballantyne CM** 2004 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 89:2697–2703
4. **Weyer C, Funahashi T, Tanaka S, Hotta K, Matsuzawa Y, Pratley RE, Tataranni PA** 2001 Hypoadiponectinemia in obesity and type 2 diabetes: close association with insulin resistance and hyperinsulinemia. J Clin Endocrinol Metab 86:1930–1935
5. **Qi Y, Takahashi N, Hileman SM, Patel HR, Berg AH, Pajvani UB, Scherer PE, Ahima RS** 2004 Adiponectin acts in the brain to decrease body weight. Nat Med 10:524–529
6. **Satoh H, Nguyen MT, Trujillo M, Imamura T, Usui I, Scherer PE, Olefsky JM** 2005 Adenovirus-mediated adiponectin expression augments skeletal muscle insulin sensitivity in male Wistar rats. Diabetes 54:1304–1313
7. **Ruige JB, Ballaux DP, Funahashi T, Mertens IL, Matsuzawa Y, Van Gaal LF** 2005 Resting metabolic rate is an important predictor of serum adiponectin concentrations: potential implications for obesity-related disorders. Am J Clin Nutr 82:21–25
8. **Stefan N, Vozarova B, Funahashi T, Matsuzawa Y, Ravussin E, Weyer C, Tataranni PA** 2002 Plasma adiponectin levels are not associated with fat oxidation in humans. Obes Res 10:1016–1020
9. **Weyer C, Snitker S, Rising R, Bogardus C, Ravussin E** 1999 Determinants of energy expenditure and fuel utilization in man: effects of body composition, age, sex, ethnicity and glucose tolerance in 916 subjects. Int J Obes Relat Metab Disord 23:715–722
10. **Lillioja S, Mott DM, Spraul M, Ferraro R, Foley JE, Ravussin E, Knowler WC, Bennett PH, Bogardus C** 1993 Insulin resistance and insulin secretory dysfunction as precursors of non-insulin-dependent diabetes mellitus. Prospective studies of Pima Indians. N Engl J Med 329:1988–1992
11. **Pajvani UB, Du X, Combs TP, Berg AH, Rajala MW, Schulthess T, Engel J, Brownlee M, Scherer PE** 2003 Structure-function studies of the adipocyte-secreted hormone Acrp30/adiponectin. Implications for metabolic regulation and bioactivity. J Biol Chem 278:9073–9085
12. **Pajvani UB, Hawkins M, Combs TP, Rajala MW, Doebber T, Berger JP, Wagner JA, Wu M, Knopps A, Xiang AH, Utzschneider KM, Kahn SE, Olefsky JM, Buchanan TA, Scherer PE** 2004 Complex distribution, not absolute amount of adiponectin, correlates with thiazolidinedione-mediated improvement in insulin sensitivity. J Biol Chem 279:12152–12162
13. **Fisher FF, Trujillo ME, Hanif W, Barnett AH, McTernan PG, Scherer PE, Kumar S** 2005 Serum high molecular weight complex of adiponectin correlates better with glucose tolerance than total serum adiponectin in Indo-Asian males. Diabetologia 48:1084–1087
14. **Ryan AS, Berman DM, Nicklas BJ, Sinha M, Gingerich RL, Meneilly GS, Egan JM, Elahi D** 2003 Plasma adiponectin and leptin levels, body composition, and glucose utilization in adult women with wide ranges of age and obesity. Diabetes Care 26:2383–2388
15. **Miyazaki T, Bub JD, Uzuki M, Iwamoto Y** 2005 Adiponectin activates c-Jun NH2-terminal kinase and inhibits signal transducer and activator of transcription 3. Biochem Biophys Res Commun 333:79–87
16. **Krakoff J, Looker HE, Funahashi T, Cummings DE, Considine RV, Tataranni PA, Knowler WC, Hanson RL** 2004 Long-term weight gain in Pima Indians: a role for ghrelin and adiponectin. Obes Res 12(Suppl):A201 (Abstract 790-P)
17. **Lindsay RS, Funahashi T, Hanson RL, Matsuzawa Y, Tanaka S, Tataranni PA, Knowler WC, Krakoff J** 2002 Adiponectin and development of type 2 diabetes in the Pima Indian population. Lancet 360:57–58
18. **Weyer C, Bogardus C, Pratley RE** 1999 Metabolic factors contributing to increased resting metabolic rate and decreased insulin-induced thermogenesis during the development of type 2 diabetes. Diabetes 48:1607–1614
19. **Stefan N, Stumvoll M, Vozarova B, Weyer C, Funahashi T, Matsuzawa Y, Bogardus C, Tataranni PA** 2003 Plasma adiponectin and endogenous glucose production in humans. Diabetes Care 26:3315–3319
20. **Leenen R, van der Kooy K, Deurenberg P, Seidell JC, Weststrate JA, Schouten FJ, Hautvast JG** 1992 Visceral fat accumulation in obese subjects: relation to energy expenditure and response to weight loss. Am J Physiol 263(5 Pt 1):E913–E919

JCEM is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.

Pediatr Transplantation 2006: 10: 187–192

Pediatric Transplantation
DOI: 10.1111/j.1399-3046.2005.00422.x

Adiponectin levels and arteriosclerotic risk factors in pediatric renal transplant recipients

Bakkaloglu SA, Soylemezoglu O, Buyan N, Oktar SO, Funahashi T, Pasaoglu H, Elhan AH, Peru H, Hasanoglu E. Adiponectin levels and arteriosclerotic risk factors in pediatric renal transplant recipients. Pediatr Transplantation 2006: 10: 187–192. © 2005 Blackwell Munksgaard

Abstract: ADPN, a recently discovered adipocytokine, has attracted great attention because of its anti-atherogenic properties. It was suggested as a protective factor for the cardiovascular system because of its close correlation with several risk factors. Our aim was to investigate serum ADPN levels in pediatric RTR and to document possible relationships between ADPN and arteriosclerotic risk factors. Twenty-one RTR, aged 16.3 ± 4.0 yr, and 23 healthy age and sex-matched control subjects were enrolled in this study. Serum lipid/lipoprotein fractions, homocysteine and ADPN levels as well as intima-media thickness of the cIMT were determined in both groups. Significantly higher serum ADPN ($p < 0.001$) and homocysteine ($p < 0.05$) levels as well as higher cIMT ($p < 0.001$) were found in RTR compared with the control subjects, whereas apolipoprotein B and lipoprotein (a) levels were not significantly different. HDL cholesterol was positively correlated with log ADPN ($r = 0.585$, $p < 0.01$). There were inverse correlations between log time post-transplantation and log ADPN as well as HDL cholesterol ($r = -0.438$, $p < 0.05$ and $r = -0.578$, $p < 0.05$, respectively). There were no correlation between log ADPN, log homocysteine, log apolipoprotein B, lipoprotein (a), creatinine clearance and cumulative steroid dose. Despite reasonable lipid profiles and remarkably elevated ADPN levels, our pediatric RTR with stable graft function displayed a risk for arteriosclerosis because of increased cIMT and mild hyperhomocysteinemia. Regarding the close positive correlation between ADPN and HDL cholesterol, it could be speculated that ADPN is a novel negative surrogate marker of arteriosclerosis. To our knowledge, this is the only report investigating levels and diverse correlates of ADPN in a pediatric RTR group. Further studies in larger groups of recipients are needed to clarify the interaction between arteriosclerotic risk factors and ADPN.

Sevcan A. Bakkaloglu[1], Oguz Soylemezoglu[1], Necla Buyan[1], Suna Ozhan Oktar[2], Tohru Funahashi[3], Hatice Pasaoglu[4], Atilla H. Elhan[5], Harun Peru[1] and Enver Hasanoglu[1]

[1]Department of Pediatric Nephrology, [2]Department of Radiology, Gazi University, Ankara, Turkey, [3]Department of Internal Medicine & Molecular Science, Osaka University, Osaka, Japan, [4]Department of Biochemistry, Gazi University, Ankara, Turkey, [5]Department of Biostatistics, Ankara University, Ankara, Turkey

Key words: adiponectin – arteriosclerosis – children – renal transplantation

Sevcan A. Bakkaloglu, MD, Department of Pediatric Nephrology, Gazi University Hospital, Besevler, Ankara, 06540, Turkey
Tel.: + 90 312 2025233
Fax: + 90 312 2129006
E-mail: sevcan@gazi.edu.tr

Accepted for publication 21 July 2005

ADPN, a recently discovered protein hormone, is the most abundant gene product of fat tissue (1). It regulates energy homeostasis and glucose metabolism with its insulin-sensitizer properties and has anti-inflammatory effects on the cellular components of the vascular wall (2, 3). There is mounting evidence that ADPN is involved in the pathogenesis of arteriosclerosis by its negative effects on TNF-α and the expression of certain endothelial adhesion molecules (4). This adipocytokine seems to play a protective role in the experimental models of vascular injury, perhaps

Abbreviations: ADPN, adiponectin; BMI, body mass index; Cho, cholesterol; cIMT, common carotid artery; CRP, C-reactive protein; ELISA, enzyme-linked immunosorbent assay; ESRD, end-stage renal disease; HDL, high density lipoprotein; PTH, parathormone; RTR, renal transplant recipients; TG, triglyceride; TNF-α, tumour necrosis factor-α.

because it suppresses the attachment of monocytes to endothelial cells (2), which is a fundamental step in experimental vascular damage as well as an early event in the atherosclertic process. Low plasma concentrations of ADPN have been documented in various patient populations at risk of cardiovascular disease such as in patients with dyslipidemia (5), essential hypertension (6), type II diabetes mellitus (7), obesity (8) and coronary artery disease (9). On the other hand, high levels of plasma ADPN are reported in uremic patients (10–12) and are suggested to protect against the risk of cardiac death as the result of its relationship to several cardiovascular and metabolic risk factors (10). Cardiovascular disease and its atherosclerotic complications is the leading cause of death in adult patients with ESRD (13, 14). After transplantation, the cardiac death rate improves substantially, but is still markedly higher than the general population (15). It was recently reported that pediatric RTR are already at risk for premature development of atherosclerotic complications and cardiovascular mortality, as well (16). To our knowledge, serum concentrations of ADPN and correlations between ADPN and atherosclerotic risk factors have not been studied in pediatric RTR. Therefore, we aimed to investigate serum ADPN levels in our pediatric RTR and to document possible relationships between ADPN and arteriosclerotic risk factors including serum lipid/lipoprotein fractions, apolipoprotein B and homocysteine levels as well as intima-media thickness of the cIMT.

Patients and methods

Twenty-one children (12 male and 9 female), aged 16.3 ± 4.0 yr, with a functioning renal allograft for a period of 26.9 ± 24.5 months and 23 healthy age, sex and BMI-matched control subjects (13 male and 10 female) were enrolled in this study. Besides routine blood chemistry, serum lipid and lipoprotein fractions, homocysteine, CRP and ADPN levels as well as cIMT were determined in pediatric RTR and compared with the controls.

Duration of previous dialysis modality was 27.94 ± 25.6 (median 23.5) months. The patients who received a renal allograft < 3 months previously and had an acute infection during the last 3 months were excluded from the study. Underlying causes of renal failure were as follows: reflux nephropathy in four, renal hypoplasia in two, chronic pyelonephritis in two, steroid resistant nephrotic syndrome in two, posterior urethral valve in one, nephrolithiasis in one, juvenile nephronophthisis in one, Henoch-Schönlein nephritis in one and unknown causes in seven. All patients, except one, were on triple immunosuppression including prednisolone, calcineurin inhibitor and azathioprine/mycophenolate mofetil. One patient was on prednisolone and mycophenolate mofetil. No rejection episode was observed during the period of the study. Eight patients were on antihypertensive medication and three patients were receiving lipid lowering agents. All patients were routinely given prophylactic calcium and vitamin D supplementation to prevent glucocorticoid-induced osteoporosis. Somatometric and descriptive features as well as detailed laboratory data are shown in Table 1 and 2.

After an overnight fasting, blood samples for the measurement of ADPN levels were drawn. Serum samples were stored at −70 °C until assayed at the Department of Internal Medicine and Molecular Sciences, Osaka University, Japan. ADPN levels were determined by ELISA as described previously (8) using the kits by Otsuka Pharmaceutical Co, Ltd., Tokushima, Japan (intra- and interassay co-efficients of variation were 3.3% and 7.4%, respectively). Besides ADPN, serum lipid profile, apolipoprotein B, lipoptotein (a) and homocysteine levels were also determined in RTR and control subjects. Routine biochemical parameters, hemoglobin, CRP, ferritin, PTH were studied in RTR. Routine biochemical parameters were studied with Abbott Aeroset System and Abbott kits (Wiesbaden, Germany). Apolipoprotein B, lipoprotein (a) and CRP levels were determined with nephelometric method (Array Protein System; Beckman, Ramsey, MN, USA). Serum homocysteine levels were studied with high performance liquid chromatography (Chromsystems, Munich, Germany). Cell Dyn 4000 Analyser (Abbott) was used for hemoglobin measurement. Ferritin and PTH levels were studied with DPC Immulite 2000 Analyser (DPC Biermann, Bad Nauheim, Germany).

Ultrasonographic measurements were performed in all individuals by the same radiologist using GE Logic 9 (Milwaukee, WI, USA) ultrasound system and a 10-MHz

Table 1. Somatometric data and blood pressure levels of the patients and controls

	Transplant recipients	Controls	p
Sex	12 Male, 9 female	13 Male, 10 female	NS
Age (yr)	16.3 ± 4.0 (16.5)	15.3 ± 7.7 (16.5)	NS
Body weight (kg)	51.1 ± 19.6 (49.0)	49.2 ± 21.2 (55.0)	NS
Height (cm)	148.8 ± 13.7 (151.0)	149.5 ± 28.3 (158)	NS
BMI (kg/m^2)	22.7 ± 7.6 (21.5)	20.5 ± 3.8 (20.2)	NS
BMI SDS*	0.60 ± 0.72 (0.21)	−0.35 ± 0.41 (−0.72)	NS
Systolic blood pressure (mmHg)	116 ± 11 (110)	102 ± 12 (100)	<0.01
Diastolic blood pressure (mmHg)	77 ± 9 (75)	67 ± 8 (65)	<0.01
Mean arterial pressure (mmHg)	90 ± 10 (87)	79 ± 9 (80)	<0.01

s.d., standard deviation; SDS, standard deviation score; s.e.m., standard error of the mean, NS, non significant; BMI, Body mass index.
Values are expressed as mean ± s.d. with median in parenthesis.
*Values are expressed as mean ± s.e.m. with median in parenthesis.

Table 2. Biochemical data of pediatric renal transplant recipients

	Mean ± s.d.
Time post transplantation (months)	26.9 ± 24.5 (median 19.0, range 3.0–80.0)
Duration of previous dialysis modality (months)	27.94 ± 25.6 (median 23.5)
Blood urea nitrogen (mg/dL; n: 5–25)	18.0 ± 8.5
Creatinine (mg/dL; n: 0.5–1.4)	1.0 ± 0.2
Uric acid (mg/dL; n: 2.6–7.2)	4.9 ± 1.8
Protein (g/dL; n: 6.4–8.5)	7.1 ± 0.6
Albumin (g/dL; n: 3.5–5)	4.4 ± 0.4
Triglyceride (mg/dL; n: 50–200)	160.3 ± 98.3
Cholesterol (mg/dL; n: 110–200)	183.1 ± 40.1
HDL cholesterol (mg/dL; n: 30–80)	62.0 ± 18.7
Low-density lipoprotein cholesterol (mg/dL; n: 60–130)	91.7 ± 29.2
Very low-density lipoprotein cholesterol (mg/dL; n:10–40)	32.4 ± 19.8
Calcium (mg/dL; n: 8.2–10.6)	9.3 ± 0.5
Phosphorus (mg/dL; n: 2.5–4.5)	3.9 ± 0.9
Parathormone (ng/mL; n: 12–75)	24.6 ± 35.8 (median 4.9)
Hemoglobin (g/dL)	12.0 ± 1.5
Ferritin (ng/ml; n: 4–204)	116.1 ± 215.1 (median 16.7)
C-reactive protein (mg/dL; n: 0–6)	8.8 ± 4.8
Fasting glucose (mg/dL; n: 70–105)	104.0 ± 20.0
Creatinine clearance (mL/min/1.73 m^2)*	84 ± 20
Cumulative steroid dosage (mg/kg)	132.0 ± 71.8

s.d., standard deviation.
*Schwartz formula.

probe. Subjects were examined in the supine position with a slightly over-extended neck, after at least a 10-minute rest. cIMT measurements were taken from the far wall of the artery in two-dimensional presentation of longitudinal view of the vessel, 1–2 cm below bifurcation. Following three measurement, the results were averaged and presented as the mean of the appropriate value of both arteries. The repeatability co-efficient was 0.98 (95% confidence interval, 0.96–0.98).

Statistical analysis

Categorical variables were analyzed by chi-square test. Difference between two groups for continuous variables was evaluated by using the Student's *t*-test. Multiple regression analysis was performed to detect the variables that affect ADPN. Logarithmic transformation was used for abnormally distributed variables. Data were expressed as mean ± s.d. Any $p < 0.05$ was considered significant. SPSS version 11.0 was used for statistical analysis.

Results

There were no significant differences in age and somatometric parameters between the two groups (Table 1). When evaluated according to Hammer's standardized curves (17), BMI was found to be in the 50–75 percentile in both groups. Additionally, BMI SDS did not differ significantly between the two groups (18). Although systolic and diastolic blood pressure levels were within the normal limits (Table 1), according to the blood pressure nomograms of Turkish children (19), the levels were found to be significantly higher compared with the control subjects ($p < 0.01$, Table 1). Hemoglobin level, lipid profile including TG, Cho, lipoproteins, apolipoprotein B and lipoprotein (a) and creatinine clearance were found to be within the acceptable limits in patients. Nevertheless, serum creatinine levels were higher and creatinine clearances were lower in RTR compared with the controls (1.02 ± 0.21 mg/dL vs. 0.66 ± 0.16 mg/dL, $p < 0.001$ and 131 ± 44 mL/min/1.73 m^2 vs. 84 ± 20 mL/min/1.73 m^2, $p < 0.001$, respectively). Detailed laboratory data of the patients are shown in Table 2.

Significantly higher serum ADPN ($p < 0.001$, Fig. 1) and homocysteine ($p < 0.05$) levels as well as higher cIMT ($p < 0.001$) were found in RTR compared with the control subjects. Apolipoprotein B and lipoprotein (a) levels were not significantly different between the two groups (Table 3). There was no correlation between log ADPN and blood pressure or BMI values. HDL cholesterol levels (Fig. 2) were positively correlated with log ADPN ($r = 0.585$, $p < 0.01$). There were inverse correlations between log time post-transplantation and log ADPN (Fig. 3) as well as HDL cholesterol levels ($r = -0.438$, $p < 0.05$ and $r = -0.578$, $p < 0.01$), whereas a positive correlation between log time post-transplantation and log homocysteine ($r = 0.583$, $p < 0.01$). Regression analysis showed a significant correlation between log ADPN and HDL cholesterol ($p < 0.05$). There was positive

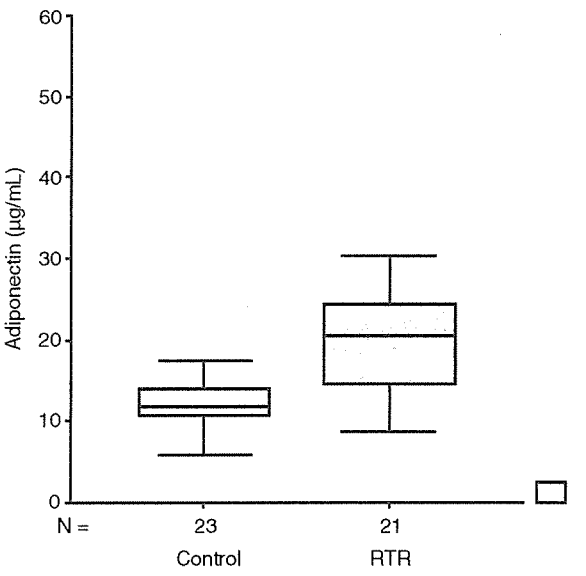

Fig. 1. Serum ADPN levels in RTR and control subjects ($p < 0.001$).

Table 3. Arteriosclerotic risk markers in both groups

	RTR (mean ± s.d.)	Control (mean ± s.d.)	p
Apolipoprotein B (mg/dL)	82.4 ± 32.1	76.5 ± 20.6	NS
Lipoprotein (a) (mg/dL)	14.0 ± 10.4 (10.3)	15.5 ± 16.9 (median: 6.0)	NS
Homocysteine (μM/L)	14.4 ± 7.2	10.3 ± 5.5	<0.05
cIMT (mm)	0.70 ± 0.09	0.51 ± 0.10	<0.001
ADPN (μg/mL)	20.7 ± 9.7	12.6 ± 4.0	<0.001
HDL cholesterol (mg/dL)	62.0 ± 18.7		

RTR, renal transplant recipients; NS, non significant; cIMT, intima-media thickness of the common carotid artery; HDL, high-density lipoprotein.

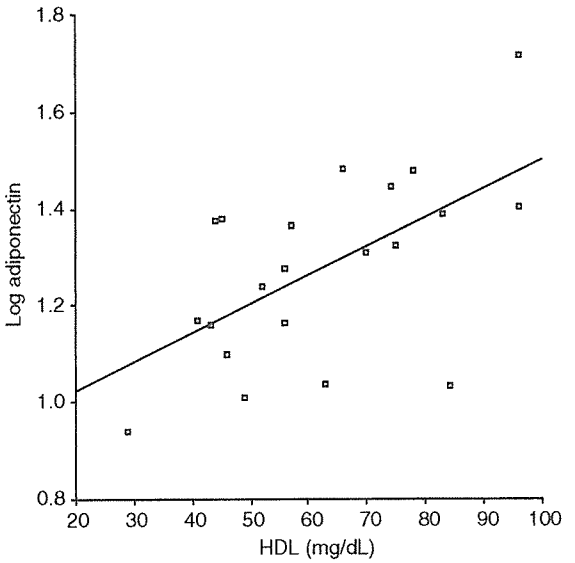

Fig. 2. Correlation between log ADPN and HDL cholesterol ($r = 0.585$, $p < 0.01$).

correlation between log homocysteine and creatinine ($r = 0.572$, $p < 0.01$). Mean arterial pressure levels were positively correlated with cIMT ($r = 0.400$, $p < 0.01$) as well as CRP levels ($r = 0.579$, $p < 0.01$). There were no correlations between log ADPN, log homocysteine, log apolipoprotein B, lipoprotein (a), creatinine clearance and cumulative steroid dose. There were no differences in ADPN, lipid/lipoprotein parameters, homocysteine and cIMT of the patients receiving antihypertensive medication compared with those not receiving antihypertensive medication.

Discussion

Cardiovascular disease and its arteriosclerotic complications are the leading cause of death in adult patients with ESRD (13, 14). Similarly, cardiovascular disease remains the major cause of mortality in uremic children (20). During the

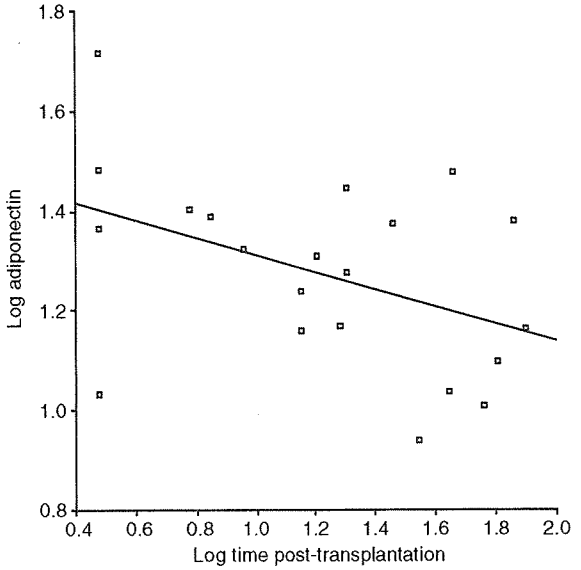

Fig. 3. Correlation between log ADPN (a) and log time post-transplantation ($r = -0.438$, $p < 0.05$).

last decade, the life expectancy of children with ESRD has increased considerably mainly because of successful renal transplantations (21). After transplantation, the cardiac death rate improves substantially, but is still markedly higher than the general population (15). Although, this particular preponderance has been associated with the presence of numerous traditional cardiovascular risk factors, e.g. hypertension, dyslipidemia, hyperhomocysteinemia, much recent interest has focused on non-traditional risk factors such as inflammation and endothelial dysfunction, hallmarks of early atherosclerotic changes in uremic subjects as well as in RTR (22–24). In addition, increased thickness of intima-media is also regarded as one of the first signs of early arteriosclerosis induced by damaging factors such as high blood pressure, dyslipidemia, hyperhomocysteinemia as well as microinflammation (25–27). Many of these atherosclerotic risk factors could be expected to partly diminish after renal transplantation. However, it was recently reported that pediatric RTR are already at risk for premature development of atherosclerotic complications and cardiovascular mortality (16). Furthermore, arteriosclerosis is nowadays recognized to be a chronic inflammatory disease that begins in early childhood (28). Therefore, we investigated diverse arteriosclerotic risk factors in pediatric RTR including ADPN, atherogenic and anti-atherogenic lipids, homocysteine, cIMT and CRP. In our patients with a well-functioning graft, TG, Cho, very low and low-density lipoprotein cholesterol levels

were within the normal limits. In addition, apolipoprotein B and lipoprotein (a) levels were found to be normal and did not differ significantly compared with control values. These results indicated a reasonable non-atherogenic lipid profile, especially in the presence of very good levels of HDL cholesterol (62.0 ± 18.7 mg/dL).

We found moderately elevated plasma ADPN levels in pediatric RTR compared with the healthy controls. Plasma ADPN concentrations in dialysis patients were demonstrated to be significantly higher than those in healthy subjects (10–12, 29). After renal transplantation, the markedly improved renal function was accompanied by a significant decrease in ADPN concentration, although the mean post-transplant concentration was still significantly higher than that in healthy subjects. Additionally, the changes in ADPN levels did not correlated with creatinine levels (29). In our study, we found no correlation between the levels of ADPN and creatinine or creatinine clearance, as well.

Plasma concentrations of ADPN are reduced in obese subjects despite restricted expression of adipose tissue (8) and are also decreased in patients with type 2 diabetes (7) or coronary artery disease (2, 9), independently of BMI. A recent report on 18 000 American men demonstrated that ADPN could be an independent negative risk factor for myocardial infarction (30). Overexpression of ADPN in apoE-knockout mice prevents atheromatous plaque formation *in vivo* (31). Despite high plasma ADPN levels, it has been reported that plasma ADPN is an independent inverse predictor of incident cardiovascular events in ESRD (10). Markedly elevated plasma levels of ADPN levels (10–12) were previously demonstrated to be positively correlated with HDL cholesterol and negatively with TG in adult patients with ESRD. Plasma ADPN levels were also reported to be lower among patients who experienced new cardiovascular events. It was concluded that plasma ADPN levels are an inverse predictor of cardiovascular outcomes among patients with ESRD. Furthermore, ADPN was suggested as a protective factor for the cardiovascular system (10). We have consistent results in our study by demonstrating close positive correlation between log ADPN and HDL cholesterol and therefore hypothesize that ADPN could be considered a positive anti-atherosclerotic risk marker like HDL cholesterol in pediatric RTR.

There was an inverse correlation between log time post-transplantation and log ADPN as well as HDL cholesterol. In addition, positive correlations between log homocysteine and log time post-transplantation as well as serum creatinine suggest that time after transplantation could bring a risk for arteriosclerosis. Additionally, we observed a tendency toward increased risk of arteriosclerosis characterized by mild hyperhomocysteinemia and increased cIMT in RTR. There was significant relationship between mean arterial pressure levels and cIMT and CRP levels. Although blood pressure levels were within the acceptable limits in RTR, higher levels compared with the control subjects were thought to contribute to the endothelial damage and inflammation in our patient group. Nevertheless, remarkably elevated ADPN levels and its close correlation with HDL cholesterol and reasonable lipid profile needs to be considered and it can be speculated that the body makes an effort to mitigate the gradually increased atherosclerotic risk with markedly elevated serum ADPN levels. Enhanced compensatory ADPN synthesis in idiopathic nephrotic syndrome and in advanced diabetic nephropathy was also hypothesized in recent publications (32, 33). In consistent with our conclusion, it was recently demonstrated that RTRs who developed diabetes mellitus after transplantation showed lower pretransplant serum ADPN concentration, which was suggested to be an independent risk factor for new onset diabetes in these patients (34).

Finally, to the best of our knowledge this is the first report to investigate serum ADPN levels and their potential correlates in pediatric RTR. Regarding close positive correlation between ADPN and HDL cholesterol, ADPN could be speculated as a novel positive surrogate marker of arteriosclerosis. Furthermore, high ADPN levels could also be the consequence of a compensatory response of the body for prevention or attenuation of arteriosclerosis. Nevertheless, the cross-sectional nature and the small number of the patients in our present study do not allow us to draw firm conclusions. Considering anti-atherogenic properties of ADPN vs. diverse factors resulting in inflammation and endothelial dysfunction, which may persist in the post-transplant period, we thought that this is an interesting topic which requires explicit study. It is our belief that further prospective, well-designed studies in larger pediatric RTR groups will help to shed new light on the value of this subject and could clarify the interaction between arteriosclerotic cardiovascular risk factors and ADPN.

Acknowledgment

The authors wish to thank Mrs. Nuray Yilmaz for her technical assistance.

References

1. 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: 286 289.
2. Ouchi N, Kihara S, Arita Y, et al. Novel modulator for endothelial adhesion molecules: adipocyte-derived plasma protein adiponectin. Circulation 1999: 100: 2473 2476.
3. Ouchi N, Kihara S, Arita Y, et al. Adiponectin, an adipocyte-derived plasma protein, inhibits endothelial NF-kappaB signaling through a cAMP-dependent pathway. Circulation 2000: 102: 1296 1301.
4. Maeda N, Takahashi M, Funahashi T, et al. PPARgamma ligands increase expression and plasma concentrations of adiponectin, an adipose-derived protein. Diabetes 2001: 50: 2094 2099.
5. Matsubara M, Maruoka S, Katayose S. Decreased plasma adiponectin concentrations in women with dyslipidemia. J Clin Endocrinol Metab 2002: 87: 2764 2769.
6. 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 75.
7. Hotta K, Funahashi T, Arita Y, et al. Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. Arterioscler Thromb Vasc Biol 2000: 20: 1595 1599.
8. Arita Y, Kihara S, Ouchi N, et al. Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. Biochem Biophys Res Commun 1999: 257: 79 83.
9. Kumada M, Kihara S, Sumitsuji S, et al. Association of hypoadiponectinemia with coronary artery disease in men. Arterioscler Thromb Vasc Biol 2003: 23: 85 89.
10. Zoccali C, Mallamaci F, Tripepi G, et al. Adiponectin, metabolic risk factors, and cardiovascular events among patients with end-stage renal disease. J Am Soc Nephrol 2002: 13: 134 141.
11. Stenvinkel P, Marchlewska A, Pecoits-Filho R, et al. Adiponectin in renal disease: relationship to phenotype and genetic variation in the gene encoding adiponectin. Kidney Int 2004: 65: 274 281.
12. Huang JW, Yen CJ, Chiang HW, Hung KY, Tsai TJ, Wu KD. Adiponectin in peritoneal dialysis patients: a comparison with hemodialysis patients and subjects with normal renal function. Am J Kidney Dis 2004: 43: 1047 1055.
13. Levey AS. Controlling the epidemic of cardiovascular disease in chronic renal disease: where do we start? Am J Kidney Dis 1998: 32(Suppl. 3): S5 S13.
14. Causes of death: United States renal data system 1998 annual data report. Am J Kidney Dis 1998: 32(Suppl. 1): S81 S88.
15. Parekh RS, Carroll CE, Wolfe RA, Port FK. Cardiovascular mortality in children and young adults with end-stage kidney disease. J Pediatr 2002: 141: 191 197.
16. Lilien MR, Stroes ES, Op't Roodt J, De Jongh S, Schroder CH, Koomans HA. Vascular function in children after renal transplantation. Am J Kidney Dis 2003: 41: 684 691.
17. Hammer LD, Kraemer HC, Wilson DM, Ritter PL, Dornbusch SM. Standardized percentile curves of body-mass index for children and adolescents. Am J Dis Child 1991: 145: 259 263.
18. Rolland-Cachera MF, Cole TJ, Sempe M, et al. Body mass index variations: centiles from birth to 87 years. Eur J Clin Nutr 1991: 45: 13 21.
19. Tumer N, Yalcinkaya F, Ince E, et al. Blood pressure nomograms for children and adolescents in Turkey. Pediatr Nephrol 1999: 13: 438 443.
20. Pediatric end-stage renal disease. United States renal data system 1998 annual data report. Am J Kidney Dis 1998: 32(Suppl. 1): S98 S108.
21. Papalois VE, Najarian JS. Pediatric kidney transplantation: historic hallmarks and a personal perspective. Pediatr Transplant 2001: 5: 239 245.
22. Kari JA, Donald AE, Vallance DT, et al. Physiology and biochemistry of endothelial function in children with chronic renal failure. Kidney Int 1997: 52: 468 472.
23. Stenvinkel P, Pecoits-Filho R, Lindholm B. Coronary artery disease in end-stage renal disease: no longer a simple plumbing problem. J Am Soc Nephrol 2003: 14: 1927 1939.
24. Hausberg M, Kisters K, Kosch M, Rahn KH, Barenbrock M. Flow-mediated vasodilation and distensibility of the brachial artery in renal allograft recipients. Kidney Int 1999: 55: 1104 1110.
25. Litwin M, Trelewicz J, Wawer Z, et al. Intima-media thickness and arterial elasticity in hypertensive children: controlled study. Pediatr Nephrol 2004: 19: 767 774.
26. Oh J, Wunsch R, Turzer M, et al. Advanced coronary and carotid arteriopathy in young adults with childhood-onset chronic renal failure. Circulation 2002: 106: 100 105.
27. Raitakari OT, Juonala M, Kahonen M, et al. Cardiovascular risk factors in childhood and carotid artery intima-media thickness in adulthood: the cardiovascular risk in young Finns study. JAMA 2003: 290: 2277 2283.
28. Personen E, Liuba P. Footprints of atherosclerotic coronary heart disease in children. Rev Port Cardiol 2004: 23: 127 131.
29. Chudek J, Adamczak M, Karkoszka H, et al. Plasma adiponectin concentration before and after successful kidney transplantation. Transplant Proc 2003: 35: 2186 2189.
30. Pischon T, Girman CJ, Hotamisligil GS, Rifai N, Hu FB, Rimm EB. Plasma adiponectin levels and risk of myocardial infarction in men. JAMA 2004: 291: 1730 1737.
31. Okamoto Y, Kihara S, Ouchi N, et al. Adiponectin reduces atherosclerosis in apolipoprotein-E-deficient mice. Circulation 2002: 106: 2767 2770.
32. Bakkaloglu SA, Soylemezoglu O, Buyan N, et al. High serum adiponectin levels during steroid-responsive nephrotic syndrome relapse. Pediatr Nephrol 2005: 20: 474 477.
33. Koshimura J, Fujita H, Narita T, et al. Urinary adiponectin excretion is increased in patients with overt diabetic nephropathy. Biochem Biophys Res Commun 2004: 316: 165 169.
34. Bayes B, Lauzurica R, Granada ML, et al. Adiponectin and risk of new-onset diabetes mellitus after kidney transplantation. Transplantation 2004: 15: 26 30.

Adiponectin Replenishment Ameliorates Obesity-Related Hypertension

Koji Ohashi, Shinji Kihara, Noriyuki Ouchi, Masahiro Kumada, Koichi Fujita, Aki Hiuge, Toshiyuki Hibuse, Miwa Ryo, Hitoshi Nishizawa, Norikazu Maeda, Kazuhisa Maeda, Rei Shibata, Kenneth Walsh, Tohru Funahashi, Iichiro Shimomura

Abstract—Patients with obesity are susceptible to hypertension. We have reported that the plasma adiponectin levels are decreased in obesity and that adiponectin has many defensive properties against obesity-related diseases, such as type 2 diabetes and coronary artery disease. The aim of this study was to determine the relationship between adiponectin and hypertension in mice. We measured blood pressure and heart rate directly by a catheter in the carotid artery and indirectly by automatic sphygmomanometer at the tail artery. Obese KKAy mice had significantly lower plasma adiponectin levels and higher systolic blood pressure than control C57BL/6J mice at 21 weeks of age. Adenovirus-delivered adiponectin significantly decreased blood pressure in KKAy mice. The direct role of adiponectin on blood pressure regulation under insulin resistance–free state was investigated in adiponectin-knockout (KO) mice. Adiponectin KO mice developed hypertension when maintained on a high-salt diet (8% NaCl) without insulin resistance. The hypertension of salt-fed adiponectin KO mice was associated with reduced mRNA levels of endothelial NO synthase (eNOS) and prostaglandin I_2 synthase in aorta and low metabolite levels of endothelial NO synthase and prostaglandin I_2 synthase in plasma. Adiponectin therapy lowered the elevated blood pressure and corrected the above mRNA levels to those of the wild type. Our results suggest that hypoadiponectinemia contributes to the development of obesity-related hypertension, at least in part, directly, in addition to its effect via insulin resistance, and that adiponectin therapy can be potentially useful for hypertension in patients with the metabolic syndrome. (***Hypertension.* 2006;47:1108-1116.**)

Key Words: hypertension, obesity ■ nitric oxide synthase ■ sodium, dietary ■ L-NAME

The cluster of hypertension, diabetes mellitus, and dyslipidemia in upper body obesity, collectively referred to as the metabolic syndrome, is a common cause of atherosclerotic cardiovascular diseases and one of the most serious threats to public health. Adipose tissue produces and secretes many bioactive substances,[1-4] conceptualized as adipocytokines.[3] Dysregulated production of adipocytokines, such as tumor necrosis factor-α, leptin, and plasminogen activator inhibitor type 1, is associated with the pathophysiology of obesity-related disorders.[1-3]

Adiponectin is an antiatherogenic[4-8] and antidiabetic[9-13] adipocytokine, identified by our group through the screening of adipose-specific genes in the human cDNA project.[14] Other groups independently cloned the mouse homologue of adiponectin as ACRP30 and AdipoQ, respectively.[15,16] Adiponectin is a plasma protein exclusively produced by adipose tissue,[14] and the plasma concentrations decreased in patients with obesity,[17] coronary artery disease,[18] type 2 diabetes,[19] and hypertension.[20] The adiponectin gene is located on chromosome 3q27, which was reported to replicate linkage with the metabolic syndrome.[21] Recently, we demonstrated that the I164T mutation of the adiponectin gene affects the prevalence of coronary artery disease and obesity-unrelated clustering of hypertension, diabetes mellitus, and dyslipidemia.[22]

Human studies of the vasodilator response to reactive hyperemia revealed that plasma adiponectin levels correlated significantly with endothelium-dependent vasodilation.[23] Moreover, adiponectin treatment suppressed apoptosis by activating AMP-activated protein kinase, Akt kinase, and endothelial NO synthase (eNOS) signaling axis in cultured human endothelial cells.[24,25] These data suggest that adiponectin is a protective factor against endothelial injury and that low production of adiponectin might relate to the pathophysiology of hypertension.

We reported previously that the adiponectin-knockout (KO) mice exhibited obesity, insulin resistance, and hypertension when fed a high-fat/high-sucrose/high-salt diet for 4 weeks.[23] In clinical studies, obesity, hypoadiponectinemia, insulin resistance, and hypertension are closely associated with one another in the metabolic syndrome.[11,19,20,26-28]

Received January 5, 2006; first decision January 24, 2006; revision accepted April 4, 2006.

From the Department of Metabolic Medicine (K.O., S.K., M.K., K.F., A.H., T.H., M.R., H.N., N.M., K.M., T.F., I.S.), Graduate School of Medicine, Osaka University, Osaka, Japan; and Molecular Cardiology/Whitaker Cardiovascular Institute (N.O., R.S., K.W.), Boston University School of Medicine, Boston, Mass.

Correspondence to Shinji Kihara, Department of Metabolic Medicine, Graduate School of Medicine, Osaka University, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan. E-mail kihara@imed2.med.osaka-u.ac.jp

Hypertension **is available at http://www.hypertensionaha.org** **DOI: 10.1161/01.HYP.0000222368.43759.a1**

Based on this background, it is important to define the direct relationship between hypoadiponectinemia and hypertension. The results of the present study showed that KKAy mice exhibited hypoadiponectinemia. KKAy mice develop maturity-onset obesity through the antagonism of the hypothalamic melanocortin system by ectopic expression of the agouti protein. The agouti and agouti-related protein compete with proopiomelanocortin-derived peptides for binding sites on melanocortin receptors to regulate food intake and energy expenditure. Furthermore, numerous studies have demonstrated that KKAy mice are good models of the metabolic syndrome, such as hypertension and diabetes mellitus.[29,30] In the present study, we showed for the first time that adiponectin replenishment improved the hypertension of KKAy mice. In addition, we induced hypertension in adiponectin KO mice by providing a high-salt diet without affecting insulin resistance. Therefore, we advance the concept that obesity-related hypoadiponectinemia contributes to the development of hypertension both directly and indirectly via insulin resistance. Our results also suggest that adiponectin therapy is potentially useful for patients with the metabolic syndrome, especially those with hypertension and insulin resistance.

Methods

Animal and Animal Treatment

KKAy male mice were purchased from Japan CLEA (Tokyo, Japan). This strain is a cross between black KK female mice and obese yellow male Ay mice, features a deregulated overexpression of the agouti gene, and exhibits severe obesity, hyperlipidemia, and insulin resistance. Adiponectin-KO (APN-KO) mice were generated as described previously and backcrossed to wild-type (WT) C57BL/6J.[10] KKAy male mice (21 weeks old) were fed normal chow during the observation period. APN-KO and WT male mice (8 to 10 weeks old) were fed a high-salt diet (8% NaCl, Oriental Yeast) or control diet (Oriental Yeast). KKAy, APN-KO, and WT mice were euthanized in the fasting (12 hours) state. N^G-nitro-L-arginine methyl ester (L-NAME), a specific NO synthase inhibitor, was added to the drinking water at 0.25 mg/mL, whereas the animals without L-NAME received plain drinking water. The experimental protocol was approved by the Ethics Review Committee for Animal Experimentation of Osaka University School of Medicine.

Blood Pressure Measurement

Systolic blood pressure (SBP) and heart rate (HR) were measured using either the tail-cuff technique with an automatic sphygmomanometer (BP98A; Softron) at the tail artery while the animals were restrained or by using indwelling arterial catheters into the carotid artery. Mice were trained to the tail-cuff apparatus at least twice. Ten readings were taken for each measurement, and a mean value was assigned to each individual mouse. The direct blood pressure measurements were achieved using a 1.4F catheter tip micromanometer (ARIA, Millar Instruments) inserted through the right carotid artery. Mice were anesthetized with isoflurane and placed on a temperature-controlled pad. Blood pressure was measured after a 30-minute stabilization period. The blood pressure was monitored for 15 minutes under restrained conditions, and then the average value of SBP was calculated and determined. The SBP levels measured by the tail-cuff method correlated well with those by the direct measurement through carotid artery catheter as reported previously.[29,31,32]

Laboratory Methods

Blood samples were collected from mice in the fasting (12 hours) state. Serum total cholesterol, triglyceride, and glucose concentrations were measured with enzymatic kits (Wako Pure Chemicals), and insulin concentrations were assayed with an enzyme immunoassay kit (Glazyme, Wako Pure Chemicals). Adiponectin concentrations were determined with ACRP30 ELISA kits (Otsuka Pharmaceutical Co). Nitrate/nitrite concentrations were measured with a Nitrate/Nitrite Colorimetric Assay kit (Cayman Chemical Company) or with a Nitrate/Nitrite Fluorometric assay kit (Cayman Chemical Company). 6-Keto-PGF1α concentrations were measured with a 6-keto-PGF1α EIA kit (Cayman Chemical Company). Plasma levels of angiotensin II; aldosterone; and urinary concentrations of epinephrine, norepinephrine, and dopamine were measured by using appropriate biochemical methods in a commercial laboratory (SRL).

Gene Expression Analysis

Total RNA was extracted using an RNA-STAT kit (TEL-TEST) according to the protocol supplied by the manufacturer, and 0.5 μg RNA was reverse transcribed using a ThermoScript RT-PCR system (Invitrogen). Real-time PCR was performed on ABI-Prism 7700 using SYBR Green I as a double-stranded DNA–specific dye according to instructions provided by the manufacturer (Applied Biosystems). We used the primers listed in the online supplement (available at http://hyper.ahajournals.org). All of the results were normalized to 36B4.

Immunoblot

The protein was extracted from the thoracic aortas of adiponectin KO and WT mice and solubilized with solubilization buffer [1% Triton X-100, 50 mmol/L HEPES (pH 7.5), 150 mmol/L NaCl, 10% glycerol, 1.5 mmol/L $MgCl_2$, 10 mmol/L NaF, 10 mmol/L sodium diphosphate decahydrate, 1% aprotinin, 5 μg/mL leupeptin, 1 mmol/L PMSF, and 1 mmol/L dithiothreitol). Whole cell lysates were resolved on 10% SDS-polyacrylamide gels, followed by electrophoretic transfer to nitrocellulose membranes (Amersham Life Science). The membranes were exposed to mouse monoclonal anti-eNOS antibodies (Transduction Laboratories, San Jose, CA) and then exposed to anti-mouse secondary antibodies conjugated with horseradish peroxidase. The bands were visualized by an enhanced chemiluminescence detection system (Amersham) and quantified by using National Institutes of Health Image analysis freeware. Band volume was determined as band intensity per area according to the instructions provided by the manufacturer.

Preparation and Delivery of Adenoviral Adiponectin

Adenovirus producing the full-length adiponectin was constructed with Adenovirus Expression Vector kit (TaKaRa). Plaque-forming units (2×10^8) of adenovirus-adiponectin (Ad-APN) or adenovirus β-galactosidase (Ad-β gal) were injected intravenously via the tail vein. Adenovirus-mediated adiponectin expression was detected exclusively in the liver using the RT-PCR method, indicating that the effect of adiponectin on other organs, including the arterial wall, were mediated by the blood stream.

Statistical Methods

Data are presented as mean±SEM. Differences between groups were evaluated by the Student t test or ANOVA with Fisher's protected least significant difference test. A $P<0.05$ denoted the presence of a statistically significant difference. All of the calculations were performed by using a standard statistical package (StatView for Macintosh, version 5.0).

Results

Adiponectin Supplementation Decreases Blood Pressure in Obese Diabetic KKAy Mice

We studied genetically obese KKAy mice, which develop a maturity-onset obesity, type 2 diabetes, and hypertension.[29] In the present study, the SBP of KKAy mice gradually increased after 13 weeks of age (13 weeks, 114±1.6; 17 weeks, 118±1.3; 21 weeks, 123±1.8; 23 weeks, 131±2.8 mm Hg). The

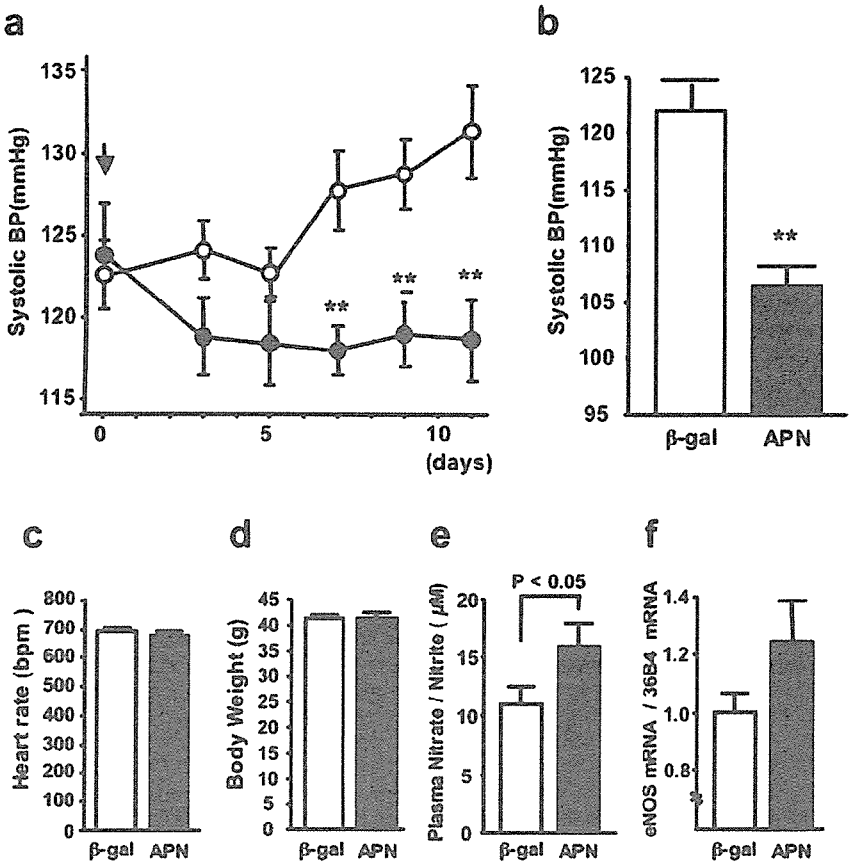

Figure 1. Adiponectin supplementation ameliorates hypertension of genetically obese KKAy mice. (a) SBP of KKAy mice treated with Ad-APN (●, n=9) or Ad-β gal (○, n=9; $^{**}P<0.01$). Ad-APN or Ad-β gal was injected intravenously via the tail vein (arrow). (b) Direct measurement of SBP on day 11 postinjection ($^{**}P<0.01$). (c) HR and (d) body weight of KKAy mice treated with Ad-β gal (□) or Ad-APN (■). Mice were fed normal chow during the indicated periods. (e) The plasma levels of nitrate/nitrite in KKAy/Ad-β gal (□) and KKAy/Ad-APN (■; $P<0.05$). (f) mRNA levels of eNOS in aorta of KKAy/Ad-β gal (□) and KKAy/Ad-APN (■). Results are mean±SEM.

SBP of KKAy mice was significantly higher than that of WT C57BL/6J mice at 21 weeks (123±1.8 versus 106±1.8 mm Hg; $P<0.01$) under normal diet. Plasma adiponectin concentrations of KKAy mice (9.3±0.4 μg/mL) were approximately half of those of WT mice (17.8±1.3 μg/mL). Before Ad-APN treatment, plasma adiponectin concentrations were 9.1±0.8 μg/mL in the KKAy/Ad-APN group and 9.5±0.5 μg/mL in the KKAy/Ad-β gal group. On day 11 after injection of Ad-APN, plasma adiponectin concentrations were 56.8±5.0 μg/mL in KKAy/Ad-APN and 9.8±1.0 μg/mL in KKAy/Ad-β gal. Ad-APN treatment significantly reduced SBP compared with Ad-β gal control on days 7, 9, and 11 postinjection (119±2.5 versus 131±2.8 mm Hg; $P<0.01$; Figure 1a). Direct blood pressure measurement also showed that SBP was significantly lower in the KKAy/Ad-APN group than in the KKAy/Ad-β gal group on day 11 postinjection (106±1.6 versus 122±2.6 mm Hg; $P<0.01$; Figure 1b). The HR (670±15.9 bpm versus 687±17.8 bpm; P value not significant; Figure 1c), body weight (41.4±0.7 g versus 41.1±0.5 g; P value not significant; Figure 1d), food intake (6.0±0.8 versus 5.9±0.6 g per day; P value not significant), fasting plasma glucose (FPG), fasting immunoreactive insulin (IRI), total cholesterol, triglyceride, angiotensin II, aldosterone, and leptin concentrations were not different between KKAy/Ad-APN and KKAy/Ad-β gal during the observation period (Table 1). The plasma concentrations of nitrate/nitrite (NO metabolites) of Ad-APN–treated KKAy mice (17.4±1.8 μmol/L) were significantly higher than those of Ad-β gal–treated KKAy mice (11.1±1.5 μmol/L; $P<0.05$; Figure 1e). The eNOS mRNA levels in aorta tended to be higher in KKAy/Ad-APN (1.24±0.16) than in KKAy/Ad-β gal (1.00±0.06), but the difference was not statistically significant (Figure 1f).

Adiponectin KO Mice Develop Salt-Induced Hypertension Without Insulin Resistance

To investigate the direct role of adiponectin on blood pressure regulation in the absence of insulin resistance, we also studied adiponectin KO mice. At the 3-week feeding of high-salt diet, SBP was significantly higher in KO mice than in WT mice

TABLE 1. Characteristics of Ad-β Gal- and Ad-APN–Treated KKAy Mice

Variables	Ad-β Gal (n=9)	Ad-APN (n=9)	P
Adiponectin, μg/mL	9.5±0.5	9.1±0.8	NS
FPG, mmol/L	10.35±0.82	11.16±0.82	NS
IRI, μU/mL	392.8±88.9	352.3±38.8	NS
HOMA-IR	183.0±20.1	197.3±17.1	NS
T-chol, mmol/L	3.53±0.28	3.36±0.17	NS
Triglyceride, mmol/L	1.55±0.16	1.67±0.15	NS
Angiotensin II, pg/mL	172.7±83.5	174.0±75.2	NS
Aldosterone, pg/mL	488.8±105.0	412.7±147.1	NS
Leptin, ng/mL	34.88±2.81	31.08±2.76	NS

HOMA-IR indicates homeostasis model assessment of insulin resistance; T-chol, total cholesterol; NS, not significant. Data are mean±SEM.

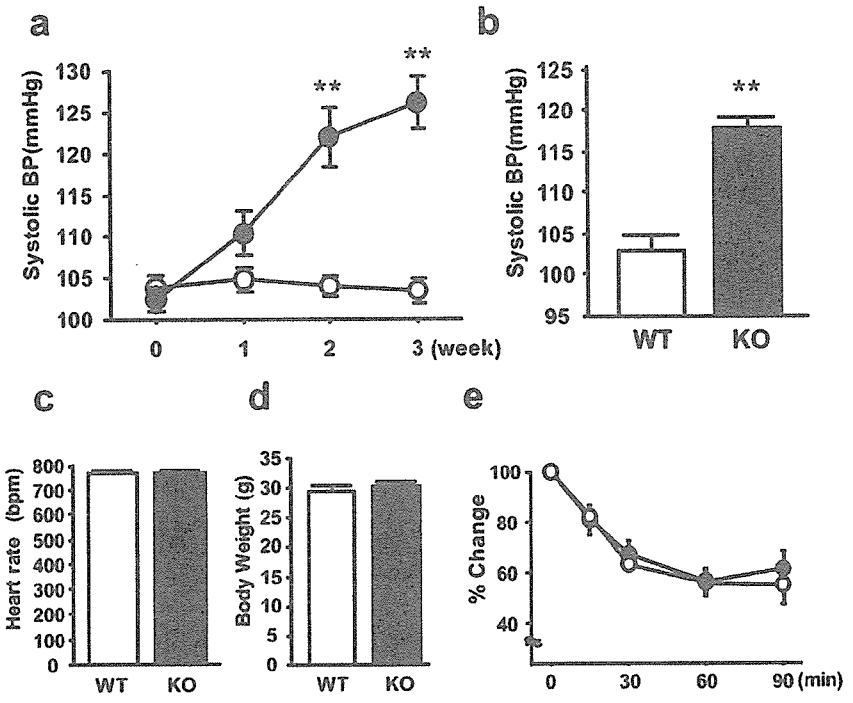

Figure 2. Salt-induced hypertension in adiponectin KO mice. (a) SBP (tail-cuff method) of WT (○ n=6) and APN-KO (● n=6) mice (**P<0.01 vs WT mice). Mice were fed high-salt diet during the observation periods. (b) SBP (direct measurement), (c) HR, and (d) body weight of WT (□ n=6) and APN-KO (■ n=6) mice at 3-week feeding of high-salt diet (**P<0.01 vs WT mice). (e) Glucose curves under insulin tolerance test of WT (○ n=6) and APN-KO (● n=6) mice at 3-week feeding of high-salt diet. Plasma glucose levels were normalized to those at 0 minutes in each group (100%). Results are presented as mean±SEM.

(126±3.1 versus 103±1.1 mm Hg; P<0.01; Figure 2a). The direct blood pressure measurement by indwelling catheters also showed that SBP was significantly higher in KO mice (118±1.2 mm Hg) than in WT mice (103.0±1.7 mm Hg) at the 3-week feeding of high-salt diet (P<0.01; Figure 2b). The HR (766±6.2 bpm versus 766±4.7 bpm; P value not significant) and body weight (30.4±0.5 g versus 29.5±0.7 g; P value not significant), FPG (5.74±0.17 mmol/L versus 5.48±0.11 mmol/L; P value not significant) and IRI (4.9±1.5 μU/mL versus 7.5±1.8 μU/mL; P value not significant) were not different between KO and WT mice during the observation period (Figure 2c and 2d).

Characterization of Adiponectin KO Mice and WT Mice

There were no significant differences in plasma Na, Cl, K, FPG, IRI, homeostasis model assessment of insulin resistance, total cholesterol, triglyceride, angiotensin II, aldosterone, and leptin concentrations, as well as in urinary volume, total urinary epinephrine, norepinephrine, and dopamine concentrations between KO and WT mice (Table 2). In addition, there were no significant differences in insulin-mediated suppression of plasma glucose between adiponectin KO and WT mice (Figure 2e).

To determine the mechanism of hypertension in KO mice, we examined the mRNA levels encoding proteins associated with hypertension. After salt overload, the mRNA levels of eNOS and prostaglandin (PG) I_2 synthase (PGIS) in aorta and eNOS in kidney were significantly lower in KO mice than in WT mice, although no significant differences were observed in the mRNA levels of inducible nitric oxide synthase, PG E synthase, endothelin-1, and adrenomedullin in aorta and renin and epithelial sodium channel in kidney between KO mice and WT mice (Figure 3a and 3b). The plasma concentrations of nitrate/nitrite as NO metabolites tended to be lower in KO mice (7.3±1.5 μmol/L) than in WT mice (10.9±1.6 μmol/L) after salt overload, but the difference was not statistically significant (P=0.09; Figure 3b). The plasma level of 6-keto-

TABLE 2. Characteristics of Adiponectin-KO and WT Mice

Variables	WT (n=6)	KO (n=6)	P
Plasma			
Adiponectin, μg/mL	15.4±0.8	ND	
Na, mEq/L	151.3±1.3	150.7±1.2	NS
Cl, mEq/L	114.3±0.3	112.3±0.8	NS
K, mEq/L	5.5±0.3	4.9±0.3	NS
FPG, mmol/L	5.48±0.11	5.74±0.17	NS
IRI, μU/mL	7.5±1.8	4.9±1.5	NS
HOMA-IR	1.8±0.5	1.3±0.4	NS
T-chol, mmol/L	2.08±0.23	2.07±0.13	NS
Triglyceride, mmol/L	0.89±0.12	0.93±0.06	NS
Angiotensin II, pg/mL	46.0±8.4	52.3±8.5	NS
Aldosterone, pg/mL	99.2±9.0	108.7±4.5	NS
Leptin, ng/mL	0.87±0.17	0.81±0.12	NS
Urine			
Urine volume, mL/d	8.3±0.9	8.4±1.0	NS
Epinephrine, ng/d	33.2±3.2	28.9±4.2	NS
Norepinephrine, ng/d	347±14.9	314±29.9	NS
Dopamine, ng/d	1272±128.4	968±132.3	NS

HOMA-IR indicates homeostasis model assessment of insulin resistance; T-chol, total cholesterol; ND, not detected; NS, not significant. Data are mean±SEM.

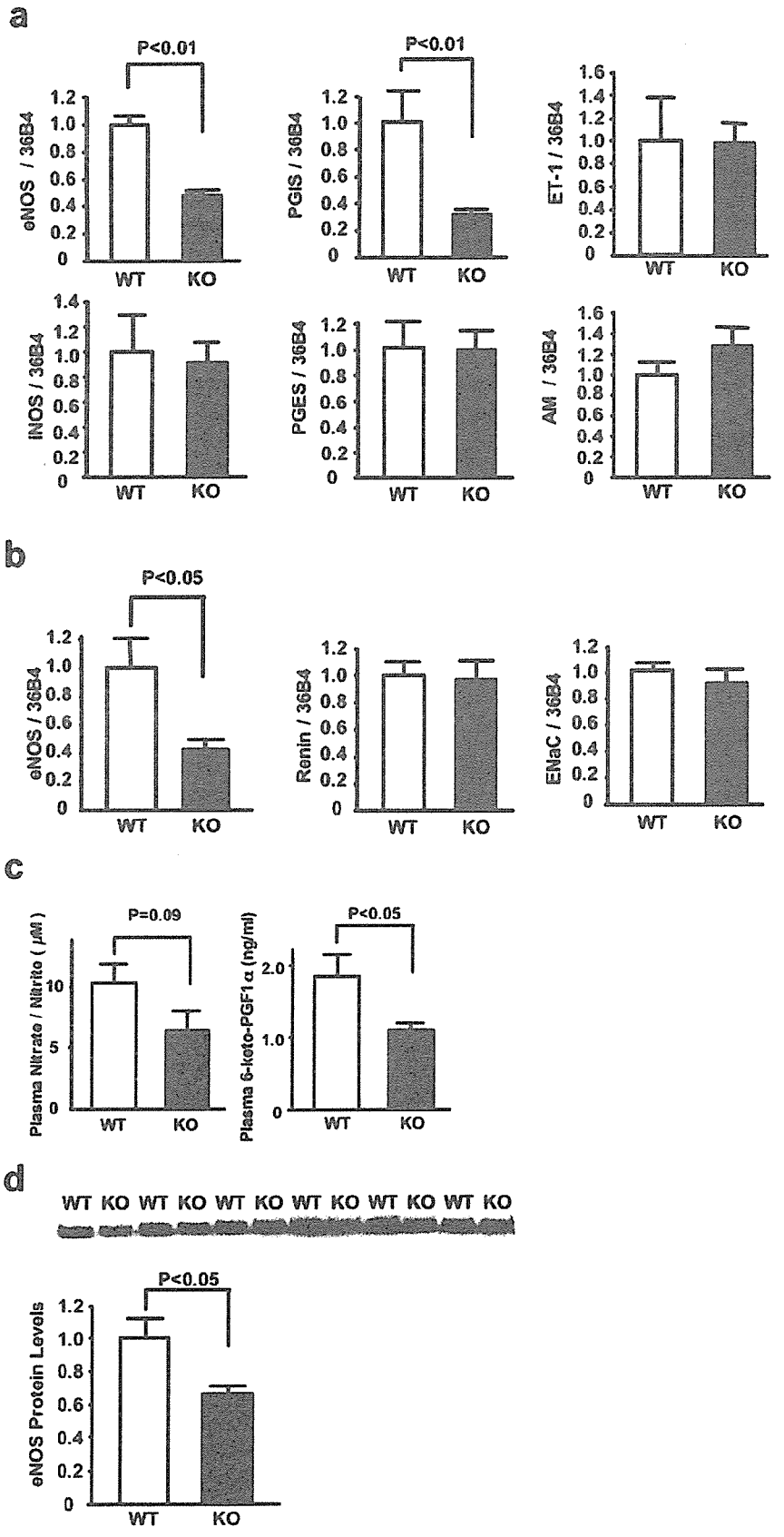

Figure 3. Impaired production of NO and PG I_2 in adiponectin KO mice in response to salt overload. (a) mRNA levels of eNOS, PGIS, inducible NO synthase (iNOS), PG E synthase (PGES), endothelin-1 (ET-1), and adrenomedullin (AM) in aorta of WT (□ n=6) and APN-KO (■ n=6) mice. (b) mRNA levels of eNOS, renin, and epithelial sodium channel (ENaC) in kidney of WT (□ n=6) and APN-KO (■ n=6) mice. (c) Plasma levels of nitrate/nitrite and 6-keto-PGF1α in WT (□ n=8) and APN-KO (■ n=8) mice. (d) Protein levels of eNOS in aorta of WT (□ n=6) and APN-KO (■ n=6) mice. Results are mean±SEM.

PGF1α, representing a PG I_2 metabolite, was significantly lower in KO mice (1.08±0.09 ng/mL) than in WT mice (1.85±0.31 ng/mL; $P<0.05$; Figure 3c). The protein levels of eNOS in aortas were significantly lower in KO mice than in WT mice (n=6 in each group; Figure 3d). The mRNA levels of angiotensinogen and leptin in white adipose tissue and angiotensinogen in liver were not different between WT and KO mice (data not shown).

Adiponectin Adenovirus Ameliorates High-Salt–Induced Hypertension and Modulates eNOS and PGIS mRNA Levels in Aorta of KO Mice

To determine the effect of exogenous adiponectin replenishment, KO and WT mice were treated with Ad-APN or Ad-β gal. After 2 weeks on a high-salt diet, Ad-APN or Ad-β gal was injected intravenously via the tail vein. SBP was measured on days 2, 4, and 6 after injection. On day 7 after injection, adiponectin levels were 10.2±0.7 μg/mL in KO/Ad-APN, not detectable in KO/Ad-β gal, 24.3±0.8 μg/mL in WT/Ad-APN, and 15.8±0.6 μg/mL in WT/Ad-β gal. Ad-APN treatment significantly decreased SBP compared with Ad-β gal control in KO mice on day 6 postinjection (108±1.9 versus 120±1.7 mm Hg; $P<0.01$; Figure 4a), whereas no effects were observed in WT mice under high-salt diet (106±3.3 versus 107±1.9 mm Hg; P value not significant; Figure 4b). In addition, the hypotensive effect of Ad-APN for elevated blood pressure in KO mice was confirmed by direct SBP measurement using indwelling catheters on day 6 after injection (104±1.5 versus 120±2.5 mm Hg; $P<0.01$; Figure 4c). Ad-APN–treated KO mice showed significantly higher eNOS and PGIS mRNA levels in aorta than Ad-β gal–treated KO mice (eNOS; 0.80±0.15 versus 0.31±0.04; $P<0.05$; PGIS; 0.84±0.27 versus 0.26±0.10; $P<0.05$). On the other hand, there were no differences in eNOS and PGIS mRNA levels between Ad-APN- and Ad-β gal–treated WT mice (eNOS; 1.03±0.09 versus 1.00±0.16; P value not significant; PGIS; 1.03±0.14 versus 1.00±0.16; P value not significant; Figure 4d and 4e).

L-NAME Has No Effect on SBP in Adiponectin KO Mice Under High-Salt Diet

To determine the in vivo effects of eNOS inhibition, we next studied the effects of L-NAME on SBP in KO and WT mice

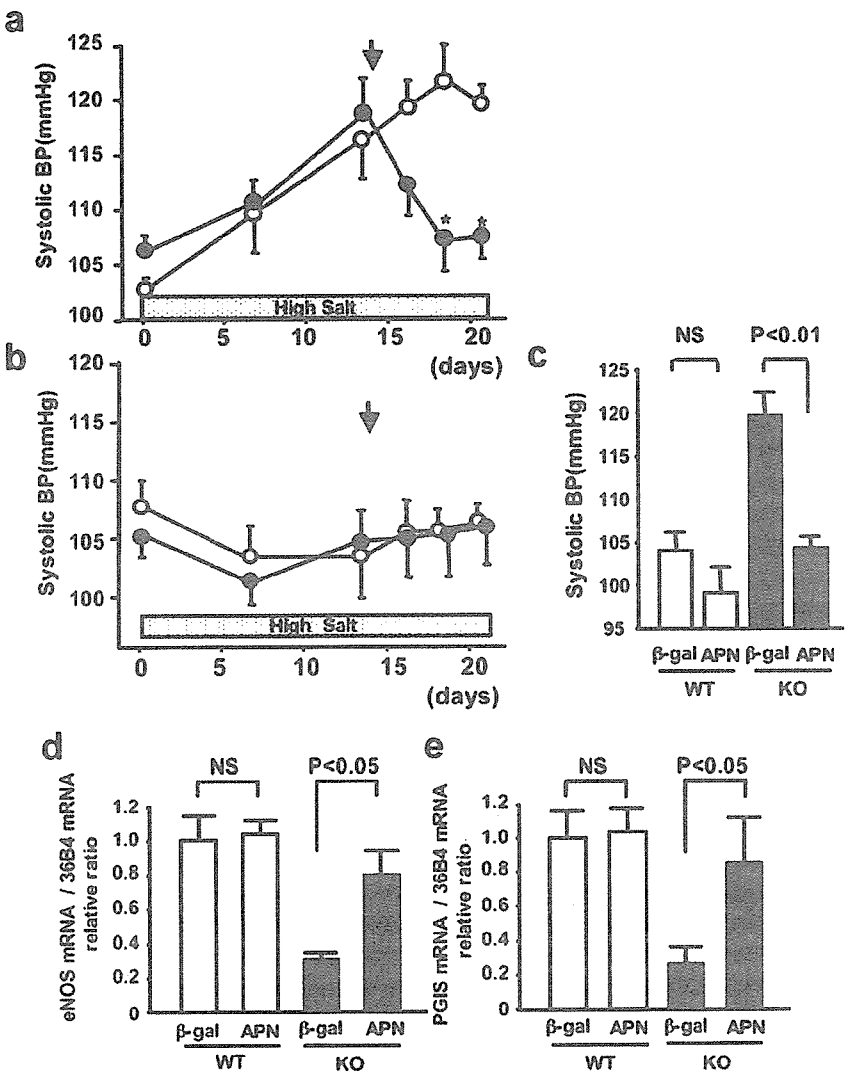

Figure 4. Adenoviral-delivered adiponectin attenuates salt-induced hypertension in APN-KO mice. (a) SBP of KO mice supplemented with Ad-APN (● n=5) or Ad-β gal (○ n=5; *$P<0.05$); (b) SBP of WT mice supplemented with Ad-APN (● n=5) or Ad-β gal (○ n=5). After 2-week feeding of high-salt diet, Ad-APN or Ad-β gal was injected intravenously via the tail vein (arrows). (c) Direct measurement of SBP on day 6 postinjection after 3-week feeding of high-salt diet (d and e). mRNA levels of eNOS and PGIS in vascular walls of WT and KO mice supplemented with Ad-APN or Ad-β gal. Results are mean±SEM.

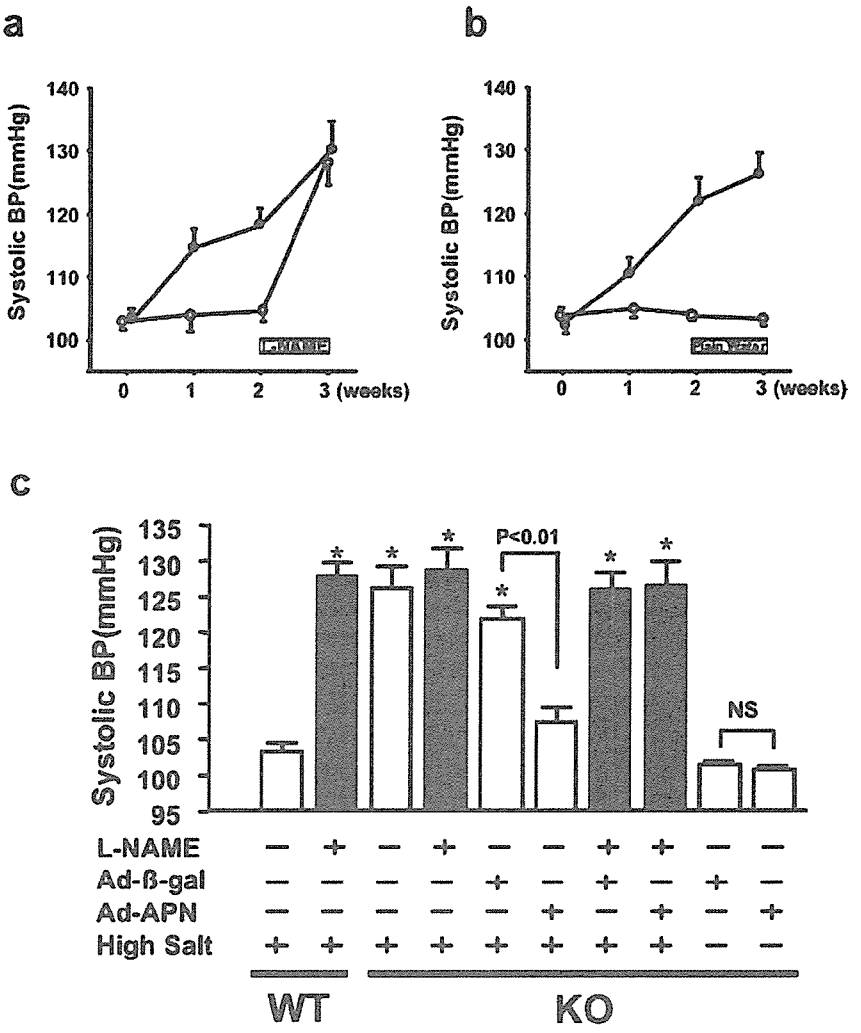

Figure 5. L-NAME had no effect on SBP in APN-KO mice under high-salt diet. SBP of WT (○) and KO (●). Drinking water containing (a) L-NAME (0.25 mg/mL) or (b) plain water was administered to WT and APN-KO mice for 1 week after 2-week salt overload. WT and APN-KO mice were fed high-salt diet during the experimental period; n=5 for each group. (c) Effects of L-NAME, Ad-APN, Ad-β gal, and high-salt diet on SBP; n=5 for each group. *$P<0.01$ vs WT mice without L-NAME under high salt. Results are mean±SEM.

under high-salt diet. One-week administration of L-NAME resulted in a significant rise of SBP in WT mice (130±2.7 versus 103±1.1 mm Hg; $P<0.01$) but did not affect the SBP of adiponectin KO mice (131±3.3 versus 126±3.1 mm Hg; P value not significant; Figure 5a and 5b). To determine whether the salt-fed KO mice developed hypertension through impaired eNOS pathway, KO mice were treated with Ad-APN or Ad-β gal under L-NAME or plain water administration after 2 weeks on a high-salt or normal-salt diet. Plasma adiponectin levels were 25.1±15.5 μg/mL in Ad-APN and not detectable in Ad-β gal. On a normal-salt diet, the SBP of KO mice was similar to that of WT mice, and no difference was observed between Ad-APN and Ad-β gal treatment (102±0.3 versus 102±0.3 mm Hg; P value not significant; Figure 5c). On high-salt diet, the SBP of KO mice was similar to that of L-NAME–treated WT mice (126±3.1 versus 128±1.7 mm Hg; P value not significant) and Ad-APN treatment significantly decreased SBP in KO mice compared with Ad-β gal treatment (108±1.9 versus 122±1.5 mm Hg; $P<0.01$). The effect of Ad-APN in KO mice was abolished under L-NAME administration (126±2.7 versus 127±3.3 mm Hg; P value not significant Figure 5c).

Discussion

The major findings of the present study were the following: (1) adiponectin supplementation reduced the SBP of spontaneously hypertensive obese KKAy mice accompanied by increased levels of plasma NO metabolites; (2) salt-fed adiponectin KO mice developed hypertension, independent of obesity and insulin resistance, accompanied by reduced mRNA levels of eNOS and PGIS in aorta and eNOS in kidney and lower levels of eNOS and PGIS metabolites in plasma than salt-fed WT mice; (3) adenoviral delivery of adiponectin improved the hypertension and reversed the reduced mRNA levels of eNOS and PGIS in aorta of salt-fed KO mice; and (4) L-NAME–induced elevation of blood pressure was not observed in KO mice.

Obesity confers a higher risk of hypertension.[26–28] Recently, numerous reports have demonstrated that dysregulated production of adipocytokines is involved in the pathophysiology of obesity-related disorders.[1–3] The adipocytokine adiponectin has antiatherosclerotic and antidiabetic properties, and the plasma adiponectin levels are significantly low in obese patients, especially those with visceral fat accumulation.[19] Accumulating evidence suggests that visceral fat obesity